STERILIZATION OF MEDICAL PRODUCTS

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Volume V

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Proceedings of the International Kilmer Memorial Conference on the Sterilization of Medical Products

Moscow, Union of Soviet Socialist Republics
September 11-15, 1989

ISBN 0-921317-36-0

Polyscience Publications Inc.
Morin Heights, Canada

Printed in Canada

Organized by:

The U.S.S.R. State Committee for Science & Technology
The U.S.S.R. Ministry of Health
Johnson & Johnson

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Preface

This volume represents the proceedings of the International Kilmer Memorial Conference on the Sterilization of Medical Products held in Moscow, Union of the Soviet Socialist Republics, on September 11-15, 1989. The conference brought together technical specialists and clinical practitioners from 22 countries and was organized with the cooperation of the U.S.S.R. State Committee for Science and Technology and the U.S.S.R. Ministry of Health.

The symposium was the fifth in a series organized as a tribute to Dr. Fred B. Kilmer, the first Director of Research at Johnson & Johnson and an early pioneer in the sterilization of medical products as well as in microbiological control of the environment.

The conference was made up of five sessions devoted to sterilization technologies, standards and basic science. The sixth session, which lasted an entire day, was devoted to the prevention and control of hospital infection and served as a forum for an interchange between U.S.S.R., European and U.S. approaches on this important global subject.

At the concluding banquet, the Kilmer Award was presented to Nina Ramkova of the All-Union Scientific Research Institute for Preventive Toxicology and Disinfection, Ministry of Health, U.S.S.R., for her contributions to the sciences of disinfection and sterilization; and to the International Atomic Energy Agency (IAEA) for advancing the transfer of radiation sterilization technology to developing countries. Accepting the award on behalf of the IAEA was Vitomir Markovic from the Industrial Applications and Chemistry Section.

New Brunswick
New Jersey

R. F. Morrissey

Moscow
U.S.S.R.

Y. I. Prokopenko

Opening Remarks
R.E. Campbell
Vice Chairman,
Johnson & Johnson, U.S.A.

Keynote Address
Lord Butterfield, M.D.,
U.K.

Opening Remarks by R.E. Campbell

Vice-Chairman

Johnson & Johnson, U.S.A.

It is a great privilege for me to have the opportunity to welcome such a distinguished group of scientists to this International Kilmer Memorial Conference on the Sterilization of Medical Products. Among us are many of the world's leading scientific investigators in sterilization and infection control. In all, you are about 300 strong, representing 22 different nations and are here to present papers and discuss advances in improving sterilization processes and technologies.

Speaking for my company, Johnson & Johnson, we are deeply honored to be able to co-sponsor this conference along with the U.S.S.R. Ministry of Health and the U.S.S.R. State Committee for Science and Technology. We thank our colleagues from the U.S.S.R. for hosting this important conference.

The objective during our four days of deliberations here in Moscow is to share technical information related to the health and well-being of people around the globe. There will be special emphasis on the prevention and control of hospital infections through microbial control practices. The science of microbiological control is of fundamental significance to public health throughout the world, so we have an important mission.

This is a non-commercial conference and many of you may be wondering why my company would co-sponsor such an event.

Since its founding more than 100 years ago, Johnson & Johnson has pioneered advances in infection control and sterilization. The development of the first readymade, ready-to-use surgical dressings by our company in the mid-1880s marked the first practical application of the theory of antiseptic wound treatment. The new concept was based on the discoveries of Sir Joseph Lister, the noted English surgeon.

Today, ours is the world's leading health care corporation, with 171 companies in 54 countries. We produce and market a broad range of consumer health care products, prescription pharmaceuticals, and products used by medical professionals.

Johnson & Johnson is highly decentralized; we have a long-term outlook in managing the business and are guided in our everyday business and social responsibility decisions by a Corporate Credo. The Credo is a reminder of our responsibilities to four constituencies: our customers, employees, the communities in which we live and work and our stockholders.

Against that backdrop you can better understand why we are pleased to be able to help provide this forum for the transfer of knowledge about the sterilization of medical products.

In addition, this conference and its stated objective are part of the legacy left to us all by Fred B. Kilmer, Johnson & Johnson's First Scientific Director, a man who served the company for 45 years. Dr. Kilmer's early goal was to awaken medical interest in Lister's findings and he accomplished this by disseminating a compilation of reports by eminent surgeons of the time. The monograph, "Modern Methods of Antiseptic Wound Treatment",

went through five editions by 1893.

Part of Kilmer's other work in the 19th century included the classic article, "Modern Surgical Dressing", published in the *American Journal of Pharmacy*. Much of that article, dealing with microbiological control of the environment and confirmation of the effectiveness of sterilization processes, still is current.

In foreseeing the trend to asepsis, Kilmer wrote: "Chemical sterilization and mechanical cleanliness are among the newer weapons that have been called to the aid of surgery. Antiseptic dressings have been made surgically clean. Antisepsis has not been abandoned, but has developed into its higher form: Asepsis—and antiseptic processes have become aseptic".

This was written at a time when many surgeons were still operating ungloved and in street clothes, in blood-spattered frock coats and with non-sterile instruments. The postoperative mortality rate was as high as 90% in some hospitals.

As a prolific and highly respected writer on scientific and medical subjects, Kilmer influenced the profession's attitude over the years through educational publications such as "Red Cross Notes", begun in 1897; "Red Cross Messenger", launched in 1908; and through "Notes and Abstracts", which he started in 1921. He was a talented writer as well as a distinguished scientist, and he produced numerous papers for professional publications and trade journals throughout his long career.

In memory of Fred Kilmer, this conference series was initiated in 1976 in the United States. Subsequent Kilmer Conferences were held in 1980 in Washington, D.C., in 1982 in Sydney, Australia, and in 1985 in Beijing in the People's Republic of China.

One of Dr. Kilmer's characteristics was his tireless search for new and improved asepsis products and procedures to bring the best possible medical care to all of humankind.

Contemporary challenges include microorganisms that are resistant to sterilization, nosocomial infection control, and the control of AIDS. And we continue to see exciting advances with new chemical sterilization technologies and developments in machine-generated X-rays. All of these, and a host of other related topics such as international regulations, will be discussed during what promises to be an outstanding conference.

Around the world, people have come to expect to live longer and healthier lives. Few scientific meetings are capable of having a direct effect on these expectations. This conference, however, is an exception. You have great potential to positively impact human health and well-being.

You have a very full and important agenda. So without further ado, let me wish you a highly successful conference.

Once again, we deeply appreciate the cooperation of the co-sponsoring USSR Ministry of Health and the U.S.S.R. State Committee for Science and Technology, which have made this conference possible.

Thank you for your kind attention.

KEYNOTE ADDRESS

The Impact of Hospital Infections on Society

Lord Butterfield of Stechford, M.D.

U.K.

Frederick B. Kilmer was born December 11, 1851 and grew up in Pennsylvania, U.S.A., where he attended Wyoming Seminary. After training as a pharmacist in New York and Philadelphia and doing various apprenticeships, Kilmer established himself in 1879 at The Opera House Pharmacy in New Brunswick, New Jersey. There he had two famous visitors: Thomas Edison who came to obtain supplies for his experiments which led to the development of the electric lamp, and Robert Johnson who established the Johnson & Johnson Company in 1886, now world famous for surgical dressings.

Johnson discovered that Kilmer was interested and well informed about Lister's antiseptic surgical techniques to prevent hospital infections after operations. In 1889, when Kilmer was only 39, Johnson appointed him the first Director of the Scientific Department of the Company. Kilmer helped to develop the market for Johnson & Johnson surgical dressings, for which America's great crop—cotton—was used for its absorbency. Following the lead of Koch, Kilmer checked the effectiveness of sterilization using the anthrax *bacillus*.

Kilmer won many honors, including recognition in England through Fellowship of the Royal Society of Arts in London. Clearly Kilmer was a seminal figure in the development of methods aimed at preventing hospital infections and it is appropriate that we honor his memory at these conferences.

At an international meeting such as this, one must stress the importance of infection not only as a cause of human distress but in terms of its economic costs to the community at large. Ways to control hospital infections have been the result of international collaboration over the years based on the exchange of information in much the same way that we are doing here.

The same spirit of international cooperation is still needed today to combat hospital infection, because it remains a major threat to man's health. Hospital infections represent a failure on the part of our medical system and such failures have, from the first, been particularly distressing and serious in teaching centers.

Historical Overview

For example, the classic epidemiological studies of Ignaz Simmelweiss in Vienna

culminated in his 1861 publication of conclusive evidence that puerperal sepsis occurred much more frequently in the hospital wards attended by the students who had just come from the dissecting rooms. Countless mothers' lives could have been saved had the students simply washed their hands. As an interesting side note, Harvard University gave a professorship to Oliver Wendell Holmes who, in 1843, recognized the contagiousness of puerperal fever.

It was the Scotsman, Joseph Lister (1749-1847), who developed and proselytized antiseptic surgical procedures. As the science of microbiology developed, his ideas were assisted through the work of Pasteur (1822-1895) in Paris and by Koch (1843-1910) in Germany. Tribute should also be paid to Metchnikoff (1845-1906) in the U.S.S.R. who made important contributions to our knowledge about how the body's defenses are raised against invading bacteria.

This new knowledge explained the way in which infections were spread and led to radical reorganization of hospitals. In England, Florence Nightingale founded the nursing profession and organized wards on the pavilion basis to isolate infections. She and others strove to refute the widespread opinion, especially among the mothers of sick infants and children in London, that hospitals were dangerous places—to stay away from if possible!

In this century there have been further developments in preventing and treating hospital infections, which have also been called hospital added-infections, cross-infections or nosocomial infections.

Regarding prevention, I should also mention the pioneering work of Leonard Colebrook, for whom I worked as an intern in the Burns Unit at Birmingham Accident Hospital. Using Bourdillon's slit sampler, which drew air from the surrounding ward or dressing station onto a slowly rotating Petrie dish (and was later incubated to show bacteria as cultures), Colebrook demonstrated the very high atmospheric bacteriological contamination that occurred when blankets were shaken or surgical dressings undone. In his Burns Unit, he established the highest achievable standards of barrier nursing, aseptic non-touch techniques for dressing changes, and so on. He was obsessional in his concern that the bandages covered the burns properly to exclude added infections. He used his bacteriological laboratory and bacterial typing to show how infections spread in hospitals. I am proud to have been a collaborator on one of his many publications in 1947.

Another major development which has extended into both the treatment and prevention of infections followed the work of Sir Alexander Fleming, a very close colleague of Colebrook's at St. Mary's Hospital in London. Credit is given to Sir Fleming for the discovery of penicillin. Penicillin was selected for development during World War II as a treatment against gas gangrene caused by anaerobic clostridium spores in soldiers' wounds.

My own interest in burns after World War II arose directly from my work with Colebrook, learning about infections and the delay infection caused in healing and recovery.

During my service in the British Royal Army Medical Corps, I led a small team of young investigators for the Scientific Adviser to the British Army Council, Sir Owen Wansborough-Jones. Our responsibility was to give him advice about injuries from nuclear explosions. Analysis showed that at Hiroshima, burns from the nuclear flash had been the largest single

medical problem, severely aggravated by the added infections which occurred there. To assess the situation, we gave ourselves measured doses of radiant heat to produce flash burns on our arms. From this work, undertaken with E. R. Drake Segar, E. E. Treadwell, and Dr. J. R. B. Dixey, we could calculate the range of flash burns from atomic bomb explosions.

We needed this information to solve the general problem of the total number of days of burn injury after the explosion of an atomic weapon of any size. Figure 1 shows the healing time of burns (vertically) against the range from the burst. The “volume” of the disc indicates the man-days of military service lost. We also calculated that if the burns became infected, the number of days of active service lost would be at least doubled or trebled. Since hands are at greatest risk for flash burns, we undertook trials to see how long it would take to bandage and protect a burned soldier’s hand from added infection. The answer was about 50 minutes per hand.

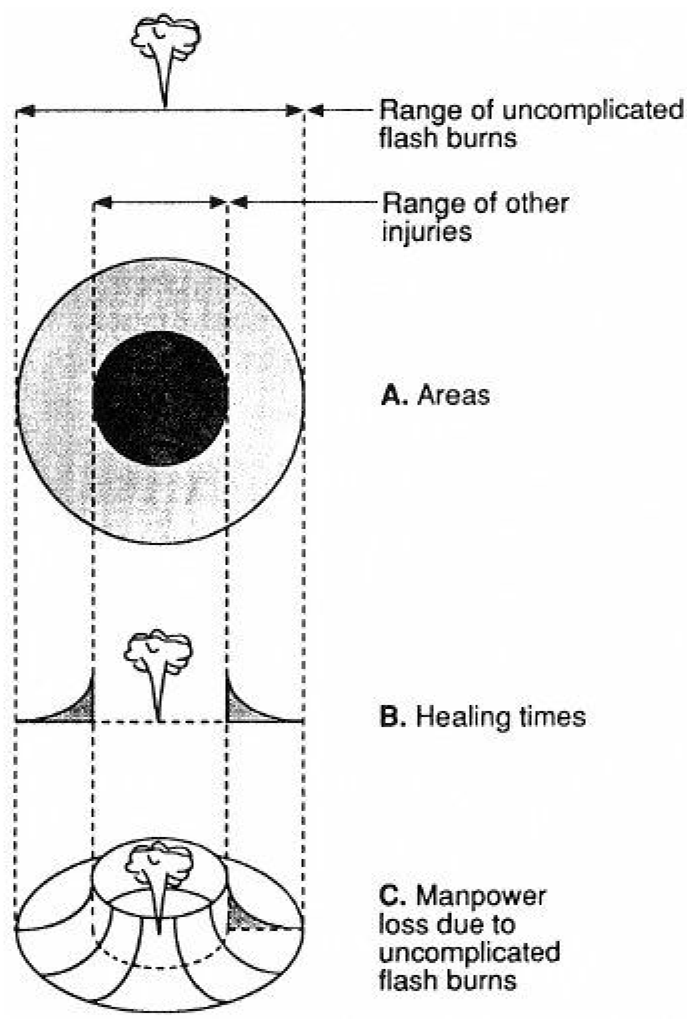

Figure 1. Healing Time of Burns

The total manpower lost in nuclear war was shown to be astronomical. This calculation quickly brought us to hope, even back in the 1950s, that politicians and military leaders would eventually avoid being drawn into nuclear war. I like to believe this experimental information about burning injuries from nuclear weapons played some small part in educating people about the scale of injuries and of the medical treatment needed in nuclear

warfare. Eventually, it was a combined group of Russian and American physicians who, by uniting themselves against nuclear war, won the Nobel Peace Prize. Incidentally, the American side was led by a contemporary of mine at Johns Hopkins Medical School, Dr. Bertram Lowns. Thank goodness, steps are underway, at last, to diminish the threat of nuclear war.

Hospital Infections Today

There is still a major battle to be won against infection in hospitals. Patients entering hospitals for treatment still incur infections. Sadly, the very success of antibiotics may have aggravated these infections, because doctors, nurses, and other workers are now much less afraid of causing infections because “they can be treated”.

As we consider the costs to society of hospital infections incurred by patients, it is necessary to keep uppermost in our minds the central human issue; that is, the risks to the patients themselves, together in certain cases with the risks to the hospital staff.

Policy makers today must consider the costs of medicine to society as a whole. The added costs incurred as a result of hospital infections have been brought into a much sharper relief because the infections extend the duration of hospital treatment. As hospitals are reimbursed in America on a case-cost basis (and may be presently in Britain), and such costs do not include those for added infection, the economic losses associated with hospital infections have attracted increased attention among hospital administrators.

It is necessary at this point to characterize hospital or nosocomial infections. First, what is meant by infection should be defined. Table I shows criteria currently used to define urinary tract infections, pneumonia, surgical wound infections, and bacteremia.

Second, where do nosocomial infections occur? As shown in Table II, the number of cases of nosocomial infections is greater in teaching institutions. This calls into mind the fears of earlier patients concerning the dangers of teaching hospitals in big cities.

Table I. Definition of Infection at Various Sites

Site	Definition
Urine:	100,000 colonies per mL urine
Wound	Pus at incision site
Lung:	Purulent sputum, new lung shadow
Blood:	Positive blood culture
Burn:	100,000 organisms/gram tissue

Mandell G.L, et al., 1985 (6).

Table II. Nosocomial Infection Rates (cases/1000 discharges) U.S.A. (1984)

Hospital Category	Infections/1000 Discharges
Non teaching	22.2
Small teaching	33.8
Large teaching	41.4

Horan, T.C., et al., 1986 (3).

However, before we put the blame on student nurses and medical students, we should note two very important factors. Nosocomial infections occur in those special services which are likely to be found in the big teaching hospitals, such as the intensive care units, the renal dialysis units, the burn units and the cancer treatment units. Teaching hospital centers are also the places to which difficult and debilitated cases are often referred. In my opinion, it is the seriousness of the illnesses of the patients referred to teaching hospitals which is responsible for nosocomial infections today.

Where do nosocomial infections arise? Urinary tract infections take the lead (Table III). Before antibiotics, physicians were very cautious about catheterizing the bladder. Now it has become a routine practice in elderly and incontinent patients. The advantages to the hospital of bladder catheterization are less urinary incontinence, less nursing support, less laundry and less odor on the wards. But a substantial proportion of patients pay the price, namely by becoming infected.

Table III. Estimates of Number of Nosocomial Infections in Various Acute Care U.S. Hospitals (1975-76)

Urinary Tract Infection	902,732
Surgical Wound Infection	510,402
Pneumonia	226,968
Bacteremia	102,950
All Sites	2,148,485

Haley, R.W., et al. 1985 (1).

The next most common nosocomial infection sites are surgical wounds. Such infections are likely caused by poor surgical techniques, contaminated air in the operating theater, and long-lasting operations which are aggravated by the patients advanced age, debilitated condition and/or poor hygiene when changing dressings. May I pay tribute to the investigations showing the importance of cleansing the hands in reducing infections undertaken by a senior member of Johnson & Johnson, Dr. Hildick-Smith.

The next most common nosocomial infection is pneumonia. Just as the elderly patient sitting in bed tends to develop urinary stasis and infection, so the post-operative case, especially the elderly patient who has difficulty clearing the pulmonary tree with coughing, gets bronchopneumonia.

Long-term intravenous catheterization creates a portal of entry for infection. The discomfort of the metal needles used 40 or 50 years ago meant that intravenous infusion was terminated as soon as possible. Now, the much more comfortable plastic catheters mean that intravenous drips for antibiotics, parental feeding, access to blood for investigations and so on, are used for a longer period of time. The risks of invasion are, thus, aggravated, particularly when there is a cut down to the vein, especially in the femoral region.

There are, of course, other sites for infections, too. Where do patients with nosocomial infections accumulate in our hospitals? Perhaps, surprisingly, they can be found on the intensive care wards. For example, in 1987 in Padua, Italy, 26.9% of the patients suffering hospital infections were from the intensive care ward. You can also find high risks of hospital infection on renal dialysis units and on wards where there is immunosuppressive

treatment for cancer or transplantation surgery. In either circumstance, additional superinfection by *Candida albicans* can cause devastating effects. It should also not be forgotten that hospital infections arise on burn wards.

The most frequent pathogens isolated in patients from the U.S.A. with hospital infections is summarized in Table IV. Infecting organisms and their relative sensitivity to antibiotics vary greatly from country to country and from hospital to hospital, so the identity and rank ordering of pathogens listed in Table IV may not necessarily be applicable elsewhere. The list of organisms is, nevertheless, formidable.

Table IV. Most Frequent Pathogens in 29,562 Isolates, U.S.A. (1984)

Pathogen	%
Escherichia coli	17.8
Pseudomonas aeruginosa	11.4
Enterococci	10.4
Staphylococcus aureus	10.3
Klebsiella spp	7.4
Coagulase negative *staphylococcus*	6.3
Enterobacter spp	5.9
Candida spp	5.5
Proteus spp	5.1
Remainder	19.9

Horan, T.C., et al. 1986 (4).

More important is the antibiotic sensitivities of the infecting pathogens (Table V). Equally important is the way antibiotic sensitivities are changing. An inevitable consequence of this is the need for the continual development of new antibiotics to ensure that clinicians are armed with the necessary weapons to defend their patients, particularly the infirm and elderly, against invading bacteria. This is true whether the patients are hospitalized or not. Of course, this implies higher costs for treatment in the future simply because of the rising expenses of research and development for new antibiotic drugs.

Table V. Antimicrobial Resistance of *Staphylococcus aureus* (%), U.S.A. (1984)

Hospital	Mathicillin	Gentamycin	Clindamycin	Chloramphenicol	Erythromycin
Non-teaching	6.0	8.6	9.1	5.4	11.9
Large teaching	11.3	10.6	10.4	7.2	18.0

Horan, T.C., et al. 1986 (5).

Economic Consequences of Hospital Infections

This leads to a discussion of the overall economic consideration of nosocomial infections. While not forgetting that individual suffering and extra burdens on the hospital staffs underlie all the statistics, what are the financial costs of hospital infections to society as a whole? Specifically, what has been added to hospital costs by prolonged hospital stay, extra tests, additional treatments and increased staff time? Early approaches simply studied the cases

of infections in hospitals at a given time and thereby determined the prevalence of infections and expressed this as a percentage of the cases in the hospital. Another, and more accurate approach, looked at the incidence of hospital infections in large numbers of hospital cases. Prevalence studies probably bias the finding in favor of the infected cases because they have longer hospital stays. Prevalence studies suggested that hospital infections cause an extra 13 days of hospitalization. Incidence studies suggested that such infections extended hospital stays by about a week.

A more scientific approach would be to establish a group of patients in whom infections had arisen and match them for all known variables (i.e., sex, age, etc.), with a group of patients who have not suffered hospital infections. Comparative costs could then be determined.

In the end, the results of all three forms of analysis—prevalence, incidence or comparative—will ultimately give the same answer, which is simply that the cost of hospital infections is far too high.

The incidence of hospital infections has been estimated to be about five per 100 admissions. Prevalence studies in 47 hospitals in various countries under the auspices of a WHO investigation, suggest a range of 3%-21%, with a median prevalence of 8.4% (Table VI).

Table VI. Rates of Hospital Infections

Year	Study	Prevalence
1985	Ortona et al.	6.75%
1987	Manderova et al.	6.6%
1988	WHO, 14 countries	8.4%
1988	Sramova et al.	6.0%

Ortona, L., et al., 1985 (9).
Manderova, E., et al., 1987 (7).
Mayon White, R.T., et al., 1988 (8).
Sramova, H., et al., 1988 (12).

The extra days spent hospitalized because of hospital infection have ranged in various studies from five to 40 for serious long-term urinary tract infections.

Bearing all these variables in mind, it is difficult to make national or international comparisons of the costs of individual hospital infection cases. Such attempts are also complicated by changing exchange rates and variations in price inflations over the years. But in Boston, Massachusetts, the cost of nosocomial infection in 1984 was estimated as $375 per case. The overall average costs in both hospitals and nursing homes for the United States was calculated to be $1,800 (Table VII).

Table VII. Estimated Costs of Hospital Infections, U.S.A. Per Case, 1985 $'s

Urinary Tract	$ 600
Surgical Wound	$ 2,700
Pneumonia	$ 4,900
Bacteremia	$ 3,100

All Cases	$ 1,800

Haley, R.W. et al., 1987 (2).

That does not seem an unreasonable figure when we look at estimates of the extra days spent in the hospital due to the various types of nosocomial infection. In American hospitals, urinary tract infections caused an extra 15 days, surgical wound infections an extra six days, pneumonia an extra six days, and bacteremia an extra seven days; this would be in line with costs of about $250-$300 per day in 1985.

When we get into estimates of the national costs, the figures become alarming (Table VIII). A 1986 estimate of the costs associated with nosocomial urinary tract infections in the United States alone suggested the total cost to be $1.8 billion per year. Another estimate published in 1987 suggested the range of cost for all nosocomial infections was $5-10 billion a year. Recent publications in the United Kingdom suggest figures of £36-76 million per year. This British figure may appear less than the cost in America but, in fact, it is in the same general range considering the high cost of medical services in America compared to Britain, the fact that the American population is over four times as large, and the rate of exchange.

Let me reemphasize, these statistics do not consider patients' suffering. For example, in an eight year study of 32,284 surgical cases in Minneapolis, only 0.3% developed surgical abscesses, however, even with the availability of antibiotics, a quarter of these patients died. We must not become too confident that we can control hospital infections and we must pay proper attention to the patient's anxiety and suffering.

Table VIII. Total National Costs of Nosocomial Infections

		All Infections	
U.S.A.	1985	$5-10 billion	(Wenzel)
U.K.	1986	£76 million	(Taylor)
		Urinary Tract Cases	
U.S.A.	1986	$1.8 billion	(Rutledge & McDonald)

Wenzel, R.P., 1985 (14).
Taylor, L., 1986 (13).
Rutledge, K.A., et al., 1986 (11).

Conclusions

What should we be doing to minimize these significant burdens of suffering and cost? We certainly must protect our nurses and hospital personnel from the consequences of hospital infections, particularly those working in the dangerous sectors. Just as our staff, particularly our young staff, risked tuberculosis in the past, so Hepatitis B is now a serious concern. Both doctors and nurses may need immunization, especially those working on dialysis units. Extra care is needed when disposing of needles in hospitals. The dreadful epidemic of AIDS among drug addicts who share needles underscores this hazard. We have recently learned how poor hospital infection control lies behind AIDS epidemics among

nursing mothers and children, first in Elista (55 cases) and then through the movement of a single AIDS infected child to Volograd (22 cases).

From the patients' point of view, attempts to reduce nosocomial infections have led some surgeons to use prophylactic antibiotic cover during operations which involve exposing the peritoneum to bowel contents. Table IX shows some savings estimates for this procedure in surgical circumstances in France.

Table IX. Use of Prophylactic Cefoxitone in Major Surgery of Upper GI Tract, France

Control Cases	–	80% infected	–	cost 1,002 French Francs
Treated Cases	–	15% infected	–	cost 470 French Francs

Renauld Salts, J.L., et al., 1985 (10).

The best way forward involves "Perestroyka"—the establishment of Infection Control Committees in every hospital under a leading microbiologist, helped by a senior nurse. If hospital infections do occur, savings can also be achieved by a proper, inexpensive, antibiotic plan, which uses oral treatment to allow the patient to be discharged from the hospital. Such plans must be agreed to by the hospital's Infection Control Committee and only in this way will they become accepted. The vigilance initiated by Holmes, Simmelweiss and Lister must not end yet. We must appreciate the ongoing battle with respect to hospital infections demands a general appreciation of the incredible costs of such infections in terms of lives, illnesses, and resources and their prevention.

The most inexpensive way to reduce the costs associated with nosocomial infections is to ensure that our nurses, doctors, and ancillary staff recognize the importance of these infections.

Let me end by saying new developments do not take away the need for vigilance about old problems, especially problems as general as infections in hospitals.

Thank you.

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Session I
Basic Science/New Technology
Chairman: Y. I. Prokopenko, M.D.

Ministry of Health, U.S.S.R. All-Union Scientific Research Institute for Preventive Toxicology and Disinfection

Modern Trends in Sterilization Science

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Since the preceding speaker, Lord Butterfield, effectively disclosed the extent to which hospital infections adversely impact society, we should make a detailed analysis of those factors inside the hospital which make infections possible. The problem of hospital infections is not a new one and a large number of researchers have already dealt with it. The present international conference on sterilization, although discussing a rather narrow area, seems to be ultimately aimed at combating hospital infection, or at least at resolving one important aspect of this problem.

Experience shows that hospital infections are, epidemiologically, still infections in which a decisive role is played by the “microbial factor” in the environment. To interrupt the pathways by which infection is carried via objects in the environment is a problem which can be solved by the decontamination approach, and which is vital for the prevention of hospital infections.

Within the discussed context, the source of infection may not always be identified. Sometimes the initial contamination of an object, such as a medical device, may take place far removed and much earlier than the very moment it comes in contact with a patient. Here, an important consideration is the process the item has been subjected to: disinfection, presterilization cleaning, and sterilization. It is also possible that the source of the infection comes from secondary contamination via the microbes present in the hospital environment (e.g., air, appliances, personnel, etc.). In this instance, the source of infection is close to the patient and in a position to widely disseminate microbe cells. No doubt, the greatest success in preventing infections will be associated with the identification and elimination of such an infection source. However, experience shows that in practice this is a difficult task. That is why valid decontamination of hospital environment objects is essential, since this would result in the lowest possible risk of secondary contamination of sterile devices and equipment.

The decontamination of hospital environments is a problem of different levels of disinfection, including antisepsis. Thus, in preventing hospital infections, the efficiency of both sterilization and disinfection processes may be important. The impact of any disinfection procedure on the probability of infectious disease outbreak will vary by individual case and will need to be considered in the risk assessment analysis.

The conference agenda includes issues of both a general and specific nature. Taking advantage of this opportunity, I should like to propose that three more issues be discussed. These are significant for the problem of the decontamination of environmental objects, in particular in the context of the hospital environment.

The first of these issues is of a general nature and concerns the problem of unity among the viewpoints that scientists have on different levels of a single process of

decontamination. Differences may be found in the quantification of the degree of decontamination and in the qualitative composition of microbial flora. Our experience, along with other associates of our Institute, with the impact of various sterilization processes on different microbes is summarized in Table I. I'd like to point out that the data in this table should not be regarded as absolute.

From left to right are listed different decontamination levels, starting with the mildest one —hygienic cleaning with a cleanser—and ending with the most stringent type, sterilization. This set includes, in an advancing way, hygienic cleansing (with a detergent/disinfectant) and skin antiseptics (resulting in hygienic disinfection of hands with a decontamination level of 99.9% and the level of sterile samples of over 50%). Then follow two disinfection levels: medium and high. These include decontamination of not only pathogenic spore formers, but also other species of pathogenic microbes. Moreover, for a high-level disinfection, all the pathogens must be eliminated, including spore-producing microbes. Sterilization may also be level-differentiated: from 10^{-6} to 10^{-3}, i.e., a cell may germinate in one sterilized unit out of every 10^6 or 10^3 units treated.

The second issue refers to the resistance of microbes to chemicals, and studies of the laws governing the shaping of cell resistance in the environment as well as mechanisms of resistance. Kinetics of decontamination and microbe mortality when exposed to different disinfectants under laboratory conditions indicates that a monoculture is heterogeneous concerning cell resistance. The microbe mortality kinetics may be plotted as a time relationship. There are cells (as a rule, most of them) which are killed in the very first minutes of the impact. However, there is a definite subset which only die after a prolonged period of time. It is this subset that is particularly important, since there is a high probability of survival of microbe cells in the environment. In practice, we have to deal with these microbe cells when disinfecting hospital environments, although in laboratory experiments the bactericidal effects are estimated for the entire cell population in the monoculture.

The experience gained shows that the so-called "residual contamination" is accounted for by the cells which have a higher resistance to chemicals than laboratory strains do. As we see it, there are two possible ways of shaping a higher resistance of the residual contamination cells: selection and induction through the genetic apparatus. The mechanism of inducing a higher cell resistance to certain chemical bactericides remains to be studied.

Our epidemiological observations of the effectiveness of preventive disinfection during a quarantine for hepatitis A in nursery schools showed that 10%-15% of samples tested positive for intestinal bacilli. For this value of residual contamination, the focal infection index was 4.0. Introduction of current permanent disinfection procedures based on the application of both chemical and physical methods decreased the percentage of positive tests ten-fold, i.e., down to 1.2%-1.5%. This residual contamination level corresponded to a focal infection level of 0.5. This example points to the significance of residual contamination and, consequently, the increased resistance of microbes to disinfectants.

Table I. The Levels of Decreasing Microbial Availability After Disinfection and Sterilization

Pathogen	Hygienic Cleansing		Skin Antiseptics		Disinfection		Sterilization
	Detergent	Detergent-Disinfectant	Hygienic Hand Dis-Infection	Surgical Hand Treatment	Medium Level	High Level	
Bacteria	80%-90%	99.99%	70%-99.9%	99.9% and 50% sterile samples	99.99%-100%	100%	1 survivor out of 10^6 items (for some items, out of 10^5-10^3 items, as the American standard allows)
Viruses					99.99%-100%	100%	
Fungi					99.99%-100%	100%	
Spore Forming Pathogens							
Spore Forming Saprophytes							

The third issue to be discussed is the resultant toxicity of materials which have been chemically treated to ensure sterility. Heat-resistant materials such as rubber and plastics are often sterilized with chemicals. However, these very materials are characterized by an ability to interact with chemicals, such as ethylene oxide and formaldehyde, resulting in the production of fragile complexes. Both ethylene oxide and formaldehyde are fairly toxic in low concentrations, even with a single exposure. The only way the material may be aerated is by degassing which takes some time. Many items in medical use are designed in such a way that it is practically impossible to effect a complete degassing. The only practical approach is to evaluate the risk of the residual chemical concentration. It is important to note that most of the methods of risk evaluation designed for and used in environmental toxicology are not applicable in this case, since they have been oriented to multiple impacts. In our case, risk is caused by a single impact or a series of impacts restricted by time limits (the period of medical treatment).

Virtually all the materials which have been sterilized using chemicals come into direct contact with the body fluids, primarily with the blood. In these cases, chemicals avoid detoxication barriers they would naturally encounter if they entered the organism via the air, drinking water, food, or through the skin. It may be assumed that in the case of parenteral application, the mechanisms surrounding liver detoxication, such as microsomal oxidases, may be activated. However, this will constitute a secondary effect after the chemical has interacted with the target tissue. This issue, as well as other sterilization-related issues of toxicology, deserve a detailed discussion.

Finally, one cannot help but discuss issues related to further development of sterilization methods, both conventional and novel. Such issues are the topics of a number of presentations. All the sterilization methods applied in medicine may be divided into two groups: a) heat sterilization, b) cold sterilization. The first group includes pressurized steam and dry hot air methods. The second group represents methods using radiation and chemicals. It is common knowledge that the simplest, most reliable, and cost-effective method of sterilization used in hospitals and clinics is heat. Research has shown that heat sterilization still has potential for development. This method's cost-effectiveness may be substantially improved by the use of microcomputers in up-to-date steam sterilizers, enabling sterilization to be conducted in a strictly predetermined way. An important

approach to updating heat sterilization, and especially steam sterilizers, is the design of the process schemes for specific homogeneous (with regard to basic materials) characteristics. This results in benefits such as less steam being spent, lower electricity losses, better preservation of sterilized items, etc. Depending upon the characteristics of sterilized materials (capillary-porous, nonporous, composite) there may be different time and heat parameters associated with steam sterilization. Recently there has been an increase in the range of temperatures used in many countries, from 105°C to 141°C. In serial-type steam sterilizers so-called “speed” schemes have been applied, using a high steam temperature (138°C to 141°C) and efficient pulsating air pumping. Sterilization time is in the order of 1 to 7 minutes. Such schemes have important implications in the hospital setting with respect to urgent surgeries, including those made under emergency conditions.

With respect to sterilization based on ethylene oxide and formaldehyde, it should be emphasized that, in spite of the fact that these methods have been regarded as “classical”, there is still potential for improvement. Such improvements include the use of microcomputers to enable a significant optimization in the sterilization process and the choice of either ethylene oxide or formaldehyde.

Solutions of sporicidal chemicals occupy a particular place in cold sterilization methods. Among the most commonly used are a 6% solution of hydrogen peroxide, acetic acid-based preparations, and a 2.5% solution of glutaraldehyde. Although the above chemicals are active sporicides, they have certain limitations, including corrosion effects, high residual toxicity and personnel toxicity, and a lack of special-purpose equipment for handling the solutions. New sporicides should be actively sought and new preparations should be developed.

A number of presentations will discuss the issue of radiation sterilization. I would like to dwell upon a few aspects related to this issue. One of them is a tendency to reduce the radiation dose while at the same time ensuring adequate sterilization. Toward this goal, industries producing sterile items should ensure both minimal contamination of the items being sterilized and an absence of highly resistant microbe species. This may be possible only under conditions of clean, from a microbiological point of view, production lines. (In such a case the radiation dose may be as low as 20 kGy, or even lower).

The effective dose of radiation may also be reduced when radiation is used in combination with other physical or chemical factors exerting bactericidal effects. However, these questions require extensive study and technological developments.

Finally, I cannot help mentioning some areas of novel development undertaken in several countries, including the U.S.S.R., that have potential applications for medical sterilization. These include low-temperature plasmas, particularly in combination with some gaseous chemical substances, super-high frequency radiation, and laser methods. Some of these methods may be upgraded to the level of industrial applications in the near future.

Acknowledgment

I would like to acknowledge gratefully the assistance of the Institute associates, Prof. N.F. Sokolova, Dr. K.A. Troshin, and Dr. L.G. Panteleeva, in the preparation of this

presentation.

Fundamental Aspects of Microbial Resistance to Chemical and Physical Agents

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Introduction

Microorganisms show a wide divergence to chemical and physical agents. 'Slow viruses' or prions are the most resistant microorganisms to biocides and to various sterilization methods. Other highly resistant organisms include bacterial spores, with Coccidia and acid-fast bacteria having above-average resistance to disinfectants (Table I) (39).

Progress towards understanding the reasons for different responses has been slow and steady rather than spectacular. It is the purpose of this paper to examine the fundamental aspects of microbial resistance to some important biocides and physical sterilization processes and to suggest ways in which additional information may be obtained. Some biocides, e.g., ethylene oxide and glutaraldehyde, are important chemosterilizers (5). Other biocides, used in combination with a physical process, are part of potentially important sterilization procedures, e.g., low-temperature steam with formaldehyde (LTSF) and hydrogen peroxide with ultraviolet radiation (18, 60, 61).

Resistance is well-defined when antibiotics and other chemotherapeutic agents are being considered. When applied to biocides and physical processes, however, it is less clearly defined. Some authors prefer to use 'resistance' and 'resistant' where there is a clear genetic association or basis, and recommend the alternative use of 'tolerance' and 'tolerate' when a genetic basis for insusceptibility has not been established. In this paper, however, the common practice with chemical and physical agents has been adopted (resistant/resistance), and is used to denote a bacterial or other organism that is not susceptible to a concentration of biocide (or the dose of a physical process) used in practice. In addition, these terms are also used to denote a microbial strain or species that is not killed or inhibited by a concentration of biocide (or the dose of a physical process) that kills or inhibits the majority of that particular organism.

Bacterial Spores

Bacterial spores are the most resistant bacteria to biocides and most physical processes (Table I). Some biocides are sporicidal, but for a sporicidal effect, much higher concentrations are necessary for longer periods of time than is needed for a bactericidal action against non-sporing bacteria (45, 46).

Table I. Comparative Responses of Microorganisms to Chemical and Physical Agents

Microorganism	Sensitivity or Resistance	Comments

Bacteria	Non-sporing bacteria are most susceptible	*Pseudomonas aeruginosa* may show high resistance, e.g., to QACs
	Acid-fast bacteria are more resistant to chemical and physical agents	
	Bacterial spores are most resistant to biocides	*Deinococcus radioduran* is the most resistant organism to UV and ionizing radiations
Fungi	Fungal spores may be resistant to biocides	Rather less resistant than acid-fast bacteria
Viruses	Non-enveloped viruses more resistant than enveloped forms to biocides	Prions are most resistant of all types to chemical and physical agents
Parasites	Coccidia may be highly resistant to biocides	Similar to bacterial spores

Bacterial spores are complex structural entities (Figure 1). Surrounding the spore core (protoplast) is the cortex around which are one or two spore coats. In some types of spores, an exosporium is present, but may surround just one dense spore coat. Located in the protoplast is the RNA, the DNA and most of the calcium, potassium, manganese, and phosphorus which is present in the spore. Also present is a substantial amount of low molecular weight (MW) basic proteins that are degraded rapidly during germination (50). The cortex consists largely of peptidoglycan, and a dense inner layer (cortical membrane) develops into the cell wall of the emergent cell when the cortex is degraded during germination. The inner (IFSM) and outer (OFSM) forespore membranes become the cytoplasmic membranes of the germinating spore or persist in the spore integuments, respectively. The spore coats consist mainly of protein.

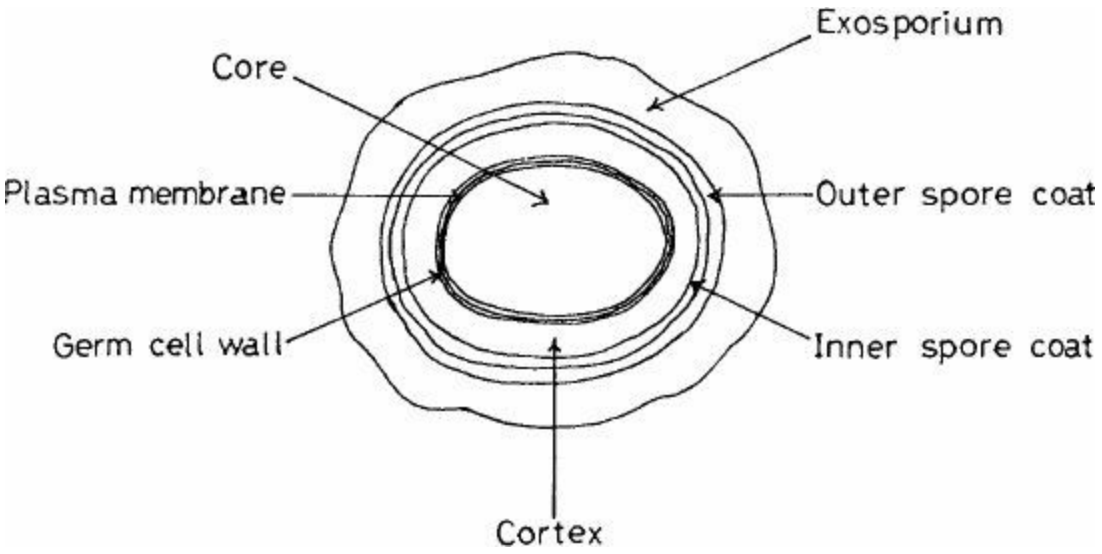

Figure 1. Structure of a 'typical' bacterial spore.

Effect of Biocides

The comparatively high resistance of spores to many biocides (Table II) is undoubtedly related to the presence of the spore coat(s) and possibly also to the cortex. Support for

this contention comes from several lines of research (42-46, 60, 61):

— a comparison of wild-type and various sporulation (Spo^-) mutants of the *Bacillus subtilis* strain 168 (38, 51);

— development of resistance of wild-type strains during sporulation (Figure 2A, 2B) and association with specific structural changes in the cells;

— a comparison of normal and coat-less spores; and

— the use of conditional cortex-less (Dap^-) mutants of *B. sphaericus* in which the amount of cortex depends on the exogenous concentration of diaminopimelic acid (Dap) (26).

Table II. Mechanisms of Bacterial Spore Resistance to Biocides

Antibacterial Agents	Spore Component Associated with Resistance
Alkali (NaOH)	Cortex
Octanol*	Cortex
Xylene*	Cortex
Lysozyme	Spore coat(s)
Chlorine compounds (hypochlorites, chlorine dioxide)	Spore coat(s)
Glutaraldehyde	Spore coat(s)
Iodine	Spore coat(s)
Ozone	Spore coat(s)
Hydrogen peroxide	Spore coat(s)
Chlorhexidine	Spore coat(s)
Ethylene oxide +	Spore coat(s)?

* Based on studies with Dap^- Mutants

\+ Conflicting data reported

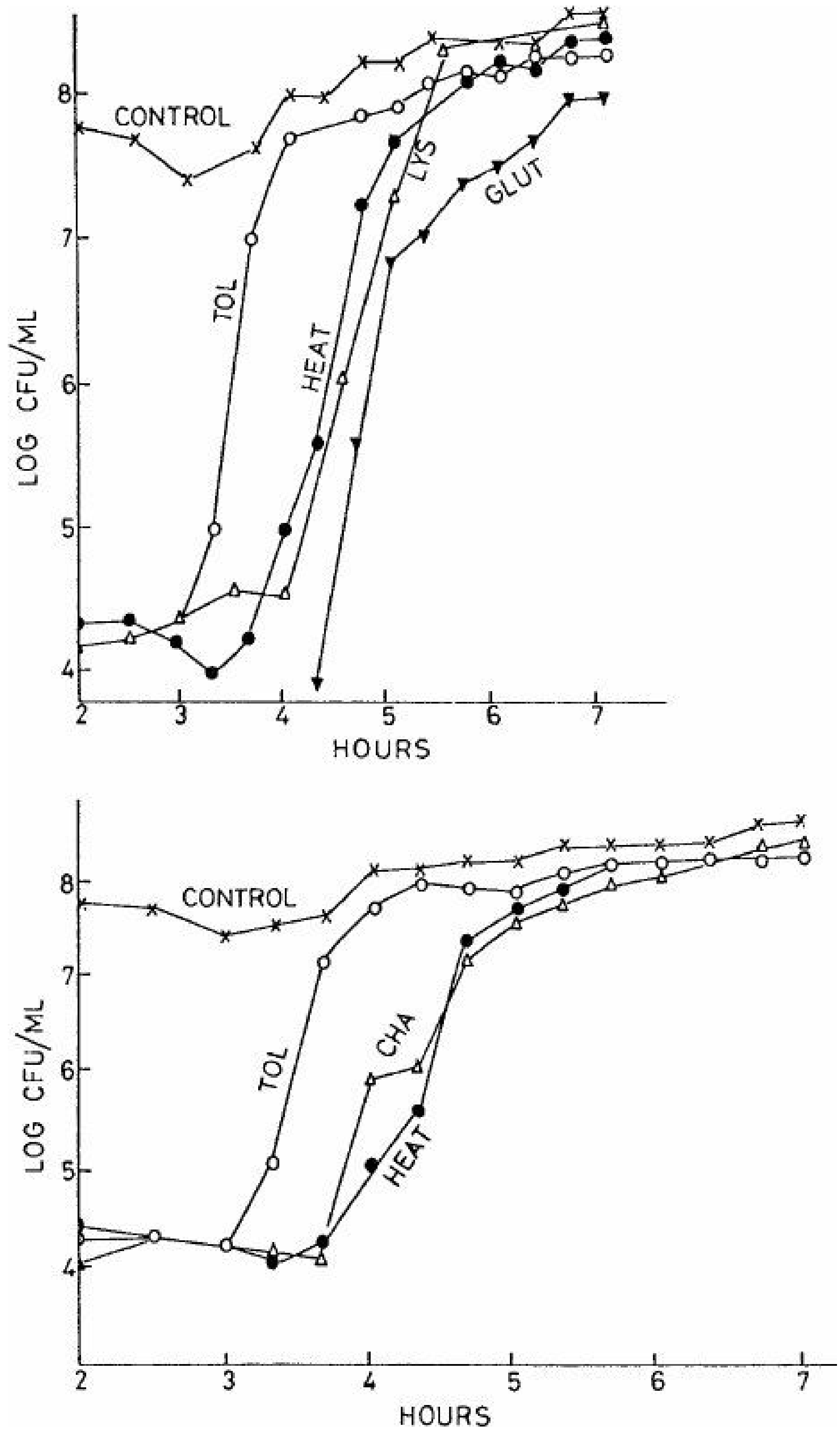

Figure 2. Development of resistance of *B. subtilis* 168 during sporulation (38, 51). Tol, toluene; CHA, chlorhexidine diacetate; Lys, lysozyme; Glut, glutaraldehyde

On the basis of these approaches, the following conclusions may be reached: resistance to toluene (not a true antibacterial agent, but a useful marker) is an early event in sporulation. Resistance to chlorhexidine develops just before the onset of heat resistance, whereas resistance to lysozyme, especially glutaraldehyde, develops at a late stage.

Jenkinson (28) proposed that lysozyme resistance was related to the synthesis of specific high MW spore coat proteins. It is unlikely that glutaraldehyde resistance can be ascribed to the same or a similar spore coat protein because the dialdehyde molecule is highly reactive and interacts strongly with amino groups in different types of protein (38). Resistance to ozone, lysozyme, hypochlorites, glutaraldehyde, hydrogen peroxide, iodine, and chlorhexidine is associated with spore coats. These offer better protection in some spores than others, e.g., in *Clostridium bifermentans*, the spore coats present a protective barrier against hydrogen peroxide but are much less effective in conferring resistance in *B. cereus*. A possible reason for this is the different coat composition found in different species, although the exact basis has not been elucidated. Reasons for the resistance of spores to ethylene oxide remain unsolved; removal of sporecoats has been claimed to decrease resistance in *B. subtilis* but not in *B. cereus* T, and mutant strains of the former have been described with defective coats but have increased resistance to the gaseous disinfectant (45,46).

The cortex may be implicated in bacterial spore resistance to octanol and xylene (26) although this conclusion is based on studies with Dap^- mutants of *B. sphaericus* and other changes in the cortex were not taken into account (60).

Effects of Physical Processes

Bacterial spores are inactivated by high temperatures and by high doses of ionizing or ultraviolet (UV) radiation. UV light is only slowly lethal to spores and thus is not considered to be a sterilization process. Examples of inactivation curves that are produced can be found in Figure 3.

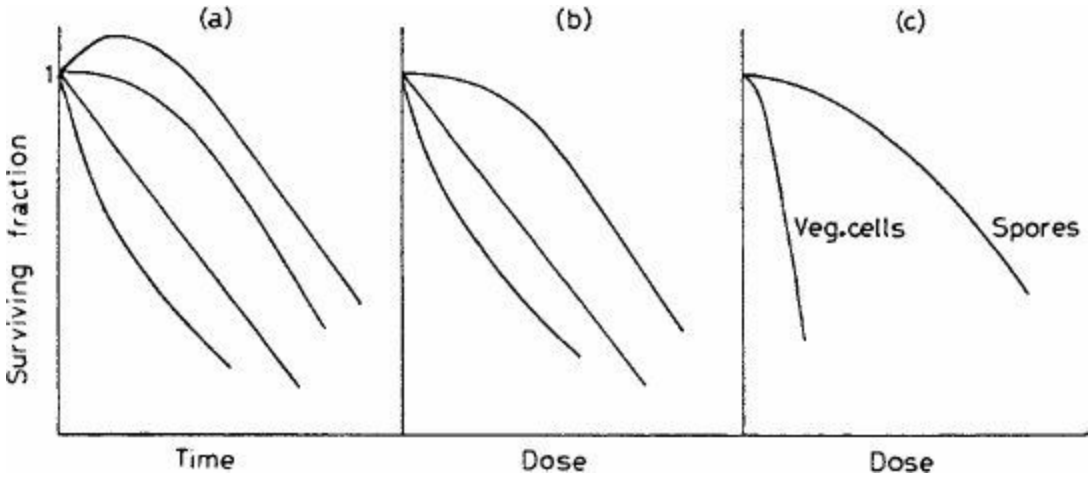

Figure 3. Inactivation curves of bacterial spores exposed to (a) moist heat, (b) ionizing radiation, (c) ultraviolet (UV) radiation.

Moist Heat

Most attention has been devoted to understanding the nature of spore resistance to moist heat. As pointed out by Lindsay et al. (30), heat resistance varies between and within

species and depends upon several factors, in particular the sporulation, heating, and recovery conditions. The exact mechanism of heat resistance remains unclear, but there is little doubt that it is associated with the water content of the core (20, 62, 63).

Various theories have been put forward in an attempt to explain the phenomenon. These generally have been of two types:

1. Partial dehydration of the spore coat. Support for this concept has come from the contractile cortex theory (involving contraction to maintain the lowered water content) and the expanded cortex theory (in which partial dehydration results by expansion of the cortex against the spore coats) leading to the osmoregulatory cortex theory. In this theory, the loosely cross-linked polymer is envisaged as being osmotically active (22). In the anisotropic cortex theory (63), it is proposed that dehydration of the core arises from cortical tension; here, layers parallel to the surface are in tension so that the swelling pressure is confined to a radial, as opposed to a uniform, direction.

Drying produces large increases in the heat stability of many non-spore proteins, and spore enzymes may be stabilized by partial dehydration.

2. General molecular stabilization of core components. This theory propounds that such stabilization results from molecular rearrangements in the core, e.g., the interaction of the specific spore component dipicolinic acid (DPA), in the form of its calcium chelate (CaDPA), with DNA and possibly, proteins (6). DPA and CaDPA affect the mobility and thermal stability (melting temperature) of nucleic acids consistent with DNA being in the A-form inside spores instead of the more normal B-form. Furthermore, studies with ultraviolet radiation have demonstrated the close proximity of DPA and DNA inside the spore coat. Because the amount of DPA/CaDPA inside the spore is much greater than the amount of RNA and DNA, it has been proposed that any uncomplexed DPA/CaDPA could stabilize enzymes and proteins (30).

Gerhardt and his colleagues (2-4, 20) have attributed the thermoresistance of spores to three main physicochemical factors that affect the protoplast (Table III). These are (a) dehydration; (b) mineralization, i.e., heat resistance decreasing with H-form spores (demineralized) and regained with Ca-form (remineralized); and (c) thermal adaptation, since spores of thermophilic species are more heat resistant than those of mesophilic or psychrophilic species. Spores of a given species are more thermoresistant when grown at maximum temperatures than when grown at optimal or minimal temperatures (2, 20). These three factors contribute to heat resistance, but the predominating factor is undoubtedly dehydration. Spores retain their heat resistance when the coats (and exosporium, if present) are removed; however, further removal of the cortex produces protoplasts that are unstable and heat-sensitive. A similar response occurs when spores are pretreated with sublethal concentrations of chlorine, which is believed to damage the cortex.

Low pH spores are more sensitive to high temperatures (acid-heat treatment). A reason for this decreased resistance may be the protonation of cortex peptidoglycan with a consequent fall in its osmotic effectiveness. These data support the contention that maintenance of the integrity of the cortex is essential for maintaining the thermoresistance of the spore. This is achieved by the cortex exerting pressure on the spore core thereby stabilizing its partly dry interior. The exact mechanism whereby the low water content of the

core is achieved and maintained has yet to be solved.

Dry Heat

The water content of spores is an important factor in determining inactivation by dry heat. Only a relatively small amount of water is needed to protect the heat-sensitive site and resistance to dry heat depends mainly on the location of water in the spore and on its association with other molecules. Presumably, molecular stabilization can also occur as described above.

Ionizing Radiation

Ionizing radiation strips electrons from the atoms of the material through which they pass. Ionizations occur principally in water, resulting in the formation of short-lived, but highly reactive hydroxyl (OH^-) radicals and protons (H^+). Damage to DNA is brought about by OH radicals which are responsible for inducing strand breakage. It is a process that has been widely used in medicine and is one method for sterilizing pharmaceutical and medical products. This process is currently being examined for its possible use in sterilizing or decontaminating certain foods.

Ionizing radiation is highly lethal to most organisms including bacterial spores. However, exotoxins are more resistant and higher radiation doses are needed to inactivate them than is needed to kill spores. Several other factors influence activity, notably the presence of oxygen during irradiation (spores are less sensitive under anoxic conditions) (42), the stage of sporulation, and pre-irradiation treatment.

Ionizing radiations induce single strand (SSB) and double strand (DSB) breaks in microbial DNA that, unless repaired, are likely to inhibit DNA synthesis or cause some error in protein synthesis, leading to cell death (34). Although not the only target, DNA is the principal one and the state of DNA in the cell is important. Several possible reasons (Table III) have been proposed for the higher resistance of spores than of non-sporing bacteria:

— the presence of a radioprotective substance (none has been found);

— protection conferred by the spore coats (coat-less spores are not more sensitive);

— state of DNA in spores. DNA in spores exists in the A-form associated with a low MW value and a partially dehydrated spore core. This type of DNA in the intact spore is more resistant to SSB and DSB than DNA existing in the natural B-form in intact vegetative cells (34). DNA extracted from spores, however, shows the same response *in vitro* to ionizing radiation as DNA extracted from non-sporing bacteria. DPA and CaDPA have been shown to stabilize DNA inside spore cores, and it is possible that this could contribute towards spore resistance; and,

— repair of damage. SSB in bacterial spores can be repaired during post-irradiation germination. It is claimed that, in the radiation-resistant *Cl. botulinum* 33A, direct rejoining of SSB occurs during or after radiation in spores existing under non-physiological conditions at 0°C and in the absence of germination (34). Such repair in these dormant ungerminated

spores may result from DNA ligase activity.

Table III. Mechanisms of Bacterial Spore Resistance to Physical Processes

Physical Process	Mechanism(s) of Spore Resistance
Moist heat	Dehydration of core (most important factor) Mineralization Thermal adaptation
Dry heat	Location of water and possible association with other molecules
Ionizing radiation	A-form DNA (more resistant to SSB and DSB) Repair of damage
Ultraviolet radiation	Genetically-controlled repair mechanisms ('spore repair' and excision repair)

Ultraviolet Radiation

UV radiation does not possess enough energy to eject an electron to produce an ion, although there is an alteration of electrons within their orbits. As such, it is a less effective process than ionizing radiation. Bacterial spores are generally more resistant to UV radiation than non-sporing bacteria (Table III; **cf**. Table I).

Exposure of non-sporing bacteria to UV light results in the formation of purine and pyrimidine dimers between adjacent molecules in the same strand of DNA (Figure 4A, forward reaction). Other types of photoproducts are found in *D. radiodurans* (Figure 4B) and in bacterial spores (Figure 4C). Unless removed, these photoproducts form non-coding lesions in DNA and death results. The spore photoproduct TDHT (5-thyminyl-5,6-dihydrothymine; Figure 4C) is identical to a product that accumulates in DNA exposed dry, or as a frozen solution, to UV radiation. UV-sensitive mutants of UV-resistant *B. subtilis* spores form the same photoproduct (TDHT) and to the same extent for a given radiation dose. It therefore follows that UV resistance is associated with the ability, and UV sensitivity with the failure, to remove TDHT.

Two genetically-controlled repair mechanisms have been described for this removal (Table III). The 'spore repair' mechanism involves the elimination of TDHT during germination, although vegetative cell growth is not required. In excision repair, TDHT disappears slowly from the large molecular weight trichloracetic acid (TCA)—insoluble fraction and appears in the TCA-soluble fraction (Figure 5B).

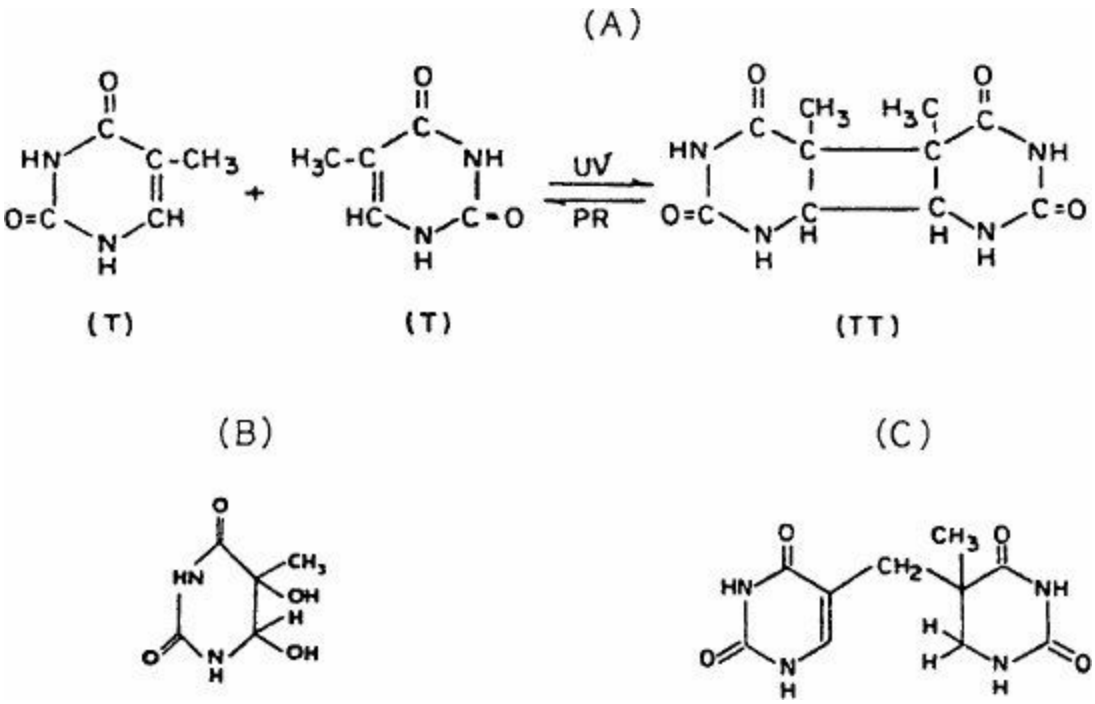

Figure 4. Ultraviolet (UV) radiation-induced photoproducts in sporing and non-sporing bacteria.

(a) Thymine dimer formation (forward reaction) in non-sporing bacteria, and photoreactivation (PR, reverse reaction)
(b) 5,6-dihydroxydihydrothymine in *D. radiodurans*
(c) 5-thyminyl-5,6-dihydrothymine (TDHT, spore photoproduct) in bacterial spores

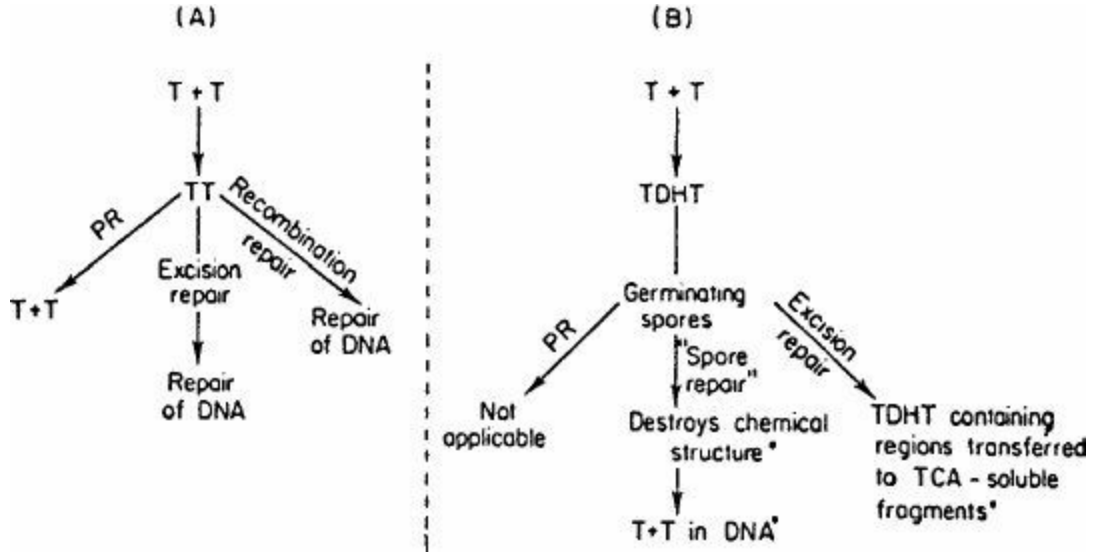

Figure 5. Repair of ultraviolet (UV) radiation-induced damage in (A) non-sporing bacteria, (B) bacterial spores.

T, thymine; TT, thymine dimer; PR, photoreactivation; TDHT, 5-thyminyl-5,6-dihydrothymine; TCA, trichloracetic acid.

Non-Sporing Bacteria

Considerable variation exists between the susceptibility of different non-sporulating bacteria to biocides. These organisms are generally not heat-resistant, although some are less sensitive to physical processes than others. A notable example is *D. radiodurans* and

ionizing and UV radiation. Non-sporing bacteria will be discussed in the context of mycobacteria, other Gram-positive organisms, and Gram-negative bacteria.

Mycobacteria

Mycobacteria are generally more resistant to biocides than other non-sporing bacteria (15, 41). Mycobacteria possess a high cell wall lipid content and their resistance appears to be related to the amount of waxy material present (41). Mycobacterial walls contain several components (37): (a) a covalent skeleton comprising two covalently linked polymers, peptidoglycan and an arabinogalactam mycolate; (b) free lipids which can be removed by neutral solvents; and (c) peptides which are removable by proteolytic enzymes.

Within the mycobacterial group, however, there are wide responses to biocides (15). Quaternary ammonium compounds (QACs), chlorhexidine, and dyes are inhibitory to *Mycobacterium tuberculosis* and to other mycobacteria, but are not tuberculocidal. Alcohols, formaldehyde-alcohol, iodine-alcohol, formaldehyde, and probably glutaraldehyde (10) are tuberculocidal. Another significant pathogen, particularly with AIDS patients, is the *M. avium intracellular* group (MAIS group). These organisms have a greater resistance to glutaraldehyde than *M. tuberculosis* (11, 24). *M. gordonae* is also highly resistant (10) whereas *M. marinum, M. smegmatis*, and *M. fortuitum* are highly susceptible (10). *M. terrae* and *M. tuberculosis* have a similar susceptibility towards disinfectants and because the former organism is relatively fast-growing with a low pathogenic potential, it has been recommended that the *M. terrae* be used for determining the tuberculocidal activity of new products (59).

Reasons for the differing resistances to biocides are unknown. Variations in response of the particular organism to a specified disinfectant have been reported (1, 9), and it is possible that differences in culture history could be associated with these variations. Differences in cell wall composition, and hence altered wall permeability, could be a primary reason for the responses of various mycobacteria, but a true assessment of the mechanisms involved must await the results of more fundamental studies.

Other Gram-positive Bacteria

Gram-positive bacteria such as staphylococci and streptococci are generally considered to be susceptible to chemical and physical agents. By virtue of its truly phenomenal ability to repair damage to DNA, *D. radiodurans* is considerably less sensitive to ionizing and UV radiations than other non-sporing (and indeed, sporing) bacteria. This organism is more than 30 times as resistant as *Escherichia coli* B/r to UV light and more than 50 times as resistant to ionizing radiations (34). Repair of UV-induced damage involves photoreactivation and dark repair mechanisms.

The light-induced photoreactivation repair mechanism is present in most, but not all (e.g., *D. radiodurans*), wild-type bacteria and is error-free since it does not cause mutation. Excision repair is a multi-enzyme process that involves removal (Figure 5A) of UV-induced pyrimidine dimers (Figure 4A). Post-replication recombination repair of UV damage works

synergistically with excision repair in bacteria that are exposed to UV radiation during exponential growth (34). A further repair mechanism is induced error-prone repair linked to the SOS response and increased mutagenesis.

The highly radiation-resistant organism *D. radiodurans* is able to tolerate large numbers of double strand breaks and can tolerate doses of ionizing radiation that produce up to 240 double strand breaks per genome. The mechanism whereby the organism achieves repair of this DNA damage is unknown.

Staphylococci are sensitive to most biocides. It must be noted, however, that the low minimal inhibitory concentrations (MICs) observed with QACs do not reflect a rapid bactericidal effect. Problems have recently been encountered with methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Some of these are resistant to QACs and to other nucleic acid-binding (NAB) agents (21, 32). The determinant for the resistance has been physically mapped on pSK57, a penicillinase plasmid detected in MRSA strains. Plasmid-bearing MRSA strains are more resistant to QACs than are plasmid-free MRSA strains, and this QAC resistance can be transferred to the latter. Two resistant genes are involved, one specifying resistance to diamidines (e.g., pentamidine isethionate), and the other to other NAB agents (e.g., QACs, acridines, ethidium bromide). By means of recombinant DNA techniques, both determinants have been cloned in *E. coli*. The manner whereby resistance to the most important of these agents (QACs) is expressed is not fully elucidated. However, resistance to ethidium bromide results from a diminished uptake by resistant cells, caused by an efflux system whereby this agent is extruded from the cell interior. A similar mechanism may be possible for QAC resistance (Table IV).

Table IV. Mechanisms of Resistance of Methicillin-resistant *S. aureus* (MRSA) Strains to Biocides[+]

Chemical*	Mechanism(s) of Resistance of MRSA Strains
Ethidium bromide	Efflux
Acidines	Efflux
QACs	Efflux (but unusually low-level resistance)
Dibromopropamidine isethionate	Efflux?
Chlorhexidine	Efflux? (low-level resistance only)

* Ethidium bromide is not used as a biocide and the acidines rarely used as such

[+] *E. coli* strains carrying recombinant plasmids (with genetic material from *S. aureus* resistance plasmids) are more resistant to all the above chemicals than are isogenic plasmid-less strains

It must be pointed out that the level of QAC resistance found to date is not high, MRSA strains being about four times less sensitive to QACs (or, in some cases, to chlorhexidine) than methicillin-sensitive (MSSA) strains. Thus, clinical problems have not, as yet, arisen by employing these biocides.

Plasmid-mediated resistance to inorganic mercury salts and to organomercurials have also been described in *S. aureus* (see Table V).

Gram-negative Bacteria

Mechanisms of resistance of Gram-negative bacteria to biocides are less well understood than are those to antibiotics, but can be considered as being either natural (innate, intrinsic) or acquired (Figure 6). Resistance to heat, ionizing and UV radiations does not appear to be a problem.

Intrinsic resistance to biocides is associated mainly with the outer membrane of Gram-negative bacteria (Table VI). The cell surface of smooth strains is hydrophilic in nature; deep rough (heptoseless) mutants, on the other hand, tend to be much more hydrophobic. Thus, wild-type (smooth) strains are most resistant to hydrophilic agents (23, 35, 47, 48). A distinction must be drawn between low MW hydrophilic molecules (< 600) which can readily enter the cell via the aqueous porins, and hydrophobic molecules which diffuse across the outer membrane bilayer. In wild-type bacteria, intact lipopolysaccharide (LPS) molecules prevent ready access of hydrophobic molecules to the cell interior, probably by shielding phospholipid molecules. In deep rough strains (which lack the O-specific side chain and most of the core polysaccharide of the wild-type LPS) and in EDTA-treated strains, patches of phospholipid appear at the cell surface, with the possibility that the head-groups are oriented towards the exterior and the side-chains towards the interior. A third pathway of entry has been proposed for cationic bactericides (23). QACs and biguanides, e.g., chlorhexidine, both interact with phospholipids and LPS, and it has been suggested that these biocides damage the outer membrane promoting their own uptake. There are, however, significant differences in cellular responses to these agents; whereas chlorhexidine is almost equally effective against wild-type and deep rough mutants, QACs are considerably more active against the former. Contamination of QAC solutions with Gram-negative rods can, therefore, be a hazard (49).

Table V. Role of Plasmids in Bacterial Resistance to Biocides

Biocide	Plasmid-specified Resistance in[+]	Biochemical Mechanism of Resistance
Ag^{+} salts	*E. coli, Salmonella typhimurium*	Reduced silver uptake
Inorganic Hg^{2+} compounds	Several types of bacteria	Mercuric reductase
Organomercurials	E. coli, S. aureus, P. aeruginosa	Hydrolase and reductase enzyme systems
Formaldehyde and formaldehyde-reducing agents	*Serratia marcescens*	Possibilities: — surface changes in cells — aldehyde metabolism
Hexachlorophane	*P. aeruginosa*	Unknown

* Plasmid-mediated resistance to various anions and cations is also known. Since these are rarely used as antibacterial agents, they are not considered here.

[+] For information on MRSA strains, see Table IV.

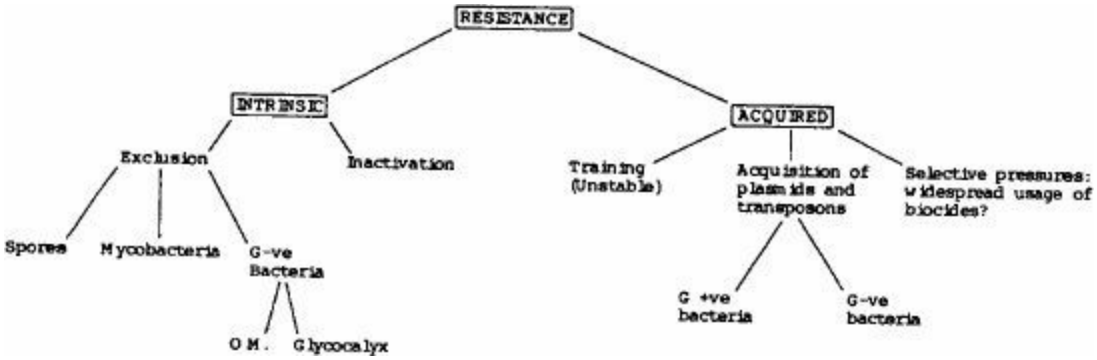

Figure 6. Resistance mechanisms of non-sporing bacteria to biocides.

Table VI. Intrinsic Resistance of Gram-negative Bacteria to Biocides: Pathways of Biocides Entry into Bacteria[+]

Pathway of entry	Application*
Hydrophilic route	Low MW (< *ca*. 600) hydrophilic biocides; hydrophobic molecules excluded
Hydrophobic route	LPS processes underlying PL in wild-type cells; in envelope mutants with defective LPS, entry of hydrophobic biocides
Self-promoted entry	Damage to outer membrane by biocides promotes its own entry into cell[+]

* LPS, lipopolysaccharide; PL, phospholipid

\+ Chlorhexidine might act in this manner, but with other cationic bactericides (quaternary ammonium compounds and diamidines) LPS appears to act as an important barrier conferring intrinsic resistance in wild-type cells.

The interaction of bacteria with surfaces is initially reversible and eventually irreversible, leading to the production of a continuous biofilm on the colonized surface. Bacteria within such a biofilm reside in a specific microenvironment that differs from cells grown in batch culture under ordinary laboratory conditions (14).

Bacteria within biofilms are much more resistant to biocides than are cells in batch-type culture. There are two possible reasons for this phenomenon: physiological changes in the cell or penetration barriers presented by the exopolysaccharide matrix (glycocalyx). The latter seems more likely and is responsible for the resistance noted to several biocides (14). Subculture of cells *in vitro* results in loss of the glycocalyx and reimposition of biocides activity (Figure 7).

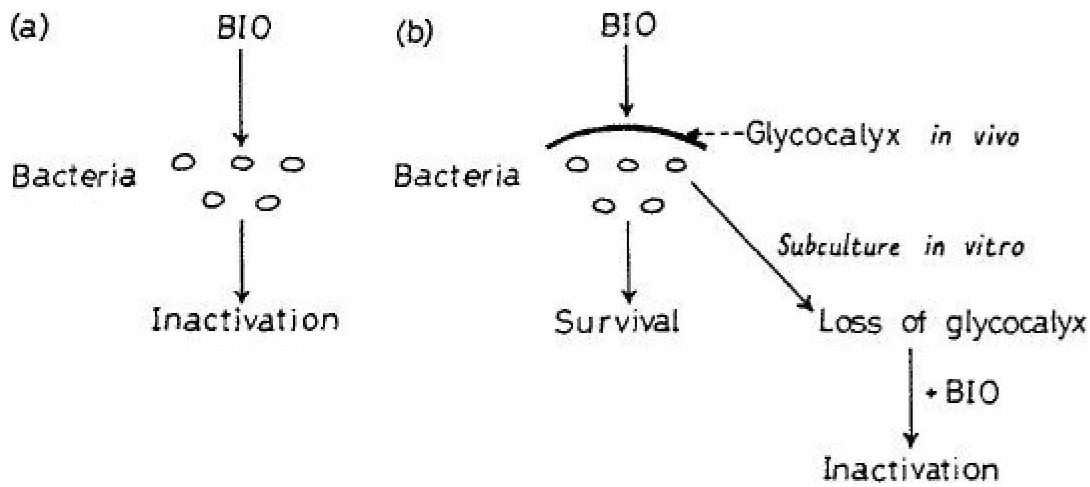

Figure 7. Glycocalyx formation and resistance to biocides

(a) No glycocalyx and bacterial inactivation by biocide
(b) Glycocalyx formation and bacterial survival on exposure to biocide
(c) Subculture *in vitro* from (b) and bacterial inaction by biocide

Acquired resistance (Figure 6) results from genetic changes in a cell and arises either by mutation or by the acquisition of genetic material from another cell, e.g., by conjugation. The development of resistant mutants by selective procedures is not an area in which many studies have been made with biocides. Resistance to chlorhexidine can be readily developed in some bacterial strains but may or may not be stable when the cells are transferred into chlorhexidine-free media. Likewise, chloroxylenol-resistant strains of *P. aeruginosa* have been prepared by repeated subculture in media containing gradually increasing concentrations of the xylenol, but the cells remained sensitive to a combination of chloroxylenol plus EDTA (47, 48). Acquired resistance involving genetic exchange is potentially more important by far. Plasmid-encoded resistance to antibiotics is well documented, but there have been comparatively few studies made with the specific aim of determining the role of plasmids in bacterial resistance to biocides (44) apart from heavy metals, such as organic and inorganic mercury compounds (53), and formaldehyde (25).

Mercury resistance is plasmid-born, not chromosomal, and may be transferred from donor to recipient cells by conjugation or transduction. Plasmids conferring resistance (Table V) are either (a) ‘narrow-spectrum’, responsible for resistance to Hg^{2+} (by reduction to, and vaporization of, metallic mercury) and a small number of organomercurials; or (b) ‘broad-spectrum’, specifying resistance to all compounds in (a) and to additional organomercurials, including the important preservatives phenylmercuric nitrate (or acetate) and thimerosal.

Plasmid-determined resistance to Ag^{+} is also known, a point of potential significance in hospital infections where silver nitrate and silver sulphadiazine (AgSu) have been used topically for preventing infection in severe burns. It is often difficult, however, to transfer silver resistance from Ag^{R} to Ag^{S} strains.

Plasmid-mediated resistance to biocides is not unknown. A correlation exists between formaldehyde resistance and specific activity of aldehyde dehydrogenase in *Pseudomonas* spp (25), and resistance to formaldehyde is associated with resistance to formaldehyde-

releasing agents. The aldehyde dehydrogenase is constitutively present in high concentrations in formaldehyde-resistant bacteria. Nevertheless, although formaldehyde resistance in *S. marcescens* and *P. putida* is plasmid-mediated and transferable, it is unlikely to depend solely on aldehyde dehydrogenase since there are also changes in outer membrane proteins in the resistant cells.

Current knowledge suggests that plasmids are not generally involved in the resistance of Gram-negative bacteria to biocides and that such resistance is intrinsic and not transferable (44). The possibility exists that resistance to antibiotics is linked to biocides resistance (47).

Molds and Yeasts

Fungi are ubiquitous, eucaryotic microorganisms, relatively few are capable of causing human diseases (13), and they show considerable diversity in size and morphology (17). Diseases (mycoses) caused by fungi are basically of three types: superficial, subcutaneous, and systemic. Some systemic fungal pathogens such as *Coccidioides immitis* and *Histoplasma capsulatum* are highly pathogenic, whereas others, e.g., *Candida albicans* and *Aspergillus fumigatus*, are opportunistic pathogens. *Candida* species are commonly found as human commensals (36), and other yeasts causing human infections include *Rhodotorula glutinis* and *Trichosporon pullulan* (52). Some yeasts and molds are significant because they can cause spoilage of food and pharmaceutical and cosmetic products.

Many biocides show both antibacterial and antifungal activity, although the latter effect may be fungistatic rather than fungicidal (16, 33). Compounds with antifungal activity include aldehydes, phenolics (notably the halogenated members), QACs, chlorhexidine, organomercury derivatives, parabens, and organic acids. Fungal spores are, however, often resistant to chemical disinfectants. Very little modern work has been undertaken to understand the route(s) of entry of biocides into fungal cells. In 1969, Miller (33) published a paper on mechanisms for reaching the site of action of fungitoxicants in fungal conidia. Most of the toxicants studied were metals, and much of the discussion centered around the role of the cell membrane, the factors influencing the efficiency of toxicants in reaching their site of action, and the nature of that site. The possible barrier role of the cell wall was dealt with briefly. More modern techniques and the possible employment of resistant and hypersensitive mutants could lead to useful information about the role of different cell wall components in conferring intrinsic resistance and about ways of overcoming this. For example, recent studies (64) have demonstrated that yeasts grown in the presence of benzoic acid tolerate higher benzoic acid concentrations, possibly because of reduced permeability to the acid.

Viruses

Effects of Chemical Agents

The effects of biocides on bacteria are reasonably well understood. Activity against

viruses, on the other hand, is less well documented, the results are often confusing, sometimes conflicting, and the mechanisms of viral responses are usually unclear. Some important recent studies by Thurman and Gerba (57, 58) address this problem. There remains, however, the question of prions and their high resistance both to chemical and physical agents.

Viruses are basically simple acellular particles that consist of a protein coat (capsid) surrounding either RNA or DNA. Disinfection may be accomplished by (a) permanently immobilizing virus particles on a cell surface; (b) blocking or destroying host cell receptors on the virus; or (c) inactivating RNA or DNA within the capsid, in each case with the intention of preventing viral reproduction in a susceptible host cell (57). Factors influencing disinfectant activity include type of virus, type of disinfectant, period of contact, presence of organic matter, and the nature of the target site, i.e., nucleic acid in some instances, and protein in others (55). The inactivation cures that are usually observed are depicted in Figure 8 (55): 'A' denotes an exponential rate of inactivation; in 'B' the presence of small percentages of viral aggregates that are more resistant to inactivation is believed to be responsible for the tailing effect; whereas the biphasic response in 'C' is considered to be the result of large percentages of viral aggregates. Subsequently, these are dispersed into individual virons or smaller aggregates that are more susceptible to the disinfectant. Curve D shows a rapid initial inactivation. Some biocides induce structural changes in virons (56). Partly because of their complexity and interaction with the environment, bacteria are considered to be more easily inactivated than viruses (57), which, in contrast, show no metabolic processes outside a host cell. Furthermore, it is likely that the complex interaction that occurs between one type of virus and a biocide does not necessarily occur with another viral type.

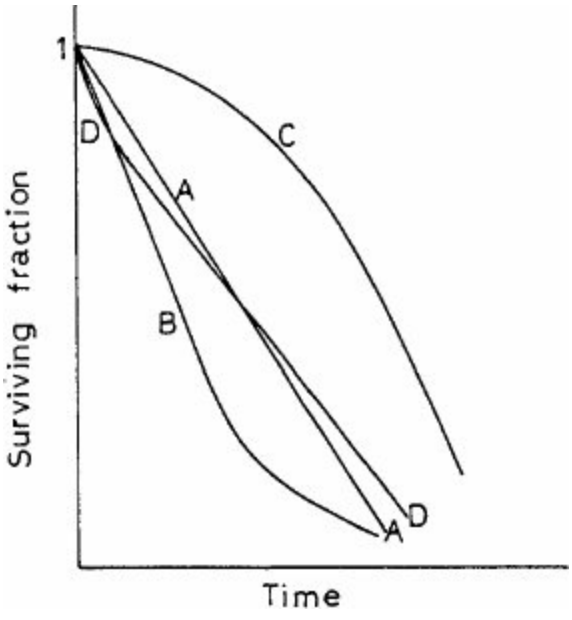

Figure 8. Typical survival curves depicting the inactivation of viruses by biocides.

(A) exponential (ideal first order reaction)
(B) shoulder followed by exponential inactivation
(C) tailing effect
(D) rapid inviral effect

Some animal viruses, e.g., rotaviruses, can withstand many commercially-available disinfectants, and only a limited number of chemical agents are effective against human rotaviruses (31, 54). A comprehensive study by Klein and Deforest (29) showed that some virucides, e.g., chlorine-releasing agents, iodine, and glutaraldehyde, were active against all types of viruses, whereas other biocidal agents were effective against some but not other viruses. Klein and Deforest distinguished between lipophilic and hydrophilic germicides, the former defined as compounds with an affinity for lipid-containing and non-lipid-containing viruses that react with some lipid compounds. Examples of lipophilic germicides are isopropanol and QACs, and viruses differ considerably in their sensitivity to them. Depending on viral size and the absence or presence of lipids, four groups of viruses are envisaged (29):

— Group A, e.g., herpes simplex, influenza, mumps and vaccinia. These are lipid-enveloped, and have a high degree of sensitivity to all biocides.
— Group B, e.g., picornaviruses, parvoviruses. These are non-lipid-enveloped, small viruses that are resistant to lipophilic germicides.
— Group C, e.g., adenoviruses, reoviruses. These are larger, non-lipid-enveloped viruses that are more resistant to biocides than Group A viruses.
— Unclassified, e.g., hepatitis A and B and hepatitis non A-non B.

The QACs are active against viruses in Groups A and C, but not Group B viruses. As pointed out earlier, glutaraldehyde is virucidal but this effect depends markedly on the type of virus; Groups A and C viruses being inactivated by a concentration of 0.02%-0.04%, but Group B viruses requiring a concentration of 1%-2%. Ethanol (70% v/v) is considered to be a powerful virucide against all viruses (29), although it is claimed elsewhere that only lipid-containing viruses are suspectable (19).

Like other retroviruses, human immunodeficiency virus (HIV) is enveloped and is readily destroyed by disinfectants such as hypochlorites, glutaraldehyde, formaldehyde, and alcohols (45). Hepatitis B virus (HBV) is more resistant to disinfectants, although less so than originally thought.

There is still, however, a comparative dearth of information about the mechanisms involved in the inactivation of viruses. Thurman and Gerba (57) consider that diffusion across the virus capsid to reach a target site (e.g., hypochlorites and viral RNA) is an important aspect of disinfection. Interaction with capsid protein is of potential significance, although consequent reduction in activity of a highly reactive virucide is then a possibility.

Effects of Physical Agents

Many types of viruses are susceptible to comparatively low moist heat temperatures (55-60°C). Some exceptions exist, however. The Herpetoviridae, herpes simplex virus (HSV), and cytomegalovirus are thermosensitive, whereas Aujeskys disease virus is more thermostable than HBV. HBV, a member of Hepadnaviridae, possesses above-average resistance (45). Viruses are less resistant than spores or *D. radiodurans* to UV radiation but often more resistant than non-sporing bacteria. It has been claimed that UV light does

not inactivate HIV (45). Viruses are inactivated, however, by ionizing radiation.

Reasons for the comparative sensitivities and resistances are largely unknown.

Resistance of Prions

Prions are responsible for spongiform encephalopathies which make the brain become spongy (27, 40). The encephalopathies have a long incubation period of several years, are characterized by pre-senile dementia and degeneration of the nervous system, and are always fatal. Examples are bovine spongiform encephalitis (BSE) in sheep and kwu (associated with cannibalism), and Creutzfeldt-Jakob disease (CJD) in humans.

The CJD agent is highly resistant to many chemical and physical agents. These include boiling, UV and ionizing radiations, ethylene oxide, alcohols, detergents, QACs, phenolics, iodine, formaldehyde (3.7% w/w), and β-propiolactone. Glutaraldehyde (5%) produces only partial inactivation, and the most effective chemical agent is 1N sodium hydroxide for 1 hour at room temperature (15 min is considered to produce partial inactivation). Autoclaving for 1 hour at 132°C is deemed to be fully effective, whereas 121°C or 132°C for 15 to 30 min is only partially effective (7, 8, 12).

Little is known about the mechanism of inactivation of prions or of the reasons why they should present such extreme resistance to chemical and physical agents.

Conclusions

This paper has discussed briefly the responses of microorganisms to several chemicals and physical inactivation processes. Wherever possible, reasons have been put forward to account for above-average resistance to a particular process, but it is clear that in many cases little is known about the basic mechanisms involved.

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Sterilization of Medical Products

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The success of surgical interventions, and even medical mass manipulations such as injections, inoculations, and blood tests, are in large part dependent on aseptic conditions, a key feature of which includes device sterilization. Nonsterility of the instruments, equipment, and materials is often the cause of hospital infections. Thus, sterilization becomes an important link in the prevention of hospital infections.

To solve practical problems associated with the sterilization of medical instruments and equipment, specific terminology, methodology, and organizational structures have been developed. As a result, a clear-cut concept of medical product sterilization has emerged, including determination of the types of items to be sterilized; standardization of major concepts in the field; treatment stages: disinfection, presterilization cleansing, sterilization; methodology and assessment of methods used to implement these stages of treatment; determination of conditions under which different stages can be successfully applied for specific groups of medical products; and concrete ways of implementing sterilization schemes dependent upon the method selected.

Sterilization should be applied to all medical products which come in contact with the wound surface, blood, injected preparations, as well as products which come in contact with the mucous membrane and can injure it. The treatment of medical products should involve the following stages:

— Disinfection, which should be implemented after the instruments have been used in festering wound operations; for administering live vaccines; and operational manipulations involving patients with an infection or undiagnosed hepatitis or patients who are pathogen or HBS-antibody carriers, or members of the hepatitis or AIDS risk group.
— Presterilization cleansing.
— Sterilization.

Thus, items to be sterilized should be subjected to at least two stages of treatment—presterilization cleansing and sterilization, and if disinfection is prescribed, three stages.

Taking into account the role of disinfection in the above-described approach to the treatment of medical products, it seems reasonable to discuss some issues concerning this stage.

It is important to standardize the concepts of disinfection and sterilization. To avoid erroneous assessment of applicability of particular methods and means for specific processes, and to effect estimation of the scheme efficiency, these concepts should be given a clear-cut definition and scientific description, as it is in the CNEA Standard 3188-81 “Medical applications products. Methods, means and schemes of sterilization and

disinfection. Terms and definitions” and in the state standard, developed on its basis 25375-82 (CNEA Standard 3188-81) “Methods, means and schemes of sterilization and disinfection of medical applications products. Terms and definitions”. According to these standards, the term disinfection is defined as the killing of pathogenic microbes present on medical products, while sterilization is defined as the process of killing any types of microbes at any development stages, present on or in medical products.

It should be noted that there is no sterilization which would be specific for the prevention of purulent-septic infections or parenterally-conveyed hepatitis or AIDS. However, in the case of infectious diseases whose spreading factor may be the medical product, sterilization is the last barrier which protects patients from the development of hospital infections.

The development of conditions under which medical products are to be sterilized includes the following scientific approaches:

- Search for novel methods as well as for improvements in existing methods of disinfection, presterilization cleansing, and sterilization of medical products;
- Study of capabilities and conditions for disinfection, presterilization cleansing, and sterilization of specific product groups or single items.

It is very important to define the methodological aspects involved in the development of conditions under which medical products must be disinfected and sterilized. In our opinion, the above-mentioned studies should be conducted with an artificial seeding, using pure test cultures which have a reference resistance to a particular disinfectant or sterilizing agent. For disinfection, these include representatives of different microorganisms (nonspore forming and sporeforming bacteria, fungi, viruses); for sterilization, these are represented by microbial spores. The test cultures should be applied to test objects (materials and products) whose physical and chemical properties enable the most objective assessment of the effectiveness of a particular sterilizing agent. In selecting the seeding rate for test objects, one should take into account the actual seeding rates of particular items or groups of same objects resulting from the production and application conditions.

To ensure that assessments of the methods, means, and schemes for disinfection, presterilization cleansing, and sterilization of medical products made by specialists from different countries are comparable, the unification of estimation methods is desirable. This should improve the information analysis and encourage an exchange of the means and equipment for the implementation of the above processes. Such efforts have been undertaken by the specialists of a number of CNEA member-states.

Methodological Approaches to Disinfection, Presterilization Cleansing, and Sterilization

In compliance with the adopted methodological approaches, validated recommendations for disinfection, presterilization cleansing, and sterilization of medical products have been developed and implemented in the U.S.S.R.

Disinfection and sterilization are comprehensive sciences. Scientific developments in

these areas are based on microbiological and epidemiological research results, and achievements in chemistry, physics, and technology. This provides a basis for the choice of disinfection/sterilization methods and agents for use in the U.S.S.R. as well as in the rest of the world.

Disinfection

Different methods may be recommended for disinfection of medical products, including physical (boiling, steam, hot air) as well as chemical means. The latter involves flushing and cleaning the products with disinfectant solutions belonging to different groups of chemical compounds. Available disinfecting compounds include those shown in Table I. The concentration and disinfection exposure times depend upon the germ species.

Table I. Disinfecting Solutions for Medical Products

Chlorine-Based Compounds
○ Chloramine
○ Dichlorine-1
○ Sulfochlorantine
○ Chlorcin
○ Neutral Calcium Hypochloride
Peroxide Compounds
○ Hydrogen Peroxide
○ Hydrogen Peroxide with Synthetic Detergents
○ Desoxon-1
Aldehydes
○ Formalin
○ Glutaraldehyde
Guanidine Derivative
○ Chlorhexidine Bigluconate (gibitan)

Presterilization Cleansing

Presterilization cleansing is an indispensable stage in the treatment of products to be sterilized. It must result in the elimination of protein, fat and mechanical impurities, the elimination of residual quantities of medications, and a reduction in the original seeding rate. Presterilization largely determines the effectiveness of sterilization by decreasing microbe resistance and decreasing the risk of pyrogenic reactions.

However, we found that at low levels of the initial microbe seeding rate, the conventional scheme of presterilization cleansing may result in an increased number of microbe seeds due to the availability of tolerated bacteria in the water. Detergents should be used for any type of cleansing of medical products, both manual and mechanized. Detergents should meet the following requirements:

— adequate cleaning effects at low foam;

— non-pyrogenic and non-toxic;

— adequate flushing from the products; and
— non-corroding.

Taking into account the above requirements, synthetic detergents which do not include a proteolytic enzyme in complex with hydrogen peroxide, may be used both with an admixture of the corrosion inhibitor (sodium oleate) and without it. “Biolot”, a preparation developed specifically for the above purpose, contains the enzyme and does not require that hydrogen peroxide be used in complex with it. This results in no corrosion effects for the products made of corrosion-vulnerable materials (metals), in a longer life-time, and a substantial economic gain.

The process of presterilization cleansing includes a number of operations, such as the ones that enable a mechanical elimination of grime by different ways: brushing, spurting with a detergent solution, ultrasonic impact, rotation effects; as well as preparatory and consequent stages. We found that it is necessary, at preparatory stages, to provide a corrosion-protective treatment, to counteract the effects of blood on metal devices which cannot be flushed with water immediately after they have been used. Toward this end, the devices should be put into the corrosion inhibitor solution, (1% solution of sodium benzoate).

Disinfection and presterilization cleansing can be combined in a single process. To this end, disinfectant-detergents must be designed, and special-purpose machines be used. In addition, the disinfection scheme should result in killing the viruses of hepatitis and human immunodeficiency. It must be run as a centralized process, ensuring epidemiological safety of the collection and transportation of used items to the treatment site.

Sterilization

The final stage of treatment of medical products is sterilization. Sterilization should result in the elimination of all microbes that are present on or in the products, both pathogens and non-pathogens—saprophytes, including the ones which are highly resistant to most of sterilization means. Since, with the exception of ionizing radiation, most means of sterilization are not effective against microbe spores, sterilization may be ensured only by this means.

For sterilization, different methods may be recommended: physical, including thermal (steam, hot air) and radiation, and chemical, including gas and/or the use of chemical solutions. The choice of the sterilization method for a specific product depends on the char acteristics of the item to be sterilized and on the sterilization method itself—its benefits and limitations. Particular aspects of each method also delineate the limits of its applicability. Those methods which can be used for the packed items may also be used for the decentralized sterilization in hospitals and clinics, and in industrial enterprises where sterile single-use products are manufactured. Those methods which are applicable to the unpacked items may be used only for the decentralized sterilization in hospitals and clinics.

Heat-Based Sterilization

Steam and hot air methods are the most widely used methods in hospitals and clinics.

The extensive use of steam is accounted for by a number of useful features which make this conventional method still widely applicable even today. The efficiency of steam sterilization is high; it is reliable and ensures sterility of not only the item surface but its inside as well. Furthermore, the comparatively low temperatures involved in steam sterilization spares the materials to be treated. Packed items may be sterilized using steam, which prevents a secondary seeding by microbes after sterilization.

For saturated steam sterilization to be effective at excessive pressure, the most important condition is the evacuation of the sterilization chamber and the items to be sterilized. To select the most adequate methods of air evacuation, a comprehensive (physical, microbiological) assessment of evacuation methods was made. Of the methods tested, (gravitational, continuous evacuation, and pulsating methods), the most promising was the pulsating method. It is this method which should be used in the design of steam sterilizers with vacuum pumps. For steam sterilizers, in which air is evacuated gravitationally and continuously, the following schemes are recommended: at the steam pressure of 0.05 ± 0.02 MPa, (which corresponds to the temperature inside the sterilization chamber of 110 ± 2°C), the sterilization exposure time is 45 min. At a steam pressure of 0.20 ± 0.02 MPa (temperature of 132 ± 2°C), the sterilization exposure time is 20 min. When sterilization is made in the pulsating evacuation mode, the sterilization exposure time is essentially reduced.

Although steam is an agent which penetrates into different points of the sterilization chamber as well as between and inside the items, to assure adequate sterilization, it is necessary to regulate the density of loading boxes with surgical clothes and dressings, rubber items, and metal instruments.

Despite all the above-mentioned benefits of steam sterilization, it has certain limitations. It results in corrosion of the metal appliances made from vulnerable metals; steam moistens the sterilized items and thus results in a deterioration of storage conditions, with a greater risk of secondary seeding of sterilized items by microbes. Furthermore, this method is not suitable for heat-vulnerable materials, including most polymers.

Sterilization by air uses hot dry air as the sterilization agent. However, when it naturally circulates in sterilizers, the items are heated slowly and to different temperatures at different points of the sterilization chamber. This results in a lower sterilization quality. For this reason, the air methods are not as widely used in the world as steam methods. Nevertheless, this limitation is not as severe since sterilizers have been designed with a forced air circulation at a speed of ≥ 1 m/sec; the extreme variations of sterilization temperature inside the loaded chamber from the set one are not greater than ± 4°C.

The impact of hot dry air on a microbe cell is not as effective as that of saturated steam. This accounts for a higher sterilization temperature required. Currently the following sterilization schemes have been officially recommended: temperature—160°C, sterilization exposure time—150 min; temperature—180°C, time—60 min. At the higher treatment temperatures used, a smaller number of different items can be sterilized: for example, no textile, rubber or polymer items may be sterilized. However, metal, glass, and silicon latex may be sterilized using the hot air method. The spectrum of materials used for packaging purposes is also narrower given the higher temperatures associated with the hot air

method. Sterilized items cannot be packed in fabrics or parchment; at a temperature above 170°C they are charred.

Nevertheless, in our opinion, the hot air method should be developed and used, since it lacks a number of limitations inherent in the steam method. When sterilization is effected by hot dry air, no moistening and metal corrosion occur. Thus, a wider spectrum of instruments can be sterilized with hot air. If all the metal instruments made in the U.S.S.R. are grouped into five categories on the basis of the materials they are manufactured from, then only three categories may be sterilized with steam, whereas four can be sterilized with hot air.

Compared with the steam method, it is even more important to observe the loading rules with respect to density and manner for most types of air sterilizers in current usage. However, a design approach has been found that eliminates this reservation. This approach enables an automatically controlled process with a time shift of the initiation of sterilization in relation to the quantity of items loaded, the item homogeneity, and the item spacing (11). In addition to the above-listed merits of the heat-based methods of sterilization, it's ecological purity should be emphasized.

Pathways to optimize thermal sterilization methods include computerization which must enable a free choice of the sterilization scheme parameters depending on the type of tems to be sterilized and their positioning in the sterilization chamber.

"Cold" Sterilization

The growing range of medical products made of heat-vulnerable materials resulted in the development and implementation of "cold" sterilization methods—radiation and chemical (gaseous and solution sterilization)—which are effective at temperatures lower than 100°C.

Radiation is the leading industrial sterilization method. A scientific basis has been developed for determining the radiation sterilizing dose in relation to the initial contamination level, the types of items manufactured, the contaminant radioresistance and a safety factor. The sterilizing dose may be calculated mathematically by formulas (2) which take into account all of the above-listed considerations as well as using a bacteriological approach based on the use of test cultures (see Equations 1 and 2).

Equation 1. Determination of the Level of Radiation Sterilization Dose (2)

$$\frac{1}{KN} = \sum_{i} \delta_i \cdot 10^{\frac{-D}{D_{10}^{i}}}$$

where:
- D = radiation sterilizing dose
- K = safety factor
- N = initial contamination

δ_i is the fraction of microbes in the microflora for which

$$D_{10}^{i-1} < D_{10} < D_{10}^{i}$$

$$\left(\delta_i = \frac{N_i}{N_0} \cdot i = 1, 2, 3, 4, 5 \right)$$

Equation 2. Determination of the Level of Radiation Sterilizing Dose

$$D = LD_{99} + D_{10} \left(-2 - \lg \frac{N}{N_0} - \lg \frac{1}{N_{0.5}} \right)$$

where: D (krads) is the dose required to obtain a safety factor equal to

$$10^{-\lg \frac{N}{N_0}}$$

LD_{99} (krads) = 690.2 $N_{0.5}^{0072}$

D_{10} (krads) = 302.4 $N_{0.5}^{0.802}$

By such methods, the sterilizing dose of radiation has been determined (gamma radiation, accelerated electrons) for a wide spectrum of products based on polymers and other materials including paper. In practically all cases the sterilizing dose does not exceed 25 kGy.

Pathways to reduce the sterilizing dose have been sought. It has been suggested that a combination of ionizing radiation and other physical and chemical agents may be used, such as a heat-and-radiation method (used for industrial sterilization of catgut) (7); magnetic-and-radiation method based on alternating magnetic field (9); and a chemical-and-radiation method using different antimicrobial agents (8, 10). The resultant synergic action of biocidal effects enables a reduction in the radiation sterilizing dose by 25%-40%.

In the case of the chemical-and-radiation method, the most technologically sound approach is the radiation sterilization of the products which contain an antimicrobial agent. In such a case, the effect is evident at a limited initial seeding rate; for example, cetylpyridine chloride (0.01%) present in eye medication films make it possible to reduce the gamma radiation sterilizing dose to 15 kGy at an initial contamination equal to or less than 10^2 microbe cells per unit (4).

With respect to gas sterilization, in addition to conventionally used ethylene oxide, a mixture of ethylene oxide with methyl bromide, (OB mixture with the weight ratio of 1:2.5, respectively) is used. In the OB mixture, methyl bromide is both a phlegmatizer and an antimicrobe agent. Thus, the total amount of the OB mixture administered into the chamber is less than that of the mixtures using other phlegmatizers (e.g., CFC, CO_2). As a result, when the sterilizing dose of OB has been administered (at a preliminary vacuum of 0.09 MPa), an excessive pressure is created, up to 9.08 MPa, which is important for the prevention of damage to air-tight products.

Relationships between the sterilization efficiency by the above gases, and the temperature and humidity conditions, level of the dose, and types of products have been

reaffirmed. Sterilization is achieved by ethylene oxide at a temperature of not less than 18°C at a dose of 1000 mg/L during 16 hrs, or by the OB mixture— at a dose of 2000 mg/L during 16 hrs. At a temperature of 35-55°C, the sterilizing dose of the OB mixture is 2000 mg/L and the sterilization exposure time is 4-6 hrs depending on the product type. The relative humidity level inside the chamber must be at least 80%. As soon as the sterilization process is over, the gas is removed from the chamber.

To ensure that the application of the items sterilized with OB and ethylene oxide is safe, in-depth physico-chemical and toxicological studies have been made, enabling the determination of kinetics of diffusion-absorption processes of ethylene oxide and methyl bromide in different polymer materials. Biological effects of these substances were identified under conditions of parenteral administration, providing a basis for the determination of residual quantities of these gases in a number of products and materials used to manufacture medical items undergoing sterilization. Maximum permissible amounts of the sterilizing agents were suggested for parenteral administration. On the basis of the data obtained, conditions have been specified for degassing (ventilation) the products with an account for the frequency and duration of contacts with the internal media of human organism.

Regulations for the degassing time are:

— for products made of glass and metal, the exposure time in the ventilated premises (at an air speed of 20 cm/s) is one day, after which the items sterilized may be used;
— 5 to 13 days for the items made of polymer materials (rubber, plastics) intended for a short-time contact (up to 30 min);
— 14 days for any products intended for a prolonged contact (longer than 30 min) with mucous membranes, tissues, and blood;
— 21 days for polymer items intended for a prolonged contact (longer than 30 min) for child use.

Formaldehyde should be thoroughly discussed in the context of its application for gaseous sterilization. Microbiological and physico-chemical research showed that gaseous formaldehyde can be useful as a sterilizing agent, and in certain respects even better than ethylene oxide. It is more effective in mixture with water vapor, achieving sterilization at a dose 100 times smaller. At above 60°C, formaldehyde has a better penetration capacity, coming through packaging (paper, polyethylene film) to the products sterilized. To sterilize by formaldehyde vapors, it seems reasonable to use a solution in ethyl alcohol, since in this form formaldehyde does not polymerize at a temperature lower than 80°C, as is the case when an aqueous solution is used. Besides, no corrosion occurs of parts or products made of metals (5). A 40% solution of formaldehyde in ethyl alcohol is used, at a dose of 150 mg/dm^3 of the sterilization chamber, the required quantity of formaldehyde solution is 375 mg/dm^3, the time of sterilization exposure is 2-3 hrs depending on the type of product sterilized.

Upon sterilization, formaldehyde vapors should be evacuated from the chamber. After the products made of glass, metals, and polymer materials (rubber, plastics) have been sterilized with the vapors of ethyl alcohol solution of formaldehyde, no additional degassing

is required. The rubber and plastic items contacting with blood should be degassed for two days under ambient conditions.

In principle, such a modification of the gaseous sterilization method appears to be possible, which uses as a sterilizing agent formaldehyde evaporating spontaneously from paraform or formalin. Sterilization, by paraform-emitted vapors of formaldehyde, of medical metal instruments with no packaging may be achieved under the following conditions: a 1 cm thick layer of paraform covers the sterilization chamber floor, with a chamber floor to volume ratio of 1:2. To obtain the relative humidity level of 95%-98%, a can with water is put into the chamber. Under these conditions and at a temperature of not lower than 20°C, it takes 5 hrs to sterilize the simplest instruments (lancets, resection knives, forceps) made of metal (1). When there was no additional moistening, no reliable sterilization effect was found for test metal products after as long as 20 hrs of treatment.

Sterilization by solutions is an auxiliary method, to be used under conditions when neither of the above-described methods is applicable. It has a number of limitations: unpacked items are sterilized, post-sterilization flushing is required, which may result in the secondary seeding with microbes. Due to these limitations, some authors think (e.g., 3), that the use of sterilizing agent solutions should be regarded as a method of aseptic preparation rather than sterilization. However, this method also has some merits: general availability, straightforwardness of implementation, etc. Thus investigations aimed at finding and studying sterilizing agent solutions should be encouraged.

Studies were conducted of the characteristics of sterilization obtained by solutions of hydrogen peroxide, desoxon-1 (acetic acid-based preparation), and glutaraldehyde. Relationships were found between the efficiency of these agents and temperature and pH of the solution, which resulted in deriving practical recommendations for the use. For the polymer products (rubber, plastics) as well as glass and corrosion-resistant metals, a 6% solution of hydrogen peroxide may be recommended at a temperature of not lower than 18°C and the sterilization exposure time of 6 hrs; at a temperature of 50°C the exposure time is 3 hrs. For desoxon-1 (1.0% for acetic acid) at a temperature of not lower than 18°C, the exposure time is 45 min.

For the items manufactured from the above materials and corrosion-vulnerable metals, a glutaraldehyde solution may be used (2.5% a.i.) with pH of 7.0-8.5, and an exposure time of 6 hrs at a temperature of not lower than 20°C. The working solution concentration of glutaraldehyde may be substantially reduced if the exposure time is much longer. For example, sterility is achieved if xenobioprostheses of cardiac valves and arteries are put into a 0.5% solution of glutaraldehyde for as long as 10 days, with the use of phosphate solution as a buffer, at a temperature of 22-25°C (6). Since sterilization is achieved if the items are unpacked, this method can be used on a decentralized basis only.

Conclusion

Sterilization is an unavoidable stage in manufacturing single-use products and with repeat use products. Thus designers and producers must provide for the possibility of sterilization for every particular product. When new products are designed, a parallel

process must be the development and investigation of sterilization conditions. If one is not able to develop up-to-date, technologically efficient, and sparing sterilization conditions for a product being designed, it cannot exist.

According to the application of medical products, the sterilization reliability coefficient is differentiated. It may be 10^3 for midwife kits, 10^6 for syringes, 10^8 for blood transfusion systems, etc. This means that one non-sterile item is permissible in the entire population of products quantified by the coefficient. However, each single item out of this population should be sterile, as determined by modern, sensitive enough methods of microbiology. The sterilization reliability coefficient is used in mathematical modelling of sterilization conditions.

Practical methods are available for the sterilization of a large variety of medical products used in hospitals and clinics (repeat products) and at industrial enterprises (single-use products). Such products include whole groups, such as medical metal instruments, rubber (on the basis of natural, silicon and fluorine rubber and latex) items and components of medical products; polymer products—probes, catheters, tubes, and blood transfusion systems; absorbent cotton; different dressings, both conventional cotton and viscous mixtures, and novel materials with additional properties, such as pain-killing, non-traumatic, antimicrobe, etc.; catgut; particular objects which are sometimes difficult for sterilization, such as plastic magazines loaded with metal clamps for surgical sewing machines made of hard polystyrene and polycarbonate, electronic cardiac stimulators and electrode cables for them, hemosorbents of different types, midwife kits, polymer containers for storing liquid and dry blood substitutes, stomatological instruments, endoprosthesis parts, heart-lung machines, rotors for the flood fractioning machines, artificial crystalline lenses, endoscopes, medical kits for flexible endoscopes, xenobioprostheses of cardiac valves and arteries.

Thus, a scientific foundation is currently available for different aspects of medical product sterilization. When medical products are sterilized in a proper way, there is no risk of the use of non-sterile products, which is an important condition for the prevention of hospital infections. To enable further improvement, continuous development efforts and improvements in existing methods should be encouraged in the field of sterilization and presterilization cleansing.

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Biological Indicators for Hospital and Industrial Sterilization

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The achievement of sterility and validation of sterilization processes are based upon statistical probability. For known sterilization processes, it has been determined that the death of microorganisms is usually a logarithmic progression. This type of progression means that the same percentage of microorganisms die every minute during sterilant exposure. In theory then, it is only with an infinite exposure time that one can be absolutely sure that all microorganisms have been killed and, therefore, all units are sterile. Theoretically, no matter how long the sterilization cycle, some fraction of the original microbial population remains viable.

As seen in Table I, if a bacterial load (bioburden) of 10^6 (1,000,000) was exposed for eight minutes at the specified conditions, the microbial population would be reduced to a level of approximately 10^{-2} or 0.01 survivors per unit. This means that after an eight-minute exposure, one viable organism would be expected for every 100 units. With this understanding of the "probability of survival concept" it is easy to rationalize that not all organisms will be killed at the identical exposure time. If a sterilization process is marginal, it would be possible to have one unit of product with viable organisms and an adjacent unit in the same sterilizer with no viable organisms.

Table I. Example of the Inactivation of a Bacterial Population

Minutes	Bacteria Living at Beginning of Minutes	Bacteria Killed in One Minute	Bacteria Surviving at End of Minute	Logarithm of Survivors
1	1,000,000	900,000	100,000	5
2	100,000	90,000	10,000	4
3	10,000	9,000	1,000	3
4	1,000	900	100	2
5	100	90	10	1
6	10	9	1.0	0
7	1.0	0.9	0.1	−1
8	0.1	0.09	0.01	−2
9	0.01	0.009	0.001	−3
10	0.001	0.0009	0.0001	−4

The concept of sterility assurance levels (SALs) or probabilities of a survivor per item (PSI) allows different values to be required for a device based on the hazard posed by its use. This concept is relatively new to the medical product industry and is generally unheard of among hospital sterilization personnel. In the medical products industry, it is generally accepted that the sterilization process for parenteral solutions and devices provide a SAL of 10^{-6} or less (fewer than one non-sterile unit per one million units processed). The validation of an industrial sterilization process assures that the specified sterilization cycle can

reproducibly yield a safe product whose quality and efficacy can be evaluated against established specifications. Ultimately, validation means that this process can become the basis for routine production with assurance that the product bioburden is killed to the extent specified in the manufacturer's design protocol. This approach allows industry to use technically equivalent methods for achieving the same end result (stated as PSI or SAL). A key ingredient to this approach for validation and routine monitoring of industrial sterilization cycles is the biological indicator (BI).

In the Central Sterile Supply Department (CSSD) of a hospital, validation of a sterilizing process is not an established practice. Cycle times for process loads are usually obtained from the sterilizer manufacturer who does not have full access to the type of product to be sterilized in each and every run nor the anticipated degree of loading of a particular hospital sterilizer. Most hospitals rely on an overkill approach to establish sterilization cycles. For this method, the hospital uses doses or periods of exposure far in excess of those required to kill the normally expected bioburden on a product. Even though the hospital's approach to sterilization quality assurance may differ significantly from that used by manufacturers, hospitals still rely significantly on the use of BIs.

A BI can be defined as a unit containing microorganisms of known concentration and resistance to a given sterilizing agent which can be expected to follow a predictable death rate when exposed to certain physical or chemical parameters. The key functional phrases in this definition are "known concentration and resistance", and "predictable death rate". BIs are critical for both hospital and industrial sterilization and as such it is imperative that they perform both predictably and reproducibly. It is essential that the same sterilization cycle is monitored by the same relative biological challenge day after day, month after month, and year after year. If BIs do not provide predictability and reproducibility, they are of little value in sterilization quality control.

Biological monitors document the efficacy of sterilization cycles to destroy living microorganisms under actual sterilization conditions. Their primary advantage is that they have the capability to integrate all parameters that affect biological efficacy. For steam sterilization they have the capability of integrating the lethal effects of exposure time, temperature, and moisture content. In ethylene oxide sterilization they integrate the lethality of exposure time, temperature, humidity, and gas concentration. In addition, factors such as packaging, loading patterns, and changes in product configuration or materials may affect the delivered lethality, and thus, affect the results of the BI.

When BIs containing sterilant-resistant microorganisms are placed in the most difficult-to-sterilize portions of the load, the user can be assured that sterilization conditions were sufficient in that run to achieve destruction of indicator organisms with a count and resistance generally much higher than the device/package presterilization bioburden. The unit serves only to demonstrate that the conditions necessary for sterilization were achieved; it cannot validate that product sterility has been obtained. This can only be determined by a proper validation program. The BI will not compensate for incompetent personnel, lack of knowledge, improper procedures or inadequate cleaning techniques.

Types of Biological Indicators

Commercial BIs that can monitor all conventional steam and ethylene oxide sterilization processes are now available. They are marketed in various package forms that maintain the integrity of the inoculated carrier and are convenient to the user. BIs of known population and resistance, in combination with good cycle development programs, validation, and good manufacturing practices, can provide sterility assurance to any predetermined probability. Manipulation of the spore concentration and resistance allows the effective monitoring of a wide range of cycles.

There are three primary types of biological monitors available for steam and ethylene oxide sterilization and each is discussed below.

Inoculated Paper Strip

This type of BI consists of spores of the desired strain inoculated onto a paper carrier. The inoculated carrier is then placed into a suitable container, usually a paper envelope, which is readily penetrable by either steam or ethylene oxide. After use in the sterilization cycle, the inoculated carrier is aseptically removed from the paper envelope and transferred to a suitable microbiological culture medium. The culture medium usually recommended for recovery of *Bacillus stearothermophilus* (steam sterilization) and *B. subtilis* sub species *niger* (ethylene oxide sterilization) is Soybean Casein Medium (60).

The primary advantage of the inoculated strip biological monitor is its relatively small size which permits the unit to be placed in small locations of devices and packages to be sterilized.

The primary disadvantage of the paper strip BI is that it must be removed from its primary package and aseptically transferred into culture medium. No matter how skilled the microbiologist or how good the aseptic technique, there is always the possibility of inadvertent contamination during transfer. This contamination may invalidate the BI test results and may take considerable time and money to prove that the bacteriological growth is not the indicator organism. In addition, a second problem associated with BI paper strips is that the culture medium may significantly affect the results obtained.

There have been several reports published recently documenting the effect of recovery medium upon BI performance (24, 21, 13). Data demonstrating the effect of recovery medium upon the performance of *B. stearothermophilus* paper strip indicators is shown in Table II. The data indicate that D-values obtained from the same lot of BI strips can differ as much as two-fold depending upon the type of Soybean Casein Digest Broth (SCDB) used.

Table II. Comparison of D-values (in minutes) at 121°C For *B. Stearothermophilus* Utilizing Soybean Casein Digest Broth from Different Manufacturers

MANUFACTURER	D-VALUE	
	AVERAGE	RANGE
A	1.0	0.8 − 1.3
B	1.2	1.1 − 1.3
C	1.4	1.3 − 1.5

D	1.9	1.6 − 2.2

Two D-Value determinations performed on each of two lots from each manufacturer.

It is important to note that the SCDBs represented in Table II were identical as to their stated ingredients, directions for preparation, and final pH. Thus, if the user of biological monitors is to obtain accurate and consistent results, both the type and manufacturer of the recovery medium should be known and controlled.

Self-Contained Biological Monitor

The primary difference between this type of monitor and the BI paper strip is that with the self-contained monitor, the recovery culture medium is an integral part of the unit. There are two primary types of self-contained monitors.

The first type of self-contained BI consists of spores of *B. stearothermophilus* suspended in a culture medium which is sealed in a glass ampule. This type of monitor is only used for steam sterilization and is usually used to evaluate the sterilization of liquids by suspending the BI in the solution container. This monitor may also be used to evaluate washer-sterilizer cycles and flash (three minutes, 134°C, gravity displacement) cycles in the hospital.

After the sterilization cycle, the BI is placed directly into the incubator (55-60°C) and observed periodically for growth. There are several manufacturers of this type of BI and nearly all of them include a pH indicator so that bacteriological growth will be more readily visible. The pH indicator has different colors at a neutral or slightly basic pH and an acidic pH. Most pH indicators change from red, blue, purple or green under neutral pH conditions to yellow under acidic conditions. The normal pH of bacteriological culture media is neutral or slightly basic. When the spores of *B. stearothermophilus* germinate and begin to grow as vegetative organisms they produce acid which, in turn, lowers the pH of the culture medium and thus, changes the color of the pH indicator.

The second and most frequently used type of self-contained BI does not put the spores in direct contact with culture medium until the sterilization cycle is complete. This type of self-contained monitor can be used for both steam and ethylene oxide sterilization cycles.

At the present time there are at least three different manufacturers of this type of self-contained BI. The products from all of these manufacturers are similar in design and consist of a vial which contains a spore inoculated paper carrier and a sealed ampule of culture media. The units are designed such that sterilant can penetrate to the inside of the vial and contact the inoculated carrier. After the sterilization cycle is over, the units are activated when the media ampule is broken and contents are released to come in contact with the spore carrier. If viable spores remain on the carrier, growth will occur when incubated at the appropriate temperature. These types of self-contained monitors also include a pH indicator to assist in the detection of growth.

The single most important advantage of the self-contained BI, as compared to the BI paper strip, is that aseptic removal of the inoculated carrier from its package and subsequent transfer to a culture medium is not required. This significantly reduces or

eliminates the potential for inadvertent contamination. In addition, the self-contained monitor reduces or eliminates the potential for adverse performance due to the quality of the recovery medium. The manufacturers of self-contained BIs should follow strict quality assurance practices in order to qualify and validate recovery media. In this manner, performance claims in terms of resistance and outgrowth will be valid. Some manufacturers of paper strip BIs have taken steps to avoid the adverse effect of culture medium by offering highly controlled medium to be used in combination with their particular strips.

The primary disadvantage of the self-contained monitor is that it is much larger than the paper strip monitor and may not be able to be placed in small locations.

BI Spore Suspensions

This type of product consists of spores suspended in solution (usually distilled water, saline, or buffer). The spore suspension is then used to directly inoculate the product to be sterilized. After the sterilization cycle, the entire product or inoculated portion of the product is cultured and incubated. An alternative method would be to use a rinsing and filtration method in order to recover the indicator organisms. The spore suspension for direct product inoculation is used exclusively for industrial sterilization. It is necessary to insure that the product onto which the spores are inoculated does not adversely affect the spores in terms of resistance, stability, or the recoverability of viable organisms. The primary disadvantage of this type of BI is that, in some types of products or materials, the direct inoculation with a spore suspension may cause atypical prolonged survival of spores under normal processing conditions (29, 57).

Industrial Use of Biological Monitors

Steam Sterilization

Different methods may be applied to sterilization validation and routine monitoring of solutions and other products such as containers and closures, packaged surgical products and devices, and various components or commodities. Different sterilization specifications may be required to moist heat sterilize such varied products. Thus, the type and application of biological monitors will also vary significantly (11, 43, 46, 52).

Regardless of the type of product to be sterilized, one of two approaches may be used in the development and use of effective steam sterilization cycles. Both of these approaches, either the Overkill Method or the Bioburden Method, may be based upon the use of biological monitors. Generally, the Overkill Method considers the inactivation of 12 logarithms of a microorganism with a $D_{121°C}$-value of 1.0-1.5 minutes. The microbial challenge will consist of selected numbers, typically 10^3 to 10^6, of moist heat resistant spores, (usually *B. stearothermophilus*) without necessarily relating the challenge population to the pre-sterilization bioburden. This method provides an overkill because the cycle conditions established to kill the microbial challenge, plus an additional safety factor, are usually more severe than those required to inactivate the product bioburden. Even

though the Overkill Method is employed, data should be obtained which indicate the typical bioburden loading associated with the product. These data do not have to be as extensive nor obtained as frequently as bioburden data associated with cycle development using bioburden methods.

The combined Biological Indicator/Bioburden Method may utilize several types of moist heat resistant spores, such as *Clostridium sporogenies, B. coagulans, B. subtilis* or *B. stearothermophilus*, as biological indicator systems.

When using the combined Biological Indicator/Bioburden Method, the microbial sterilization challenge of the product may include the requirement to inactivate the initial inoculum concentration to a pre-established logarithmic level. The relative resistance and population of the initial challenge inoculum of the biological indicator microorganism should be compared to the mean number and thermal resistance of bioburden generally associated with the product. The comparison of such data should demonstrate that the inactivation of a predetermined level of biological indicator microorganisms would ensure a sterility assurance level of no greater than 10^{-6} for bioburden in the process. The calibrated biological indicator used must ensure that the probability of a bioburden survivor is 10^{-6} or less. This method is considered to be a bioburden based cycle and hence the bioburden must be enumerated and resistance determined.

Regardless if one is using the Overkill Method or the combined Biological Indicator/Bioburden Method, biological monitors must be employed during cycle development and validation. Once the manufacturing mode has been attained, routine monitoring of the sterilization process may or may not be based upon the use of biological monitors. Where reliable process parameter control can be documented, with correlation to sterility assurance, release of batches based on delivered process parameters can be considered for terminally sterilized items. Parametric release of finished products should only be employed by firms which have a significant level of sterilization and manufacturing expertise.

If biological monitors are used for routine monitoring and product release, the selection and use of biological indicators should be consistent with the microbiological testing previously conducted. The type of BI, population level and resistance selected to routinely monitor the process should be the same as, or correlatable to, the microbial challenge used during product cycle development and validation studies. This will enable correlation of microbial inactivation between the routine process cycle and the data used to establish and verify the cycle.

The choice of biological monitors for use in industrial steam sterilization will vary significantly depending upon the product type and whether the Overkill or Bioburden Method is employed. If the Overkill approach is utilized, the monitor should have a sufficient population and D-value to assure total survival following an exposure to an F_o of 5 minutes and total kill to an F_o of 15 minutes. If the combined Biological Indicator/Bioburden approach is employed, the population and resistance of the biological monitor should be correlated to bioburden data plus an additional safety factor.

Ethylene Oxide Sterilization

Biological validation and monitoring of ethylene oxide sterilization is similar to steam sterilization in that either an Overkill or Bioburden Method of cycle development/validation can be utilized. The two sterilization processes differ in that there are significantly more process variables that can affect the efficacy of ethylene oxide sterilization as compared to steam sterilization. Due to the significance of variables in ethylene oxide (EO) sterilization, the industry has developed a more standardized approach for monitoring and validation. Thus, the potential for these variables resulting in sterilization failure have been minimized (5, 6, 15, 18).

Traditionally, Overkill Methods have been used to establish EO sterilization cycles. The microbial challenge normally consists of 10^6 ethylene oxide-resistant spores, typically *B. subtilis* var. *niger*, without necessarily relating the challenge population to the presterilization bioburden. This method provides an overkill because the cycle conditions established to kill the microbial challenge, plus an additional safety factor, are more severe than those required to kill the presterilization bioburden. Even though the Overkill Method is employed, data should be obtained which indicate the typical bioburden loading and resistance associated with the product. These data do not have to be as extensive nor obtained as frequently as bioburden data associated with cycle development using Bioburden Methods.

Although not commonly used in EO sterilization, the Bioburden Method is an alternative to the overkill approach. This approach requires significant data relative to the bioburden load and resistance. Based on bioburden population limits and/or resistance determinations, sterilization cycle parameters should be selected to achieve destruction of the presterilization bioburden to a level of 10^{-6} or less. This approach should only be employed by firms which have a significant level of sterilization and manufacturing expertise.

Biological monitors should be employed during cycle development and validation regardless if one uses the Overkill or the Bioburden Method. In most cases, routine monitoring and product release are also based upon the use of biological indicators. Where reliable process parameter control can be documented and correlated to sterility assurance, release of batches based on delivered process parameters can be considered for terminally sterilized products. Due to the number of variables involved in EO sterilization, this is only feasible for advanced state-of-the-art equipment but it is not in general practice.

The types of biological monitors used in EO sterilization in terms of population and resistance are much more standardized than those in steam sterilization. Typically, the Overkill approach employs a BI with 10^6 spores of *B. subtilis* var. *niger*. These spores typically have a D-value of approximately 3 minutes under exposure conditions of 600 mg EO/liter, 54°C and 50%-70% relative humidity. The biological monitor should have a minimum survival time of 15 minutes and a maximum kill time of 60 minutes under these same exposure conditions. An example of cycle selection for Overkill Method is the selection of a cycle that demonstrates a 6 logarithm reduction of the above referenced BI at one-half the sterilization cycle EO exposure time. If the combined Biological Indicator/Bioburden approach is employed, the population of biological monitor is correlated to the population and resistance of the bioburden plus an additional safety factor.

BI paper strips, self-contained type monitors, and spore suspension for direct product inoculation are commonly employed during development and validation of the sterilization cycle. For the purpose of routine monitoring and product release, paper strips or self-contained units are commonly used because of their relative ease of use. Regardless, the BI type should be consistent with the microbiological testing previously conducted. As for steam sterilization, the population level and resistance to the sterilization parameters of the routine monitor selected should be the same as, or correlatable to, the microbial challenge used during product cycle development and validation studies for EO sterilization.

Hospital Use of Biological Monitors

The manner in which hospital sterilizer cycles are validated and routinely monitored differs significantly from the methodologies employed by industry. In hospital CSSDs, validation of a sterilization process for a particular product is not an established practice. In addition, the hospital CSSD has little or no data relative to the bioburden associated with the product to be sterilized. There is also little information regarding the influence that mixed product loads have on the sterility assurance level obtained. Thus, most hospitals rely on the sterilizer manufacturer to establish sterilization cycles using an extreme overkill approach.

In order to improve and standardize hospital sterilization quality assurance, several organizations have published standards and guidelines (1, 4, 9, 10, 33). These guidelines are all very similar with respect to the recommended frequency and type of monitoring which should be employed. Due to the fact that the recommended monitoring frequency is significantly lower in the hospital, as well as the fact that hospitals do not effectively monitor bioburden levels or control sterilizer loading, there is greater assurance that a particular sterility assurance level will be achieved in an industrial setting. Thus, it becomes even more critical in the hospital setting that recommended practices are followed. Simply stated, the fewer the control points in a sterilization process, the more critical each one of these points becomes.

Biological Indicator Performance Standards

Suggested performance characteristics for steam and EO BIs, as listed in the United States Pharmacopeia (61), are shown in Table III. The recommended limits provide a wide range of resistance parameters that can be used for determining the suitability of a particular monitor. In addition to suggested performance characteristics for a suitable BI, the individual monographs for steam and ethylene oxide sterilization paper strip biological monitors give more specific performance characteristics.

Table III. USP XXI Suggested Performance Characteristics of Biological Indicators

	EO 600 mg/L 54°C 60% RH	STEAM 121°C
Typical D-Value	3.0	1.9
D-Value Range	2.6 − 5.8	1.5 − 3.0

Survival Time	(Labeled D-Value)	× (Log_{10} Labeled Spore Count per Carrier −2)
Kill Time	(Labeled D-Value)	× (Log_{10} Labeled Spore Count per Carrier +4)

In these monographs it specifies that the resistance of BIs shall be defined in terms of both D-value and survival/kill times. The D-value can be defined as the time required at a specific set of conditions to reduce the microbial population by 90% or one logarithm (Figure 1). The D-value determination described in the U.S. Pharmacopeia is the Spearman-Karber methodology (48).

Based upon the population and D-value, survival and kill times are described for the paper strip BI. The survival time is determined when 100% of the BI samples demonstrate growth when exposed to the same test conditions that were used to determine the D-value. The kill time should result in none of the samples demonstrating growth. The survival time and kill time for both steam and EO BIs are determined by the formulas listed in Table III.

Using these formulas, the survival time would result in approximately 1.0 × organisms surviving per BI exposed. The kill time would result in approximately 1.0×10^{-4} organisms surviving per BI, or stated differently, approximately one BI demonstrating growth for each 10,000 units exposed.

The labeled survival and kill times are of particular value since most BIs for routine monitoring are used and examined for a growth/no-growth response. The test for survival and kill times provides some assurance for the consistent performance of an individual production lot of monitors. If the monitors were not sterilized in a log-linear fashion as anticipated, they would not be expected to pass this test.

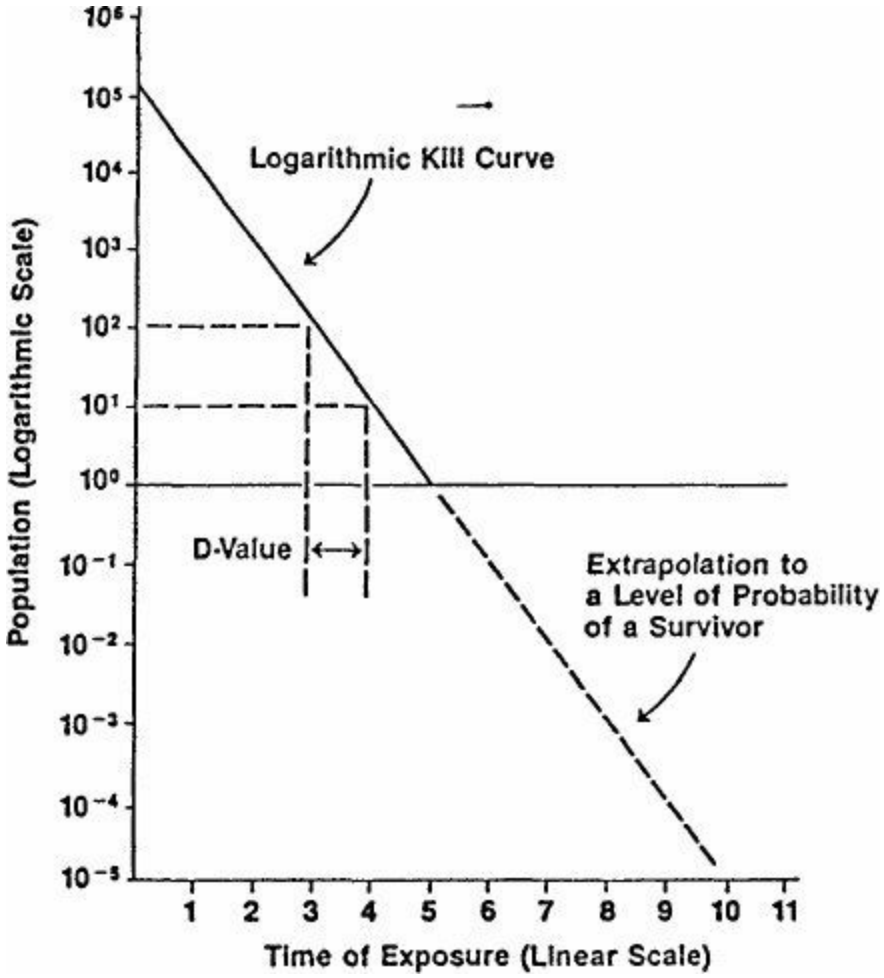

Figure 1. Determination of D-Value using the Spearman-Karber Methodology

As stated previously, in order for the BI to provide its maximum value, it must perform reproducibly under a given set of conditions and as such, the production and quality control of the biological monitor must be closely controlled. Listed in Table IV are factors that may affect the standardization of a BI and selected references that relate to those factors.

In order to accurately and precisely monitor the performance characteristics of BIs, it is necessary that both the recovery conditions and the test equipment be adequately controlled. In 1981, due to the need for standardization of equipment for evaluation of BI resistance, the Association for the Advancement of Medical Instrumentation (AAMI) developed and approved standards for steam and EO Biological Indicator-Evaluator Resistometer (BIER) vessels (7, 8). Performance standards for the steam and EO BIER vessels are listed in Tables V and VI, respectively.

Collaborative studies, jointly sponsored by the U.S. Food and Drug Administration and the AAMI, were undertaken in 1986 and 1987 to determine the variability of BIER vessels. All participants in these studies had BIER vessels which conformed with AAMI guidelines (7, 8).

Table IV. Factors Affecting Standardization of a Biological Indicator System

FACTOR	REFERENCES

Strain Selection	6, 11, 30, 40, 61

Spore Propagation Procedures	3, 22, 23, 25, 26, 28, 31, 32, 35, 44, 62, 63
Harvesting and Cleaning Procedures	3, 22, 23, 25, 26, 28, 31, 32, 35, 44, 62, 63
Indicator Preparation	22, 23, 31
Storage and Maintenance	2, 16, 19, 20, 27, 31, 38, 53, 54, 55, 56, 69
Resistance Data Analysis	12, 36, 41, 48
Use of Biological Indicators	9, 14, 17, 18, 37, 39, 49, 51, 60

Table V. Performance Criteria for BIER/Steam Vessels

1. The time to target temperature (with corresponding saturated steam pressure) shall be reproducible at the site monitored.
2. The time to target temperature (e.g., 121 ± 0.5°C, 29.7 psia) shall not exceed 10 seconds.
3. The time to exhaust shall be reproducible and shall not exceed 5 seconds.
4. Pressure monitoring devices shall show that vessel pressure is within ± 3.45 kPa (± 0.5 psi) of the saturated steam pressure.
5. Temperature control devices shall control the temperature of saturated steam to within ± 0.5°C.
6. Temperature monitoring devices shall be capable of determining saturated steam temperature to within ± 0.5°C.
7. Timers shall be graduated so that fractions of a minute can be read with a precision of ± 1 second. Timers shall have a repeatability of ± 1 percent.

Table VI. Performance Criteria for BIER/EO Gas Vessels

1. The time-to-gas-concentration (at test temperatures and percent relative humidity) shall be reproducible at the monitored site.
2. The time-to-gas-concentration for the exposed BI units shall not exceed 60 seconds.
3. The ethylene oxide gas conditions to test for (1) and (2) above shall be 600 mg (± 30 mg) EO per liter at 54°C (± 1°C) and 50 to 70 percent relative humidity.
4. The initial time-to-exhaust of the EO from the exposure chamber shall be less than one minute and reproducible (± 10 sec.); after the end of the exposure period, items must be removed immediately from the test vessel.
5. Pressure-vacuum monitoring devices shall show vessel pressure with a precision of ± 0.84 kPa (± 6.35 mm Hg).
6. Temperature control devices shall control the temperature to ± 1.0°C at a specific monitored site(s).
7. Temperature monitoring devices shall be capable of determining temperature to within ± 0.5°C.
8. Vessels shall be operated with a timer that has a precision of ± 1.0 second.

The first of these studies was designed to evaluate the performance of EO BIER vessels. Seventeen BIER-EO vessels were included in the study and ten were manufactured by the Joslyn Valve Corporation. Each participant was provided with the same lots of BIs and prepared sterile SCDB in order to eliminate variability caused by different BI types or different recovery medias. All resistance testing parameters were specified and all D-values were calculated by the same (end-point) methodology.

Shown in Table VII is a summary of the data obtained from all participants in the study. Statistical analysis of the data was performed by G. S. Oxborrow, C. W. Twohy, and C. A. Demetrius. At the 95% and 99% confidence intervals, the percent variability that could be expected were 18% and 28%, respectively. The range of all data points used to compute

the averages was 1.40 minutes. Thus, even though all of the vessels were supposed to conform to AAMI standards, a great deal of variation in the D-value from the same lot of BIs was obtained. The small standard deviation for each individual BIER unit reflects the reproducibility of a given unit as well as the consistency of BI samples.

Table VII. EO/BIER Vessel Collaborative Study Summary of Data from all BIER Vessels

Test Unit	Average D-value (Min.)	Standard Deviation (Min.)	D-value Range (Min.)
1	2.48	0.04	0.11
2	2.46	0.18	0.45
3	2.47	0.08	0.20
4	3.03	0.20	0.54
5	2.50	0.12	0.30
6	2.55	0.02	0.06
7	2.37	0.07	0.17
8	2.48	0.17	0.47
9	2.49	0.14	0.38
10	2.63	0.13	0.33
11	2.01	0.05	0.13
12	2.96	0.15	0.37
13	2.45	0.10	0.28
14	2.46	0.05	0.12
15	2.41	0.09	0.20
16	2.48	0.04	0.10
17	2.18	0.07	0.18

Mean of means = 2.49
Standard deviation of means = 0.23
Range of means = 1.02
Range of all points = 1.40 (low = 1.93, high 3.33)

Tables VIII and IX show a comparison of data obtained from Joslyn Valve BIER vessels and non-Joslyn units, respectively. The standard deviation obtained between the Joslyn units was 0.15 while it was 0.23 between the non-Joslyn units. Thus, the largest variance for all BIER vessels was due to the non-Joslyn units.

Table VIII. EO/BIER Vessel Collaborative Study Summary of Data from Joslyn BIER Vessels

Test Unit	Average D-value (Min.)	Standard Deviation (Min.)	D-value Range (Min.)
1	2.48	0.04	0.11
2	2.46	0.18	0.45
6	2.55	0.02	0.06
7	2.37	0.07	0.10
9	2.49	0.14	0.38
11	2.01	0.05	0.13
13	2.45	0.10	0.28
14	2.46	0.05	0.12
15	2.41	0.09	0.20
16	2.48	0.04	0.10
17	2.18	0.07	0.18

Average of means = 2.39
Standard deviation of means = 0.15
Range of means = 0.54

Range of all points = 0.82 (low = 1.93, high = 2.75)

Table IX. EO/BIER Vessel Collaborative Study Summary of Data from Non-Joslyn BIER Vessels

Test Unit	Average D-value (Min.)	Standard Deviation (Min.)	D-value Range (Min.)
3	2.47	0.08	0.20
4	3.03	0.20	0.54
5	2.50	0.12	0.30
8	2.48	0.17	0.47
10	2.63	0.13	0.33
12	2.96	0.15	0.37

Average of means = 2.68
Standard deviation of means = 0.23
Range of means = 0.56
Range of all points = 0.99 (low = 2.34, high = 3.33)

The second collaborative study was designed to evaluate the performance of steam BIER vessels as well as variability caused by the recovery media. Thirteen BIER-steam vessels were included in the study and six were manufactured by the Joslyn Valve Corporation. Each participant was provided with the same lots of BIs, prepared sterile SCDB and dehydrated SCDB (powder) in order to evaluate the effect of the same media prepared at different sites. In addition to the media provided, each collaborator provided one additional media lot of their choice. The dry media was prepared by each collaborator for the test. All parameters of the resistance testing were specified and all D-values were calculated by the same (end-point) methodology.

A summary of the data obtained from all participants in the study is shown in Table X. Media A was the prepared media provided to each participant and had the greatest variance. Medias B and C had the same variance and there was a significant mean difference noted between all media combinations at the 95% confidence interval. As was the case in the first study, the standard deviation for all Joslyn vessels was significantly less than all other vessels (data not shown).

The data obtained from the BIER collaborative studies demonstrated that several factors can significantly affect the resistance value of the BI. Unfortunately, the interaction of these factors is not completely understood. Use of a single BIER vessel may significantly reduce this variability, but the media type must also be controlled. These data indicate that users of biological monitors should be aware of the difficulty in obtaining identical results to those specified on the labels of BIs.

Table X. Steam/BIER Vessel Collaborative Study Summary of Data from All BIER Vessels

Test Unit	Average Media D-Value		
	Media A	Media B	Media C
1	3.07	2.70	2.79
2	2.55	2.60	1.90
3	3.31	3.30	2.10
4	1.97	2.11	2.14

5	1.68	2.17	2.25
6	2.91	2.14	1.85
7	3.26	2.61	2.62
8	3.19	2.27	2.20
9	2.20	2.45	1.85
10	3.34	2.72	2.41
11	2.75	2.47	2.06
12	2.63	2.88	2.27
13	2.49	2.50	1.89
Range	1.68-3.34	2.11-3.30	1.85-2.79
Mean	2.72	2.53	2.18
Standard Deviation	0.53	0.34	0.34

Qualification/Validation of the BI

Regardless of the indicator type chosen, population, expiration data, storage conditions, resistance performance characteristics, culture media, and conditions for use (such as temperatures and time of incubation) should be specified. For commercially available BIs, this information should be provided in the labeling. Included with the labeling should be a certificate of performance which should detail the following information:

- The BI organism(s)
- Lot number
- Viable spore population
- Resistance performance characteristics under specific test conditions
- Expiration date
- Recommended storage conditions

As stated previously, it may be difficult for the user to duplicate resistance data specified on the label, but one should attempt to confirm reproducibility between and among lots. Admittedly, this is nearly impossible for the hospital setting.

Conclusions

The biological indicator is a key tool in sterilization quality assurance. By itself, the BI cannot ensure sterility of the product, but when used in combination with Good Manufacturing or Good Hospital Practices, it allows effective means of developing adequate levels of assurance. Due to the knowledge that many factors can affect the performance of BIs, it is imperative that both manufacturers and users maintain adequate controls in the use and evaluation of biological monitors. An adequate sterilization quality assurance program must include personnel training, satisfactory facilities, equipment, and material flow systems, as well as adequate monitoring devices. Only by controlling all of the variables can one ensure the predictability and reproducibility of sterility assurance. Stated briefly, sterility cannot be ensured only by end product testing or BI monitoring, but must be achieved by the implementation of a total set of controls.

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Impact of Sterilization on Toxic Properties of Medical Polymers

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The introduction of items derived from polymer materials into medical practice necessitated the development of methods and conditions for their sterilization at the manufacturers, which would not negatively impact hygienic affinities and at the same time meet the modern requirements of asepsis.

At the present time, our native medical industry has put into mass production a number of disposable medical items. They include systems for blood taking and transfusion, components of extracorporeal blood circulation units, containers for continuous blood and its components preservation, subclavian and intravenous catheters, needles for injection, bougies, probes, and prostheses for various purposes.

A wide range of polymers with various chemical structures have been used for medical devices, the choice of which is dependent upon the purpose(s) of the item to be manufactured, its stability to the biological media, its physical and function features, its ability to resist the sterilization and disinfection effects, and its ability to be processed with minimal changes in physical, chemical, and mechanical features. The vinyl polymers, polyolefins, the materials based on styrene, acrylate, polyamides, fluor-polymers, polyethers, polycarbonates, polyurethanes, and silicone rubbers have been widely distributed.

One of the most important technological steps in medical device manufacturing is sterilization. Some requirements for the sterilization methods used in the industrial conditions are as follows:

— provisions for the sterility of items to be packaged;
— possibility of many sets of products to be processed; and
— possibility of having a continuous process.

Problems associated with the development of sterilization and disinfection methods for polymer-based medical devices include determinations of the adequacy of the sterilization method and the preservation of the biological “inertness” or harmlessness. Any change in the physico-chemical affinity of the polymer material under the influence of temperature, chemical, radiation, and the other processes by which sterilization is achieved, is inevitably concomitant with a change in the product’s hygienic characteristics. The toxic affinity of the item to be sterilized can be defined both in terms of the potential for destruction of the product because of instability and by the potential for absorption of the sterilizing and disinfectant agent(s) by the material.

Heat, radiation, and gas sterilization methods are the most frequently used in the industrial manufacturing of medical polymer-based devices both in the U.S.S.R. and abroad.

Radiation Sterilization

The most common sterilization method for disposable medical items derived from polymer materials in the U.S.S.R. and abroad is radiation.

The main features of this sterilization method are: the possibility for sterilization of thermolabile materials; processing of items in paper, glass or plastic packages; and the possibility of inclusion for sterilization into the continuous industrial process.

The radiation sterilization method for various disposable items related to blood collection and transfusion provides an interesting perspective. The “disposable” nature of the item is determined by two considerations. First and most important is the ability to exclude the possibility of infection transmission by reused items. The second consideration, limited largely to repeat use items, is the radiation stability rate of the polymer material(s).

The main source of ionizing radiation used for sterilization processing of medical polymer-based items is the radioactive isotope, ^{60}Co, and linear electron accelerators.

Upon exposure of polymers to ionizing radiation, various physico-chemical transformation takes place. The character and extent of these transformations depends on the chemical nature of the polymers, its phase state, condition, and the radiation dose.

In many countries the effective sterilization dose is 2.5 Mrad. In most countries the effectiveness of a sterilization dose of 2.5 Mrad has been verified at many enterprises. The interaction of the ionizing radiation with the polymer beyond a consideration of the polymer structure are related to energy absorption and the transition of polymer molecules into an activated state. Upon exposure of the polymer to ionizing radiation two main competitive processes can take place—cross-linking and destructive processes of macromolecules in radiation. These processes can proceed simultaneously and their speed ratio depends on the chemical nature and polymer structure and also on the radiation conditions. Accurate prediction of the outcome of radiation action on the polymer is limited, therefore accumulation of extensive experimental data is necessary to reliably determine the nature of the radiation-chemical interaction. The mechanism(s) underlying such an interaction may be dependent on the chemical structure of the main chain, the nature of groups substitution, the regularity of macromolecular structure, crystallinity, etc.

The influence of radiation on polyvinylchloride has been given great attention. Polyvinylchloride (PVC) plastics are widely used in disposable medical items. They are used in the manufacture of tubes and blocks for extracorporeal blood circulation, blood transfusion systems, containers for continuous storage and blood separation, and bioproducts. A great number of papers have been devoted to PVC radiation-chemical transformation, an analysis of which shows that there is no unified opinion regarding the mechanism underlying the radiation-induced polymer injury.

The primary mechanism of action of PVC radiolysis is the most arguable problem. Based on data from some foreign investigators, the primary mechanism of PVC radiolysis is a disrupture of the C-C link. According to this hypothesis, the alkyl radicals formed as a result of radiation combine with free chlorine atoms resulting in the formation of hydrogen chloride. Investigation of the radiation oxidation problems and the concomitant reactions acquires a special importance in the case of medical plastic devices. Here it is important to bear in

mind the formation of the low molecular products which may be able to migrate into a contacting medium, and also the change of the surface state of a polymer material which is in contact with the biologically active media.

PVC takes an intermediate place between the cross-linking and destructive polymers in radiation action. The polymer cross-linking takes place at a radiation dose of 80-100 Mrad, which is higher than the dose used for sterilization. In general we observe the destruction processes at all steps of radiation and in the period of post-radiation storage. It is necessary to take into consideration that most medical items derived from PVC-plastics have a prolonged term of use and storage (2-5 years). Multiple investigations have demonstrated the acceleration of PVC decomposition after exposure to ionizing radiation.

The negative influence of radiation sterilization on the hygienic affinities of PVC-based medical items has been highlighted in some foreign papers. Polish researchers investigated the effects of sterilizing PVC tubes with accelerated electrons at a dose of 3.0-4.0 Mrad. A result of this action was the accelerated migration in the modelled media of the zinc stabilizer and a considerable increase of blood protein deposition on the surface of the drain tubes.

German investigators have shown that increasing the sterilization dose above 2.5 Mrad resulted in reductions in the stability of PVC-plastics, as well as negative influence on an isolated frog heart preparation as well as on other biological objects.

So, the available literature suggests that the appearance of the toxic affinities of the PVC medical items by the ionizing radiation used in the sterilization process should be stipulated.

This conclusion is supported by the results of a series of investigations conducted at the toxicology department VNIIIMT (All-Union Research Institute for Medical Engineering) with the PVC-plastic medical items exposed to radiation sterilization.

In these experiments, some PVC-plastic containers for continuous storage of blood and bio-products were exposed to sterilization with accelerated electrons and radiation in doses 2.5, 3.5, and 5.0 Mrad. The aim of this experiment was to discover possible changes in the toxic affinities of the items as a result of the sterilization dose and to investigate the influence of various methods of radiation sterilization (accelerated electrons and radiation) on the hygienic properties of the plastic. Analysis of the toxicological data from these experiments yielded the following results. When animals were tested with the extracts from containers sterilized by accelerated electrons and radiation dose 2.5 Mrad, they did not show any differences in physiological or biochemical indices when compared to animals in the control group. Animals exposed to extracts from containers sterilized with accelerated electrons and radiation in doses 3.5 and 5.0 Mrad, showed a number of differences when compared with animals in the control group: decrease in body weight; changes in liver, kidney, nervous system functions; and change in body reactivity. The weight coefficients of the internal organs were also changed. The pathological investigations showed remarkable changes in the microstructure of the internal organs and signs of albuminoid-fatty degeneration of the internal organs were increased. No differences were noted in the toxicological effects of the two methods for radiation sterilization (accelerated electrons and radiation) when similar doses were used on the PVC-plastic.

In order to confirm the conclusions made from these experiments, we have studied the toxicologic effects of the PVC-plastic sterilized by the fixed doses of radiation and accelerated electrons at 2.5, 3.5, and 5.0 Mrad. The results of this investigation also showed that an increase in the sterilization dose up to 3.5 Mrad remarkably decreased the hygienic affinities of the plastic and the toxic effects of the extracts on the experimental animals were increased in accord with an increase in the sterilization dose.

Heat Sterilization

Heat sterilization is widely used in medical practice as a method of decontamination due to its reliability, low energy exposure, and ease of operation. The other advantage of heat sterilization is that the sanitary-hygienic characteristics of the polymers exposed to heat, with the correct selection of the method and condition, are, as a rule, better when compared with the other methods of sterilization.

The main methods of the heat sterilization are air (180°C, 60 min) and steam (120°C, 45 min). In the manufacture of medical polymer-based devices, heat sterilization is used less often than other “cold” methods because of the high thermolability of many materials.

Two main events have been observed during polymer heating. The first event is connected with gradual transition of the linear polymer into a viscid-fluent state. This process, if one does not consider any possible changes in geometric form or size, is reversible and determined only by temperature. The second event is characterized by chemical reactions in the polymers leading to its destruction. This event is irreversible and depends on temperature, time, medium, and affinity of the polymer material.

The action of heat on the polymers, as with any other kind of energy, results in undesirable changes in properties. Heat destruction of the polymers is characterized by disintegration of the molecular chains into low molecular fragments and also by splitting of lateral groups and interaction of fragments. It is considered that the polymers obtained by polycondensation are usually destroyed within the law of incident, and the polymers obtained by polymerization are destroyed by the mechanism of depolymerization. The polymers with quaternary atoms of carbon in the molecular chains and low value of heat polymerization, as a rule, are degraded into monomers. The monomers are not always formed by the destruction of polymers containing the secondary and tertiary atoms of carbon and a high value of heat polymerization. Accumulation of monomers in the process of heat sterilization is an extremely undesirable event which results in the deterioration of hygienic features.

The influence of heat sterilization on polymers affinities can be considered with the devices derived from PVC-plastic.

PVC heat destruction is accompanied by hydrogen chloride release and formation of single- and conjugated double-links. The admixtures of iron, copper, and zinc salts exert a great influence on the process of PVC heat destruction.

As a result of polyene structure formation in the process of destruction, the polymer is strained, rigidity is increased, and mechanical durability is decreased. The dehydrochlorination process is observed at the temperature 100°C. With further

temperature increases, these processes are intensified.

Investigations were conducted with PVC-plastic containers used for conservation and prolonged preservation of blood and its components that were sterilized by autoclaving in two modes: (a) temperature 110°C, 60 min; (b) temperature 110°C, 60 min with drying at the temperature 100°C during 75 min. The results of these investigations showed that sterilization using more rigid temperature-time intervals remarkably deteriorated the hygienic features of the containers. This conclusion was made on the basis of sanitary-chemical investigations of the extracts from some items, an observed increase in bromination and pH, and the results of toxicological experiments. With respect to the latter, experimental animals exposed to containers' extracts sterilized under more rigid conditions, showed some changes in peripheral blood count, fermentative liver function, changes in the plasma histamine content, and histological changes in internal organs.

Sterilization of PVC-plastic used in the packaging of injection solutions of some medicinal preparations (by autoclaving at the temperature 120°C, 30 min) also produced changes in the hygienic characteristics of the items. Deterioration of the material stability was increased with prolongation of the post-sterilization period. There were also increases in pH values, oxidation, zinc ion content, and a vinyl-chloride monomer in the items' extract.

So, the results of these experiments indicate that one should be cautious in considering the suitability of saturated steam sterilization for some polymer items.

On the other hand, if the polymer possesses high thermostability, this method is easy and can be used on-site in clinics. Primarily, these involve silicone elastomers, vulcanized by silicon hydrasides peroxides. A wide range of diagnostic and therapeutic instruments, as well as disposable and reusable items such as catheters, probes, bougies, tubes for tracheotomy, hoses, airducts, and endotracheal tubes have been sterilized by heat.

Our investigations have shown that the levels of migration for chemical compounds released from the items sterilized by heat have been low enough. Identification using the chromatographic and spectral methods showed that among the products migrated were some admixtures involving vulcanized agents in rubbers and also products of its transformation. The results of toxicological investigations on these products at the levels they were discovered did not exert any negative action on the body. The opportunity for repeated heat sterilization for reusable items extends the product's usefulness. It was shown that the toxicologic and hygienic characteristics of items derived from silicone elastomers were preserved after sterilization at 100°C.

Chemical Sterilization

There are two ways of chemical sterilization. Gas, and with the help of aldehydes (glutaraldehyde and formaldehyde).

The search for an effective gaseous sterilizing agent has led to widespread use of ethylene oxide. Ethylene oxide is widely used for sterilization of polymer items with various constructions and purposes. The lethal effect of ethylene oxide on all kinds and forms of microorganisms has been adequately proven.

It is known that during the sterilizing process accumulation of the gas takes place on the

surface and inside of materials. The extent of such gas depends on its pressure, temperature, and humidity. For achieving the greatest contact of the gas with the microorganisms and consequently for improving the quality of the sterilization, these parameters should be increased to the levels that leads to result in increased gas absorption by materials. The amount of gas absorbed depends on affinity of the material sterilized. As a rule, desorption of gas proceeds slowly and its duration is variable, taking from several hours up to some weeks and months. This is also determined by characteristics of the gas and the materials to be sterilized.

The problem of removing of residue amount ethylene oxide and its derivatives from polymers is a problem in modern technology of gas sterilization.

There have been some reports about serious complications, including inflammation and necrosis following the application of endotracheal and tracheotomic tubes pre-sterilized with ethylene oxide. Burns to the face and hands were observed following application of masks and gloves when residue amounts of ethylene oxide were not removed. Cases of blood hemolysis in contact with medical PVC tubes pre-sterilized with ethylene oxide have been described. In general, despite the rapid introduction of gas sterilization into practice, data about remarkable health dangers of ethylene oxide and derivatives often formed in the sterilization process (ethylene chlorohydrin and ethylene glycol) have accumulated.

For example, we have conducted experiments that illustrate the influence of gas sterilization with ethylene oxide on hygienic features of the medical items. Dialysers with membranes of cellulose sterilized with ethylene oxide were investigated. Extracts from these items showed unfavorable influences on animals with the following signs: the weight of the animals lagged behind controls as well as changes in the weight of the heart, liver and spleen were noted. In our opinion, these effects were the result of the action of residue amounts of the sterilizing agent absorbed by the polymer on the body.

Toxicological investigations of some membrane oxygenators (type “Silar”) manufactured on the basis of co-polymer polyacrylate with polydimethylsiloxane after ethylene oxide sterilization showed some changes in the ratio of protein fractions in blood serum, hemoglobin content, and changes in the weight coefficients of internal organs of the experimental animals exposed to membrane extracts. These effects may be also explained as action of ethylene oxide since the non-sterile membrane does not possess toxic activity.

However, one cannot but admit that gas sterilization is an effective method. Problems with its application can be characterized in two groups: rate setting of residue amounts and efficiency of decontamination for medical items. Introduction of a biological control using highly sensitive objects, for example sex cells of mammalia, is very expedient. We have developed such techniques and have introduced them into industry.

Glutaraldehyde sterilization, widely used in other countries, has been distributed in our country. Glutaraldehyde is used as a tannic and sterilizing agent, mainly for cardiac valves and blood vessels made from biological tissues. Simultaneously it is used as a solution for preservation. Since 1962 when the sterilizing properties of glutaraldehyde were discovered, various concentrations of its solution have been proposed (from 20-25% to 2%). In our country it was shown that processing of xenobioprostheses of cardiac valves with 0.2% solutions and for a second time, 0.5% solution, had a good sterilizing effect.

The toxic features of glutaraldehyde have been well studied. It is assigned to the third class of danger according to a classification system adopted in my country (moderate toxic substances). The main signs of intoxication are lesions of the nervous system, changes in liver function, and changes in blood following single and repeated introduction into animals.

Contact of a 2.5% glutaraldehyde solution with the skin and mucosa of eyes causes irritation and heavy lesion of eyes. Glutaraldehyde in high doses possesses embriotoxic and gonadotoxic activity.

The main problems associated with the application of glutaraldehyde are as follows:

1) determination of the optimal concentration of a glutaraldehyde solution and processing conditions for the items;
2) development of optimal conditions for washing following glutaraldehyde for the various kinds of items; and
3) development of standards for residue amounts glutaraldehyde.

Results have shown that the optimal concentration of glutaraldehyde for sterilization and preservation of xenobioprostheses of cardiac valves and vessels is 0.5% solution.

In determining the washing conditions for items exposed to glutaraldehyde, it is necessary to note that it should be found empirically for each kind of item. Thus, this process is influenced by not only glutaraldehyde but also by some other substances which are added for hemocompatibility and anticalcium. A cardiac prosthesis treated with heparin and washed according to the standard technique of the U.S.S.R. Ministry of Public Health did not produce any toxic effect on animals at a glutaraldehyde concentration of 2 mg/L. At the same time the analogous prosthesis treated by sodium dodcilsulfate or EDTA-zinc and washed with the same technique produced remarkable toxic effects on biological test-objects and animals. The concentration of glutaraldehyde was equal to 4.56-5.93 mg/L.

The results showed that the toxic effects of glutaraldehyde are not evident in extracts with residual contents of 2 mg/L and lower. In some cases, glutaraldehyde was removed completely by strengthening the conditions used for washing.

With respect to the use of formaldehyde for sterilization of medical devices, it is necessary to note that, despite a lesser toxic danger, the problems with formaldehyde are the same as those with glutaraldehyde, i.e., optimal conditions of washing and determination of standards for residue amount.

Conclusion

Up to the present, the problems of interaction of compounds with polymers have not been extensively studied. Such investigations are needed.

The process of sterilization as a final step in the manufacture of medical polymer-based devices are corroborated by the results of toxicological investigations. Thus, the development of sterilization methods for medical polymer-based devices should be conducted in conjunction with determinations of potential danger with a goal of optimizing methods which preserve the biological compatibility of the materials and devices.

Low Temperature Plasma: A New Sterilization Technology for Hospital Applications

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Introduction

Low temperature gas sterilization has been in use for some time as an alternative to heat sterilization (both moist and dry). Although the predominant technology employed in low temperature sterilization is ethylene oxide (EO), formaldehyde is used in some applications, especially in various health care institutions outside the United States. As the use of sophisticated heat- and/or moisture-sensitive medical devices has grown, so has the need to sterilize instruments with means other than the steam autoclave.

Radiation sterilization, mostly involving gamma irradiation (cobalt 60), has been in commercial use for just over 30 years (2). However, because of the large investment necessary to build gamma irradiation facilities and the stringent technical controls required for safe operation, the technology has been limited to large industrial applications. It is basically beyond the reach of most health care institutions. Furthermore, the detrimental effects of gamma irradiation on the bulk properties of some nonmetallic materials places limitations on the use of the technology in applications which require repetitive processing of reusable nonmetallic devices.

At the same time that the desire for low temperature processes (such as ethylene oxide) has increased, so too have concerns about potential EO-induced problems. The recent intensified awareness of the role of chlorofluorocarbons (CFCs) in the depletion of the earth's ozone layer of protection is but one example. This problem along with the widely expressed concerns about the potential effects of the mutagenic and carcinogenic characteristics of both ethylene oxide and formaldehyde, have quickened the pace of efforts to find alternatives to current low temperature gas sterilization technologies.

In the search for alternatives, it is appropriate to start with some concept or characterization of the hypothetical ideal sterilization system. Since our focus in this report is on applications in hospitals and other health care institutions, the boundaries of such an ideal sterilization system are discussed from that prospective and can be summarized as follows:

— **Quick turnaround time**: Instrument turnaround time with modern hospital steam sterilizers (pre-vacuum) is about one hour, including drying and cooling time. The ideal sterilizer should at least be equivalent.

— **Low temperature**: At operating temperatures between 45° to 55°C, ethylene oxide has been shown to be compatible with a wide range of materials. The target for an ideal system should, therefore, be in or below this temperature range.

— **Versatility**: The technology should be adaptable for a wide range of applications,

not only with respect to materials and instruments, but also with regard to sizes and sites of use.

— **Toxicity**: Toxicity is a subject, of course, which should be of paramount concern with any new technology. Safety should be with respect to both operators and patients as well as to the environment in general.

Among the many alternative systems being investigated is plasma technology. It may be helpful to note that the plasma which is the subject of this paper is not the same as the plasmas encountered in biology or the medical sciences, but rather that state of matter usually derived from gases or vapors.

What follows is, first, a description of several fundamental features of plasmas and, subsequently, a discussion of some experimental results from studies on plasma sterilization conducted at Johnson & Johnson Medical, Inc., over the last few years. Finally some key factors relevant to the successful commercialization of plasma technology for hospital sterilization will be highlighted. This report will not discuss in any detail the features of any particular product.

Plasma Technology

Plasma is defined as a fourth state of matter, distinguishable from solids, liquids and gases. It can be produced through the action of either very high temperatures or strong electric or magnetic fields, and it is normally composed of a reactive cloud of ions, electrons, and neutral species (3). In a plasma, the concentrations of positive and negative charge carriers should be approximately equal. This condition is satisfied when the dimensions of the discharged gas volume are significantly larger than the Debye length, λD. The Debye length (3) is defined as:

$$\lambda D = \frac{\xi_o \, kT_e}{ne^2} \; 1/2$$

D represents the distance over which a charge imbalance can exist.

Where:
- Λ = the difference of the discharge gas
- λD = Debye length
- ξ_o = the permitivity of free space
- k = the boltzmann constant
- T_e = the electron temperature
- n = the electron density
- e = the charge on the electron

There are two categories of plasmas generally of interest in plasma chemistry. One category includes those plasmas described as high temperature plasmas, produced by arcs or plasma jets. Temperatures of these plasmas are normally greater than 5,000°K and they are not of interest to our present discussion. Our interest lies in the second category, which includes those plasmas described as glow discharge or low temperature plasmas.

Low temperature plasmas are characterized by the following features (3):

— Average electron energies in the range of 1-10eV

— Electron densities in the range of $10^9 - 10^{12}$ cm^{-3}
— Ambient temperature of the plasma is not in equilibrium with the electron temperatures; usually T_e/T_{am} is in the range of 10-100, where T_e is the electron temperature and T_{am} is the ambient temperature of the plasma.

Background of Plasma Sterilization

The application of plasma technology to instrument sterilization has been investigated by a number of researchers for at least the last 20 years. One of the earliest disclosures on the use of plasma as a sterilizing agent was in a 1968 patent assigned to the Arthur D. Little Company (16). The process involved the use of a pulsed high pressure/high temperature plasma for sterilization. The first application of plasma to low temperature sterilization was reported in 1972 in a separate patent granted to Arthur D. Little (1). That process involved the use of low pressure, low temperature plasmas based on halogen gases for the sterilization of container surfaces.

Other disclosures in the patent literature on the use of plasma include the work by the Boeing Company (12, 13) on the development of a flow-through plasma sterilization system for medical devices; by Boucher (8) on the combination of plasma and aldehydes in a process described as “seeded plasma”; and by Motorola (5,6) on sterilization through sealed porous packaging, and on the use of pressure pulsing of the plasma to enhance sporicidal activity in apertures and narrow lumens.

While there are a number of other development efforts not cited here, the above examples highlight the increasing interest in using plasmas for instrument sterilization.

Why Plasma?

From a fundamental point of view, the selection of plasma as a potential sterilization technology can be justified by a number of the inherent attributes of glow discharge plasmas which approach the features desired for an ideal sterilization system.

Through the proper design of the system as well as the appropriate choice of precursor gases or vapors from which to generate the plasma, a fairly low ambient temperature can be achieved even in a highly reactive environment. The highly reactive environment enhances the potential for rapid sterilization.

Further, because the active species are created only when power is applied to the system, and immediately eliminated when the input energy is withdrawn, the very active species which can cause rapid inactivation of microorganisms will not be present as a potential source of toxicity and environmental hazard when the process is terminated. Additionally, through appropriate selection of the precursor gases or vapors, the opportunity to achieve nontoxic residues or by-products can also be realized.

Another area of potential advantage for glow discharge plasmas is that their effects are generally restricted to the surfaces of materials so, inherently, they should be free of the problems associated with the effects of gamma irradiation on the bulk properties of some materials.

Experimental Studies

It is appropriate to consider the important practical issues involved in instrument sterilization with plasmas. In the following sections, issues such as the importance of the selected active species, precursor gases, decomposition or by-products, packaging, the methods of plasma generation, and key process parameters will be discussed. The discussion that follows is based on the experimental results of research by Lin (15) and Dr. Paul Jacobs at Johnson & Johnson Medical, Inc. It should be noted that, at present, there is relatively little in the public literature on the theoretical aspects of plasma sterilization and such theory is not the focus of this report.

Active Species and Parent Gases

As indicated earlier, the precursor gas or vapor that one selects for plasma generation should be a critical factor in sterilization since it dictates not only the antimicrobial species susceptible to sterilization efforts, but also toxicity-related factors such as chemical residues and by-products from the process.

It is obviously desirable to select those precursors whose end products are simple, nontoxic compounds such as O_2, H_2O, and H_2. Of course, we also want to have present in the plasma those active species known to have high sporicidal activity such as $\cdot OH$, $HO_2\cdot$, $O\cdot$, and $H\cdot$.

In order to evaluate the relative sporicidal activity of these species, the microwave discharge apparatus illustrated in Figure 1 was used to generate specific radicals, using oxygen, hydrogen, nitrous oxide, and nitrogen dioxide in various combinations as precursor gases (15). It should be noted that plasmas based on monotomic inert gases such as helium and argon were not included in these experiments because at low temperatures their plasmas are apparently lower in sporicidal activity than the species under consideration here.

The sterilization chamber consisted of a pyrex vessel which connected to a vacuum pump for drawing vacuum to pressures in the range of 0.1 to 0.5 torr (or 0.13 to 0.67 hektopascal). Primary gas was introduced into the chamber at one end, where the gas could be acted upon by the microwave discharge system consisting of a Raytheon PGM 10×1 microwave power generator which produced 10 to 100 watts of energy at 2.45 GHz. A secondary gas inlet was also provided for some of those experiments that required a mixture of gases to obtain the appropriate species, as shown in Figure 1.

Figure 2 illustrates the comparative activity of these active species against *Clostridium sporogenes* spores at room-temperature, and at various distances from the source of generation of the radicals. The experiments were conducted at a pressure of 0.5 torr and at a power level of 100 watts. Approximately 10^6 spores were inoculated onto glass slides and in all cases were exposed to the active species for 30 minutes. The flow rate of the gases was set at approximately 0.1 ml/minute. As shown in the plots, sporicidal activity decreases for all species as the distance from the source of generation increases, as expected. As the radicals interact with the walls of the reaction vessel and with other

molecular species in the vessel, their concentration downstream is expected to be lower, resulting in lower sporicidal activity. The curves also suggest that the oxygen-containing radicals may be more potent than hydrogen for sporicidal activity at room temperature. The results of similar experiments conducted at 60°C indicated that the relationship between sporicidal activity and distance from the source is similar to the results at room temperature. However, the higher temperature data also indicate that the magnitude of temperature effects may be different for different active species. For instance, the increase in temperature appears to have the most beneficial effect on H activity. Activity at 12.5 cm distance was improved by a factor of about 100 (or two logs), while the corresponding improvement of ·OH activity is almost negligible. These temperature effects, which are consistent with the known activation energies for these species, are further illustrated in Figure 3. As shown, O· appears to be the most sporicidal at both temperatures. However, the data also indicate that under the appropriate conditions all the species evaluated could be quite sporicidal. The significant fall-off of the activity of the ·OH radical with distance is also consistent with the known reactivity of hydroxyl radicals.

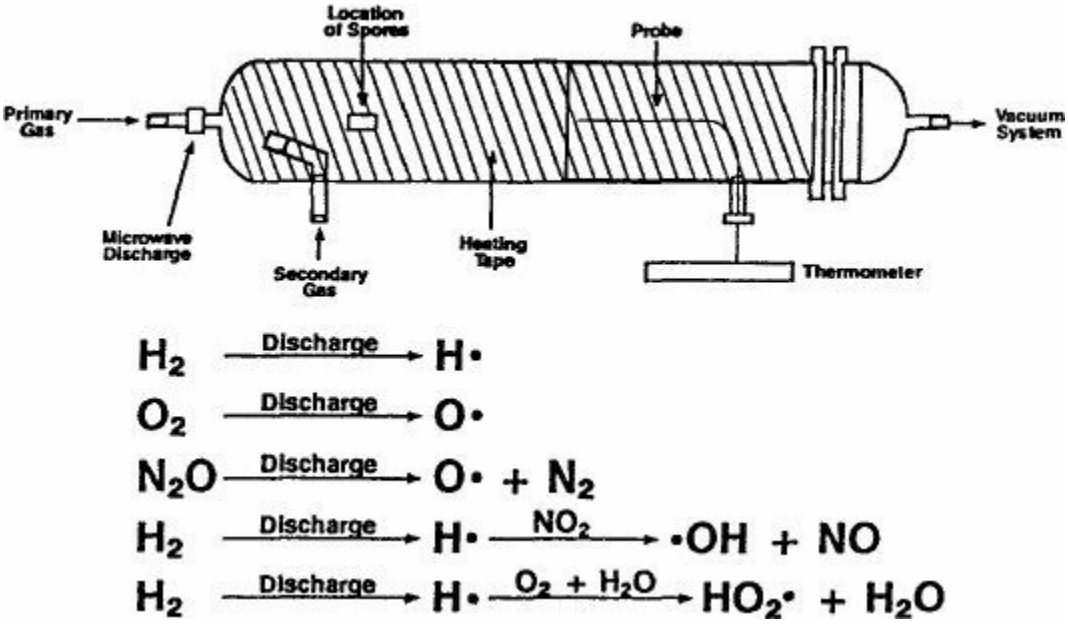


Figure 1. Microwave discharge system for generation of active species

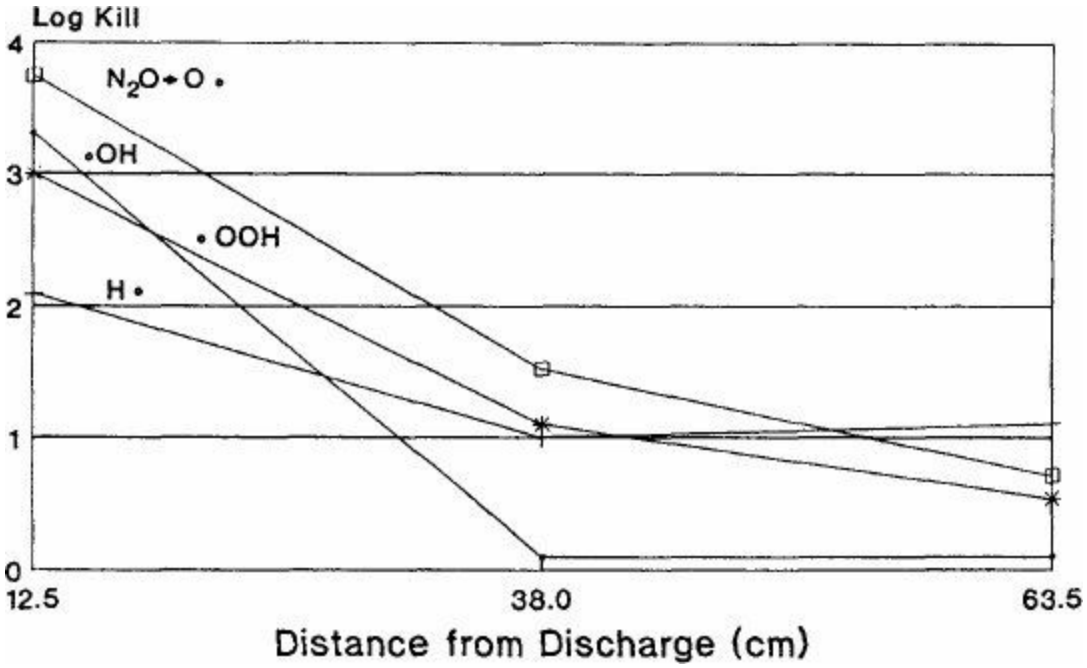


Figure 2. Comparative activity of various radicals at room temperature against *Clostridium sporogenes*

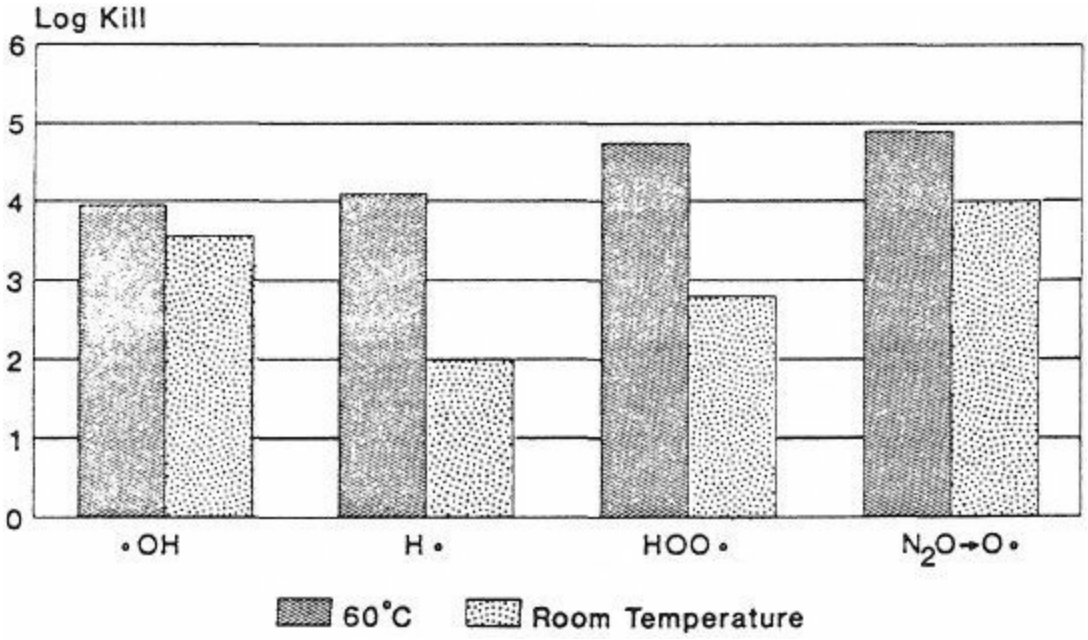


Figure 3. Comparative sporicidal activity of radicals at 60°C and room temperature against *Clostridium sporogenes*

In another series of studies with the same experimental apparatus, the resistances of several organisms to the various radicals were evaluated at 60°C. It was determined that the *Bacillus subtilis* var. *niger* spore was consistently more resistant to all the radical species than were *Clostridium* and other spores tested. The experiments to be described in the following sections were thus conducted using *B. subtilis* as the indicator organism.

Turning to the subject of precursors, it should be recalled that our interest is in selecting those compounds which provide the desired active species *and* which can result in simple decomposition or by-products. For evaluation of candidate precursor gases or vapors, an

inductive radio frequency (RF) discharge system was used, as illustrated in Figure 4. In contrast with the microwave discharge system, the RF discharge is capable of producing a broad spectrum of radicals and other reactive species in the plasma. From practical considerations, the RF discharge system is also superior to the microwave system for scaling up, partly because of its much broader usage in many applications in plasma chemistry. RF discharges are also less difficult to sustain at low pressures. The apparatus was used to study the comparative sporicidal activity of various precursors including water vapor, hydrogen, oxygen, nitrous oxide, and hydrogen peroxide. For these tests, about 10^5 *B. subtilis* (var. *globigii*) spores on paper discs and in polyethylene packages (Tyvek) were placed in the center of the chamber which was then evacuated. Depending on the precursor, an aqueous solution or gas was introduced into the chamber and the pressure maintained at approximately 1.5 torr. The gases or vapors were then allowed to flow into or remain in the chamber for approximately ten minutes before plasma generation was initiated. (The reason for this procedure will be discussed in a later section). The concentration of hydrogen peroxide was about 0.2 mg/liter of chamber volume. The plasma was generated at 2.49 MHz frequency and at a lower level of 150 watts, with a power pulse ratio of 1:2 (one millisecond on and two milliseconds off). Plasma was maintained in all cases for 15 minutes before the process was terminated and the spore samples assayed for survivors.

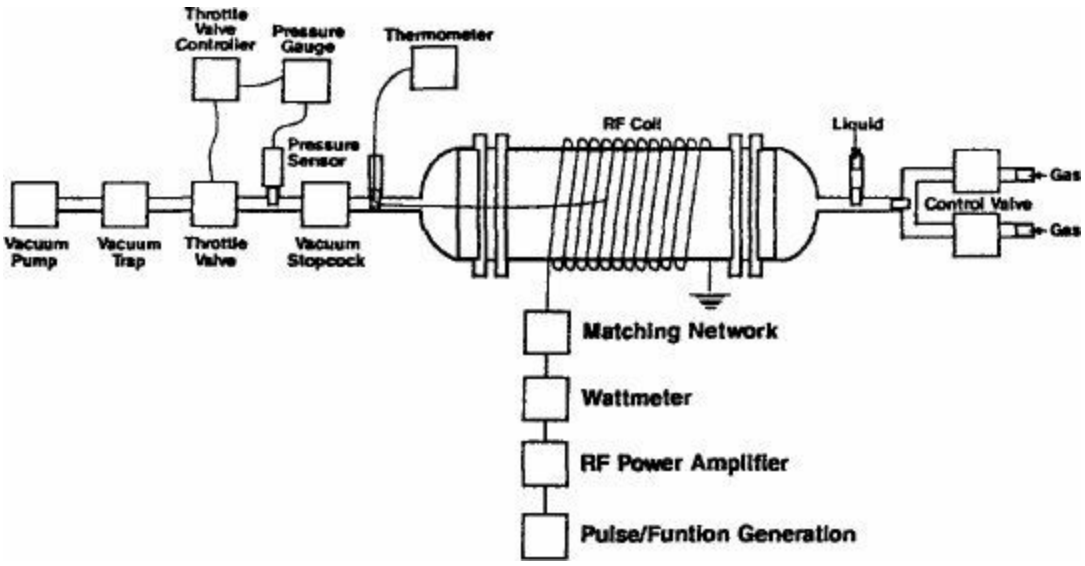


Figure 4. Schematic diagram of RF discharge apparatus

As shown in Figure 5, the hydrogen peroxide plasma has significantly higher sporicidal activity than the plasmas from the other precursors, achieving a greater than five-log reduction (total kill) in spore population. The next most effective precursor, N_2O, achieved less than one-log reduction under the test conditions.

Why is hydrogen peroxide plasma so effective compared to the other plasmas studied? While no theoretical models yet exist to totally explain the difference, the results are consistent with a number of the known characteristics of hydrogen peroxide and its dissociation or activated species, as described below.

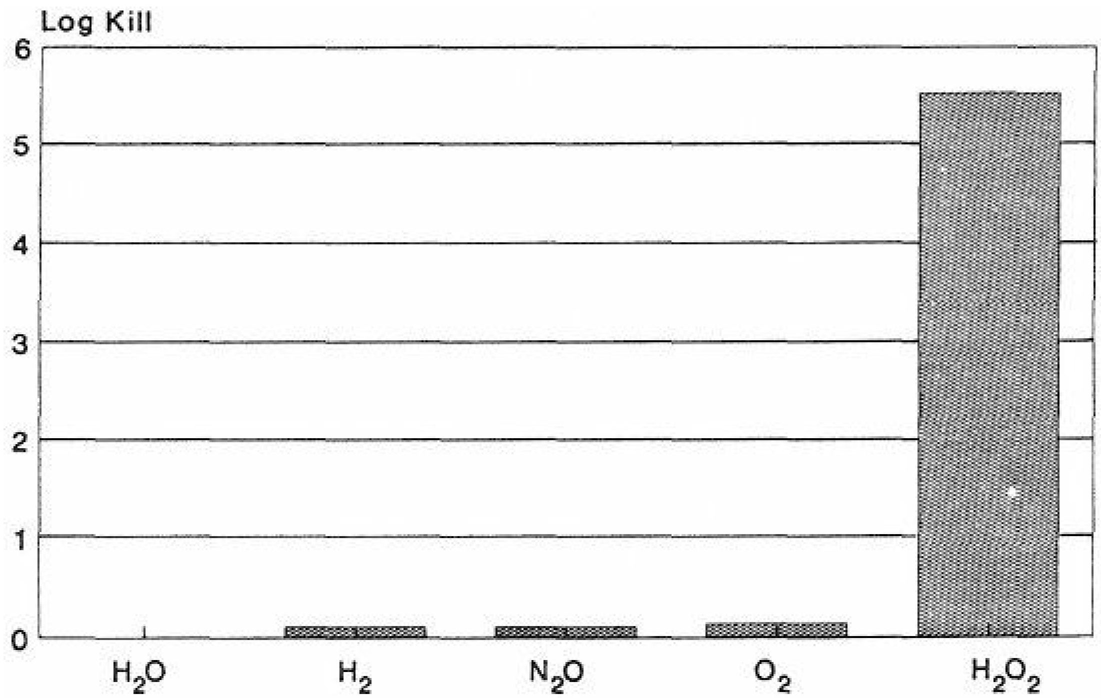


Figure 5. Sporicidal activity of various plasmas against *B. subtilis* (var. *globigii*)

First, the bond energy involved in generating ·OH radicals from hydrogen peroxide is relatively low—about 51 Kcal/mole compared to about 119 Kcal for H_2O, for instance. Thus, for the same power input, it is much easier to generate a rich reactive mix from hydrogen peroxide than from most other precursors.

Secondly, the active species generated from hydrogen peroxide are among the most reactive known activated species, particularly the ·OH and $\cdot O_2H$ radicals, UV radiation, and a number of other oxygen containing radicals of known high oxidative potential. Thus, while N_2O has a somewhat lower bond energy (40 Kcal/mole) associated with the formation of the O· radical, the reactive mix in a nitrous oxide plasma is potentially not as rich as that in a hydrogen peroxide plasma.

Based on the known dissociation products of H_2O_2, the mechanism for the generation of the highly reactive mixture in a hydrogen peroxide plasma can be simplified as illustrated below:

$$\text{H-O-O-H} \xrightarrow[\text{1-10eV}]{e^-} \text{HO}\cdot + \cdot\text{OH}$$

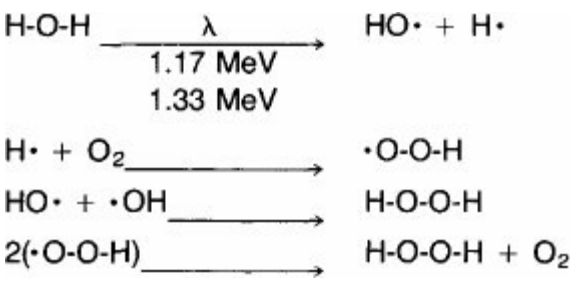
$$\text{HO}\cdot + \text{H-O-O-H} \longrightarrow \text{HOH} + \cdot\text{O-O-H}$$

$$\text{H-O-O-H} \xrightarrow{e^-} \text{H-O-O-H}^*$$

$$\text{H-O-O-H}^* \longrightarrow \text{H-O-O-H} + \text{Visible and UV Radiation}$$

The chain of events resulting in the glow discharge hydrogen peroxide plasma is started by the accelerated electrons which result from the strong electric field imposed on the chamber by the RF system.

The sequence of events is similar to the suggested chain of events involved in the secondary reactions during sterilization by gamma irradiation, as illustrated below (7):



The reactivities of the key radicals to be expected in a hydrogen peroxide plasma have been discussed by a number of researchers (9-11, 14).

From the results of the experimental studies described above, in conjunction with the generally postulated characteristics of various free radicals, hydrogen peroxide is— among the list of other candidate gases and vapors—a particularly suitable precursor for plasma sterilization. Beyond its sporicidal characteristics, hydrogen peroxide plasma also has significant advantages with respect to the probable decomposition products which, because of the simplicity of the structure of H_2O_2, are predominantly O_2, H_2O, and H_2, as desired for an ideal sterilization system.

Packaging

Of importance to any terminal sterilization system is the method of preserving sterility until the sterilized item is delivered to the point of use. This means that it generally is necessary to be able to sterilize instruments after they have been sealed in a package designed to preserve sterility. To evaluate the potential effect of such packaging on the sporicidal activity of various radicals, comparative tests were conducted in the microwave discharge apparatus shown in Figure 1 using both packaged and unpackaged *B. subtilis* spores. The package was made from a porous nonwoven fabric sold under the tradename of Tyvek, a microfiber polyethylene material produced by DuPont and used widely for sterile packaging. The tests were conducted in a similar manner to those described previously which evaluated the comparative sporicidal activity of the same radicals against *C. sporogenes*. As shown in Figure 6, the presence of the package substantially inhibited the activity of all the radical species. The packaging resulted in the loss of sporicidal activity by as much as three logs.

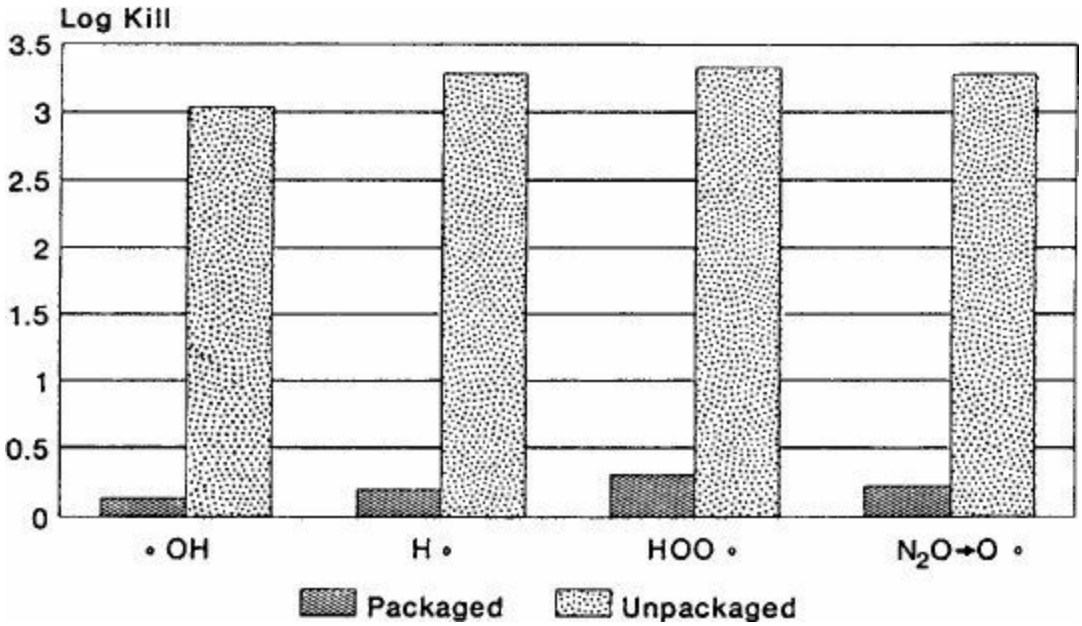


Figure 6. Comparison of sporicidal activity of free radicals between packaged and unpackaged *B. subtilis* (var. *niger*) spores

At the boundaries of the package, collisions and other interactions between the active species and neutral molecules, or more importantly the “walls” of the package, or with other activated species, would cause a significant proportion of the active species to return to ground state, and thus have only a small fraction of the generated species penetrating the package to effect the inactivation of spores. The package is then an effective barrier to the penetration of free radicals.

The problem of packaging has been a major issue for many of the previous efforts to exploit plasma technology for sterilization in a broad range of applications where packaging is necessary.

In evaluating various approaches to the problem, it was discovered that, under appropriate conditions, plasma can be generated within packages with a high enough concentration of free radicals and other active species to cause inactivation of a large population of spores. This, however, required that the precursor be allowed sufficient time to diffuse through the package to the sites of microbial contamination *before* initiating the generation of plasma at the power level required to achieve sterilization. With this approach, the negative effects of the interactions at the boundary of the package are significantly reduced. To illustrate the importance of this “pretreatment” time for diffusion, an experiment was conducted in the RF discharge apparatus shown in Figure 4 for generation of hydrogen peroxide plasma. Spore samples on paper discs in Tyvek packages were assayed for survivors after being subjected to diffusion times ranging from one to ten minutes, followed by exposure to plasma at 150 watts with a 1:2 power pulse ratio for 15 minutes. The hydrogen peroxide concentration in the chamber was approximately 0.2 mg/liter, and the pressure was maintained at about 1.5 torr. Figure 7 shows the dramatic effects of the diffusion or “pretreatment” time on the sporicidal activity of H_2O_2 plasma. As expected, the effect of the diffusion time apparently plateaus after some period; this period may be influenced by the package and load in the chamber. For comparative purposes, Figure 7

also includes results obtained when no hydrogen peroxide was introduced in the system and when hydrogen peroxide was introduced as in the previous experiments but plasma was not activated for the entire 25 minutes of exposure time. The plots illustrate that little activity is achieved under these two latter conditions, thereby highlighting the significant role of the active species generated in the hydrogen peroxide plasma.

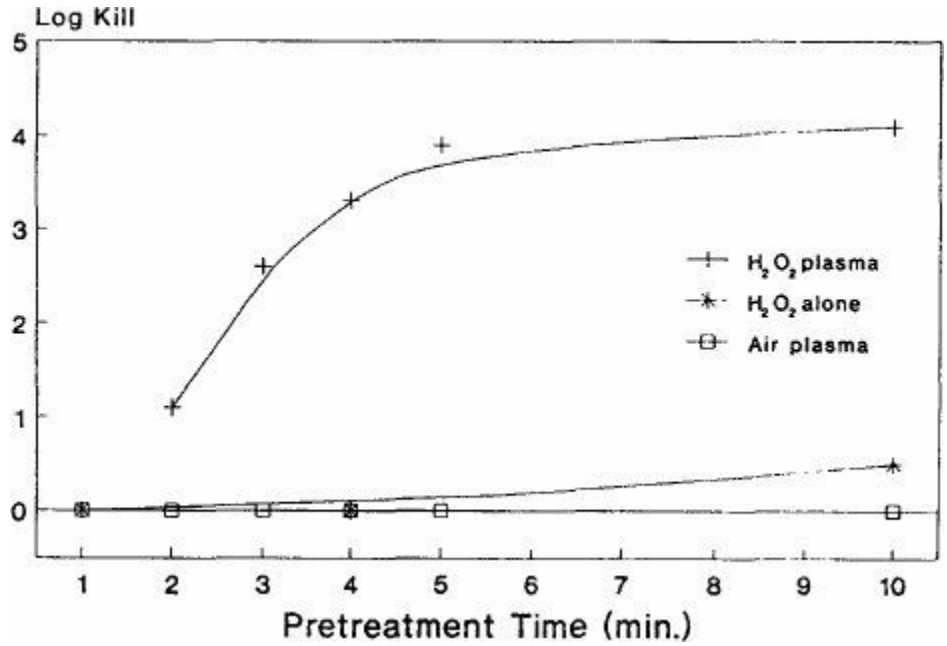


Figure 7. Effect of H_2O_2 pretreatment time on sporicidal activity of H_2O_2 plasma

Plasma vs. Heated Vapor

Depending on process parameters and method of generation, plasmas can be associated with varying degrees of heat. Because of the known sporicidal activity of hydrogen peroxide itself, it is useful to separate the sporicidal effectiveness of plasmas from the potential action of heat and hydrogen peroxide. Comparative tests were conducted in the RF discharge system. In the experiments involving no plasma an infrared heat lamp was used to heat the sterilization chamber at about the same rate of temperature increase as obtained with the RF plasma. The experiment included the determination of sporicidal activity as well as the depletion of H_2O_2, which should be expected in a plasma environment. This depletion was determined spectrophotometrically by measuring the amount of H_2O_2 on carrier paper discs before and after the plasma or heat treatment.

Tyvek-packaged paper discs were first subjected to hydrogen peroxide pretreatment at a concentration of about 0.2 mg/liter at three torr for about 15 minutes, and subsequently subjected to plasma at 150 watts and a pulse ratio of 1:2 at 3.89 MHz, or alternatively to infrared heat, for five, ten and 15 minutes. The treatment pressure in both cases was about 1.5 torr.

Sporicidal tests were conducted with Tyvek-packaged *B. subtilis* (var. *globigii*) spores, and the initial spore population was about 10^6. Figures 8 and 9 are plots of the data on both the sporicidal activity and hydrogen peroxide depletion for heated vapor and plasma,

respectively.

In comparing the two figures, it is evident that the action of hydrogen peroxide plasma is dramatically different from that of heated hydrogen peroxide. The kinetics appear to be different. As expected, the plasma rapidly deactivates spores and at the same time rapidly depletes H_2O_2 (in sustaining the plasma) and thereby reduces the level residuals. Thus, within five minutes of plasma treatment, total kill has been achieved. By contrast, as seen in Figure 8, minimal sporicidal activity was obtained with the heated hydrogen peroxide within five minutes. Even though the sporicidal activity improved after 15 minutes, total inactivation was not achieved and there was no reduction of H_2O_2 residuals. The plasma within 15 minutes had removed about 95% of the H_2O_2 on the paper disc.

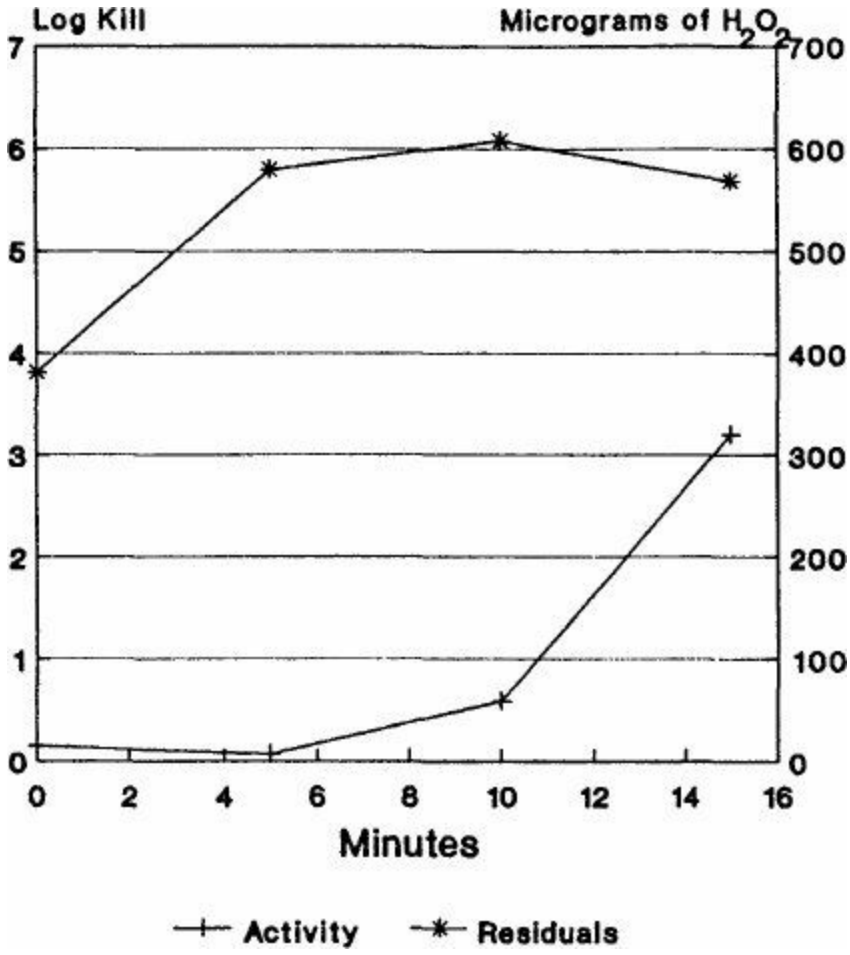


Figure 8. Sporicidal activity and H_2O_2 residuals of heated H_2O_2

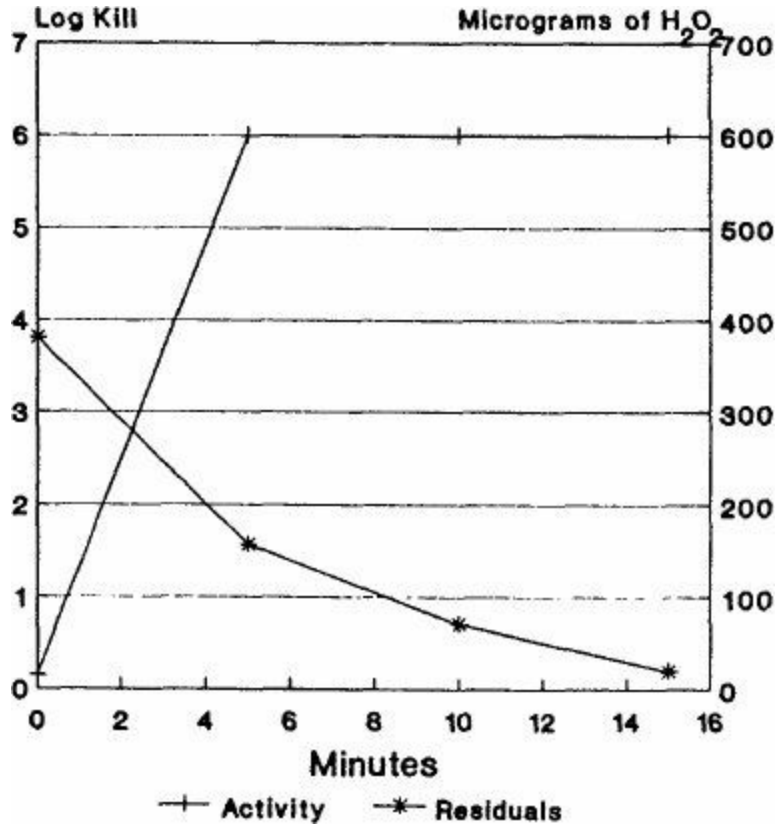


Figure 9. Sporicidal activity and H_2O_2 residuals of H_2O_2 plasma

Process Parameters

Having discussed some of the fundamental aspects of plasma sterilization, we should briefly mention some of the key process parameters and design issues relevant to any attempts to commercialize the technology.

Plasma Generation

There are many types of plasma generators, some of which, for example, are summarized by Bell et al. (4). Of most active interest in low temperature sterilization applications are probably RF and, to a lesser extent at this point, microwave-generated plasmas. Of these, RF systems are the most familiar and perhaps the most flexible from a design point of view. However, even after selecting the method of inducing the plasma, there are still a number of design choices one must make. For example, a major factor in the design of RF systems is the method of coupling the RF energy into the reaction or sterilization chamber. One can, for instance, select either a capacitively coupled system or an inductively coupled system, depending on the particular needs of the application. There are also several types of chamber configurations for various applications, including cylindrical and parallel plate reactors. Finally, a choice can be made to operate within either a primary or secondary plasma environment. Frequency is also a factor to be considered. The design of the plasma chamber and generation parameters influence plasma uniformity, efficiency, and effects on materials.

Concentration of Precursor

Since the precursor provides the raw material for the active species, it is expected that the higher the concentration, the more reactive species will be available for sporicidal action. The maximum concentration is, of course, limited by the pressures required for efficient generation and maintenance of the plasma. Figure 10 illustrates the effect of increasing H_2O_2 concentration on the sporicidal activity of the plasma. Packaged *B. subtilis* spores were subjected to ten minutes of pretreatment at one torr pressure followed by plasma at 200 watts and a 1:2 pulse ratio for 15 minutes. The effect of increasing concentration was as expected. For control purposes a similar experiment was run for the same total duration, but without the plasma. Again as shown in the H_2O_2 plot, very little activity was achieved without plasma.

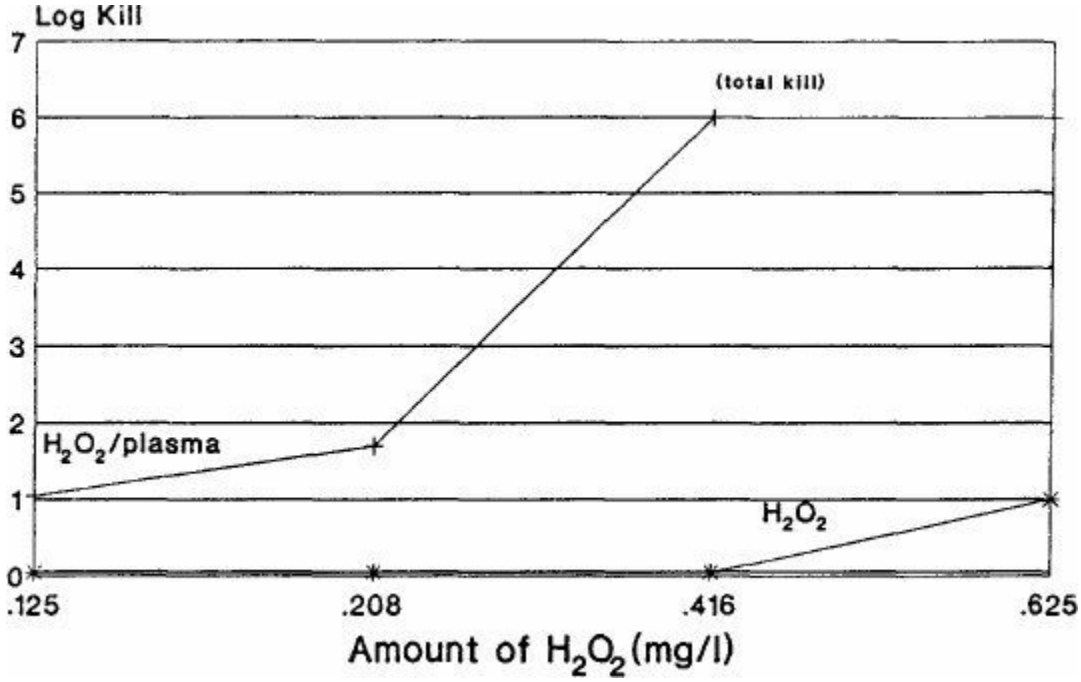


Figure 10. Effect of H_2O_2 concentration on sporicidal activity of H_2O_2 plasma with Tyvek-packaged *B. subtilis* (var *niger*)

Plasma Power

As expected, sporicidal activity increases as RF power is increased. Here as well, it should be noted that since increased power is usually associated with higher temperatures, and perhaps increased potential for detrimental surface effects, it is often necessary to increase power levels only within well-defined limits.

Pressure

The influence of pressure on the sporicidal activity of plasmas is not as direct as those of precursor concentration and RF power levels. From the results of the experiments conducted by Lin (15), it is evident that for a given set of other plasma parameters (especially frequency), there is a specific pressure range in which sporicidal activity is

maximized. For most applications and frequency considerations, the optimum pressure range is probably below two torr. It should also be noted that the temperature of the plasma tends to increase as the pressure increases.

Frequency

The frequency used to generate the plasma was demonstrated to have a definite effect on the temperature, optimum pressure and thus, the sporicidal activity of the plasma (15). Both temperature and sporicidal activity tend to increase with frequency. Experiments at Johnson & Johnson Medical, Inc. with frequencies ranging from below one MHz to microwave frequencies appear to confirm this general trend.

Conclusion

In conclusion, while there is presently very little in the literature on the fundamental aspects of plasma sterilization, and while there is yet no theoretical models to explain the sporicidal activity of plasmas, there are sufficient empirical data to indicate that, under certain conditions, plasmas can be used as highly effective and practical methods for instrument sterilization. Furthermore, plasmas do not appear to be associated with many of the problems inherent in current gaseous sterilization systems. Undoubtedly, research efforts will continue to expand our understanding of the fundamentals of plasma sterilization technology and to broaden its potential application beyond the hospital setting.

Since plasmas occur at low pressures, an important limitation of plasma sterilization is that it cannot be used for the sterilization of liquids or other “wet” products such as biological tissues to be preserved for implants. Moreover, to process cellulosic materials with high affinity for H_2O_2 such as paper and linens, it is necessary to use concentrations of H_2O_2 and/or processing times that diminish the potentially unique attributes of plasma sterilization as a rapid process with no toxicity problems.

Based on the general principles presented in the above discussion, a new hydrogen peroxide plasma sterilization system is under development at Johnson & Johnson Medical, Inc. In our laboratory, testing has shown that the system has a broad spectrum of antimicrobial activity, as well as a surprisingly wide range of material compatibility, both in terms of physical effects and biocompatibility.

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Session II
Sterilization Standards
Chairman: P. Doolan
Becton Dickinson and Company, France

Sterilization Standards in the U.S.S.R.

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In compliance with the documents of the International Standardization Organization, all the world countries, including the U.S.S.R., assume standardization to be "the activities aimed at attaining an optimal degree of consistency in a particular area by introducing guidelines for the existing or potential task". Standardization is a most important component of the overall management of the national economy, aimed at improving the level of technology, product quality and cost-effectiveness, intensification of national production forces, and a greater production efficiency and development of a rational commodity nomenclature.

The main objective of standardization in the U.S.S.R. is the development of regulatory documents and technological rules, which establish the up-to-date requirements products must meet when manufactured for the needs of the national economy, Soviet population and defenses and export, the manufacturing and applications standards, as well as monitoring procedures for compliance with these documents. Soviet standards are developed on the basis of close relations with the development of International Standards (International Standardization Organization, Council for Mutual Economic Assistance). International Standards are either directly converted into state standards, or taken into account when the latter are developed. In the U.S.S.R., standards have been given the status of compulsory documents whose non-compliance is prosecuted.

Development of standards is an indispensable component of the work on new or upgraded products. Standards are, as a rule, based on the results of research and development and design efforts. The conventional life-time of a standard is five years. New developments may result in amendments which are introduced on a legal basis.

Standards may be distinguished into two categories: All-Union state standards or Branch standards.

In addition to the above-mentioned standards–in line with their development or to complement them–methodological documents may be written. For example, in the area of health care, sanitation norms, methodology guidelines, recommendations, rules, and individual techniques are developed.

The state standards are authorized by the U.S.S.R. State Committee for Standardization. The branch standards and methodological documents which are in force for sections of the national economy are authorized by the relevant departments. One of the prerogatives of the U.S.S.R. Health Ministry, which is mandated by law, is the authorization of regulatory documents.

A number of standards are in force in the area of sterilization in the U.S.S.R. These can be divided into four groups. The first group are Terminology Standards. These include the definition of concepts concerning the sterilization and disinfection of medical products, as

well as the design of sterilization equipment. By means of example, consider the state standard “Methods, means and schemes of the sterilization and disinfection of medical products. Terms and definitions”. This standard defines the terms and gives definitions to the methods, means, and schemes of sterilization and disinfection of medical products to be used in medical practices. These are compulsory for use in science, technology, and production. This standard specifies particular aspects such as:

— sterilization/disinfection method;
— sterilizing agent/disinfectant;
— sterilization/disinfection means;
— sterilization/disinfection scheme; and
— sterilization/disinfection parameters.

The standard also includes the definitions of terms characterizing sterilization methods (e.g., steam, air, radiation, gas sterilization, sterilization by solutions), and sterilization schemes (e.g., sterilization time, temperature, solution concentration, gas dose, irradiation, sterilization control—bacteriological, physical, thermal, chemical).

The same group of standards also includes the state standard “Steam, air and gas sterilizers. Terms and definitions” which specifies the terms and definitions for main concepts for steam, air, and gas sterilizers used in medical institutions that are to be applied in science, technology, and production.

The second group of standards specifies the requirements sterilizers must meet to ensure adequate sterilization. Such standards are represented by the state standards “Medical steam sterilizers. General specifications and testing procedures”, “Medical air sterilizers. General specifications”, and others.

The former standard describes the classification of steam sterilizers as related to the mechanical impact they are subject to (stationary, portable), the position of a load port (lateral, vertical), the concept of loading and unloading, the type of heating and basic sterilization schemes, falling into the categories of single program units, double program units, or multiprogram units. The range of the sterilization chamber volumes is specified as varying from 4 to 1300 dm^3. The standard describes specifications, including the long-range ones, and sets the dates of implementation for some of them.

The standard, “Medical air sterilizers. General specifications” includes comprehensive technical characteristics for air sterilizers and the requirements for them, with the dates of implementation specified. Similar to the standards governing steam units, this standard also includes categorization with respect to the position of the loading port, the shape of a sterilization chamber and the loading and unloading procedure used. The range of loading volume of the chamber is specified as 1-1300 dm^3. Specifications include the degree of automation, blocking, process indication, recording of the sterilization process parameters, mechanization, material handling mechanization, sterilizer heating, forced cooling with a bacteria-excluding filter, etc. Acceptance rules are also indicated as well as the testing procedures to check main technical characteristics of sterilizers. The standards include the sterilizer classification, sterilization cycle, basic sterilization schemes and relevant times of sterilization exposure.

The third group of standards defines the methods, means and schemes of sterilization treatment (disinfection, presterilization cleaning, sterilization) of medical products for multi-use purposes. This group includes the state standard “Sterilization and disinfection of medical products. Methods, means and schemes”. This standard is compulsory for those institutions where medical devices are used, such as hospitals and outpatient clinics, schools, daycare centers for children, etc., as well as for those organizations and industries which design and produce medical devices. The standard formulates indications for effecting presterilization cleaning, disinfection, and sterilization. The order in which presterilization cleaning is made is described, detergents and rules for their use are given, including corrosion inhibitors, and methods, means and schemes of sterilization and disinfection are described.

Physical and chemical methods may be used to effect sterilization. Particular methods and means are recommended. The choice is based on the properties of materials, type of surface treatment, and design characteristics. As a further development of this standard, for example, related to medical metal devices, to aid the designers and manufacturers, the standard “The resistance of medical metal devices to the means of disinfection, presterilization cleaning, and sterilization” may be used. This standard divides all the medical devices conditionally into five groups on the basis of the materials used for manufacturing, galvanic coating, and design particularities. For each group, a respective disinfection and sterilization method may be recommended. The standard was developed on the basis of the case-studies for specimens which were exposed to particular sterilization/disinfection methods to test their resistance. An availability of such a standard may improve the development of regulatory documents and production instructions for medical products.

Practically all the documents (state and branch standards, specifications of particular items) include the requirement for sterilization/disinfection resistance for medical products. When regulatory and production documents are developed, the principle of standardization of specifications is observed, based on the actual conditions of medical product use in practice. In such connection, there is a need to standardize an indicator such as “The resistance of medical products to the means of sterilization and disinfection”. Standardization of this requirement makes a designer select such a material and provide such a surface machining that a product would not corrode, its metal and paint coat would not peel, and its functional properties would not fail. This requirement is standardized in all types of regulatory and production documents (CNEA standards, state and branch standards, specifications), including requests for proposal (RFP) for the development of new products.

In basic standards for medical products, this requirement has been specified as “the products and parts thereof, which are to be disinfected, must be resistant to the impacts stated in the regulatory and production documents on the disinfection methods. The parts of the products which come in contact with the wound and mucosal surface must withstand the following cycle of treatment: presterilization cleaning, disinfection, and sterilization”. This has been reflected in the state standards “Medical instruments, apparatus, and equipment. General specifications” and “Medical metal devices. General specifications”.

The fourth group of standards may include branch standards specifying the requirements for sterilization of particular products. By means of example, consider the branch standard "The resistance of medical metal devices to the means of disinfection, presterilization cleaning, and sterilization. Classification. Choice of method", which is used as a basis for the development of instruction manuals authorized by the U.S.S.R. Health Ministry. As such, the following documents may be mentioned:

— "Instructions in methods of presterilization cleaning…";
— "…of the use of corrosion inhibitors…";
— "…of sterilization of dressing materials, surgical coveralls, surgical instruments…";
— "…of cleaning and sterilization of dental devices";
— "…of some hemosorbents…"; and
— "…of xenobiological prostheses…".

In addition to the above-described regulatory and production documentation for the multiuse products where the requirement of resistance to the means of sterilization and disinfection are standardized, there is also a regulatory and production documentation for single use medical products in sterile packaging. These products are additionally characterized by the indicator, "sterility shelf life".

When medical products are sterilized on an industrial basis, both the Soviet state standard for disinfection and sterilization, and the special-purpose regulations developed for industries producing sterile products are used. In this country, an order is in force, issued jointly by the U.S.S.R. Health Ministry and the U.S.S.R. Ministry of Medical Industry, which is aimed at achieving an overall sanitary (including microbiological aspects) control throughout the production line. This enables sterilization of products with the minimal number of microorganisms, and helps to ensure adequate sterility.

Quite a number of sterility regulations deal with the methods of sterility control. These concern the sampling procedures aimed at sterility control at industrial enterprises and in hospitals and clinics, as well as the methods of controlling sterility of numerous multiuse and single use appliances, and for recording control data. The U.S.S.R. Health Ministry authorizes the methods of controlling sterility for each particular single use product included in the production documentation for such a product.

There are also instructions in methods, which concern certain methodological aspects of sterilization research, such as "The instructions in methods of estimating resistance of bioburden, which contaminate medical products under conditions of production and applications, to sterilizing agents" and "The instructions in methods of studying gaseous sterilizing agents for the purposes of hygienization and content control thereof, in medical products".

Thus, the standards for sterilization of medical products and the regulatory and production documentation developed on the basis of and in support to such standards regulate different aspects of sterilization. These must ultimately ensure adequate sterility of both multiuse and single use medical products and effectively prevent outbreaks of hospital infections.

AAMI National and International Standards Program

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I would like to express my appreciation to the organizers of this conference, the U.S.S.R. State Committee for Science and Technology, the U.S.S.R. Ministry of Health, and Johnson & Johnson, for the opportunity to discuss AAMI's national and international standards programs. Conferences such as this make an important contribution to the advancement of medical technology through the sharing of technology knowledge and experiences.

As you know, I am Michael J. Miller, executive director of the Association for the Advancement of Medical Instrumentation (AAMI). I have been Executive Director of AAMI for 20 years.

Overview

In my presentation, I will describe the Association for the Advancement of Medical Instrumentation and, particularly, its national and international standards programs; the development and nature of voluntary medical device standards in the United States; the differences between U.S. voluntary standards and other national and international standards; AAMI's expanded international standards program activities; and AAMI's medical device sterilization standards program.

AAMI, Membership Service Organization

AAMI is a membership organization of 5,000 managers, users, and developers who are united by a desire to share their considerable knowledge to improve the development, management, and use of medical technology. AAMI's members — engineers, technicians, physicians, scientists from many disciplines, managers, academicians, and researchers — represent a variety of institutions, including manufacturers, health care facilities, universities, government agencies, and research institutions.

AAMI serves its membership and the field by providing standards on equipment safety, performance, development and use, technical publications, educational programs, and a number of other services that foster effective equipment development, management, and use.

AAMI initiated and sponsors certification programs for health care engineers. While AAMI is well known for many of its services, such as its annual meeting, exhibit program, and publications, such as *Biomedical Instrumentation Today*, a bimonthly journal, it has made a unique contribution to medical technology through its standards, which provide baseline safety and performance requirements for many types of widely used technologies.

The Importance of Standards

Let me begin by stating why standards are important. Standards provide a common language for the important communication among device developers, government, and users. This common language enhances device development, evaluation, improvement, safety, efficacy, and appropriate use. Manufacturers accept voluntary standards as marketplace regulations and design equipment according to accepted standards to compete effectively and serve their customers. Purchasers, evaluators, and users accept standards because they often are an inexpensive and effective basis for ensuring safety, efficacy, effective management, and reasonable cost.

Standards are an important medium of education and information in that they provide the user and manufacturer with fundamental and widely accepted elements of device safety, performance, and testing. They are also important for reconciling the medical technology expectations of the developer, user, and regulator.

Standards are becoming a primary medium for international trade. Events in Western Europe and the rest of the world make it clear that standards will soon determine how, if, and when medical devices will be traded among different countries of the world. In effect, standards will become the international language of trade and establish the parameters of competition and trade among countries of the world. In Western Europe, for example, EC standards will determine whether or not specific medical devices can be purchased and shipped from other countries.

Impetus to U.S. Voluntary Standards

In the United States, the Food and Drug Administration (FDA), acting under the authority of the Medical Device Amendments of 1976 (the U.S. law governing medical device regulation) has provided a major impetus to voluntary medical device standards.

Under the medical device law, FDA's authority provides for three basic areas of regulation: general controls (such as good manufacturing practices, recall of hazardous products, good labeling of products, etc.); mandatory standards for certain types of products; and premarket clearance of products that present particular risks.

The medical device law outlines a very complicated and expensive scheme by which the FDA identifies the need for, develops or commissions someone else to develop, and promulgates mandatory standards. Because of the expense and administrative burden associated with mandatory standards, the FDA has deferred to voluntary standards to fulfill the regulatory aspects of its program that pertain to medical device standards. The FDA has made several efforts to develop and promulgate a mandatory standard, but to date these efforts have been unsuccessful.

After the passage of the Medical Device Amendments of 1976, the FDA published its list of needed standards. A number of organizations, with AAMI in the forefront, began to work on many of the high priority standards.

Another impetus to voluntary standards was a series of FDA contracts for standards development during the 1970s, a time when the agency had a significant amount of funding

for this type of activity. Several of AAMI's most comprehensive standards are based on the contract work performed for the FDA, including AAMI standards for cardiac monitors, defibrillators, and electrosurgical devices.

Much of the standards work that AAMI initiated and conducted in the 1970s and 1980s was based on needs expressed by the FDA, industry, and professions for voluntary standards that would reduce or eliminate the possibility of regulation.

Thus, the standards work that AAMI has completed, and the manner in which the work was completed, has been greatly influenced by the U.S.'s and FDA's concerns about safety and industry's concern about the FDA's intended use of voluntary standards.

Other Impetuses to Voluntary Standards

Other major incentives to voluntary standards are:

(1) The increasing importance of international standards to world trade. The U.S. has more reasons now than ever before to develop carefully thought-out positions on domestic voluntary standards to ensure an effective posture in international standards setting.
(2) The need for basic safety and performance recommendations as a means of enhancing the manufacturer's marketplace and assuring the user of the quality of the product.
(3) The need to assure patients and users of safe environments and procedures for medical technology use.
(4) Government agencies' willingness to defer to voluntary standards rather than utilize resources for mandatory standards.

Recognition of the Value of Voluntary Standards

The U.S. seems to be moving toward a national policy that recognizes that voluntary medical device standards are often a more acceptable and cost effective approach to device safety than regulatory standards. Congress is now considering an amendment to the Medical Device Amendments of 1976 — proposed by the Department of Health and Human Services — that would preclude FDA from developing and using mandatory standards if there are adequate voluntary standards being adhered to by the industry. Although FDA has not used mandatory standards as a primary means of regulation, the agency does use voluntary standards in a number of regulatory contexts, including approving of foreign devices for import, enforcing good manufacturing practices, administering the 510(k) process (the process by which the FDA concludes that a device is similar to another device and, therefore, needs less regulation), and evaluating premarket approval applications.

Voluntary standards are de facto regulations in the marketplace. Consequently, AAMI standards are included in purchase specifications by most hospitals and other health care facilities and AAMI standards are a primary frame of reference in a regulatory or voluntary context wherever fundamental aspects of medical technology are specified.

I will mention other uses of AAMI's voluntary standards later in this presentation.

Differences in U.S. and Other Countries' Standards

One of the major differences observed between AAMI standards and standards written in other countries is that the standards of other countries are often regulation oriented and written by persons who do not have a practical, day-to-day, working knowledge of medical devices. As a result, the standards of other countries tend to be much more constrictive and rigid. AAMI makes a serious effort to involve experts with practical working experience with medical devices in its national and international standards activities.

AAMI standards are specifically directed to health care providers, technology managers, and industry. User oriented standards have the advantage of leaving latitude for technological advancements while still maintaining a high degree of patient and user safety. AAMI standards are used in a number of contexts and, therefore, must be flexible. They are used in the design and manufacturing of equipment, and by device users to guide purchase decisions or assess equipment already used. AAMI standards also address environmental safety issues.

Standards can be developed for very different reasons. For example, standards in the U.S. are often developed for safety, while some countries' standards are developed to affect national and international trade. These differences in orientation often make the harmonization of standards very difficult.

Safeguards Built into AAMI Standards

Conferences in 1969 and 1972, co-sponsored by AAMI, the National Institutes of Health, and FDA and the medical device amendments, fostered many of the policies and safeguards on which AAMI standards are based. The perception existed in the 1970s that voluntary standards would likely become mandatory standards and should be written with this possibility in mind. In addition, many of the safeguards fostered by the law seemed very appropriate for voluntary standards which are often de facto marketplace regulations.

These influences (the recognition that voluntary standards could become regulations) resulted in AAMI's recognition that the *need and rationale* for voluntary standards must be clearly established before new standards development work was undertaken. Careful thought about whether a standard is needed and what exactly is needed can preserve resources and produce better standards. This thinking process is codified in AAMI standards. Similarly, AAMI requires that each standard have a *rationale statement* for important provisions to ensure accurate interpretation. These statements tell the user of a standard why a provision was written and how it is to be used.

AAMI has been instrumental in promoting the concept of rationale in domestic and international standards. It is AAMI's policy to state why a standard is needed, why specific provisions are included, and why they are written the way they are. Other national and international organizations have adopted the concept of rationale reluctantly, presumably because they fear that too much detail could increase their liability, and because rationale requires much additional work. Inclusion of rationale statements has materially enhanced the value and use of standards since the user of standards not only has safety,

performance, and testing criteria, but also has the rationale or justification for the specific aspects of each requirement.

The flurry of activity stimulated by FDA's push for standards also resulted in an awareness of what adverse effects unneeded standards could produce and how standards could be misused. One of the best examples is the planned use of a draft AAMI pacemaker standard by a foreign government even though the standard was outdated and, in some respects, incomplete.

Another important safeguard now built into AAMI's standards is a clear explanation that the *user has an important responsibility* to make sure that the standard is still relevant and appropriate to the user's needs. AAMI carefully emphasizes to the potential user of a standard that although the standard represents a national consensus of experts in a general context, each provision of a specific standard must be carefully evaluated by the user to ensure relevance to the user's specific needs.

This caveat is also important in the context of converting voluntary standards to regulatory standards. The regulator's needs are often quite different from the needs of other users of standards, and the regulator's development and "enforcement" process is also quite different. Many companies were quite concerned when an AAMI dialyzer reuse standard was adopted by the federal government to determine eligibility for cost reimbursement. Their concern was based on the differences in how the standard was promulgated, revised, and intended to be used by the private sector and the government.

AAMI has insisted that all of its standards provide *test methods* to ensure that there are objective criteria for demonstrating adherence to safety and performance requirements. Industry has supported the concept of test methods since it provides manufacturers with predictability about how their devices will be tested by government and health care facilities. Predictability is an important factor in maintaining a stable marketplace and an effective relationship between the industry and the user of medical devices.

AAMI uses the concept of *"referee" test methods* in its standards, which permits manufacturers to employ tests comparable to those described in the standards, provided that equivalence with the referee tests can be demonstrated in terms of comparability of test results.

Another important AAMI policy is to *avoid design standards* unless absolutely necessary, since they are restrictive to device development. Instead, AAMI has established performance requirements, an approach which provides maximum flexibility to the device designer. AAMI standards state performance goals to be achieved and leave the manner of achievement of these goals to the device design.

Elements of AAMI's Voluntary Consensus Standards System

The safeguards I have just described are important elements in setting good standards and determining essential contents of a standard. I will describe some other concepts fundamental to effective standards setting.

I believe that AAMI's standards setting system has produced some of the best standards in the world and that a number of countries have already acknowledged the

excellence of AAMI standards, either by adopting them as regulations or by deferring to these standards instead of regulating.

First, an important ingredient in a good standards system is balanced participation from experts from all fields, and experts who have a practical working knowledge of medical devices. It is fundamental to good standards development that no interest group or groups dominate the standards writing process. All of AAMI's committees include people from both the health care professions and the industry who are very closely involved with the design, research, development, and use of medical devices. If standards are developed by users, academic or research persons alone, this can lead to standards that exceed the bounds of existing technology. As a result, these standards can be very restrictive or impractical.

Second, AAMI's standards system requires *due process* which ensures that all viewpoints will be heard and all viewpoints and comments will be resolved. The AAMI voluntary consensus standards system includes a process by which appeals can be registered and resolved at several points in the development of a standard. This ensures that the standard cannot be approved as a national standard unless technical resolution has occurred and all minority viewpoints have been heard and resolved.

AAMI's system requires that at least once, and often twice, AAMI's standards be subjected to a "public review" process whereby all interested organizations and individuals have an opportunity to review and comment on standards. In the U.S., this not only results in better standards, it also provides legal protection against restraint of trade lawsuits.

Third, industry strongly participates and supports the AAMI standards effort. I cannot conceive of an effective voluntary standards development process without the active participation and support of the medical device industry. Standards without industry participation are likely to be impractical and of little use. Industry participation is essential to assist users in defining what is practical, effective, attainable, and useful.

Fourth, AAMI has strong government and user participation in its standards development process. Government and users must understand what it takes to develop a standard, what a standard means when it is developed, what the limitations of a standard are, and in short, how to effectively use a standard. I believe that participation by government representatives has been instrumental in the FDA decision to utilize voluntary rather than mandatory standards. This determination by the FDA has led to flexible voluntary standards that have not only ensured safety, but have also enhanced innovation.

AAMI's International Standards Program

Before I discuss AAMI's sterilization standards, let me discuss what is happening in the area of international standards. In 1988, AAMI's corporate members made it clear that the integration of Western European countries, representing a market of 320 million plus consumers, could present a major opportunity for U.S. industry to expand its sales. But this could also be a formidable challenge. Unlike the U.S. voluntary system, under the European Community system, or EC system, standards will become regulations and thereby have an enormous influence on the competitive position of companies and countries outside the EC, including the U.S. If a company cannot meet EC standards, it cannot ship its products to EC

countries.

Our members are concerned that the standards adopted by the EC could be developed in organizations such as the European Committee for Standardization and its electrically-oriented sister organization, CENELEC. These are regional Western European organizations, with either limited or no U.S. participation. Future standards developed in these regional organizations will be given regulatory status and are considered to most likely have an adverse affect on U.S. companies.

On the other hand, the U.S. is an influential member in two international standards organizations, the International Organization for Standardization (ISO) and the International Electrotechnical Commission (IEC), which are based in Europe and have members from around the world. Representatives of the EC, CEN, and CENELEC have publicly announced that they will defer to ISO/IEC standards if available and if the meet their needs. Otherwise CEN and CENELEC will write standards, with the financial support of the European community, to enforce EC directives. I should add that CEN and CENELEC are not likely to initiate new standards work if it is anticipated that required and needed standards will soon be available through ISO or IEC.

As you know, manufacturing one product that has to meet the combined requirements of two different standards produced by different countries can increase production costs and adversely affect competitiveness. Thus, U.S. companies could be placed at a competitive disadvantage if EC standards set requirements and manufacturing practices that our technical experts and regulators believe to be overly restrictive and are, therefore, not required or commonly followed in the U.S.

A task force appointed by the AAMI Board of Directors developed a strategy and outlined a program that would help AAMI's industry members compete internationally. This strategy is based on the assumption that the EC and other world governmental bodies will use and conform to international standards developed within IEC and ISO and also defer new work if needed standards are slated for or are being developed by ISO/IEC committees. Our objective is to work with the EC by giving them the standards they need to accomplish the integration of Western Europe, but to develop these standards in a context that is in their interests as well as our own, namely through ISO and IEC. In fact, we have already received informal assurances that AAMI standards and international involvement would be welcomed by Western European interests. We realize that international standards will have an effect that extends beyond Western Europe and, in fact, will have far reaching economic and trade effects throughout the world.

The international standards program developed by AAMI has been endorsed by the Health Industry Manufacturers Association, representing U.S. industry, the Food and Drug Administration (a very strong regulatory body responsible for the regulation of medical devices), and the professions. AAMI's intention is to initially assume a leadership position in international standards for electromedical devices and safety; sterilization technology; cardiovascular implant devices; and biocompatibility and toxicity testing procedures.

The American National Standards Institute, the U.S. member of ISO, has designated AAMI as the administrative secretariat of a new and proposed international technical committee on medical device sterilization. Thus, AAMI will play an important administrative

role in the development of both national and international standards in the area of medical device sterilization. AAMI will be proposing as a basis for international standards its complete inventory of medical device sterilization standards in the areas of radiation, steam, ethylene oxide, and other technologies, since there are now virtually no international standards.

The medical device areas we chose to initially concentrate on in the international sphere, in terms of obtaining a leadership role, are those in which the U.S. through AAMI has a reputation for contributing significant standards and committee expertise necessary for their development. For example, AAMI's experience as the secretariat administrator of an ISO subcommittee that is responsible for developing international standards for pacemakers and other cardiovascular medical devices has been most favorable. AAMI has taken its applicable domestic standards to the international subcommittee and convinced other countries that these documents are useful points of departure for international standards. As a result, much of the content of international draft and final standards for pacemakers, heart valves, oxygenators, dialysis equipment, and vascular graft prostheses reflects AAMI standards and technology.

Additionally, AAMI standards, particularly its medical device sterilization standards, have been accepted and are used by national and international government agencies. In particular, the FDA, U.S. Health Care Financing Administration, Japan and the United Nations International Atomic Energy Agency, defer regulatory action based on the existence of AAMI voluntary standards and either adopt them as regulations or incorporate them into government sponsored user education programs. The FDA, for example, has deferred mandatory standards regulation of cardiac monitors because the AAMI voluntary standard provides adequate protection to the patient through the adherence of the U.S. industry and because voluntary standards are much less restrictive and expensive than regulatory standards.

AAMI's leadership in international standards will be asserted by assuming responsibility for the administrative secretariat for international standards activities, both nationally and internationally, such as U.S. technical advisory groups and international committees, and subcommittees and working groups for standards development. AAMI's international standards work will be funded primarily by AAMI members, but it will also be supported by government agencies and trade organizations.

I will now briefly summarize each of the standards that AAMI has developed in the medical device sterilization area.

Difference Between Recommended Practices and Standards

AAMI develops two types of standards documents: recommended practices and product standards.

Recommended practices pertain to the procedures or policies that should be undertaken by users of products or processes to make sure that the proper product or process has been selected, that it will be used safely and effectively, and that the environment in which a product or process is used is safe.

Product standards, generally developed for the use of manufacturers, include recommended design and performance characteristics essential for the safety and efficacy of the medical device and include recommended tests of these characteristics.

AAMI has begun to publish compilations of its technical documents. Our current compilation of national standards and recommended practices for sterilization was published in July 1988 and will be published in a revised form in 1990. The 1988 edition includes all 14 final AAMI documents in the field of sterilization.

AAMI Recommended Practices for Sterilization

Process Control Guidelines for Gamma Radiation Sterilization of Medical Devices, Approved 1984

This AAMI recommended practice provides guidelines for processing medical devices through gamma radiation facilities, including recommendations for preirradiation, establishment of process specifications, general operating guidelines, dosimetry systems, and dose-setting methodologies.

Guideline for Industrial Ethylene Oxide Sterilization of Medical Devices, Approved 1988

This guideline provides the essential elements of good operating practice for gaseous ethylene oxide (EO) sterilization of medical devices. The manufacture of a safe and sterile health care product requires attention to product considerations and to sterilization methods, facilities, and controls. The guideline covers the interrelationship of these factors to ensure product safety and effectiveness. Sterilization process development and validation, and the control of routine EO sterilization, in-house or by contract, are addressed.

This guideline is for all manufacturers who sterilize with EO or who contract to perform EO sterilization of medical devices.

Determining Residual Ethylene Oxide in Medical Devices, Approved 1986

The American National Standard describes several analytical methods for measuring residual ethylene oxide (EO) in gas-sterilized medical devices. This recommended practice includes descriptions of:

(1) Methods of sampling EO-sterilized objects and how to store and handle them;
(2) Two general methods of extracting EO from medical devices (exhaustive and simulated use), as well as extraction fluids and conditions recommended for performing the extractions;
(3) The full details of analytical methods which have been found suitable for determining the EO context of medical device extracts; and
(4) How the analyst should treat and interpret the data to permit calculation of residual EO levels and approval of product release.

The summary of results of “round robin” laboratory tests and statistical analysis of the data are also included.

Determining Residual Ethylene Chlorohydrin and Ethylene Glycol in Medical Devices, Approved May 1988

This American National Standard covers test methods for determining ECH and EG residue levels in medical devices. Its content essentially parallels that of the standard for EO residues.

Automatic, General-Purpose Ethylene Oxide Sterilizers and Ethylene Oxide Sterilant Sources Intended for Use in Health Care Facilities, Approved 1987

This standard covers minimum labeling, safety, performance, and testing requirements for ethylene oxide sterilizers which are intended for general-purpose use in health care facilities and which have automatic controls. For purposes of this standard, a “general-purpose” ethylene oxide sterilizer is defined as a chamber-type sterilization system that injects water vapor to adjust humidity during the cycle, generally employs excursions in pressure from atmospheric levels, and is intended to sterilize a wide range of medical items. This standard also covers labeling, product composition, and container requirements for ethylene oxide sterilant sources. Referee test methods and a glossary are also included, as well as an appendix explaining the rationale for the provisions of the standard.

Good Hospital Practice: Ethylene Oxide Gas-Ventilation Recommendations and Safe Use, Approved 1987

This was the first AAMI Good Hospital Practice in the field of EO sterilization, focusing on ventilation recommendations and safe use. It includes definitions of equipment and procedures, ventilation recommendations, and other guidelines intended to help reduce personnel exposure to EO, and to ensure compliance with federal regulations. It also includes a highly practical guide for hospital personnel responsible for selecting the equipment or services that will be used at their facilities to measure airborne EO in the workplace or to assess worker exposure to airborne EO. The guide explains the conceptual approaches to monitoring, describes most of the kinds of equipment and services which are currently available, and summarizes certain advantages and disadvantages of the equipment and services.

Good Hospital Practice: Performance Evaluation of EO Sterilizers — EO Test Packs, Approved 1985

This is the second AAMI Good Hospital Practice related to EO and evaluates the performance of EO sterilizers by means of EO test packs. It defines a test pack with biological indicators, or BIs, that can be used to challenge sterilizer performance as part of

qualification testing by the manufacturer and for purposes of installation testing and periodic quality assurance testing in health care facilities. The test pack is simple and cost-effective enough to be used for routine monitoring of inhospital sterilization cycles as well. It is also important to note that the testing procedures recommended in the Good Hospital Practice are designed to minimize occupational exposure to EO.

The principal subjects that the document covers are:

— Composition and use of challenge packs in qualification, installation, and QA testing;
— Test pack composition and use in routine biological monitoring;
— Aeration of test packs; and
— Recalls.

Guideline for Industrial Moist Heat Sterilization of Medical Products, Approved 1987

This guideline provides the essential elements of good manufacturing practice for moist heat sterilization of medical products. The manufacture of a safe and sterile health care product requires attention to product characteristics and to sterilization methods and controls. This guideline helps ensure product safety and effectiveness by providing recommendations for the use of moist heat in sterilization process development, validation of the sterilization process, and control of routine sterilization.

This guideline is directed to all industrial manufacturers and contract organizations who sterilize medical products with moist heat. In addition, certain other users of moist heat processes, specifically laboratories required to meet current Good Manufacturing Practice (GMP) or Good Laboratory Practice (GLP) requirements, are included.

Good Hospital Practice: Steam Sterilization and Sterility Assurance, Approved 1988

This is the first sterilization guideline that AAMI published. It is intended to provide hospital personnel with guidance to ensure a safe, efficient environment and a degree of assurance in achieving and maintaining sterility in items undergoing the steam sterilization process. It covers the use and maintenance of gravity air removal sterilizers, pre-vacuum high-temperature sterilizers, and high-temperature pulsing sterilizers.

Specific subjects addressed are:

— Design considerations, including traffic control and physical facilities;
— Personnel considerations, including qualifications, training, hygiene, and attire;
— Processing recommendations;
— Care and maintenance of sterilizers; and
— Quality control by means of mechanical, chemical, and biological monitoring.

Good Hospital Practice: Steam Sterilization Using the Unwrapped Method (Flash Sterilization), Approved 1986

The Flash Sterilization Guideline addresses many of the same issues as the steam

sterilization and sterility assurance document but relates them to the emergency practice of flash sterilization. This document describes the procedures necessary to ensure the sterility of devices and materials steam sterilized by the unwrapped method with either gravity displacement or pre-vacuum sterilizers.

The document emphasizes that the wrapped method of steam sterilization is preferred and that flash sterilization should not be used as a convenience to compensate for inadequate inventory or failure to anticipate need. It also stresses the importance of using equipment that meets all the standards for other hospital steam sterilizers.

AAMI Standards for Sterilization

Hospital Steam Sterilizers, Approved 1988

This standard applies to steam sterilizers intended for use in health care facilities and which have a volume greater than two cubic feet (5.6 liters).

It covers minimum labeling, construction, performance, and testing requirements for steam sterilizers which have automatic controls, utilize an external steam source, and provide means for automatically recording time and temperature.

Selection and Use of Chemical Indicators for Steam Sterilization Monitoring in Health Care Facilities, Approved 1988

This document covers fundamental concepts of sterilization, variables that affect steam sterilization, performance characteristics of currently available types of chemical indicators, questions that users should ask in selecting products, use and interpretation of chemical indicators, and attributes of an effective sterility assurance program.

Biological Indicators for Ethylene Oxide Sterilization Processes in Health Care Facilities, Approved 1986

This standard establishes minimum production, labeling, and performance requirements for biological indicators (BIs) intended for use as monitors of ethylene oxide sterilization cycles in health care facilities.

Biological Indicators for Saturated Steam Sterilization Processes in Health Care Facilities, Approved 1986

This standard establishes minimum production, labeling, and performance requirements for biological indicators (BIs) intended for use as monitors of saturated steam sterilization cycles in health care facilities.

BIER/EO Gas Vessels, Approved 1982

This standard establishes requirements for ethylene oxide exposure vessels used to evaluate the resistance performance of biological indicators (BIs) that are intended for use in monitoring ethylene oxide sterilization cycles. These vessels are designated Biological Indicator-Evaluator Resistometer vessels using ethylene oxide (BIER/EO gas vessels).

Included within the scope of this standard are minimum performance and construction requirements for BIER/EO gas vessels, with the objectives of:

(1) Assuring that the exposure unit is effective in determining the resistance performance of biological indicators;
(2) Assuring that lots of biological indicators can be effectively compared; and
(3) Assuring the safety of trained personnel using the equipment.

BIER/Steam (B/S) Vessels, Approved 1981

This standard establishes requirements for saturated steam vessels intended for evaluation of the resistance performance of biological indicators (BIs).

It includes minimum construction and performance requirements intended to (a) help assure that the exposure chamber is effective in determining the resistance performance of B/S; and (b) help assure the safety of trained personnel using the equipment.

Future Work

I would also like to mention the sterilization documents that have not yet received final approval, but are the focus of our present efforts.

Process Control Guideline for Electron Beam Radiation Sterilization of Medical Devices, Approval expected in 1990

This guideline covers procedures for radiation sterilization of medical devices processed in electron beam facilities, including pre-irradiation handling, irradiation, and post-irradiation handling, as well as dosimetry guidelines and methods of establishing an auditing radiation dose.

Good Hospital Practice: Guidelines for the Selection and Use of Reusable Rigid Sterilization Container Systems, Approval expected 1990

This recommended practice covers cleaning and decontamination considerations, preparation and assembly of containers, sterilizer loading, matching container system with sterilization cycle, storage, transport, and maintenance.

Chemical Sterilants and Sterilization Methods — A Guide to Selection and Use, Approval expected 1990

This technical information report addresses general safety and performance

characteristics of chemical sterilants, mechanisms by which manufacturers obtain government clearance for the marketing of their products, questions that users should ask of chemical sterilant manufacturers when choosing a product, and general guidance on the selection and use of chemical sterilants.

Good Hospital Practice: Handling and Biological Decontamination of Reusable Medical Devices, Approval expected 1990

This recommended practice covers guidelines for the safe handling, cleaning, and biological decontamination of reusable items used in patient care within health care facilities.

Guidelines for the Use of Industrial Ethylene Oxide and Steam Biological Indicators, Approval expected 1990

This recommended practice covers the use of biological indicators to monitor industrial steam and EO sterilization processes, including applicable sterilization equipment, BI selection, appropriate use of BIs for qualification/validation, routine monitoring, and revalidation of sterilization processes.

Conclusion

In conclusion, I believe that AAMI can make a significant contribution to international standards on medical device sterilization because it already has an excellent inventory of standards that establish essential requirements for safety, efficacy, and testing in the context of the design of equipment, industrial sterilization processes, user practices, and environmental safety.

AAMI and the United States are willing to share this knowledge with the world to ensure that international standards reflect a practical working knowledge of medical device design, development, and use.

If international standards continue to reflect a practical working knowledge of medical devices developed by balanced international committees not only will world trade and competition be favorably affected, but patients all over the world will be favorably served and the quality of life will be enhanced.

IAEA Standards Applicable for Sterilization

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Introduction

This session deals with state-of-the-art reviews on sterilization standards, and the previous speakers have dealt with standards criteria and guidelines in the U.S.S.R., European Economic Community, including the United Kingdom, and the North American AAMI guidelines. The valuable information that has been presented can be regarded as reflecting both the practices and procedures as well as the features of the regulatory standards that are currently employed in the sterilization processing of the majority of the medical products being produced worldwide. Whatever relevant development that might be forthcoming in the future promulgation of regulatory standards in the national health-care systems of interested Member States is likely to bear a major dependence on the data and information that has already been provided.

My presentation is on IAEA standards related to sterilization. The International Atomic Energy Agency (IAEA) is an international organization with the objective "to accelerate and enlarge the contribution of atomic energy to peace, health and prosperity throughout the world". In keeping with these defined service objectives and with particular regard to the emphasis of the programs for the benefit of the developing Member States, the IAEA's activities in the current context aim to promote the applications of "microbicidal effects of ionizing radiation energy" to help achieve effective sterilization of indigenous medical supplies and to attenuate the risks of nosocomial disease during clinical interventions. The scope of these IAEA programs thus becomes limited to technology transfer and should remain engaged in strengthening the required skills and capabilities of the recipient developing Member States. In addition, the IAEA programs should strengthen indigenous manpower and optimize the utilization of existing facilities in an attempt to properly undertake radiation sterilization processing of duly manufactured medical products to meet the intended clinical quality standards and to sustain the health and safety of consumer patients. The Member States receiving guidance and assistance from the IAEA programs are expected to consider the options and the principles of the process criteria and standards made available to them. They then freely decide which aspect(s) of the various standards should be included or excluded, either partially or totally, in the context of the stipulations of their national health regulatory policies for the formulation of national guidelines standards.

Regulatory Control Aspects of the Sterilization Process and the Products

Like all other sterilization processes, radiation sterilization processing and the resultant sterilized medical products need to fulfill the appropriate validation criteria, as stipulated and

implemented by the relevant national health regulatory authority, such as the Food and Drug Administration, Pharmacopoeia Commission, or any other equivalent national body. The purpose of this regulation is to impose the strictest possible quality control on the radiation-processed medical product to ensure that the desired objective pertaining to consumer safety is fulfilled. Often times, radiation sterilized items may be consumed beyond the national boundary of production. Under such circumstances, the items must comply with the regulatory requirements of the consumer country as well. Such compliance can be facilitated by the availability and dissemination of the regulatory guidelines for standardization to all levels involved in their coordination and successful implementation.

The need for such guidelines has been recognized since the early development of radiation sterilization processing of pre-packed medical supplies. As early as 1967, an IAEA expert group, in cooperation with the World Health Organization (WHO), recommended the basis for an International Code of Practice for radiation sterilization of medical products. As further experiences accumulated through the process operation, including those from a growing number of Member States in developing regions, IAEA, along with representatives of the national health regulatory authorities in the Member States and with WHO, have undertaken periodic revision of these guidelines. One such revised IAEA guideline is currently awaiting publication. Radiation sterilization is an integral part of the total manufacturing process and all relevant elements involved in the manufacture of medical products such as Good Manufacturing Practice (GMP), Good Radiation Practice (GRP), and consideration of material compatibility with radiation processing among others, are inherently associated with quality assurance (QA) actions and standards formulation. The IAEA guidelines have attempted to provide up-to-date technical guidance on all of these matters; it also provides additional references for consultation. Relevant excerpts from this document on some of the above aspects follow.

Good Manufacturing Practice (GMP) in the Manufacture of Medical Products

Exposure of medical products to a validated and controlled sterilization process is not the only factor involved in assuring that the product is safe and suitable for their intended use. The primary manufacturer must assure that all steps in the manufacture of the items to be sterilized are carried out in accordance with relevant Codes of, or Guides to, Good Manufacturing Practice. Where conformity to standards of GMP is not yet mandatory or where no national code or guide exists, it is essential that manufacturers of medical products comply scrupulously with an existing authoritative code or guide, such as:

(a) U.S. Code of Federal Regulations, Part 820, Good Manufacturing Practice for Medical Devices: General, 1981.
(b) Guide to Good Manufacturing Practice for Sterile Medical Devices and Surgical Products, HMSO, U.K., 1981.
(c) Code of Good Manufacturing Practice for Sterile Therapeutic Devices, Commonwealth Department of Health, Australia, 1986.
(d) EUCOMED Guides to Good Manufacturing Practice for Sterile Medical Devices

and Surgical Products, EUCOMED, 1986.

(e) Manufacture and Handling of Industrially-Sterilized Medical Devices for Single Use, National Bureau of Health and Welfare, Australia, 1982.

Such codes or guides describe all activities necessary to ensure that a manufactured product meets requisite standards with regard to their quality, safety and performance, and generally elaborate upon the following basic principles of GMP:

- An integrated system of manufacture and quality assurance.
- Separate management responsibilities for production and quality assurance.
- Suitable premises, equipment and materials.
- Trained personnel.
- Documented procedures for manufacture and quality assurance.
- Appropriate batch and product records.
- Adequate handling, transport and storage.
- A recall system.
- A system for auditing the operation of GMP.

Selection of the Sterilization Dose

It is a basic assumption that the product to be sterilized is manufactured under conditions that comply fully with the requirements of GMP. In the present context, it is particularly important that practices be implemented, and actions taken, which ensure that the number of microorganisms on product items destined for radiation-sterilization processing is consistently low. A dose of 25 kGy (2.5 Mrads) has been found to be an effective sterilizing dose. It is generally believed that this dose provides maximally a sterility assurance level (SAL) of 10^{-6}. Where it is not feasible to generate data on the radiation resistance of the natural microbial population present on product items, a minimum sterilizing dose of 25 kGy (2.5 Mrads) can be used. The guidelines of the Association for the Advancement of Medical Instrumentation (U.S.A.) (AAMI) recommends basing the selection of a sterilizing dose on knowledge of the resistance of the natural microbial population present on the items to be sterilized, and on a reasoned selection of maximal SAL. Dose selection using this approach are described in Methods 1 and 2 Appendix B of the AAMI Process and Control Guidelines for Gamma Radiation Sterilization of Medical Devices. Basic requirements in applying this approach are:

1. Access to a cobalt-60 radiation source capable of delivering accurate doses in the range of 1.0 to 18 kGy (0.1 to 1.8 Mrads).
2. Access to competent microbiological laboratory services.
3. Performance of sterilizing dose auditing procedures at a frequency to be determined by the primary manufacturer.

Good Radiation Practice (GRP)

The radiation facility is considered a part of the product manufacturing processing. The different sections include irradiator commissioning, dosimetry, process validation, routine process control, organization and personnel training, and recordkeeping. Together, they comprise Good Radiation Practice (GRP).

Process Validation

The establishment of materials compatibility, the determination of the required minimum sterilizing dose, the establishment of the product loading pattern, dose mapping, including the identification of the minimum and maximum dose zones, and the establishment of the cycle timer setting constitute the validation of the sterilization process for a specific product.

Materials Compatibility

Prior to selecting the radiation sterilization process for a medical product, it is important to consider the effect that radiation will have on the materials that comprise the product or its components. For instance, some plastics such as polystyrene can readily accept an irradiation dose of 200 kGy or more while others, such as polyoxymethylene or polytetrafluoroethylene (PTFE, teflon) easily degrade with only 1-15 kGy. The degradation effects observed can be characterized as embrittlement and/or discoloration of the material. After selecting the materials, they should be evaluated in terms of their radiation stability by exposing them to at least the maximum anticipated radiation dose. Following irradiation, these materials should be subjected to radiation stability studies over a one-year period of time, if practical, using physical tests designed to evaluate degradation effects caused by irradiation. It should be recognized that radiation effects on materials are cumulative and re-sterilization (by any method) should be avoided.

Significant Factors in the IAEA Program Implementation

Almost all of the cobalt-60 facilities installed in the developing Member States through IAEA support are administered by the respective governmental establishments, such as the Atomic Energy Commission or the Ministry of Science and Technology. Consequently, their operation involves the service sterilization of medical products from local manufacturers. This implies features of organization and management, including education and technical guidance of the manufacturers, necessary for adherence to standards criteria.

IAEA's objectives of providing guidance to Member States in need, as well as for generating an awareness of the status and state-of-the-art practice for radiation sterilization compared to conventional alternatives, are of key importance to the fullest development of the health and welfare potential. IAEA-sponsored regional programs, such as the Regional Cooperation Agreement (RCA) for the countries in Asia and the Pacific region, have set the pioneering model in this regard. The promotional role of IAEA, including the guidance towards the standards and maintenance of a quality status for sterile medical supplies in the developing Member States, have been fostered through:

(a) National Executive Management Seminars on Industrial Radiation Sterilization of Medical Products for the dissemination of the principles significant for the technology introduction and standards criteria setting.
(b) Regional Training Courses with emphasis on sterility assurance and quality control criteria and guideline principles.
(c) Workshops to provide hands-on training for technicians at the medical products manufacturers and engineers for irradiation facilities.

International Dose Assurance Service (IDAS)—An International Effort through the IAEA Program for Process Dosimetry Standardization

Accurate dosimetry in routine radiation processing is a key requisite for good radiation practice (GRP) and in turn contributes to the technical criteria for “standard and quality assurance” of irradiated finished products. Since 1977, the Agency’s Dosimetry Section program has coordinated investigations at selected institutes in the Member States aimed at performance analysis of a number of high-dose dosimeters, such as alanine, ceric-cerous, radiochromic dye films, perspex, clear and red. The reason for the choice of the above dosimeters is their high stability and radiation dose-effects quantification within a very wide range of absorbed radiation doses, spanning in the upper limit, doses of 100 kGy or even more.

Such internationally coordinated research has resulted in the implementation since 1985 of an industrial radiation-processing (dose measurement) standardization and quality control (QC) service, using alanine/ESR system for the Member States in need, and designated as International Dose Assurance Service (IDAS). Through this IAEA activity, a gap in the sphere of international standardization criteria for dosimetry and dose assurance for large radiation sources has been narrowed and the scope of effective applications of radiation processing has been further broadened to the health care field.

To date, over 30 institutes located in 22 developing and developed Member States have used the IDAS, and the participation list is growing. IDAS is provided within the framework of an agreement between the Agency and the Member States concerned, and the confidentiality of the results is maintained. Further details of information on IDAS can be obtained from Mr. J.W. Nam of the Dosimetry Section of the Agency’s Division of Life Sciences. The availability of such a standardization instrument could provide a basis for national authorities to grant regulatory approval of irradiated products and could even serve as the basis for international clearance for free trade.

Future Outlook and Conclusions

The balance sheet for the rapid advances of radiation sterilization technology worldwide and those in the technologically-less-advanced Member States served to a large extent through IAEA-sponsored programs, are sufficiently suggestive of the further potentials for health-welfare contributions. This immediately implies the continuing need for actions to help ensure the necessary adherence to quality assurance standards. In many of those countries

currently involved in the introduction of radiation-sterilization technology for medical disposables, there are preponderant practices for sterilization by toxic ethylene oxide gas. Furthermore, gross inadequacies often exist in the national health authority standards to safeguard both the consumer patients and the occupational workers in the medical manufacturing facilities. Current activities related to technological transfer along with the dissemination of safety principles and awareness generation to the regulatory authorities have still greater roles to play and hence, these should be further strengthened through regional coordination.

International meetings such as the Kilmer Memorial Conference which are attended by leading experts from multidisciplinary fields provide a major force for attaining goals related to the upgrading of health-welfare, particularly among developing countries. This is accomplished by providing cross-transfer of valuable guidance and ideas for implementation.

USP Guidelines on Sterilization and Sterility Testing

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Introduction

In order to understand what the impact of USP requirements and guidelines might be on American pharmaceutical manufacturers, as well as distributors and users of pharmacopoeial articles, some knowledge of the pertinent laws and regulations of the United States is essential. An appreciation of how the standards set forth in the compendium are utilized by regulatory compliance bodies is also important. Certainly, the subjects of sterility test requirements and sterilization guidelines afford good examples of the intricacies and nuances involved in the interaction of science and law with respect to public standards in the United States.

The Law

The fundamental law is entitled “Federal Food, Drug, and Cosmetic Act, As Amended”. The Secretary of Health and Human Services has charged the Food and Drug Administration (FDA) with responsibility for administering and enforcing the Act. The FDA has promulgated a number of regulations to implement the provisions of the Act. Among the most important of these are the so-called Current Good Manufacturing Practice regulations (2). These are to be addressed later under the summary of the FDA’s position regarding sterility testing of final products.

Within the Act, the USP is mentioned by title or as an official compendium (10) in four major sections: definition of terms, adulteration prohibitions, misbranding prohibitions, and administrative provisions. Among the definitions of a drug (8) or a device (9) is that such articles are monographed in the USP. If an article is purported to meet the requirements of USP and fails to do so, it would be considered adulterated (11). Now the meaning of the term “adulteration” is not limited to filth. For instance, if an article in USP were to have a specification for a lower limit of water and the test for water gave results below the lower limit, the article would be adulterated under the law. Misbranding can involve the misuse of established names for drugs (12) and devices (13), as well as aberrations in labeling and packaging (14). The title of a USP monograph is an official, established name. If a manufacturer uses such a title on a label even without a USP designation, the material so labeled is considered to be compendial grade. If it then does not meet USP standards, it is misbranded. The administrative sections of the Act relate to the process of designating official names (15) and the participation of the FDA in the USP standard-setting process (16).

Briefly therefore, the standards contained in the USP have the force of law in the United States. The USP is recognized as an official, standards setting body. Since it is not a

government agency, however, it has no compliance enforcement authority. Such authority rests with the Food and Drug Administration.

The USP

An understanding of USP's legal status does not necessarily enlighten a practitioner as to the nature and purpose of the compendium. These are frequently misunderstood by otherwise knowledgeable academicians, industrialists, and regulators. The USP is not, and does not purport to be, a Quality Control-Quality Assurance manual. The tests contained therein are referee methods designed for arbitration or litigation purposes. Although a number of such tests are not state-of-the-art, they are official until replaced by more advanced methodology using a system of due process of proposal and review. A practitioner may use USP methods for quality control and assurance purposes, but such use is not stipulated as a requirement. Furthermore, the USP is not, and does not claim to be, a manual of manufacturing methods. Reference may be made to manufacturing methods by way of information, but such references do not constitute official standards.

The USP is, and purports to be, a compendium of standards to which any and all portions of a lot or batch of a compendial article must conform throughout its useful shelflife. The concept of "any and all portions" is of fundamental concern where destructive analyses are involved.

The General Chapters in USP fall into two broad categories: mandatory and non-mandatory. The former are themselves standards which provide the details of the official referee methods. For example, Chapter 71, entitled, "Sterility Test" is a mandatory chapter. Chapter 1211 entitled, "Sterilization and Sterility Assurance of Compendial Articles" is non-mandatory, and its purpose is to provide background rationale for the design, use, etc., of the sterility tests as well as information on manufacturing of aseptically filled and terminally sterilized articles. Chapter 1211 does not contain official standards. However, if a drug substance or dosage form monograph cites a section of such chapter, that section becomes a standard for the article involved. A note of convenience to users of USP: chapters numbered below 1000 are mandatory and above 1000 are non-mandatory. The staff at USP has yet to face the editorial crisis in determining which way to classify Chapter 1000.

Sterility Tests

The development and revisions of the highly technical sections of Chapter 71 are relatively straightforward. Issues involving media, transfer technique, state-of-the-art equipment, and other procedural details, although subject to debate among experts in microbiology, are generally resolved fairly promptly. The major problem has been with the section of the Chapter concerned with interpretation of sterility test results (6), particularly as they might be applied to an assessment of the condition of the entire lot of product from which the samples tested were taken. This concern, of course, arises when one attempts to utilize the test for quality control and assurance purposes with major reliance being

placed on test results for lot release. A fundamental consideration dictates against such use: the statistical inadequacy of the sample size coupled with the high probability that microbiological contamination may not be uniformly distributed throughout the lot of product. The test as written contains an important caveat:

> "Note — Where stability testing is used as **part** of an assessment of a production lot or batch or as one of the quality control criteria for release of such lot or batch, see Sterilization and Sterility Assurance of Compendial Articles 1211".

This tells the practitioner that the method as written is intended for referee purposes and not as a quality control test. The sample sizes are specified as a matter of practicality—obviously one does not consume an entire batch or a significant portion thereof to achieve statistical satisfaction. However, the sample sizes are minimal: regulatory laboratories are free to extend their tests in arbitrations and litigations. One must not forget that the "any and all concept" pertains. If the practitioner wishes to use the test as a control method, other factors come into play, and these are covered in the non-mandatory Chapter 1211 under the sections headed, "Sterility Testing of Lots" and "Performance, Observation and Interpretation" (7).

Other Compendia

The interpretation section of USP Chapter 71 contains five key elements or statements relating to: retests, investigations, passing, failing, and use of the test for release of batches or lots. There is further elaboration of each of these elements in Chapter 1211. It is interesting to compare the contents of the interpretation sections of the British Pharmacopoeia (1), the European Pharmacopoeia (3), and the State Pharmacopoeia of the U.S.S.R. (5).

STATEMENTS ON	INTERPRETATION ELEMENTS			
	USP XXII (*U.S.A.*)	BP 88 (*British*)	EP 2 (*European*)	SP X (*U.S.S.R*)
(1) Retests	X	X	X	X
(2) Investigations	X			
(3) Passing	X	X	X	
(4) Failing	X	X	X	X
(5) Batches	X			

The fact that USP XXII covers more elements does not make its interpretation section better than those in the other compendia. The proliferation of statements is a reflection of the interaction of science and law alluded to earlier, particularly where the enforcers of the law may not fully appreciate the differences between referee usage and quality control usage. The silence on the part of the U.S.S.R. Pharmacopoeia as to passing the test is most intriguing. After some thought about this, it would appear that this "one-sided" process does make decision making less agonizing.

The descriptions of the materials tested vary somewhat from compendium to compendium:

MATERIAL DESCRIPTIONS	
USP XXII	– "The article tested".
BP 88	– "The preparation being examined".
EP 2	– "The product to be examined".
SP X	– "The preparation".

Many practitioners in the United States interpret the USP description to mean that the results of the sterility test apply only to those samplings of the lot actually tested and not necessarily to the entire lot. This interpretation would conform to the referee test concept, especially where probability is a dominating factor.

Regulatory Position

The FDA frequently uses meetings of scientific and trade associations to articulate policy on regulatory issues. At the Second Annual Joint Conference between the FDA and the International Society of Pharmaceutical Engineers in 1983, a representative of the Agency (4) stated with regard to the use of the sterility test for end product control testing that, "… despite its limitations, the sterility test remains a last chance means of detecting, in a cumulative way, all the significant production errors or microbial anomalies which can cause sterility failure in a batch. The bottom line is, lot by lot end product sterility testing remains a current good manufacturing practice. The sterility test is still required and thorough investigation is needed for retests".

Whether or not one is of the opinion that the test, by itself, is inadequate as a quality control method, regulations state, in effect, that it must be run (2). The interlocking writeups in Chapters 71 and 1211 represent USP's effort to accommodate the regulatory situation.

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9. Ibid: Sec. 201 (h)(I).
10. Ibid: Sec. 201 (j).
11. Ibid: Sec. 501 (b).
12. Ibid: Sec. 502 (e)(3)(B).
13. Ibid: Sec. 502 (e)(4)(B).
14. Ibid: Sec. 502 (g) and (h).
15. Ibid: Sec. 508 (a) and (b).
16. Ibid: Sec. 707.

DISCUSSION

Sterilization Standards

Comment by Mr. Doolan, Becton Dickinson and Company, France

Thank you very much, Mr. Schmitz, for that stimulating discussion regarding the use and limitations of the sterility test. I would like to invite Mr. Komandor from Poland who has asked for a few minutes to address the preservation and storage of tissues for transplantation—that is, the problem of tissue sterilization.

Invited Comments by Mr. Komandor, Poland

Many specialized tissues are used in reconstructive surgery or for wound dressings throughout the world. In defining sterilization, mention is always made of the effects of the process on the numbers of microorganisms, but nothing is typically said about the influence of the sterilization process on the material which is being sterilized. In certain instances, the effects of the sterilization process are visible. For example, the mechanical properties of bone are greatly diminished following sterilization; when this same material is used after freezing, deleterious effects on mechanical resistance are not as severe. As another example, the deposition of calcium on implanted fibrous connective tissue increases rapidly when the tissue is sterilized prior to implantation. Furthermore, when collagen material which is used for implantation or for wound dressing is first subjected to radiation sterilization, it is then absorbed very quickly after implantation; however, the same material, if sterilized by heating, is stable. It, therefore, seems appropriate to introduce into the definition of sterilization, consideration of the effects of the sterilization process on the material being sterilized.

Comment from the Floor

I think one of the issues which deserves consideration, and has not been covered very well this week, is how to adequately sterilize a number of the biologically active items which are now used to protect patients. Consider artificial skin, for example. It must be recognized that these immune modulators may not survive traditional sterilizing procedures. Sterilization becomes a challenge as more complex support and actual maintenance of biologic activity are required.

Question for Mr. Miller, Association for the Advancement of Medical Instrumentation, U.S.A.

How will the development of voluntary standards in the U.S. be coordinated with the activities of the European Commission, CEN, and EUCOMED? For instance, what about AAMI's position on biological indicators?

Answer by Mr. Miller, Association for the Advancement of Medical Instrumentation, U.S.A.

The first effort was to propose an international committee on sterilization within ISO. This has already occurred. Over time, there are a number of different requirements in national standards that will have to be reconciled. I think the existence of this international committee is the most constructive step to ultimately reconcile standards from all the countries throughout the world and to resolve technical differences in this area as well as in others.

Question for Dr. Belova, All-Union Scientific Research Institute for Preventive Toxicology and Disinfection, Ministry of Health, U.S.S.R.

Can you explain the process by which the standards you referred to are prepared in the U.S.S.R.?

Answer by Dr. Belova, All-Union Scientific Research Institute for Preventive Toxicology and Disinfection, Ministry of Health, U.S.S.R.

The Soviet standards are developed based upon research and development projects which are conducted by the research institutes and development laboratories and checked using actual experimental samples. For example, in sterilization facilities, the sterilizers, steam, and air are monitored during the evaluation of experimental samples. When the results corresponding to the requirements are obtained, these requirements are then included into the standards. The requirements for chemical control are developed similarly. They are developed by the research institute and checked in practice. Only after being confirmed are they included into the standards which then become the law.

Session III
Ethylene Oxide Sterilization
Chairman: A. Parisi, Ph.D.
Baxter Pharmaseal, USA.

Introduction Ethylene Oxide Sterilization

A. Parisi, Ph.D.

Baxter Pharmaseal, USA

When Wurtz reported the discovery of ethylene oxide in 1859, I am certain that he had no idea of the significant role this chemical would play, not only as a basic chemical used in the synthesis of more complex organic compounds, but as the most widely used commercial method of sterilizing medical products in the latter 20th century. This chemical has made the disposable medical device industry as it is known today possible, permitting manufacturers to use low-cost thermo plastics which would otherwise be unsuitable to the use of steam under pressure.

It took about 64 years after its discovery before the biocidal activity of ethylene oxide was first reported. Cotton and Roark in 1928 reported on the activity of ethylene oxide as an insecticide. It was not long after, in 1940, that Griffith and Hall applied for the first patents for the use of ethylene oxide for the destruction of microorganisms in natural products such as gums and spices. These patents also provided the first description of an industrial sterilization process. The process described employed vacuum chambers and used pure or 100% ethylene oxide gas as the sterilization agent. Aided by the wartime need to provide effective field hospital sterilization for supplies, considerable activity took place in the 1940s and 50s with experimentation that demonstrated that laboratory plastics, medical and biological preparations, hospital bedding, plastic bandages, medical instruments, surgical implants, and many other items could be successfully sterilized by this chemical agent. Still today, ethylene oxide is the primary chemical agent used in hospitals to sterilize items that cannot be sterilized by steam. Additionally, those industries which supply medical devices still use ethylene oxide for products that are sensitive to the heat of steam sterilization or contain materials that are degraded when exposed to radiation sterilization.

The basic sterilization processes described and defined by early investigators such as Griffith and Hall, Kaye, Phillips, Earnst, Kereluk, and others have changed little. Essentially, two classes of processes developed: 1) the deep vacuum which uses pure or 100% ethylene oxide cycle; and 2) soft cycle or shallow vacuum cycles which use ethylene oxide blended with a safety diluent gas. From the 1940s through to the 1970s, sterilization developed as a science along with the evolution of the understanding of the probabilistic nature of the achievement of sterility. Early research focused on the understanding of the mechanics of achieving reliability in providing microbial lethality. This included gaining knowledge about the interrelationships between gas concentration, temperature, humidity, exposure time, and the need to present *product* to the process in a consistent manner, as well as the need to present the *process* to the product in a consistent manner. The latter two concepts address the issues of pre-sterilization conditioning and chamber environment uniformity. Recognition emerged that there were factors that limited ethylene oxide sterilization effectiveness with products having features such as mated surfaces, long

lengths of tubing, and sterility maintaining protectors, as well as sterile barrier packaging materials with poor or variable porosity. With the understanding of the probabilistic nature of microbial inactivation emerged the concepts of biological indicators, process validation, and in some instances, parametric process release in place of the concept of absolute sterility demonstrated by product sterility testing.

A diversity of equipment evolved over the years for providing the sterilant to the product. The equipment has ranged from the vacuum vessels of Griffith and Hall, to simple atmospheric pressure metal chambers and gas-tight plastic bags reported by Phillips. Regardless of the size of the sterilizer, the elements of the process to be controlled are identical. An ethylene oxide sterilizer is basically a pressure vessel with a capacity to evacuate air, warm and moisturize the product uniformly, add the sterilant gas to a predetermined concentration, maintain a uniform and stable temperature, and finally, remove the sterilant after a specified time interval.

The vessels themselves have changed little over the past 30 years. The major design changes have been primarily to ensure the homogeneity of the sterilizer's internal environment by the use of recirculating devices such as fans and external recirculation loops. These aid in reducing temperature variability and cold spots which have slower rates of microbial lethality. The other major change in providing a reproducible process resulted in the evolution of sterilizer control systems from those employing electromagnetic sequencing devices to those using the current technology of microprocessors and computers. These provide ongoing sensing of established parameters and the ensuing feedback to the control devices.

In 1928 when Cotton and Roark first reported on the antimicrobial effects of ethylene oxide, they also reported that "ethylene oxide is not highly toxic to man." How little they knew. As evidence accumulated which demonstrated not only the acute toxic effects of ethylene oxide, but its mutagenic and carcinogenic potential, the need to protect the workers and the patients increased. Concern over the impact of ethylene oxide on the environment was heightened by legislation enacted by various states in the USA, notably, California. This was compounded by the worldwide concern over the effect of the most commonly used diluent gas employed in the soft vaccum cycles and the effects of these chlorofluorocarbons (CFCs) on the protective ozone layer.

This new awareness has resulted in many instances of optimized cycles which use less gas. Less gas means lower product residues and, therefore, faster product release, less post-cycle worker exposure, and less impact on the environment. The need for lower factory emission has resulted in the emergence of technologies to abate these emissions by reclaiming the sterilant, converting the ethylene oxide gas to a non-toxic product such as a glycol or by incineration of the non-CFC compounded sterilants. The planned reductions in CFC availability and its attendant increased cost has resulted in searches for alternatives to ethylene oxide. A need has arisen for alternative diluents to the CFCs. Carbon dioxide has been used as a diluent for many years but concerns over potential stratification within the chamber, as well as equipment limitations due to the high operating pressures commonly encountered, have limited its desirability in many parts of the world. A heightened interest has arisen in the use of nitrogen as a safety diluent.

In our program this afternoon, the program committee has assembled an outstanding group of speakers who will be addressing the current issues surrounding ethylene oxide and expanding on a number of the areas I've discussed. Our speakers, Dr. Young and Mr. Danielson, will provide indepth discussions of current activities and of the future prospects and possible limitations to the use of ethylene oxide as a medical product sterilant for both industrial and hospital environments. As I mentioned above, evidence has accumulated demonstrating not only the acute effects from ethylene oxide exposure, but its human mutagenic and carcinogenic potential, and, as a result, the need developed to protect the environment, the worker and the patient. Mr. Jorkasky will address the U.S.A. experiences with worker exposure studies and the regulations restricting the use of CFCs. Risk assessments from patient exposure to ethylene oxide and its byproducts as residues on medical products and the impact of this information on the formation of standards in the U.S.A. and U.S.S.R. will be discussed by Drs. Roderick and Zayeva.

Following the formal presentations, there will be a panel discussion to provide the opportunity to address issues from you, the audience, as well as answer any questions that may develop from the information presented by our speakers.

Ethylene Oxide: Current Status and Future Prospects

Jack H. Young, Ph.D.

Penn State University at Erie, U.S.A.

Introduction

Over the last forty years, ethylene oxide (EO) has evolved as the predominate method for sterilization of heat and moisture sensitive medical devices. During this period scientific studies and developments in EO sterilization practices have focused on not only sterilization efficacy, but also addressed critical issues associated with worker exposure to EO and potential environmental impact of sterilant discharge. The more recent developments have dramatically changed EO equipment and facility designs, as well as worker safety practices. In addition, old regulatory standards have been revised and new standards established to reflect new scientific knowledge concerning potential toxic effects. No other sterilization chemical or process has been as extensively evaluated. Concerns over EO toxicity have significantly increased basic research directed towards identification and development of alternative low temperature sterilants. Currently no single chemical or process appears to be a suitable direct replacement for EO.

This paper will summarize the most critical issues associated with EO sterilization:

— toxicity and regulation standards;
— minimization of worker exposure; and
— control of sterilant discharge to the environment.

In addition, the current status of efforts to develop alternative low temperature sterilization processes will be summarized and their potential for replacing EO discussed.

EO Chronology

Although first discovered and described in the last century (43), ethylene oxide was not recognized as a sterilant until Gross and Dixon (18) obtained a United States patent entitled “Method of Sterilization”. The pioneering work of Phillips and Kaye (24, 25, 36, 37) in the late 1940s established the general effects of time, humidity, temperature, and EO concentration on sporicidal activity (Figure 1). These studies spurred activities to develop the basic information needed to advance EO as a widely accepted commercial sterilization process. Diluents such as carbon dioxide and fluorocarbons were added to produce sterilization mixtures less flammable and explosive than 100% EO. During the 1950s, studies were conducted to determine the concentration of EO and other residuals in medical products following EO sterilization. These studies showed that residual EO in devices could cause skin lesions (40) and hemolysis of blood (21).

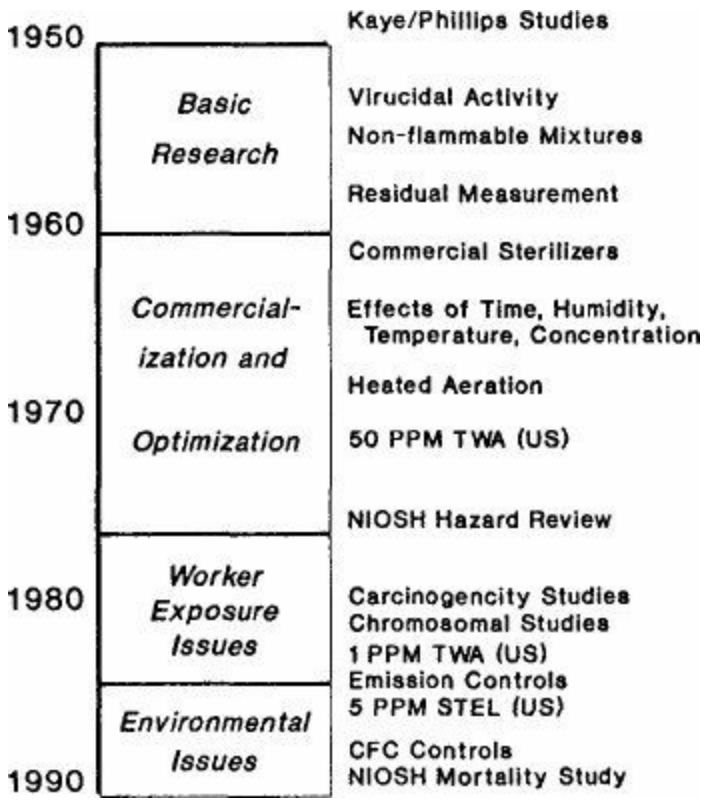

Figure 1. EO Chronology

In the early 1960s, commercial sterilization equipment was being manufactured and used by hospitals and medical device manufacturers. Sterilization research centered on optimization of process parameters to assure sterility, minimize degradation of items due to EO sterilization, and to increase productivity. Heated aeration chambers were introduced to accelerate the release of residual EO following sterilization (35).

In 1971, the United States Occupational Safety and Health Administration (OSHA) (31) required employers to assure that employee exposure to EO did not exceed 50 parts of EO per million parts of air (50 ppm), determined as an 8-hour time-weighted average (TWA). The 50 ppm level was based on limited data from a six-month animal inhalation study and a study of employees exposed for 10 years or more to estimated levels of 5 to 10 ppm with no reported adverse effects. During the early 1970s, inhalation exposure to ethylene oxide was rarely, if ever, monitored. No regulations or guidelines existed for maximum permissible levels of EO residuals in medical devices, but general guidelines requiring aeration of items for seven days at room temperature or 12 hours at 120°F were being advocated for hospitals.

In 1977 the National Institute for Occupational Safety and Health (NIOSH) recommended a 75 ppm maximum permissible exposure limit for 15 minutes (16). This was based upon studies showing changes in genetic material of cells in at least 13 biological species and NIOSH's concern that EO might increase frequency of mutations in exposed populations. Concerns were raised that EO might be a potential human carcinogen, but no data were available. This initiated a period during which numerous studies were conducted to determine actual worker exposure levels and to assess the potential health hazards resulting from EO inhalation exposure. During this period permissible exposure limits were

lowered, employee exposure monitoring became routine, standards were proposed for maximum residual levels in devices, additional safety features were added to sterilizers, and work practices were changed to minimize worker exposure.

Toxicity and Current Exposure Standards

With respect to potential carcinogenicity, two animal inhalation studies were especially significant and influenced the lowering of inhalation exposure limits in the U.S. Lynch and colleagues (30) conducted a chronic two-year inhalation study with male rats and monkeys exposed at 50 and 100 ppm. EO-exposed rats generally showed incidents of gliomas (brain tumors), peritoneal mesothelioma (a testicular tumor), and leukemia. Whereas none of the monkeys showed any evidence of leukemia, neuropathological evaluations (in conjunction with findings in the rats) indicated possible affects on the central nervous system.

Snellings et al. (41) also conducted a two-year chronic inhalation study. They exposed male and female rats to EO concentrations of 10, 33, and 100 ppm and concluded that EO exposure increased the incidence of mononuclear cell leukemia and peritoneal mesothelioma. OSHA utilized the data from Snellings et al. (41) to conduct a quantitative assessment of risk and set a new inhalation exposure standard (Figure 2). Based on a 45-year working lifetime, OSHA estimated 12 to 23 excess deaths per 10,000 workers exposed to 1 ppm TWA. Corresponding values for a 50 ppm TWA exposure are 634 to 1,093 deaths per 10,000. Figure 3 compares these estimated EO risks to documented risks for other activities. The estimated risk for 50 ppm TWA is higher than the risk associated with manufacturing jobs and even such high risk occupations as fire fighting. It is important to emphasize that these estimated EO risks are based on rat carcinogenicity data (an unverified no-threshold model for cancer development) and significant extrapolations to estimate risk to humans over a 45-year lifetime. This quantitative risk assessment was used to establish public health policy and does not prove EO to be carcinogenic, rather it assumes it to be carcinogenic and assesses the risk if this presumption is correct. Risk assessment may lead to control of a toxic substance even though probability of harm to an individual is small and studies to assess the risk are incomplete.

Available human epidemiological data in 1984 were extremely limited in size of population studied, estimates of exposure levels, and potential for exposure to other toxic chemicals. Although definitive human cancer data were not available, OSHA concluded that enough data existed to consider EO a potential human carcinogen and lowered the maximum permissible exposure limit to 1 ppm TWA (32). They also established regulations for worker exposure monitoring and medical surveillance. In 1988, OSHA (34) established an additional requirement that no worker be exposed to greater than 5 ppm averaged over a 15 minute sampling period. This is generally referred to as an excursion limit or short-term-exposure limit (STEL). Other countries have established similar inhalation exposure limits (Figure 4).

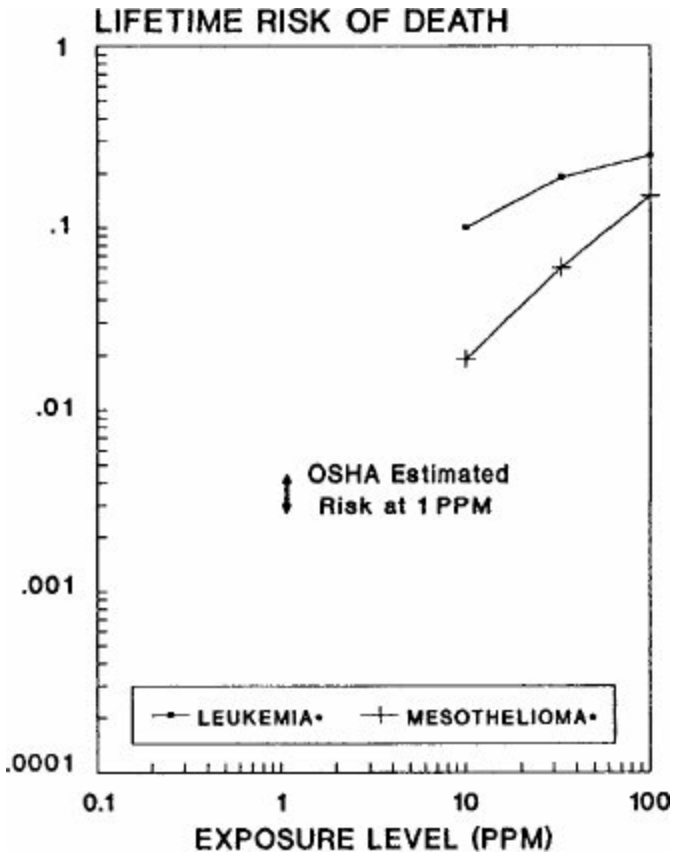

Figure 2. EO Inhalation Cancer Risk

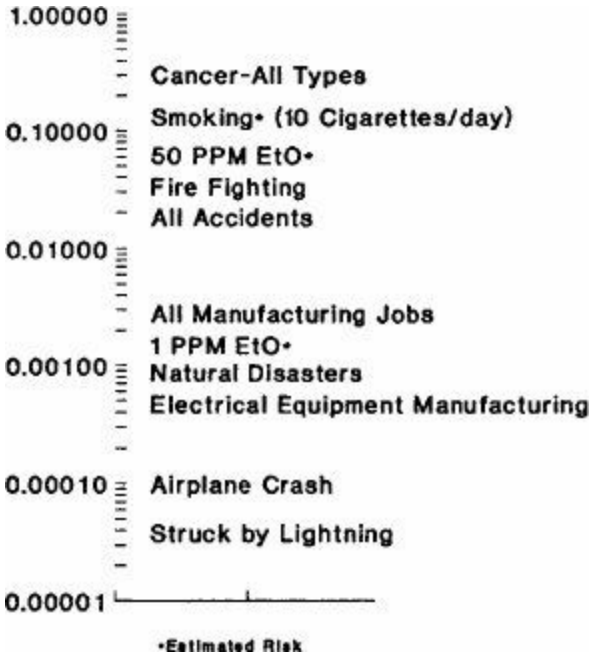

Figure 3. Lifetime Risks of Death

Country	8 Hour TWA	15 Minute STEL
Belgium	1 PPM (1984)	
Canada (Ontario)	1 (1987)	10 PPM (1987)
Denmark	1 (1985)	
France	5 (1982)	10 (1897)
Italy	30 (1978)	
Netherlands	50 (1985)	
United Kingdom	5 (1987)	
United States	1 (1984)	5 (1988)
U.S.S.R.	0.5	

Figure 4. EO Exposure Limits

In addition to inhalation, EO exposure may result from contact with medical devices containing residuals resulting from EO sterilization. In 1978, the U.S. Food and Drug Administration (12) proposed regulations for maximum acceptable levels of ethylene oxide, ethylene chlorohydrin, and ethylene glycol (Figure 5). This proposal has never been finalized, but most of the U.S. medical industry follows it as a general guideline. The lifetime risk of death due to cancer from medical devices containing the 1978 maximum EO levels was estimated by Cyr et al. (6) to be lower than the risk associated with the 1 ppm inhalation standard.

Medical Device	Residual Level
Implant	
Small (<10 Grams)	250 PPM
Medium (10-100 Grams)	100
Large (>100 Grams)	25
Intravenous Devices	5
Intraocular Lenses	25
Devices Contacting:	
Mucosa	250
Blood	25
Skin	250
Surgical Scrub Sponges	25

Figure 5. Proposed Maximum EO Residual Levels

Since 1984, a number of human epidemiologic studies have been initiated to better assess potential cancer risks. Gardner et al. (14) studied 2876 exposed workers and found no clear excess of leukemia and no increase in stomach cancer. Kiesselbach and colleagues (26) followed over 2500 industrial workers and found no significant difference in standardized mortality ratios between exposed and control groups. These studies do not exclude the possibility of EO being a human carcinogen, but suggest that the risk of cancer associated with current exposure limits may be small. NIOSH is conducting a cohort

mortality study for leukemia which includes 21,000 workers representing 248,000 years of exposure. Results from this study should give the best evidence to date concerning the possibility that EO is a human carcinogen.

In addition to potential carcinogenic effects, EO has reversible effects resulting from acute overexposure. These include eye and respiratory tract irritation, nausea, vomiting, diarrhea, vertigo, and headaches. Other studies have reported that EO exposure may result in human cytogenetic (15, 33, 44), reproductive (20), and neurologic effects (11, 17, 28). Anyone involved in the use of ethylene oxide should be knowledgeable of current toxicity findings.

Minimizing Worker Exposure

An effective program for EO exposure control includes all of the following:

— method for determining exposure levels;
— training and monitoring of employees for proper work practices, including emergency procedures;
— properly operating sterilizers and aerators equipped with EO safety features.

Numerous references (2, 7, 19) give guidelines for workpractices, room ventilation when sterilizers and aerators are present, employee training programs, and emergency procedures/equipment. These recommendations will not be summarized here, but an overview of current monitoring technology and sterilizer safety features will be presented.

In the mid to late 1970s, very few users of EO were monitoring worker exposure levels. The only available monitoring devices were bulky infrared spectophotometers costing between $5000 and $10,000 or air sampling devices which required wearing an air pump and sending the collection tube to an analytical laboratory for analysis.

Small, light-weight, passive diffusion badges are currently available for monitoring personnel exposure to EO. These devices clip to the worker's uniform near the breathing zone and do not require an air pump. Some badges can be analyzed on site, whereas others have to be sent to an analytical laboratory. Badges can be used to monitor either 8-hour TWAs or 15 minute STELs. Commercially available badges can monitor 8-hour TWAs down to 0.1 to 0.2 ppm accuracies of ± 25% at 1 ppm and ± 35% at 0.5 ppm which are the OSHA (32) requirements for monitor accuracy. Personnel monitoring is usually required by governing regulatory agencies, but is also valuable in identifying workers with poor work practices.

It is recommended that fixed location monitoring devices (area monitors) be used to supplement personnel monitoring. They monitor the instantaneous EO concentration at given locations and most can be used to trigger an emergency alarm if concentrations exceed a preset level. Sensing probes are located in areas where EO leakage might occur and typically include EO storage area, sterilizer doors, aerator doors, safety valves, and sterilizer gas exhaust lines. Examples of area monitors are gas detection tubes, gas chromatographs, and infrared spectophotometers. Since area monitors vary significantly in technical sophistication and cost, users should be aware of the advantages and

disadvantages of each type of monitor. The Association for Advancement of Medical Instrumentation (1) provides good general guidelines on various types of area monitors.

Sterilizer designs have evolved such that today's sterilizers contain numerous features to assure the safe use of ethylene oxide. Common features include special cycles, interlocks, local ventilation at selected locations on the sterilizer, and redundant components (Figure 6). All safety features are designed to further minimize the potential for worker exposure or to alert workers to an abnormal condition which might lead to exposure.

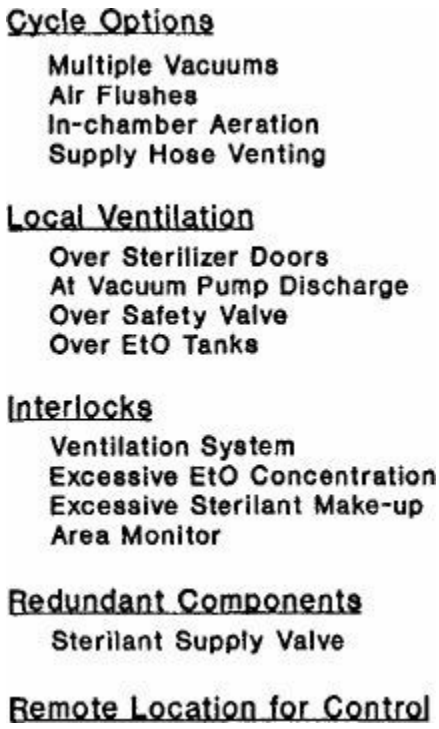

Figure 6. Sterilizer Features for Minimizing EO Exposure

During the sterilization phase of a typical cycle, concentration within the chamber is in the range of ≥ 650 mg EO per liter of chamber volume which equates to 273,000 ppm of EO. Consequently, EO chamber concentration must be dramatically reduced prior to a worker opening the door to remove the load. This can be accomplished by pulling a vacuum on the chamber and then filling the chamber with sterile air. This sequence is repeated a number of times and many sterilizers permit the operator to select the number of such vacuum pulses. EO can also be removed by continuously flushing the chamber with sterile air. Ideally, one would like to open the door only when all the EO is removed not only from the chamber but also from the items which have been sterilized. Some sterilizers have control systems which permit sterilization and aeration within the same chamber. This is usually a cycle option used when productivity requirements permit.

A special cycle can be used for venting the EO supply line through the sterilizer chamber when changing EO supply tanks. Since EO has a high vapor pressure and the supply lines contain liquid EO, high vapor concentrations result when a line containing liquid is opened. By vaporizing the liquid and venting it through the chamber, the normal sterilizer method of exhausting EO can be used and release to the general work area does not occur.

Sterilizers usually have their own air blower for providing local ventilation on the sterilizer where EO might be released. These locations include the door area, safety valve, vacuum

pump discharge, and near the EO tanks. The sterilizer can be electrically interlocked to the general room ventilation and sterilizer ventilation systems so that a cycle cannot be initiated unless both systems are functioning. Additional interlocks monitor the chamber pressure during the sterilization phase to ensure that an excessive among of EO is not put into the chamber due to a component failure or chamber leak. If such an event occurs, the EO supply valve is closed, the operator warned, and the chamber is evacuated. An area monitor can be interfaced to the sterilizer so that a high EO concentration in the area around the sterilizer can be used to sound an alarm and abort the sterilization cycle.

It is recommended that all critical components such as EO supply valves whose failure could result in a substantial release of EO, should have a backup. Some sterilizers have a control panel which can be located remote from the chamber, thereby permitting operating of the sterilizer from a distance. This could be valuable in the case of an emergency.

The importance of both engineering controls and employee work practices in an overall program for minimizing exposure is illustrated in Figure 7. These data show the average 8-hour TWA exposures associated with varying degrees of engineering controls and work practices in U.S. hospitals (8). Effective engineering controls and good work practices resulted in less than detectable levels whereas no engineering controls and poor practices resulted in unacceptable levels.

Control of Sterilant Discharge

Once released to the atmosphere, EO degrades to less harmful byproducts, but this process has been estimated to require three to seven months (5, 9). In addition, chlorofluorocarbon 12 (CFC-12), the most common diluent gas used in EO sterilant mixtures, is very stable and has been linked to destruction of atmospheric ozone. Consequently, use of EO requires that attention be paid to disposal of the sterilant gas and diluent.

Emission Control

In 1985, the U.S. EPA (10) published its intent to list EO as a hazardous air pollutant. This would require the EPA to set standards for the maximum quantity of EO which could be released to the atmosphere. Although no national standards have yet been set, various states and local authorities have set EO emission limits. The three most common technologies for controlling emissions are acid hydrolysis, catalytic oxidation, and reclamation.

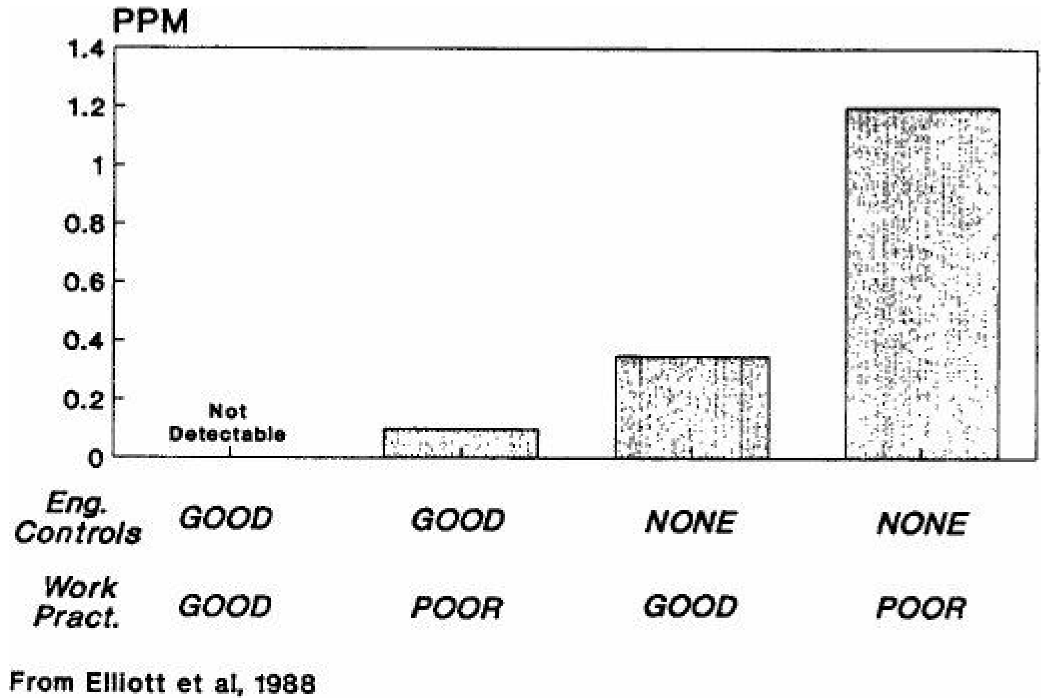

Figure 7. Hospital 8-Hour TWA

Acid Hydrolysis

EO can be converted to ethylene glycol by reaction with a strong acid such as sulfuric acid. Conversion efficiency of commercial equipment is 99% or greater. The resulting ethylene glycol has to be neutralized with a basic solution prior to disposal or sale.

Catalytic Oxidation

At temperatures of 150° to 180°C, ethylene oxide can be converted to carbon dioxide and water when in the presence of oxygen and a catalyst. Catalytic systems have efficiencies of 99% or greater, but can only handle EO concentrations below typical chamber sterilization concentrations. Consequently, significant dilution with air is required prior to the sterilizer exhaust entering the unit. This technology is most appropriate for hospital-sized sterilizers and exhaust from aeration rooms.

Reclamation

A third alternative is reclamation and reuse of the sterilant. The sterilizer effluent is passed through a dryer to remove water vapor used to humidify the load. It is then compressed and recondensed back to a liquid. If it is being reused as a sterilant, the reclaimed mixture has to be reblended since the load may selectively absorb EO or CFC. This reblending can be done on or off site depending on the economics involved.

Elimination

In 1987, 24 nations agreed to a structured reduction in CFC production such that by 1998 the production level would be ≤ 50% of the 1986 level. More recently, these nations have agreed to the complete elimination of all CFCs by the year 2000. In the U.S., an excise tax has been imposed on CFCs, causing the price of the most widely used mixture, 12% ETO/88% CFC 12, to increase in cost by a factor of 300% with further increases expected. Currently, there are three available options for eliminating CFC usage (carbon dioxide mixtures, 100% EO, and reclamation) with a fourth option (12/88 direct replacement) expected in the mid 1990s.

Carbon Dioxide Mixtures

A 10% EO/90%CO_2 mixture is non-flammable and has been used for sterilization. Changing from the CFC mixture to CO_2 requires higher operating pressures to achieve the same EO concentration. This can have an effect on product integrity and some sterilizers are not designed to operate at these higher pressures. In addition, polymerization of EO and problems assuring a uniform mix of EO and CO_2 within supply tanks are common. Other EO/CO_2 mixtures are available but they are flammable.

100% EO

100% EO is gaining wider acceptance as a sterilant even though it is flammable. It offers the advantages of containing no CFCs and requiring a vacuum vessel, not a pressure vessel, since sterilization concentrations can be achieved at sub-atmospheric pressure levels. This largely eliminates concerns about EO leaking from the sterilizer into the work area. Sterilizers designed for 12/88 usage can not be readily adapted for 100% usage as special engineering requirements exist for handling flammable and explosive substances. In addition, safety requirements exist for the type of facility needed to house a 100% sterilizer and for storage of the sterilant. Therefore, changing to 100% usually requires a large capital investment.

Reclamation

A third option is reclamation of CFC which has the major advantage of allowing a reduction in both EO and CFC emissions. CFC emissions can be reduced 50% to 90% through the use of reclamation. Currently this is a viable option for larger industrial users, but not individual hospitals and small industrial companies.

EO/Hydrochlorofluorocarbon Blend

The ideal replacement for CFC mixtures would be one that could be used in existing sterilizers with little or no modifications required and which has no adverse effects on the wide range of items currently being sterilized with the 12/88 mixture. An EO/hydrochlorofluorocarbon (HCFC) blend is being evaluated as a direct replacement. The

HCFC molecule has 2% of the ozone depletion potential of CFC-12. The new mixture would be nonflammable and nonexplosive but will not be commercially available until the mid-1990s. Other non-ozone depleting mixtures are under investigation, but due to the extensive testing required, no direct replacement is anticipated prior to 1994.

Alternative Low Temperature Sterilants

Concerns over potential EO toxicity and costs associated with handling sterilant discharge have provided greater impetus for identification and commercialization of new low temperature sterilization processes. Ideally, one would like a vapor phase sterilant capable of processing the wide range of items currently sterilized by EO and which require no or minimal changes to existing device packaging, equipment, and sterilization techniques. Unfortunately, no such sterilant has been identified, let alone commercialized, but significant progress has been made on alternative sterilants for selected applications.

For specific products, alternatives to EO have existed for a number of years. For example, it has been estimated that EO accounted for 70% of U.S. industrial sterilization processes in 1978 versus 10% for radiation (4). In 1987, sterilization with EO decreased to 50% whereas radiation had increased to 30%. Some companies are designing new products and re-designing selected older products to be compatible with radiation. Switching to radiation is not a viable approach for many industrial products or general hospital sterilization.

Figure 8 compares EO to other low temperature sterilants. These sterilants are either currently being used to process items previously sterilized with EO or are under investigation for future commercialization. There is a definite trend away from alkylating agents such as EO and formaldehyde to oxidizing substances such as hydrogen peroxide, chlorine dioxide, and ozone. Most reports suggest that the sterilizing effect of these oxidizing agents is due to hydroxyl free radicals (42) as opposed to the agent itself. The effectiveness of these free radicals can be seen by comparing the required sterilization concentration to that of EO. Typical EO cycles are run with concentrations of 400 to 600 mg/L or higher, whereas many oxidants require concentrations of 10 mg/L or less.

Chlorine Dioxide

Rosenblatt (39) has reported that *Bacillus subtilis* spores exhibit a D-value as low as 10 minutes when exposed to chlorine dioxide gas at 27°-30°C, 10 mg/L and 80% relative humidity. D-values increase significantly when the relative humidity is below 50%. Chlorine dioxide degrades silk, uncoated aluminum foil, and unbleached paper. Chlorine dioxide gas cannot be shipped or stored and must be generated on-site. Based on its chemical and physical properties, one would expect it to have better penetration characteristics than the other oxidants currently being evaluated. Preliminary results indicate excellent compatibility with polyethylene, polyvinyl chloride, polypropylene, cellulosic, silicon rubber, and 316-L stainless steel. OSHA has established an 8-hour TWA of 0.1 ppm.

Agent	Mechanism	Temperature	Concentration
Ethylene Oxide	Alkylation	30-60 C	400-1200 mg/l
Chlorine Dioxide	Oxidation	30	10-50
Hydrogen Peroxide	Oxidation	4-60	.5-5
Hydrogen Peroxide Plasma	Oxidation	30-40	.2-10
Plasma	Dependent on Gas	30-40	
Ozone	Oxidation	30	8-10%
Peracetic Acid Liquid	Oxidation	50-55	.2%

Figure 8. Low Temperature Sterilization Processes

Hydrogen Peroxide

Rickloff (38) has shown that *Bacillus stearothermophilus* spores exhibit a D-value of 0.3 minutes when exposed to 2 mg/L hydrogen peroxide vapor at 35°C. Sterilization has been achieved at temperatures as low as 4°C. These data also show *B. stearothermophilus* to be more resistant than *B. subtilis*. Cellulosic materials are not recommended for sterilization by hydrogen peroxide since they readily absorb the vapor. Hydrogen peroxide does not have the excellent penetration characteristics of EO, but has the advantage of non-toxic degradation products—water and oxygen. Initial sterilization applications being evaluated include gloveboxes, freeze dryers, and dental instruments. The 8-hour TWA in the U.S. is 1 ppm.

Lin (29) has shown that hydrogen peroxide vapor plasma is an effective sterilant. A D-value of less than 3 minutes was obtained for *B. subtilis* spores exposed to plasma generated from 0.42 mg/L of hydrogen peroxide vapor.

Plasma

The plasma of a number of different gases (i.e., argon, nitrogen, and oxygen) have been evaluated for sterilization efficacy (3, 13, 22). In all cases, a deep vacuum has to be drawn (typically absolute pressures of 1 torr or less) and radio-frequency or microwave energy introduced to generate the plasma which results in electrically charged and neutral gaseous species. The mechanism of kill for plasma has not been well established and most probably depends on the type of plasma generated. In the case of oxygen, free radicals of atomic oxygen and excited molecular oxygen are formed. Consequently, strong oxidizing species must exist in many plasma processes. Historically, the ability of plasma to penetrate through materials and into crevices has been a question.

Plasma is routinely used to remove thin proteinaceous films and other contaminants from

surfaces. Therefore, close attention has to be paid to the effect of plasma on surfaces of items being sterilized. A major potential advantage of plasma is the absence of toxic residuals within the sterilizer or on sterilized items. If a sporicidal chemical is used in conjunction with plasma, the plasma can be used to degrade the chemical, thereby eliminating residuals.

Ozone

Ozone has been used to treat drinking water and liquid seepage for a number of years and has recently been proposed as a vapor phase sterilant (23). Ozone has the advantage of having one of the highest oxidation potentials. Karlson (23) has reported a D-value for *B. subtilis* of 4 minutes when using 8% by weight ozone at ambient temperature. This high humidity ozone sterilant can be generated on-site from oxygen. Materials such as steel, brass, iron, and natural rubber can be effected by prolonged exposure to this humidity and strong oxidant. The 8-hour TWA in the U.S. is 0.1 ppm.

Liquid Peracetic Acid

Industrial sterilization processes require a method of maintaining sterility following the process, but certain items within hospitals are used immediately after sterilization within the operating room. Consequently, liquid sterilants provide an alternative to EO for items such as flexible endoscopes. A 0.2% liquid peracetic acid solution has been reported to be appropriate for this application (27). A buffered solution at 50°-55°C is circulated through and around the item for 12 minutes. Use of liquid sterilants requires thorough rinsing to assure removal of the sterilant. The major disadvantage of any liquid sterilant is that packaging cannot be used to maintain the item sterile following sterilization.

EO Replacements

One of the most frequently asked questions concerning any new sterilant is “Is it a direct replacement for ethylene oxide?” In all cases the answer is no. Changes in sterilization equipment, device packaging or the device itself are required with the new sterilants. In some cases, the switch from EO is not possible for technical and/or cost considerations.

When considering any new sterilant, it is helpful to view a sterilization process as consisting of three sequential steps: sterilant penetration, biological kill, and sterilant removal (Figure 9).

During the sterilant penetration phase, one would like a sterilant with high vapor pressure, high molecular stability, and polarity. All of these properties aid in a sterilant’s ability to penetrate and diffuse through device packaging and the device itself. EO is excellent in this regard, whereas most proposed sterilants fall short of EO.

PROPERTY	IDEAL	EO	H_2O_2
Sterilant Penetration			
Vapor Pressure (mm Hg)	High	600	2
Boiling Point (C)	Low	11	150
Molecular Stability	High	High	Low
Biological Kill			
Concentration (mg/l)	Low	600	3
Temperature (C)	Low	50	25
Time (Minutes)	Low	30	10
Sterilant Removal			
Non-toxic Degradation Products	Yes	No	Yes
Molecular Stability	Low	High	Low
Boiling Point (C)	Low	11	150

Figure 9. Sterilant Properties

The second step is the actual achievement of sterility. Ideally, a sterilant should be rapid at low temperatures and concentrations. Many of the new sterilants discussed previously are significantly better than EO in this respect. For example, hydrogen peroxide can sterilize in less time, at a lower temperature, and with 0.5% the concentration of EO. Of course, hydrogen peroxide has to be able to penetrate to the site requiring sterilization before its rapid biological effectiveness can be utilized.

The final step is sterilant removal. The ideal sterilant would be unstable, have a low boiling point and non-toxic degradation products so that residuals could be easily driven off or degraded to safe components. As with biological kill, the new sterilants show great potential in this area. Many of these new sterilants degrade through short-lived intermediate species to non-toxic chemicals, whereas EO and its residuals, ethylene glycol and ethylene chlorhydrin, are stable and difficult to remove from the product.

From the above discussion it becomes clear that none of the new sterilants will be a direct replacement for EO in the next few years. Evaluation of new sterilants requires a trade-off analysis of penetration, kill effectiveness, residuals, material compatibility, toxicity, handling safety, regulatory requirements, and cost. Sterilant development is a costly and time-consuming process best accomplished by limiting the evaluation to a selected application or group of products. Consequently, it is most likely that a number of new sterilants will be used for limited applications and gradually one or two of these sterilants will gain wider acceptance and potentially result in a general purpose sterilant. As with EO, this process will require a substantial period of time. We should be seeking the ideal

sterilant, not a replacement.

Summary

Over the last 15 years, EO sterilization practices have dramatically changed. This has been driven by concerns over toxicity issues associated with the local work environment as well as the global environment. Technology exists for the safe and effective use of EO, but cost and uncertainty over future regulatory actions have spurred significant activity to identify new low temperature sterilants. Although the new sterilants are not seen as a direct replacement for EO, their use for specific applications will grow. EO will remain the primary low temperature vapor sterilant for at least the next 5 to 10 years.

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Ethylene Oxide Environmental, Worker Exposure and Chlorofluorocarbon Regulations

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In this chapter, I will discuss the occupational and environmental concerns regarding ethylene oxide (EO) and chlorofluorocarbons (CFCs). I represent the Health Industry Manufacturers Association (HIMA) and will also discuss U.S. regulatory activities on EO and CFCs. I have been working on these issues for close to ten years, staffing HIMA Committees, and dealing with the U.S. Regulatory Agencies.

To further outline my presentation, I will discuss HIMA and its involvement in sterilization issues, recent activities of the U.S. Occupational Safety and Health Administration regarding worker protection, and the U.S. Environmental Protection Agency regarding environmental protection, and the conduct by the National Institute for Occupational Safety and Health and HIMA of major epidemiologic studies on Industrial EO Workers.

I appreciate the fine general introduction to EO which Dr. Young provided. I will go into greater depth on several aspects.

Let me put into perspective how EO is regulated in the United States. The Occupational Safety and Health Administration (OSHA), a part of the U.S. Department of Labor, has a specific standard for EO exposure in the workplace, as well as standards for general workplace safety. The Environmental Protection Agency (EPA) regulates EO as a pesticide, requires the reporting of EO inventories and environmental EO emissions, and requires the reporting of any new health effects information on EO. The Department of Transportation (DOT) regulates bulk shipments of EO. The Food and Drug Administration (FDA), in the U.S. Department of Health and Human Services, regulates product sterilization in general and EO residues in products. Individual states can regulate worker, environmental, and product exposure to EO and environmental emissions of CFCs. Finally, on the research side, organizations such as the National Institute for Occupational Safety and Health (NIOSH), the National Cancer Institute (NCI), and the National Toxicology Program (NTP) can conduct research on EO which could result in recommendations for regulatory activity by OSHA, EPA, and FDA.

I will not address the issue of ethylene oxide residues—Drs. Rodricks and Zayeva will address this subject later in this volume.

Health Industry Manufacturers Association (HIMA)

For those of you who don't know HIMA, the Health Industry Manufacturers Association is a trade association representing U.S. manufacturers of medical devices, diagnostics, and hospital information systems products. HIMA has approximately 320 member companies representing about 90% of the volume of medical products manufactured in the United

States.

About 170 companies, approximately half of the HIMA membership, market sterile products. About 50 companies use EO within their facilities for product sterilization. The remainder of the companies sterilize in-house with another method (radiation, steam, dry heat) or use contract sterilization services (EO, Cobalt-60 radiation, accelerated electrons, steam). HIMA estimates that 50% to 60% of the volume of manufactured medical products are sterilized using EO—this translates into approximately 15-20 billion health care items annually. The remaining 40% to 50% of the volume of products is sterilized predominantly by Cobalt-60 irradiation, with a small portion sterilized by accelerated electrons, steam, dry heat, and other methods.

HIMA has been involved with issues concerning EO since the association was founded in late 1974. Why so much effort on one chemical? EO is a highly effective sterilizing agent. Currently it is the only available method for effectively sterilizing products composed of certain materials that are sensitive to heat, moisture, or radiation. EO has been an important factor in the decrease of hospital-acquired infections. Additionally, the development of life-saving medical procedures and products that rely upon an effective sterilization process employing EO has resulted in enormous benefits to the U.S. and World Healthcare Systems.

Because of its very nature as a sterilant, i.e., killing microorganisms, EO may have adverse health effects on higher organisms. Such effects have been shown in animals; there is inconsistency in the data regarding human health effects. Regardless, since 1975, HIMA has encouraged companies to use EO safely. HIMA has also held meetings and published extensively on the safe use and control of EO. A brief chronology of HIMA's activities on EO appears in Table I.

Table I. HIMA's Activities Regarding EO

1977	EO Update Meeting/Proceedings
1978	Comments to EPA and FDA
1980	Safe Use of EO Meeting/Proceedings
1981	Board Policy — Reduce Potential EO Exposure
1981	Monitoring EO Meeting/Proceedings
1982	EO Worker Safety Issues Meeting/Proceedings
1983	HIMA Supports 1 ppm Permissible Exposure Limit
1983-1984	EO Compliance Seminar
1985-1989	EO/Worker Right-To-Know Compliance Seminars
1988	Comments to EPA/OSHA
1988	Sterilization in the 1990s Meeting/Proceedings

Other organizations in the U.S. are concerned about EO. HIMA belongs to an organization called the Ethylene Oxide Industry Council (EOIC), which represents EO producers and users. The EOIC has been very active in responding to regulatory initiatives and supporting research on EO. The American Hospital Association has also been an active

voice in the continued use of EO. Various labor unions such as the AFL-CIO, and consumer groups such as the Public Citizen Health Research Group, as well as NIOSH, have long been involved in the EO issue. The unions and consumer groups have been proponents of stricter worker and environmental standards for EO.

The Occupational Safety and Health Administration (OSHA)

As I mentioned earlier, the U.S. Government has broad authority to regulate EO. OSHA was created in 1970 by the Occupational Safety and Health Act. OSHA has broad regulatory authority to ensure safety in the workplace environment. The agency can impose standards and guidelines for workplaces in general, as well as exposure standards for specific chemical substances.

In 1971, OSHA adopted the 1968 American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value (TLV) of 50 parts per million (ppm) EO as its permissible exposure limit (PEL). The ACGIH is a non-regulatory body of government representatives and academicians that develops recommended workplace exposure limits called TLVs. Its TLVs are often adopted by OSHA as regulatory standards, or what we call PELs.

In the late 1970s and early 1980s, the safety of the 50 ppm PEL was brought into question, based upon various animal studies, especially those suggesting EO as an animal carcinogen. In the early 1980s, the ACGIH lowered its TLV for EO to 10, then 5, and then 1 ppm. Likewise, many EO producers and users established internal worker standards well below the 50 ppm PEL.

In 1984, after a lengthy rulemaking proceeding, complete with legal challenges and appeals, OSHA promulgated a revised worker standard of 1 ppm as an eight-hour time-weighted average. The 1 ppm PEL was supported by HIMA and the EOIC. This is the PEL in force today. In addition to setting a 1 ppm PEL, the standard also contained requirements for exposure monitoring, identification of regulated areas where there is the potential for EO exposure above the PEL, medical surveillance, respiratory protection, signs and labels, communication of hazards, and emergency procedures. The standard also sets an action level of 0.5 ppm—half the 1 ppm PEL. If a company meets the 0.5 ppm action level, then the frequency with which a company must perform exposure monitoring and medical surveillance is reduced.

Let me point out four important aspects of the standard. The standard requires exposure monitoring that is representative of the employee's breathing zone. This is called personal monitoring. OSHA recommends a standard methodology which is a charcoal tube method, but a company can use any validated method as long as it meets the established accuracy requirements. Most companies are using charcoal tube methods such as the OSHA method, the Quazi-Ketcham method, and the ASTM method, which is actually a modification of the Quazi-Ketcham method that was developed by the Ethylene Oxide Industry Council. Many companies are also using passive dosimeters to perform personal monitoring. Some companies are also performing area monitoring to supplement personal monitoring and for leak detection. They use direct reading instruments connected to gas

chromatographs to detect area levels of EO—these instruments are becoming increasingly sensitive, measuring accurately and reproducibly to 0.1 ppm and below.

The standard also requires warning labels in regulated areas. Although OSHA acknowledged that EO was a *suspected* human carcinogen, the standard requires warning labels for regulated areas to read, “Cancer Hazard and Reproductive Hazard”.

The standard requires that potential exposure to EO be eliminated or reduced through engineering controls or workplace practices. OSHA has a hierarchy for chemical exposure control—first, a company should eliminate or reduce potential exposure through engineering controls or through changing workplace practices. Where these controls are not feasible or workable, then respirators are used to supplement control. OSHA prefers positive pressure, air-line supplied respirators. In the EO standard, OSHA acknowledged that there are certain exposure situations in the medical products industry where engineering controls and work practices are not feasible—these include retrieving biological indicators and quality samples, changing tanks, and maintenance. In these situations, an employer does not need to prove that engineering controls and work practices were not feasible—OSHA acknowledges this and allows the supplemental use of respirators. You will note, however, that “unloading sterilizers”, a task which is recognized as one in which there is the potential for EO exposure, is not included in OSHA’s list. Therefore, for workers that unload sterilizers, employers must first show that engineering and workplace controls were not feasible before using respirators to supplement control. Or, in other words, in the situation of unloading sterilizers, unlike the other tasks mentioned above, the burden of proof is on the employer to show that respirators were needed to supplement engineering controls and workplace practices.

Finally, the standard contains a section on downstream labeling. This states that, if the product has the potential to expose customers downstream to EO levels greater than the PEL, the product must be appropriately labeled. Therefore, OSHA requires a manufacturer to have objective data showing that its products do not have the potential for releasing levels of EO above the PEL at the distribution and customer levels. Consequently, employers are monitoring workers around products in transit, at distribution sites, and in hospitals to determine if, for example, a box of EO sterilized product is capable of releasing EO above the PEL.

What effect has the 1984 revision of the PEL to 1 ppm had on HIMA members? A 1987 survey of the HIMA membership revealed that:

— The number of companies conducting EO sterilization in-house dropped by one-third (from 132 to 95 facilities) and that the number of sterilization chambers also dropped by one-third (from 351 to 242). Several manufacturers, realizing that they could not meet the new exposure standard without building a new facility, ceased operations, consolidated sterilization operations, or sent product to contract sterilizers to be processed.

— Industry spent $45.9 million dollars (net present value) to meet the ppm PEL, which translates into $78,000 per facility.

— The population potentially at risk decreased dramatically. HIMA estimated that, in 1987, the total possibly exposed workforce numbered 1,814, down 85% from the

11,832 possibly exposed workers in 1983.

— HIMA also estimated that 98% of workers possibly exposed to EO were in compliance with the 1 ppm PEL. Additionally, HIMA also found that 97% of the workforce was also below 5 ppm on a short-term basis.

Let me speak a little more about the short-term exposure issue. In 1984, OSHA decided not to establish a Short-Term Exposure Limit (STEL) for EO since the scientific evidence did not support it. A STEL is a limit not to be exceeded over a 15-minute period. OSHA was sued, and after lengthy litigation, promulgated an Excursion Limit (EL) of 5 ppm not to be exceeded over a 15-minute period. An excursion limit is different from a STEL in that the former is based on good industrial hygiene practice and the latter is based upon scientific evidence. OSHA promulgated the 5 ppm EL since it had data from its consultants that the limit was both technologically and economically feasible. HIMA opposed the EL, stating that industry's extensive database revealed that the existing 1 ppm PEL was sufficient to protect workers from high-level short-term exposures. More importantly, in its comments, HIMA requested that the task, "unloading sterilizers", be added to the list of tasks for which supplemental respirator use is allowed. OSHA declined HIMA's request. Therefore, as with the 1 ppm PEL, a company must first show that engineering controls and workplace practices are infeasible to meet the 5 ppm EL before using respirators to supplement control for workers unloading sterilizers.

OSHA's 5 ppm Excursion Limit was issued in April 1988, and employers had to be in compliance with all EL requirements by December 1988. One of the concerns in the EL issue has been whether it can be adequately monitored. OSHA states in the final EL rule that its OSHA charcoal tube method is an accurate and reproducible method for EL monitoring and that any validated method can be used as long as it meets OSHA's accuracy requirements. There is not much information in the open literature about the adequacy of charcoal tube or passive dosimeters for short-term or EL monitoring. The EOIC has sponsored two studies with an independent consultant to take a look at the feasibility of EL monitoring by various methods under laboratory and field conditions. The studies showed that the methods were feasible under laboratory conditions, but that the results were inconclusive in the field — one of the problems is that the sampling period is so short. I am aware that individual HIMA companies and monitoring equipment manufacturers are performing any number of studies, especially modifications of existing charcoal tube methods, to show the feasibility of existing exposure monitoring methods for short-term monitoring. We should expect to see some articles in the literature on this subject shortly.

In conclusion, OSHA is routinely conducting workplace inspections at medical product facilities and enforcing the 1 ppm PEL, the 5 ppm EL, and the attendant requirements.

OSHA is also working on other activities that will affect EO users. For some time, OSHA has had in place its hazard communication or "Worker Right-to-Know" standard, which requires employers to notify workers of the hazards of the chemicals with which they work. For several years, the agency has also been working on revisions to its respiratory protection standards, especially how respirators are fit-tested. Any changes to these standards will be incorporated into the respiratory protection provisions of the EO standard.

U.S. Environmental Protection Agency (EPA)

The U.S. Environmental Protection Agency (EPA) is also concerned about environmental emissions of EO. For several years, under authority of the Clean Air Act, the EPA has been considering whether to list EO as a hazardous air pollutant and control its emissions from facilities. The EPA has determined that it is feasible to control EO emissions from sterilizers through EO scrubbers, incinerators, and conversion and reclamation systems. The EPA is currently studying whether emissions from aeration rooms, that is, the exhaust from off-gassing product in warehouses, can be controlled by EO catalytic conversion systems. The EPA expects to propose a federal emissions standard for EO by late 1990, with a final rule in place by early 1992. This rule would likely require the control of sterilizer emissions; it could also require control of emissions from warehouses in which sterile product is off-gassing.

It is likely that other events in the U.S. will accelerate the federal regulation of EO emissions. In its next session, the U.S. Congress will consider legislation to significantly change the Clean Air Act, expediting the process by which potential hazardous pollutants, such as EO, are regulated. Additionally, now that U.S. companies must report their inventories and emissions of hazardous chemicals annually, there has been much community and public scrutiny of the amount of EO being released from facilities into the atmosphere.

EO and Chlorofluorocarbons (CFCs)

The same scrutiny being paid to EO is also being paid to CFCs. CFCs have been implicated in depletion of the earth's protective ozone layer, which results in an increase in ultraviolet radiation, a distinct threat to the earth's ecology and human health. There has been an international effort, called the Montreal Protocol, to reduce CFC production and consumption and to seek alternative chemicals and substitutes worldwide.

Although the U.S. EPA has imposed production limits which will reduce the amount of CFCs produced and consumed domestically over the next ten years, Congress is considering further measures that would, in the short term, impose a tax on each pound of CFC produced, and, in the long term, eliminate all CFCs by the year 2000. Several U.S. manufacturers, including some medical product manufacturers, have voluntarily moved to eliminate the use of all CFCs or to adopt substitute chemicals.

Although the Medical Products Industry uses only 4% to 6% of the CFCs produced annually, it is still a major consumer of these chemicals. The EPA is looking for ways the Medical Products Industry can reduce CFC use, find substitutes, or control emissions. CFCs are used to dilute pure EO, mitigating its explosive potential. CFCs are also used to clean particulate matter off medical products. There are alternatives to the EO/CFC mixture in sterilization, namely 100% EO and EO/Carbon Dioxide mixtures. However, these have limitations. Pure EO is a fire and explosion hazard. The EO/Carbon Dioxide mixture requires higher pressures and can polymerize. Alternative sterilization methodologies, such as Cobalt-60 or accelerated electrons can be used, but these methods are not always compatible with the product or component materials.

Several U.S. chemical manufacturers are working on substitute CFCs to blend with EO for sterilization. However, these companies have stated that due to the extensive toxicity testing required, these substitutes may not be available until at least 1992. In the meantime, medical manufacturers that continue to use CFCs in sterilization or solvent cleaning will find them more costly and difficult to obtain.

Various states within the United States have also taken action with respect to controlling EO workplace and environmental exposure, as well as use of CFCs. In the absence of Federal Regulation, states and local communities often develop their own regulations. The state of California, for example, has EO and worker right-to-know standards which may include requirements beyond those of the Federal Government. California also plans to impose an EO emissions standard in the absence of a federal emissions standard. Additionally, California has also moved forward with its Safe Drinking Water and Toxic Enforcement Act of 1986, known as California Proposition 65, which states that a company has a duty to provide warnings if it has the potential to expose anyone to carcinogens or reproductive hazards in the workplace, through the environment, or through product exposure. EO is listed in California as both a carcinogen and a reproductive hazard, and therefore, the state requires warnings in the workplace for EO exposure beyond that required in the Federal OSHA Standard. Several other states are considering California Proposition 65-type measures, so companies are facing a variety of federal and state requirements, often conflicting, for workplace and environmental exposure to chemicals.

Several states and local communities have also moved to ban the use of CFCs. The city of Irvine, California has enacted an ordinance banning the use of CFCs in the community, effective July 1990. CFCs used in the manufacture of drugs and medical products are exempted at this time.

As I mentioned earlier, both Federal OSHA and the state of California consider EO a human carcinogen. However, even after all of these years and countless studies on EO, there is still disagreement and controversy in the scientific and regulatory communities about the human carcinogenic potential of EO.

Epidemiological Studies of Chronic EO Exposure and an Increase in Cancer

Epidemiologic studies conducted by Hogstedt et al. (3-6), Morgan et al. (8) and Stolley (9) suggest an association between chronic exposure to EO and increased risk of cancer. Increased incidences of stomach cancer, leukemia (3-6), pancreatic cancer, Hodgkins Disease (8), and breast cancer (9) have been associated with occupational exposure to EO.

I must emphasize, however, that in all of these epidemiologic investigations, knowledge of exposure to EO is limited, exposure to other industrial chemicals is often documented, and the number of observed tumors in the study population is small. Additionally, the types of tumors found at increased incidence were not consistent from study to study, indicating that each study lacked sufficient statistical power to detect a small increase in cancer incidence.

Four additional human epidemiological studies have been issued recently. The Union

Carbide Corporation and NIOSH jointly conducted a long-term epidemiology study of chemical production workers at its Kanawha Valley, West Virginia facility for the years 1940-78. In its 1987 notification to the government, Union Carbide stated that, as a whole, death rates for the ethylene oxide cohort were consistent with rates for the U.S. male population. There was, however, a slightly increased incidence of leukemia and pancreatic cancer in workers assigned to the ethylene chlorohydrin unit, at which potential for exposure to EO was judged to be low.

Last year, abstracts were issued from three European epidemiology studies: Gardner et al. from the United Kingdom (2) on chemical production and hospital sterilizing workers; Kiesselbach et al. from West Germany (7) on chemical plant workers; and Bisanti et al. from Italy (1) on licensed EO handlers. Based upon these abstracts, the Gardner and Kiesselbach studies reported no total cancer excess, with non-statistically significant observed and expected rates for leukemia and stomach cancers. Bisanti, however, reported higher-than-expected rates for digestive, respiratory, and urinary cancers. These studies are expected to be published jointly in the near future — full papers will likely give greater information upon which to make conclusions, especially in the case of the Bisanti study.

In the United States, both NIOSH and HIMA are conducting human epidemiologic studies on workers in the medical products sterilization industry. NIOSH is a part of the Centers for Disease Control in the U.S. Public Health Service which is part of the Department of Health and Human Services. It is a research body, not a regulatory body. NIOSH can make recommendations on workplace exposure limits to OSHA, who may then choose to adopt the recommendations as legally enforceable standards.

NIOSH has long been interested in EO. It has sponsored animal studies and, as mentioned earlier, is now conducting a human epidemiologic study. In 1981, NIOSH issued a current intelligence bulletin in which it recommended that exposure to EO be reduced as low as possible. Since 1983, NIOSH has recommended that the eight-hour exposure limit for EO be 0.1 ppm, one-tenth of the existing PEL, with a 5 ppm ceiling which cannot be exceeded for any period of time.

Back in 1981, NIOSH proposed to conduct a study of industrial EO workers to determine if there was an increased incidence of leukemia or other cancers due to EO exposure. NIOSH chose the medical sterilization industry in which to conduct an EO exposure study since the population was relatively free of exposures to other industrial chemicals. NIOSH performed surveys of over 40 industry facilities to determine if the study was feasible. In 1984, NIOSH determined that there were sufficient person-years of exposure and statistical power to conduct the study at ten companies representing 14 facilities. The study covers workers for 30 years — from the early 1950s to the early 1980s —and includes a cohort of over 20,000 exposed individuals, representing over 250,000 person-years of exposure. Study results are expected late in 1990. This is an extremely important study, since it is the largest and most comprehensive human epidemiologic study on industrial EO exposure to date.

Since nine of the ten companies in the study are HIMA members, the Association has had a consulting epidemiologist auditing NIOSH's conduct of the study as well as performing

a limited parallel analysis of the same database that NIOSH is using. The results of HIMA's parallel study are expected in early 1990.

If the HIMA and NIOSH studies are negative, that is, they find no correlation between EO exposure and cancer, the focus of EO research may shift to other potential health effects. If the study is positive (i.e., a correlation between EO exposure and cancer), then industry's continued use of EO could be jeopardized. NIOSH could recommend to OSHA that the permissible workplace exposure limit for EO be reduced further, the media may raise concerns over EO use and control in the workplace and the availability of alternate sterilants, and the specter of litigation may increase.

HIMA's "Sterilization in the 1990s" Conference

HIMA is preparing its members to deal with the results of the NIOSH and HIMA studies. One significant program we have already held was a "Sterilization in the 1990s" conference in Washington, D.C. The conference was attended by over 400 members of industry, the government, and academia. It was an open forum on the future of sterilization, especially the feasibility of new sterilization methodologies. It had sessions on:

— Sterilization Methodologies and Materials
— The Regulatory Environment
— Biohazards Control and Decontamination
— Sterilization Program Strategic Planning
— Workshops on EO/CFC Emissions Control, Contract Sterilization, Sterility of *In Vitro* Diagnostic Products and the Hazard Assessment of EO Residues.

I would now like to share with you some conclusions drawn from that meeting. Dr. Briggs Phillips, a noted authority on medical sterilization, stated in his keynote address that "EO is absolutely essential to our overall goal of infection prevention", but added that "we will have to change our way of using it". Dr. Phillips went on to describe an ideal automated process, in which product is sterilized with no worker exposure, EO emissions are trapped before they reach the environment, and product is off-gassed completely. Many companies in the U.S. are working on such automated systems; however, these will take extensive resources in terms of time and money to implement.

Dr. Zory Glaser of the U.S. Food and Drug Administration echoed Dr. Phillips comments by stating that "EO is an essential ingredient for sterilization".

A host of speakers described the future of sterilization and predicted that over the next ten years:

— Cobalt-60 irradiation sterilization will continue to increase in popularity as the supply of the isotope remains plentiful and prices stabilize;
— The energy levels and penetrating ability of accelerated electrons will increase dramatically; and
— New sterilization applications will be found for chlorine dioxide, vapor phase hydrogen peroxide, ozone, and gas plasma ionization.

Industry commentators concluded at the end of the meeting that EO will likely remain the predominant sterilant for the next five to ten years, that no new "optimal method" for sterilization will be developed, and that several of the new sterilization methodologies will find "product or niche-specific" applications.

HIMA has prepared a comprehensive meetings proceedings, available from our publications department.

Conclusions

In closing, let me summarize:

— EO is an essential sterilant — many life-saving products could not be sterilized without it. Due to its essential nature, we expect it will continue in use for the indefinite future. Industry is studying alternative sterilization methodologies and component materials/packaging and using these where appropriate. However, several of the alternate technologies may only find product-specific applications.

— In the United States, EO is highly regulated. OSHA enforces a 1 ppm eight-hour time-weighted average permissible exposure limit and a 5 ppm 15-minute excursion limit, with attendant requirements for monitoring, medical surveillance, respiratory protection, signs and labels, communication, and emergency procedures.

— Even though there is controversy in the scientific and regulatory communities regarding EO's potential for adverse health effects in humans, the U.S. medical products industry has moved to significantly reduce and/or eliminate EO exposure. Industry is investigating automation of the EO sterilization process to reduce/eliminate the potential for worker, environmental, and product exposure.

— 1990/1991 will be important years for EO since both NIOSH and HIMA plan to release human epidemiologic studies on EO exposure. The results of these studies could have significant impacts on the further regulation of EO.

— 1990/1991 will also be important since EPA will likely propose to control EO emissions from sterilization operations, as well as to further limit CFC production and use.

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Ethylene Oxide Residuals: Toxicity, Risk Assessment and Standards

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Introduction

Risk assessment has become a central component of regulatory decision-making in the United States. Decisions about the need for establishing limits on human exposure to potentially toxic chemicals, and the magnitude of those limits, are typically based on a three-step process. The first step involves development of basic scientific knowledge and data concerning the health effects of chemical agents and the extent of human exposure to them. In the second step, all relevant knowledge and data are evaluated to arrive at an estimate of the size of the health risk posed under specific conditions of exposure. *Risk assessment* is the term used to describe this second step. Under the third step—*risk management*—decisions are reached about the need for, and extent of, risk reduction necessary for public heath protection. Risk management is distinct from risk assessment and is in the hands of policy-makers who must take into account the requirements of applicable laws. Depending upon those requirements, risk managers may consider only the size of the risk (such as applies, for example, to food additives), may consider both risks and benefits (drugs, medical devices, pesticides), or may take into account risks and the technology available to reduce them (drinking water or food contaminants, air pollutants)[1]. Risk reduction comes down to setting limits on human exposure to ensure the risks associated with those exposures do not exceed specified, very low levels.

Although this three-step process is not always perfectly executed, it has come into use in the regulation of foods, cosmetics, pesticides, consumer products, industrial chemicals, air and water pollutants, hazardous wastes, and occupational exposures. There is as yet no record of its use in regulation of chemical components of medical devices, but the experience to be described regarding human exposure to ethylene oxide (EO) residues on medical devices suggests the U.S. Food and Drug Administration encourages its adoption for this class of regulated materials.

Science Policy Choices in Risk Assessment

The three-step decision-making scheme, and particularly the risk assessment component, became firmly embedded in the chemical regulation process of the United States following the 1983 publication of a study by an expert committee of the National Research Council (NRC). The NRC committee examined the history of the use of risk assessment, endorsed its continued use, and urged common adoption of certain basic definitions and procedures by all regulatory agencies. The committee also devoted several

of its recommendations to issues of *science policy*. What the committee meant by this phrase, in the context of the risk assessment process, is essential to an understanding of the risk information to be presented below in connection with EO residues on medical devices.

The basic problem in risk assessment is the need to describe risks that are too small to be measured directly with the tools of epidemiology or even with studies in experimental animals. Although some would argue that a risk need not be a public health concern if it is not sufficiently large to be directly measurable, there is a long history of regulatory policy that has sought to limit risks to very low, and currently immeasurable, levels to ensure that they never rise to detectable levels in human populations.

Because of the concern about low levels of risk, particularly those affecting very large populations, risk assessors are forced to engage in various forms of extrapolation beyond available data. The typical case is represented by the situation in which data on adverse health effects are available from studies in small groups of experimental animals at high levels of exposure, and the concern is over possible risks to very large numbers of people exposed to the same agent at much lower levels. At least two forms of extrapolation are needed to answer this risk question:

— extrapolation from experimental animals to humans; and
— extrapolation from high-to-low exposures (doses).

There are some scientific bases for these two forms of extrapolation, but they are by no means established with the degree of empirical verification usually sought by scientists, either in general or for specific chemicals. Indeed, several alternative approaches are available for each form of extrapolation, and their application can yield substantially different risks for the same exposure situation.

Because of the need to provide answers about the possible magnitude of risks that cannot now be directly measured, but to ensure that such risk information is not accorded the same scientific status as risks that can be empirically verified, the NRC committee urged the regulatory agencies to make clear that their choices of extrapolation models are based on certain policy considerations (buttressed, of course, with as much support as current science can offer), and that the uncertainties associated with their application be explicitly recognized and taken into consideration in the risk management process. (Although the objective of taking uncertainties into account in the risk management process is widely accepted, I believe this is rarely done in a systematic way. Risk assessors are partly at fault, because they often do not present uncertainties in a readily useable form. Risk managers, like most of us, are not comfortable with uncertainties and would rather deal with the simplest possible expression of risk. We have much to do to improve the current situation).

The principal EO health concern is carcinogenicity. This effect has been documented in animal studies. Epidemiological studies in highly exposed workers provide suggestive evidence of an association between EO exposures and certain human cancers, but do not establish a causal relationship.

Partly for scientific reasons and partly for policy reasons—i.e., the need for prudence in

public health matters when science is uncertain—a science-policy choice is made to treat EO as a probable human carcinogen. Characterization of EO risks to the general population exposed to residues on medical devices should include recognition of the nature of this and other such choices in the risk assessment. As will be seen, assessment of human exposures to EO residues on medical devices also requires the imposition of assumptions where data are lacking, and science-policy choices are needed at several steps in the assessment process. As long as such science-policy choices and their attendant uncertainties are explicitly recognized, risk managers can make informed judgments about whether action is needed and the form of that action, or whether additional data should be gathered to reduce uncertainties before action is taken.

As a final and critical introductory point, the nature of the science-policy choices that are typically made by regulatory agencies in the United States should be discussed. In general, these choices are designed to avoid understating human risk. If a range of scientifically plausible, alternative data sets, extrapolation models, or assumptions is available to complete a step of the assessment, and there is not clear scientific preference among them, regulatory policy generally adopts those that yield the *highest* estimate of risk (such choices are sometimes labelled conservative ones). Because many such choices are needed to complete a risk assessment, the cumulative effect is almost certainly an upper limit on the human risk. The actual risk is unlikely to be higher than the upper limit, is most likely lower than it, and could in many cases be zero. All discussions of risks estimated using regulatory methodology should acknowledge their conservative nature.

Although alternative risk assessment methodologies are available for EO residues on medical devices, the one applied in this paper conforms to that which the U.S. FDA has found acceptable for their regulated agents. A critical assumption is that EO cancer risks exist at all exposures (no-threshold model) and that they increase in direct proportion to received dose (linear model). Other models will generally show less risk than that presented here.

Components of Risk Assessment

The necessary components of every complete risk assessment are set forth in Figure 1. These four components and their relations were first described by the NRC committee, and now have been almost universally adopted by risk assessors. It is useful here to discuss the last component — risk characterization.

At present, risk characterization—the final output of a risk assessment—is expressed in one of two forms. For carcinogens, the upper limit on the lifetime probability of cancer resulting from specified levels and conditions of exposure is typically obtained as follows:

Carcinogenic Risk = (Risk per Unit Dose) × (Lifetime Average Daily Dose, LADD)

The Risk per Unit Dose (also called Potency) is obtained from the Hazard and Dose-Response Evaluations, and the LADD from the Human Exposure Assessment (see Figure 1). For non-carcinogenic forms of toxicity, the Margin of Safety (MOS) separates the estimated Average or Maximum Daily Human Dose (ADD or MDD) of the agent from the

estimated No-Observed-Effect Level (NOEL) for toxicity. The NOEL is obtained from the Hazard and Dose Response Evaluation, and is the highest dose at which no effect is found. The ADD or MDD is derived from the Human Exposure Assessment. Thus, Note that the only true risk estimate is that associated with carcinogens. The MOS is the rough indicator of the size of a possible risk, and risk management decisions concerning the adequacy of a given MOS for public health protection are based on policy and scientific precedents that have been long established.

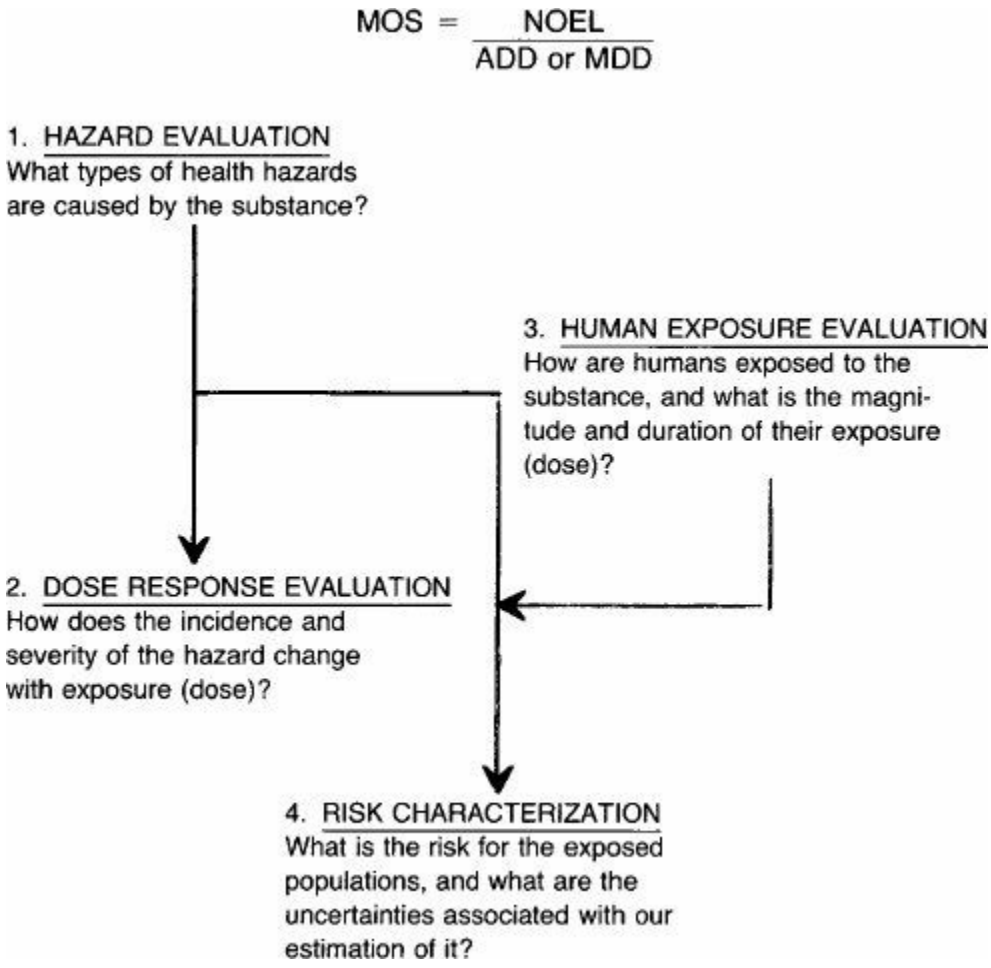

Figure 1. Components of Risk Assessment **(NRC, 1983)**

Quantitative and qualitative descriptions of the strengths and weaknesses of all components of the data base, and the many other uncertainties that typify most risk assessments, are essential ingredients of the risk characterization step. Unfortunately, risk assessors have not yet mastered the discipline of thorough risk characterization, and they are perhaps discouraged from doing so because risk managers would prefer to deal with simple numerical estimates. Perhaps the greatest deficiency in the current practice of risk assessment and management is that the risk assessment uncertainties are not well described by assessors and are virtually ignored by managers. Some needed improvements in this area are now being worked upon, but discussion of them is beyond the scope of this paper.

Significant Risk Decisions

As stated earlier, decisions about risk acceptance in different circumstances are not part of the risk assessment. Although the size of the risk is not the only consideration in risk management decisions, certain patterns have emerged in the past 10-15 years. However, there has been little attempt to develop rigorous, analytic support for these decisions (13).

Except where statutes forbid acceptance of any cancer risk (notably the Delaney Clause of the U.S. Food, Drug, and Cosmetic Act), regulatory agencies have generally found lifetime cancer risks < 10^{-6} (one in one million) as negligible and unworthy of regulation. Lifetime cancer risks in the range of < 10^{-4} down to 10^{-6} have been accepted in many circumstances, usually because of technical constraints on risk reduction, or because offsetting benefits were judged to be exceedingly high. The typical MOS for non-carcinogenic forms of *chronic* toxicity is usually required to be at least 100, although smaller margins have frequently been accepted for occupational exposures, or where overriding health benefits are a factor. A MOS > 100 has been required in instances in which there are deficiencies in the data or where hazards are especially severe.

Precedents for significant risk decisions specific to medical device components are not available.

Summary of Hazard and Dose-Response Data on Ethylene Oxide

The acute toxicity of EO in humans has been described in a number of case reports and, to a more limited extent, in case-control studies. Case reports indicate that headache, nausea, vomiting, dyspnea, and respiratory irritation can result from inhalation of EO vapors. Dermal contact with EO has caused skin burns and possibly sensitization. Hypersensitivity reactions have been observed in patients using sterilized plastic tubing for hemodialysis or cardiac catheterization and in health platelet donors. The exposures at which the above effects occurred are not well known but are undoubtedly very much higher than those associated with residues on medical devices (4).

The effects of repeated, low-level exposure to EO in humans are not well documented. Case reports indicate that such exposures may be associated with neurological effects (headaches, lethargy, numbness, sensorimotor neuropathy, seizures), ocular effects (primarily cataracts), and hematological effects (21).

In animal studies, repeated exposure to EO resulted in neurotoxicity, pathologic changes in the lungs, kidney, liver, and testes, and hematological effects (21). Subtle neurologic effects have been observed in monkeys and mice at air concentrations as low as 50 ppm (16, 18) and decreased body weight gain has been reported in rats at 33 ppm EO in air (17). A dose of 2 mg/kg/day is the no-observed-effect level (NOEL) for non-neoplastic effects of chronic EO exposure in air, based on an inhalation exposure of rats to 10 ppm EO for six hours per day, five days per week (17).

EO has been shown to produce adverse reproductive and developmental effects in experimental animals. Exposure of female animals has resulted in maternal toxicity, depression of fetal weight gain, fetal death, and fetal malformations (21). EO (100 ppm in air) administered by inhalation to rats in a one-generation study caused longer gestational periods, decreased litter size, and decreased number of implantation sites (15). No adverse

effects were observed at 33 ppm. In male animals, repeated EO exposure at several hundred ppm has resulted in testicular degeneration and adverse effects on sperm (21). In an epidemiologic study of Swedish sterilizing staff nurses, Hemminki et al. (6) reported an association between EO exposure and spontaneous abortions, at levels speculated to be as low as 0.1 ppm. Based on experimental data showing adverse effects on reproduction and development in EO-exposed rats (15), a dose of 9 mg/kg/day has been used as a NOEL for the reproductive effects of EO. This dose corresponds to an exposure to 33 ppm EO in air for six hours per day, seven days per week.

EO is a direct-acting mutagen. The chemical has been shown to induce gene mutations in bacteria, fungi, higher plants, *Drosophila*, and cultured mammalian cells without the use of a metabolic activation system (21).

EO has also been shown to induce dominant lethal effects in rodents; chromosomal aberrations in higher plants, *Drosophila*, and rodents; micronuclei in rodents; and sister chromatic exchange (SCE) in rodents and humans (21).

The demonstration of mutagenic potential of EO strengthens the weight of the evidence for its carcinogenicity. Current scientific understanding of the relationship between mutagenicity and carcinogenicity or the health significance of germ cell mutations is insufficient, however, to establish quantitatively the significance of EO's mutagenic activity to human health risk. It is not possible, therefore, to conduct a quantitative risk assessment on the available mutagenicity data for EO.

The carcinogenicity of EO has been assessed in both animals and humans. Epidemiologic studies suggest an association between chronic occupational exposure to EO and increased risk of cancer. Investigators have reported increased relative risks of stomach cancer, leukemia, pancreatic cancer, Hodgkin's disease, and breast cancer among EO-exposed workers. Because of shortcomings in all of the epidemiologic investigations, including difficulty in quantifying exposures, these data provide only limited evidence of human carcinogenicity (4, 20).

The evidence of the carcinogenicity of EO from animal studies is more definitive. Three long-term inhalation studies performed at exposure levels ranging from 10 to 100 ppm show statistically significant increases in the incidence of tumors (including mononuclear-cell leukemia, peritoneal mesothelioma, and tumors of the brain, lung, Harderian gland, uterus, mammary gland, and hematopoietic system). IARC (8) has concluded that there is sufficient evidence for the carcinogenicity of EO in experimental animals.

Estimation of the Carcinogenic Potency of Ethylene Oxide

An estimate of the carcinogenic potency of EO was derived based on methods consistent with those used by FDA for other substances. The mathematical model for low-dose extrapolation of cancer risk used by FDA is the Gaylor-Kodell linear proportional method. The Gaylor-Kodell method assumes linearity of the dose-response relationship at all dose levels below the lowest dose tested. In the Gaylor-Kodell method, the 95% upper confidence limit (UCL) on the maximum likelihood estimate (MLE) of risk at the lowest test dose is extrapolated linearly to the background risk level (i.e., the risk level at zero dose).

The Gaylor-Kodell method as applied by FDA does not directly provide a unit cancer risk (UCR, or measure or carcinogenic potency), but rather is intended to provide an upper-bound estimate of lifetime cancer risk at the predicted level of human exposure. A UCR can be generated, however, using the mathematical relationship that applies to other linear extrapolation models:

Upper-bound on Lifetime Risk =
(Lifetime Average Daily Dose) X (UCR)

All available experimental data from cancer bioassays on EO were evaluated in order to select the most appropriate data set for risk extrapolation. Development of a quantitative estimate of cancer risk was based on the incidence of mononuclear cell leukemias and brain gliomas (combined) in the Fischer 344 female rat as reported in the Bushy Run Research Center study (17). This data set provided the highest estimate of lifetime risk, and is the data selected by the U.S. EPA (21) in their risk assessment for EO.

Data from the pharmacokinetic study conducted by the Bushy Run Research Center (19) were used to determine the systemic doses resulting from inhalation of EO vapor in the cancer bioassay. Tyler and McKelvey (19) found the total systemic dose resulting from exposure of male Fischer 344 rats to air concentrations of 10 and 100 ppm for six hours were 2.7 and 20.2 mg/kg, respectively. The mid-dose level (33 ppm) was estimated to correspond to a systemic dose of 7.2 mg/kg by linear interpolation.

Applying the Gaylor-Kodell model to data on the incidence of mononuclear-cell leukemias and brain gliomas (combined) in female rats yields a lifetime risk estimate for EO that corresponds to a UCR of 4.8×10^{-2} $(mg/kg/day)^{-1}$. Note that the units for the UCR are risk (unitless) per unit of dose in mg/kg/day.

We emphasize that UCR estimates based on experimental animal data are only approximate indications of risk in exposed populations. Quantitative risk assessments typically incorporate a series of conservative assumptions. In the current risk assessment for EO, the unit cancer risk is based on a 95% upper confidence limit on risk using the data set providing the highest estimate of cancer potency. The resulting estimate of cancer potency is therefore considered to be an upper bound. A summary of toxicity values for EO is presented in Table I.

Table I. Summary of Toxicity Values for EO

Cancer Potency	Chronic Toxicity	Reproductive Toxicity
UCR = 4.8×10^{-2} per mg/kg/d	NOEL = 2 mg/kg/day	NOEL = 9 mg/kg/day

Human Exposure Assessment

Ethylene oxide is the sterilant of choice for many medical devices, some of which cannot be effectively sterilized with radiation, steam, or any other alternative. Many types of disposable and reusable medical devices, ranging from the common adhesive bandage to pacemaker systems and dialyzers, are sterilized with EO by the manufacturer or custom

sterilization companies (Table II).

Table II. Examples of Medical Devices that may be Sterilized with Ethylene Oxide

Bags, blood administration	Pacemakers and accessories
Bags, IV administration	Prostheses, breast
Bandages, adhesive	Reservoirs, cardiotomy
Catheters, intravascular	Sponges, gauze and cotton
Catheters, urological	Syringes, hypodermic
Dialyzers and accessories	Syringes, insulin
Filters, blood	Tips, surgical suction
Gloves, surgical	Tubes, infant feeding
Lenses, intraocular	Tubing, blood or IV
Needles, hypodermic	Valves, heart, prosthetic

After sterilization, some of the EO may remain absorbed on the surface of the device or dissolved into its materials of construction. These residues dissipate with time at a rate depending on the materials of construction, the degree of ventilation of the product during storage, the type of product packaging, and other factors. During sterilization, some of the EO may react with water vapor to form ethylene glycol (EG) or with chlorinated compounds to form ethylene chlorohydrin (ECH), either of which may also remain on the device. Manufacturers hold their products for a period prior to shipping to allow dissipation of EO and, to some extent, the other two substances.

This section deals with the potential exposure that might be incurred by patients or other end-users of devices, not by medical personnel or employees of manufacturers or sterilizing companies. Furthermore, it covers only the potential exposures associated with medical devices delivered sterile to hospitals, physicians, or the patient, not the risks of devices that are sterilized or resterilized in the hospital or clinic.

Methods for estimating exposure to EO residues on sterilized medical devices have not been well developed. We know of only one other attempt to do so (12). Novel methods were needed to estimate patient exposure to residues of EO on medical devices.

Two distinct ways of estimating exposures were employed. One, the “composite individual” method, is designed to characterize exposure to the entire U.S. population for the purpose of assessing the overall carcinogenic risks of EO. The other, the “specific procedures” method, is designed to estimate the risk of cancer or other toxic effects from exposure to medical devices in the course of specific medical procedures.

Composite Individual

A “composite individual” is a hypothetical person who uses exactly his or her share of all the medical devices sold in the United States every year. For example, if 6 billion adhesive bandages are sold annually in the U.S. and the U.S. population is 240 million, then the composite individual uses 6,000/240 or 25 bandages per year. Over a 73-year lifetime, about 1,800 bandages would be used. The corresponding numbers for infrequently used devices such as intraocular lenses are considerably lower; even when we multiply the annual use rate by the expected lifetime of 73 years, the composite individual may still “use”

considerably less than one device over a whole lifetime. The composite individual thus represents a compromise or average between people who use one or more devices of a specific type over a lifetime and those who use none at all. Table III shows a random sample of device use by the composite individual from a set of about 75 devices for which we have estimated exposures (4).

Table III. Examples of Use of Medical Devices by a Hypothetical Composite Individual

Device Type	Lifetime No. of Uses
Bandages, adhesive	1,800
Clamps, umbilical cord	1
Drapes, surgical	45
Gloves, surgical	19
Pacemakers and accessories	0.003
Sponges, surgical	142
Tubes, endotracheal	9
Tubing, blood or IV	15

Note: In this and succeeding tables, the numerical values presented may appear precise, as they often are taken directly from computer printouts. The accuracy of the numbers usually would support no more than one significant figure, however.

Device Use

To derive these estimates, we first estimated the number of devices used in total across the United States in the course of a year. No comprehensive source of data on the use of medical devices in the United States came to our attention. We therefore estimated annual usage through a combination of complementary approaches.

For a very limited number of devices, the Census of Manufacturers (3) tabulates data on number of units sold, total sales in dollars, or both. Some information on unit or dollar sales can also be found in the files of trade publications that serve the biomedical product industry. If prices of devices can be obtained from catalogs or industry sources, they can be divided into total dollar sales volume to estimate unit sales. Although not every unit sold is necessarily used on a patient, sales volume is a satisfactory surrogate for use volume for many types of devices. The principal exceptions involve items that have widespread use for other than medical services, such as the use of hypodermic needles in biological and chemical research, the use of surgical-quality gloves in laboratories, or the use of absorbent towels in hospitals for general purposes instead of patient contact.

Device use can also be estimated (independently, in part) from statistics on disease rates and medical procedures. The National Center for Health Statistics (NCHS) (11) publishes data on the number of surgical, diagnostic, and therapeutic procedures undertaken in short-term care hospitals (see Table IV for some examples). To obtain an estimate of the number of devices of a given type used per year, we can add the numbers estimated to be used in various procedures. For each procedure, that number is the product of the annual number of procedures performed and the number of devices of the given type used in each procedure. Estimates of device use per procedure are sometimes obvious (one pacemaker system per pacemaker implant operation) but are best provided

by medical professionals. We asked active hospital nurses and nurse supervisors to estimate the average number of devices used in approximately 70 different procedures.

Table IV. Example Procedures Performed in Short-Term Care Hospitals

	Annual Number of Procedures (thousands)	Lifetime Procedures per Person	Dose (MMD) per Procedure (mg/kg)
Appendectomy	294	0.09	0.11
Arthroscopy of knee	237	0.07	0.06
Bypass anastomosis for heart revascularization	202	0.06	0.53
Cesarean section and removal of fetus	814	0.24	0.14
Dilation and curettage of uterus	744	0.22	0.07
Excision or destruction of intervertebral disc	203	0.06	0.12
Insertion of cardiac pacemaker system	170	0.05	0.21
Left heart cardiac catheterization	220	0.07	0.07
Partial excision of large intestine	166	0.05	0.13
Repair and plastic operations on the nose	235	0.07	0.08
Total cholecystectomy	484	0.15	0.13
X-ray of urinary system	480	0.14	0.05
IV administration	20,000*	6.00	0.09

Sources: NCHS (12)
*ENVIRON estimate

The NCHS does not report data separately on certain common procedures, such as anesthesia, that are considered a routine part of another procedure, or on procedures that are rarely performed inside the hospital, such as dialysis or insulin injections. The frequency of these kinds of procedures can sometimes be estimated from the prevalence in the population of persons with the corresponding disorders (kidney failure and diabetes, respectively).

Exposure per Use

Estimates of the numbers of medical devices sterilized with EO each year are combined with estimates of the exposures obtained from one use of each of the devices to estimate the aggregate exposure to the composite individual. The mass of EO received over a lifetime is the product of the number of uses per lifetime and the mass absorbed into the body during each use. To match the information about toxicity, however, we need instead the dose (or more precisely, the dose rate) received in an average day. Dose is defined as the number of milligrams (mg) of EO received per kilogram (kg) of body weight. The lifetime average daily dose (LADD) is just the total number of mg received over a lifetime by our hypothetical composite individual divided by 26,650 (the number of days in a 73-year lifetime) and by 60 kg (the assumed average weight of a person over a lifetime, including both men and women). With a little additional arithmetic, we can express the LADD as:

$$LADD = \Sigma (D_d \times N_d)/(60 \times 365 \times P), \quad (1)$$

where D_d is the dose, in mg, from one use of devise d,
N_d is the number of devices used annually,
60 is the weight of the composite individual in kg,
365 is the number of days per year,
P is the U.S. population, taken to be 240 million, and
the units of LADD are mg/kg/day.

Next we estimate the absorbed dose, in milligrams, per use of each device. The quantity of EO *available* for exposure, in micrograms, is equal to the residue on the device (ppm by weight) times the weight of the device, in grams. The actual *absorbed* dose is estimated by applying a "reduction factor" that accounts for the tendency of EO to escape to other media (air or fluids leaving the body) or to remain on the device after use because the time of contact with the patient was insufficient for total absorption. The absorbed dose (mg) is given by:

$$D_d = F_r \times R_d \times m_d, \quad (2)$$

where m_d is the weight of the device, in g,
R_d is the residue of EO on the device, in ppm, and
F_r is the reduction factor applicable to use of such devices.

No data base of residues on devices at the time of use is known to be available. Although data are gathered about the residues on devices prior to release for sale, to our knowledge, a comprehensive compilation of these levels has never been attempted. Furthermore, little is known about the average shelf life of devices, and dissipation of EO during storage is correspondingly difficult to estimate. We therefore made a strong (and probably substantially conservative) assumption about residue levels to arrive at a plausible risk assessment. We relied on the levels proposed by the FDA (1978) as limits for residues. These proposals, which have never been finalized by the FDA, are shown in Table V.

Table V. FDA-Proposed Residue Limits For EO on Medical Devices

	Concentration Limits (ppm)
	EO
Implant	
Small (< 10 grams)	250
Medium (10-100 grams)	100
Large (> 100 grams)	25
Intrauterine device	5
intraocular lenses	25
Devices contacting mucosa	250
Devices contacting blood ("ex vivo")	25
Devices contacting skin	250
Surgical scrub sponges	25

We were also unable to find any systematic compilation of device weights. For easily

obtained devices, we weighed typical samples. We also obtained estimates from manufacturers or judged them from similarity to other devices for which weights were known.

Most uncertain of all are the values of the reduction factors. We were unable to find direct information on the transfer of EO from sterilized medical devices to patients or even to experimental animals. Some measurements of “dissipation curves” have been made to understand how fast EO escapes from sterilized devices as a function of storage conditions (1, 2, 7, 10, 14, 22), and these may indicate the rapidity with which the sterilant would move into the air from devices applied to the skin. Very few experiments have been conducted to measure the transfer of EO from devices to fluids (9), such as would occur in the course of transfusions, IV administration, or dialysis involving tubing sterilized with EO.

The rates of transfer appear to vary significantly, depending on materials of construction and other factors. Many dissipation curves show that the rate of loss drops as the EO source is depleted, as would be expected from the physical chemistry of the condition. This behavior can be represented in terms of “half-lives” ranging from a few hours or less to more than a week.

This variation is less important than it first may seem because those devices that transfer EO rapidly to the body also rapidly lose EO between release for sale and actual use. In the absence of measured values for the reduction factor, we assumed that the transfer would occur at a rate of about 3% per hour of contact, which is reasonably consistent with a half-life of 24 hours.

Time of contact of devices with patients ranges from a second or two for a gauze sponge to many years for implants. We assumed that any device remaining in place longer than 36 hours transfers all of its EO residues, either to the patient or to external fluids (air, urine). Transfer for shorter periods of contact is assumed to be proportionally lower. We obtained estimates of time of contact from our nurse consultants, from industry sources, or from our own observations.

Devices applied to the skin or mucosa are assumed to transfer less EO to the body than a device used invasively for the same period. Table VI shows assumed reduction factors by type of exposure for several periods of exposure.

Table VI. Assumed Transfer Reduction Factors

	Exposure		
Time of Contact	Blood or Internal Organs	Mucosa or External Conducts	Skin
1 second	7.0×10^{-6}	3.5×10^{-6}	2.0×10^{-6}
1 minute	4.0×10^{-4}	2.0×10^{-2}	1.3×10^{-4}
1 hour	2.5×10^{-2}	1.3×10^{-2}	8.0×10^{-3}
1 day	6.0×10^{-1}	3.0×10^{-1}	2.0×10^{-1}
Prolonged	1.0	5.0×10^{-1}	3.3×10^{-1}

When all of these data are combined, a data base of exposures per use of each device can be constructed. Table VII gives a sample of estimated exposure per use from the data

described above. The data used to derive these estimates are substantially uncertain; most of the necessary assumptions are in the direction of overestimating the exposure per use. In particular, use of the FDA-proposed residue limits may have little relationship to the true values of residues on devices.

One can also compute the contribution to the LADD for the composite individual from these doses by multiplying by the number of exposures per lifetime and dividing by body weight and number of days per lifetime. These results are also displayed in Table VII for the selected devices.

Specific Procedures

The appropriate measure of exposure for chronic toxic effects other than cancer is not the LADD but the daily dose over a shorter period. The appropriate duration depends on the specific type of effect under consideration. For example, in the case of birth defects (teratogenesis), the critical period may be measured in days; for most other chronic effects, it is much longer.

Table VII. Selected Estimates of Exposure Per Use and Corresponding Lifetime Average Daily Dose

Device type	Exposure per Use	LADD* (ng/kg/day)
Bags, blood administration	0.042	0.19
Bandages, adhesive	0.008	7.82
Catheters, intravascular	0.125	2.34
Dressings, surgical	0.825	6.19
Gloves, examination	< 0.001	0.02
Lenses, intraocular	< 0.001	<0.01
Oxygenators, blood	7.500	0.30
Protheses, breast	12.500	0.07
Sponges, laparotomy	0.075	0.39
Tubes, infant feeding	0.088	0.32
Tubing, drainage	1.250	7.50

*LADDs for the composite individual are so small that they are best expressed in mg/kg/day.

To be conservative, we assumed that all the dose from all the devices used in a medical procedure could be accrued in a relatively short time, generally one day unless we knew that several devices were used over a period of several days. The maximum daily dose, MDD_P from a given procedure, p, is thus estimated by

$$MDD_p = \Sigma (n_{dp} \times D_d)/60 \quad (3)$$

Where n_{dp} is the number of devices of type d used per procedure,
D_d is the dose per use of device d, as defined earlier, and
60 is the weight of the average person, in kg. The units of MDD are thus also mg/kg/day.

Discovering which types of devices are typically used in which procedures and how many of each are typically used was by no means a simple endeavor. Sometimes the answer is obvious, such as the association of an umbilical cord clamp with childbirth and the

typical birth involving exactly one clamp. Most are not obvious, however, because the rate of wastage is unclear and the rate of replacement is not obvious. In some cases, a rough idea of use can be obtained by counting the contents of a prepackaged sterilized kit destined for use in a specific type of procedure, but even here, the degree of redundancy is not obvious. Therefore, most of the identities and numbers of devices used per procedure were provided by our nurse consultants.

Using these estimates with the estimates of exposure for one use of each device, we can estimate the total exposure associated with all the devices used in a specific procedure and the corresponding maximum daily dose, MDD, by applying the above equation.

The information on exposures for specific procedures can also be used as a semi-independent check on the exposure estimated for the composite individual. The number of procedures accomplished per year in the U.S. is multiplied by the expected number of years in a lifetime (73) and divided by the population of the U.S. (240 million) to obtain an estimate of the expected number of procedures for the composite individual over a lifetime (11). That estimate can then be multiplied by the exposure from all the devices used in the procedure to obtain an estimate of the lifetime exposure from the expected number of procedures. For example, about 120,000 mastectomies are performed annually; that projects to a lifetime risk of mastectomy of 0.121 × 73/240 or 0.04 (one in 25). The contribution of mastectomies to lifetime exposure for the composite individual is obtained by multiplying the exposure for one mastectomy by the factor 0.04.

These estimates can be summed over all the types of procedures considered to obtain a second estimate of lifetime dose for the composite individual. This estimate is not entirely independent of our earlier estimate, because the same estimates of exposure per use of one device are employed and because our estimates of the annual number of devices used were sometimes obtained from the annual number of procedures performed. The procedure method will tend to underestimate total exposure because not all procedures can be included in a list of reasonable length and because uses of medical devices occur outside hospitals, especially in doctors' offices and homes. These omissions may result in underestimation by a factor of two to three.

Risk Characterization

The nature of any risks posed by exposure to residues of EO on medical devices can be understood by combining the results of the dose-response assessment and the exposure assessment.

Cancer

The analysis of the risks of cancer from exposure to EO residues assumes conservatively that some risk of cancer exists at any non-zero level of exposure. For low exposures, the lifetime risk is assumed to increase linearly with increasing total dose, expressed in daily terms as the LADD.

For this assumed dose-response relationship, the constant of proportionality is the UCR

or potency for EO, which was given in Table I as 0.048 $(mg/kg/day)^{-1}$. Under these assumptions, the upper bound on lifetime risk, R, of developing cancer from EO on medical devices is

$$R = 0.048 \times LADD \tag{4}$$

This equation can be evaluated either for all devices combined [(LADD from Eq.(1)] or for all the devices used in a specific procedure [dose from Eq. (3) averaged over a lifetime (LADD = MDD/26,650)]. For the composite individual, the upper bound on lifetime cancer risk computed by this procedure is about seven in a million (7×10^{-6}). This figure applies to all medical devices sterilized with EO; the risks from any one device or from all the devices used in any one procedure are much lower. For example, the cancer risk from a bypass operation, which is the procedure with the highest aggregate dose, is estimated as 0.048 × 0.53/26,650 or about one in a million.

Chronic and Reproductive Toxicity

The risks of health effects other than cancer may be evaluated by comparing the MDD [Eq. (3)] with some criterion of minimal toxic potential, in our case the NOEL as given in Table I. The ratio of the NOEL or LOEL to the MDD is defined as the margin of safety, MOS.

$$MOS = NOEL/MDD \tag{5}$$

If the margin of safety drops below unity, concerns about possible health effects in humans should be raised. If the margin of safety exceeds the uncertainty factor usually applied to a NOEL derived from animal experiments to estimate a safe exposure in humans, typically a factor of 100, then essentially no concern at all is justified. In between, the acceptability of the margin of safety must be judged by the quality and quantity of data used to fix the NOEL, the likelihood that sensitive groups of humans may be more susceptible to the effects of EO than any of the animal species and strains tested, and the degree of conservatism invested in the estimate of the MDD.

In our investigation of the risks of EO, we found no instance of a MOS that fell below 1.0. In the aforementioned example of the bypass operation, the MDD was about 0.5 mg/kg, assumed to be delivered in one day. That dose is four times lower than the NOEL (2 mg/kg/day). Furthermore, the NOEL was for chronic exposure; a single day of dosing is not likely to be as dangerous.

The MOS is always larger for reproductive effects, because the NOEL is 9 mg/kg/day. In that the reproductive effects on which the NOEL was based were observed after exposure during pregnancy, the procedures of concern are those likely to be performed on pregnant women who are not terminating the pregnancy. These also tend to have much smaller MDDs than for a bypass operation.

Because children weigh less than 60 kg, the same exposure (if measured in mg) produces a larger MDD. The difference is greatest for newborns, who weigh about 3 kg.

For them, the exposures due to childbirth itself may be most important. Using extreme assumptions that are unlikely to prove true for most infants, we estimated the MDD to approach 2 mg/kg/day, the NOEL for chronic effects. Under our assumptions, however, these doses would persist no more than three days, and the application of a chronic NOEL would appear to be adequately protective. In this and other cases where the estimated MOS is less than 100, however, further research is indicated to reduce the current high level of uncertainty about the true exposure levels.

Summary and Conclusions

In this quantitative risk assessment of the potential hazards of EO residues on sterilized medical devices, the risk of both cancer and other chronic toxicity have been assessed, both for a hypothetical composite individual and for persons undergoing a variety of specific medical procedures. The cancer risk for the composite individual has been estimated conservatively to be seven in a million from exposure to EO in all the medical devices studied. Furthermore, exposures from specific procedures have been estimated to be lower than the minimum levels causing chronic health effects, often substantially so.

The techniques described in this chapter were developed specifically to assess the risks of EO. The basic concepts and many of the key data are also applicable, however, to other potentially toxic substances present in medical devices. The principal requirement would be to develop the concentrations of the substance in the various device types and the corresponding transfer reduction factors. In conclusion, quantitative risk assessment is a valuable tool in the evaluation of the potential hazards of medical devices.

Acknowledgements

Thanks go to the Health Industry Manufacturers Association for supporting this work and to our colleagues at Environ, especially, Susan Rieth, Andrew Fragen, and Dr. Resha Putzrath for their contributions.

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Ethylene Oxide Residuals: Toxicity, Risk Assessment and Standards

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Sterilization of thermolabile medical products made of polymer materials by gaseous chemical agents is currently one of the most promising types of “cold” sterilization practiced in medicine. However, the dose adsorption capacity of polymers for gases, and the possibility of residual amounts of these chemicals on the medical product, poses an actual threat of adverse events when in contact with the sterilized items (8).

Experimental results and actual observations have verified the risk involved with the use of items sterilized by ethylene oxide (EO) (9). Thus, a necessary requirement for the safe use of medical products sterilized by EO is degassing to ensure that residual amounts of EO are safe. In other words, all sterilizing agents, including EO, may be used provided that appropriate regulations are developed for medical products on the basis of experimental toxicology studies.

Currently no unified methodological approaches are available for the study and regulation of sterilizing agents. Therefore, no common worldwide regulatory standards exist for particular agents, including EO, although there are national regulations for EO in every country that have varying specifications (1, 3, 5).

For a number of years, we have studied the toxicity of a variety of promising sterilizing agents: ethylene oxide, mixture of ethylene oxide with methyl bromide, formaldehyde, glutaraldehyde, hydrogen peroxide, and mixtures based on hydrogen peroxide and desoxons. These studies have involved evaluations of the physico-chemical and toxicological aspects of the above agents.

On the basis of the results obtained from such studies, we developed methodological approaches to the investigation of sterilizing agent toxicity and safety regulation for medical products. The methodological scheme developed address the following major aspects: toxicological investigation with the sterilizing agent, its physico-chemical properties within the gas-polymer system, assessment of the sterilized item, rationale for the permissible residual amounts (PRA) of the sterilizing agent, and the development of a degassing scheme for the medical products until the recommended safe residual levels are attained (Figure 1).

Toxicity Studies of Sterilizing Agents

- General (specific and remote) toxicological effects
- Duration of contact and contact pathway
- Acute and subacute toxicity
- Local irritation
- Sensitization effects
- Gonadotoxic and embryotoxic effects
- Chronic toxicity
- Hematologic effects
- Mutagenic effects

Medical/Technical Specifications of Medical Product(s) Suitable for Sterilization with Agent Under Investigation

- Limiting criteria (i.e., pathway, time of impact)
- Contact duration
- K_{stor} for medical product(s)
- Human body weight
- Material, shape, and weight configuration of medical product(s)

Physico-Chemical Evaluation of "Polymer-Gas" System

- MAC calculated
- Development of chemical methods to control for sterilizing agent and other components in polymer
- Study of adsorption and diffusion properties
- Estimation of adsorption and diffusion coefficients
- Differentiated K_{stor}

Assessment of Sterilized Product

- Criteria for contact duration
- Classification of medical products
- Toxicological/Hygienic studies of extracts
- Sanitary/chemical studies of extracts
- Experimental method: single or repeated contact
- Simulation method: short or long term
- Sterilizing agent MAC of medical product

Development of Medical Product Degassing Schemes

Figure 1. Relational Diagram for the Toxicological/Hygienic Investigation of Sterilizing Agents Used for Medical Products

Toxicological Evaluations

The program of toxicological research is determined first by the specificity of the effects of the sterilizing agent under evaluation on man, and second, by the actual conditions under which the sterilized products come in contact with a patient (e.g., pathway, duration, number of contacts). Using animal tests, the general toxicity, including both specific and remote effects of the sterilizing agents are studied, taking into account the polytropic action

of most sterilizing agents on the organism. Basic toxicological parameters are evaluated in acute, subacute, and long-term studies involving the potential pathways of the sterilizing agent into the organism (e.g., the stomach, subcutaneously, intramuscularly, intraperitoneally, in the blood, as well as by skin or mucous membrane contact).

During the initial assessment of toxicity, mean lethal doses (LD_{50}), species sensitivity, and toxic impact threshold are determined, and the agent's cumulative, stimulating, and sensitizing properties are studied. When labor-intensive remote effects are studied, such as embryotoxic, gonadotoxic, and mutagenic effects, rapid methods are generally used.

It is possible to estimate the lethal impact for the limited number of pathways by which the sterilizing agent may get into the organism. At the stage of comprehensive toxicological evaluations, the thresholds and ranges following subacute and chronic exposure are determined, taking into consideration both specific and remote effects. A subacute experiment takes 1.5-2 months while a long-term one takes 4 months. Animals are tested throughout the exposure periods using sufficiently sensitive tests.

On the basis of the parameters obtained, i.e., LD_{50} values following various exposure routes, dose ranges for acute, subacute, and long-term effects, the risk of using a particular sterilizing agent can be classified into one of four categories (Table I).

Table I. Classification of Agent Toxicity by the Extent of Effects on the Organism

Indicators	Classes of Danger			
	I	II	III	IV
Maximum allowable concentration (MAC) of toxic agents in the air of working area (mg/m^3)	<0.1	0.1-1.0	1.1-10.0	> 10.0
Mean lethal dose when administered into stomach (mg/kg)	< 15	15-150	151-5000	>5000
Mean lethal dose when applied to the skin (mg/kg)	< 100	100-500	501-2500	>2500
Mean lethal concentration in the air (mg/m^3)	<500	500-5000	5001-50000	> 50000
Coefficient of potential poisoning by inhalation (CPPI)	>300	300-30	29-3	<3
Acute effect zone	<6.0	6.0-18.0	18.1-54.0	>54.0
Chronic effect zone	< 10.0	10.0-5.0	4.9-2.5	>2.5

Class I = Extremely dangerous agents
Class II = Highly dangerous agents
Class III = Moderately dangerous agents
Class IV = Slightly dangerous agents

To determine the limiting biological effect, a comparison is made of the general toxicity, specific, and long-term effects of threshold amounts of sterilizing agents.

As to the potential pathways by which sterilizing agents may get into the organism, we comply with the following recommendations. The intraperitoneal route is used for the medical products which come in contact with blood and wound surfaces. For the items having contact with other types of tissues, the subcutaneous pathway is used as the main

pathway.

Taking into account the respective threshold level for toxicologic effects (Lim) (acute, subacute, or chronic), a 100-fold storage coefficient, and the mean human body weight (50 kg), the values of the daily maximum permissible dose of a sterilizing agent may be calculated for man under conditions of a one-time, repeated, or long-time contact with the sterilized product using the formula:

$$D_M = \frac{Lim_{(acute,\ subacute,\ or\ chronic)} \times 50\ kg}{J_s\ (100)}$$

where:

D_M is the maximum permissible dose on a daily basis for one-time, repeated, or chronic impact by sterilized items;

$Lim_{(acute,\ subacute\ or\ chronic)}$ are the threshold values of acute, subacute, and chronic effects;

J_s is the storage coefficient (the ratio of threshold values to safe ones); and

50 kg is the mean body weight.

On the basis of the obtained values of the daily maximum permissible doses for different sterilization agents, their maximum allowable concentrations (MACs) are calculated for medical products in compliance with the classification scheme developed. The classification takes into account three main indicators: pathway, time of impact, and duration of contact with man.

Physico-Chemical Evaluations

Physico-chemical investigations of sterilizing agents in “polymer-gas” systems involves the study of adsorption and diffusion properties, including determination of the adsorption and diffusion coefficients and extraction kinetics, as well as the development of chemical methods for detection of residual amounts of both sterilizing agents and attached migrating components. This research establishes the rationale underlying criteria regarding the duration of contact between the sterilizing agent and the organism, on the basis of the agent’s diffusion velocity in the stimulated environment.

We suggest that a short-time contact be defined as the time for which not more than 10% of the total agent content of the product enters the environment and a long-term contact defined as the time when over 10% enters the environment. Additionally, physico-chemical investigations enable one to recommend materials with optimal adsorption and diffusion properties, and to calculate the time for optimal degassing of sterilized products.

The hygienic aspects of the research includes substantiation of the MAC of the sterilizing agent in the product. This includes sanitary, chemical, and toxicological studies of the extract compositions obtained from the sterilized product. Animal experiments are used to test the safety of the extracts obtained from a number of sterilized items (medical products of different applications) which contain the MAC of a sterilizing agent, in particular EO. Such research permits development of regulations for the sterilizing agent which take into account components migrating to the extract. The above is in agreement with published data that the side-product content of the sterilizing agent polymer reaction is, by several orders of magnitude, lower than the sterilizing agent content of the polymer (2, 4).

Thus, we propose that the main principles of hygienically-based regulation of chemical sterilizing agents' content of medical products involve the following (10):

— standardization for the sterilizing agent taking into account the possible impact of the entire complex which migrates from the product which is sterilized;
— standardization for the agent per unit product; and
— establishment of differentiated MACs in relation to the duration, time of impact, and the pathways of contact between the product and man.

EO Toxicity

On the basis of the above-described methodological approaches, a study of EO toxicity was made and it was hygienically standardized for medical products. It was shown that, on the basis of an acute experiment, EO may be classified as either a moderately dangerous agent or a slightly dangerous agent, depending upon whether a subcutaneous or intraperitoneal administration route, respectively, was used (6). The classification of agent toxicity is presented in Table II.

Table II. The Classification of Agent Toxicity for Subcutaneous and Intraperitoneal Administration Routes

Toxicity Class	Toxicity Degree	Mean Lethal Dose (mg/kg)	
		subcutaneous	intraperitoneal
I	Extremely Toxic	< 0.3	<0.2
II	Highly Toxic	0.4- 15	0.3- 10
III	Moderately Toxic	16- 150	11- 100
IV	Slightly Toxic	151-1500	101-1000
V	Practically Non-Toxic	1501-4500	1001-3000
VI	Relatively Harmless	>4500	>3000

The values of mean lethal doses of EO for the potentially dangerous pathways into organisms are within the range of 120-320 mg/kg. The sensitivity of mice, rats, and guinea pigs to EO does not differ substantially.

The behavioral signs associated with acute EO poisoning in animals is characterized by lethargy, asthenia, and paresis of extremities. In experiments with rats in which EO was administered intragastrically, subcutaneously, intraperitoneally, and intravenously, acute impact thresholds were established, equal to 15, 5, 0.5 and 0.5 mg/kg, respectively (Table III).

Table III. Acute Toxicity and Impact Thresholds for EO in Rats Administered via Potentially Dangerous Routes

Administration	LD_{50} (mg/kg)	Lim_{acute} (mg/kg)	LD_{50}/Lim_{acute} Ratio
Intragastrically	320 ± 35.9	15.0	21.3
Subcutaneously	187 ± 12.8	5.0	37.4
Intraperitoneally	144 ± 9.7	0.5	288.0
Intravenously	154 ± 7.6	0.5	308.0

The tabulated data indicate that the acute EO toxicity following the intraperitoneal and intravenous administrations has similar values and is higher than that for the subcutaneous and intragastric administrations. The hazard of acute poisoning (with respect to the acute impact zone) is of a similar consistent pattern.

Similarly, experimenting with rats, the chronic toxicity (3-4 months) of EO in the rat was estimated for the two pathways: subcutaneoulsy (0.1, 1, 40 mg/kg) and intraperitoneally (0.01, 0.1, 5 mg/kg) using a large number of sensitive indicators. The results of this investigation established that the thresholds for EO's toxic effects following chronic exposure are 1 mg/kg following subcutaneous administration and 0.1 mg/kg following intraperitoneal administration. Thus, EO is a highly toxic agent.

Acute and chronic exposure to EO resulted in changes in the nervous system, liver, kidney, and in the morphological composition of peripheral blood. The changes were of phased type and exhibited the dose-time dependence.

In addition to the study of the general toxicity of EO, remote effects of its exposure were also studied, namely its gonadotoxic, embryotoxic, and mutagenic effects. Conventional methods were used to estimate gonadotoxic and embryotoxic effects. The mutagenic effect was studied with the use of the following methods: micronuclear, metaphase analysis, the method of dominant lethal mutations, and the anathelophase analysis of liver cells (7). The investigations show that the remote safety hazards (long-term effects) of EO are associated with higher levels than are its general toxicologic effects. For example, the minimal effective dose of EO demonstrating a mutagenic effect, determined by the anathelophase method for rat liver cells with subcutaneous administration for three months, is five times as high as its general toxicity threshold (chronic impact) and is equal to 5 mg/kg.

The impact thresholds for EO determined by the acute and chronic toxicity experiments were used as a basis for the calculation of the maximum permissible daily doses (D_M) for one-time and repeated contacts, respectively, and for the subsequent establishment of EO's MAC in sterilized medical products with different durations of contact (Table IV). As seen in this table, the most dangerous contact with EO is that via the blood.

Table IV. Maximum Permissible Daily Doses (D_M) of Ethylene Oxide, for One-Time and Repeated Contact Following Subcutaneous and Intraperitoneal Administrations

Administration	$Lim_{(acute)}$ (mg/kg)	$D_{M(acute)}$ (mcg)	$Lim_{chronic}$ (mg/kg)	$D_{M(chronic)}$ (mcg)
Subcutaneously (with mucous membrane)	5.0	2500	1.0	500
Intraperitoneally (with blood)	0.5	250	0.1	50

To calculate the sterilizing agent MAC of a sterilized product, however, the time of the contact between the product and man must be taken into account, and this time varies widely. As a basis for the delineation of the contact time and its classification as single short-time and single long-time, as well as repeated contact, a physico-chemical factor was used, that is, the diffusion rate of the sterilizing agent from the specified product into the

simulated environment.

All medical products to be sterilized via a gaseous method involving EO may be divided in three groups. The first group includes single-use items that are used on a short-term basis, such as catheters, endoscopes, probes, single-use syringes, etc. The second group includes single-use items that are used on a long-term basis (from several hours to several years) such as cardiac pacemakers, blood transfusion systems, long-term catheters, and probes. These items may yield up to 100% of the adsorped gas and thus their MACs are more stringent, i.e., lower by an order of magnitude than are those for the items included in the first group. The third group includes items subjected to repeated contact, such as dialyzers, blood transfusion systems, etc.

Table V shows the values for EO for specific groups of medical products; these MACs take into account the length of exposure and pathways of application. The values of MAC are given in mcg and essentially represent the values of maximum permissible daily doses (numerator), with the denominator including the values reduced per gram of material, by dividing the MAC value for a particular product by its weight.

Having estimated the MAC values for a sterilizing agent, one can determine the degassing time necessary to achieve them, and, thus, ensure the safe application of the sterilized medical products. On the basis of the MAC values recommended by us for EO, V. Herzog (GDR) performed a preliminary calculation of the EO desorption time for some EO-sterilized products, enabling a safe application of these products in clinical practice. The results are given in Table VI. Different desorption times are shown to be dependent on the application conditions and the product materials. Some items may be used immediately after sterilization, while others have to be degassed for 3 or 18 hours.

Table V. Permissible Residual Amounts of Ethylene Oxide (in mcg) for Various Groups of Medical Products[a]

Products	Medical Product Application		
	One-Time		Repeated Use
	Short-Term	Long-Term	
Ureteral Catheters	25000 2500	2500 250	500 50
Stomach Probes	2500 500	2500 50	500 10
Duodenal Probes	25000 630	2500 62.5	–
Endoscopes	2500 –	–	–
Intravascular Catheters	2500 2500	250 250	50 50
Intratracheal Tubes	25000 850	2500 85	500 15
Cardiac Pacemakers and Electrode Wires	–	250 2.0	–
Blood Testing and Transfusion Systems	–	250 5	50 1

Syringes	2500/500	–	50/10
Spiral Contraceptives	–	2500/6250	–
Artificial Crystalline Lens	–	2500/–	–
Heart-Lung Machine	–	250/0.063	–

[a] Numerator is for the entire product; denominator is for 1 g of material

In conclusion, a scientifically substantiated system of hygienic standardization regarding the use of ethylene oxide in which differentiated MAC values are determined on the basis of the agent's toxicity ensures a safe application of the EO-sterilized items.

Table VI. Approximate Calculation of the Desorption Time Ensuring Safe Application for Some Medical Products that are Sterilized by Ethylene Oxide

Area of Application item[a]	Times of Use, Duration	Material	Mfr	Desorption Time in Hrs[b]		
				0	3	18
I. Angiography/Radiography						
Arterial Catheter	Single, < 30 min	Polyurethane	Wigon, FRG	x		
(Wigoflexpur)	Single, > 30 min				x	
	Multi-Use					x
Troacar-Catheter Substitute	–//–	Polyvinyl-chloride	Wigon, FRGX			x
						x
						x
Electrode Hydrocatheter	–//–	Polyethylene	VEB Ultrasonic Halle, GDR	x		
				x		
						x
II. Urology						
Urocatheter	–//–	Polyvinyl-chloride	Rusch, FRGX		x	
						x
						x
Curved Catheter	–//–	Polyamide	Rusch, FRG		x	
						x
						x
Thiemann Catheter	–//–	Natural Rubber	Rusch, FRG		x	
					x	
						x
III. Anesthesia						
Trachealtubus with a Bottle	–//–	Polyvinyl-chloride	Portex, UK			x
						x
						x
Laringectomitubus	–//–	Natural Rubber	Rusch, FRG		x	
					x	
						x
Laringoflex	–//–	Latex Rubber	Rusch, FRG		x	
					x	
						x
IV. Implantation						
Intraocular Lenses	–//–	Polymethyl-methacrylate	Hellgrebe, GDR	–		
					x	
				–		
V. Gastroenterology						
Duodenal Probe	–//–	Polyvinyl-chloride	Medicinal Plastics, Lichtenberg, GDR		x	
						x
						x

[a] Aseptically packed items were sterilized by ethylene oxide and desorped in the MGB 9.532 — type apparatus at an ethylene oxide level of ca. 1000 mg/L, at a temperature of 55°C, relative air humidity of 75%, for 4 hours

[b] Desorption was performed at the same temperature, at an air change of 60 times an hour in the sterilizer immediately after the sterilization process has been over. As a basis, the data cited by Lyarsky et al. (10) were used, on the permissible amounts of ethylene oxide.

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The Use of Ethylene Oxide in the Hospital Setting

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Introduction

Volatile or gaseous disinfectants have been used in the health care industry for more than 100 years. Ethylene oxide and formaldehyde vapors are the most frequently used because their sporicidal properties elevate them to a gaseous sterilant status. Ethylene oxide, however, has become the preferred gaseous sterilant in the United States. Ethylene oxide sterilization equipment was first introduced to the health care industry in the early 1950s by the American Sterilizer Company and Castle Company. With this introduction came the ability to design equipment capable of operating at a lower temperature and relative humidity and with larger capacity chambers for both hospitals and industrial use.

Since that time technological advances in equipment design for safe use, both for the patients and the employees who operate the equipment, have brought about an increased use of ethylene oxide in health care institutions. The most prominent equipment designs are:

1) Equipment designed to use a mixture of ethylene oxide-fluorinated hydrocarbons. The most common mixture is 12% ethylene oxide and 88% dichlorodifluoromethane (Freon 12). This is a non-flammable mixture available in tanks or cylinders. Equipment of this type are generally in the range of 4 to 50 cubic feet capacity.
2) Equipment designed to use 100% ethylene oxide. The ethylene oxide is available in unit dose cartridges or canisters, and contain 67 to 140 grams. Equipment of this type are less than four cubic feet capacity.

Benefits to the Health Care Field

The most important characteristic of ethylene oxide gas sterilization is its efficacy of sterilizing heat-sensitive and/or moisture-sensitive materials, that is, materials that may be damaged or destroyed by other methods, such as saturated steam under pressure sterilization, dry heat sterilization, liquid chemical sterilization, or other gaseous sterilant mixtures. This characteristic has brought about widespread use of ethylene oxide gas sterilization in both health care facilities and industry.

Though the use of ethylene oxide in the industrial setting has increased the use of disposable, one-time-use medical devices in the health care facilities, there still is a need for its use in reprocessing reusable medical devices. An advantage of ethylene oxide gas sterilization is its ability to sterilize complex device assemblies and machines. This results in greater assurance of product safety in hospital infection control programs.

Ethylene oxide is a toxic chemical and has mutagenic effects on plants, bacteria, seeds, and laboratory animals. Concern also exists that ethylene oxide may be carcinogenic.

These possible hazards from occupational exposure to ethylene oxide were brought to the forefront in the late 1970s and early 1980s. The emphasis placed on employee safety in the workplace and their exposure to toxic substances resulted in a decline in the use of ethylene oxide for a short period. However, with advances in equipment design, work practices, and engineering controls, its use continues to be a viable part of our infection control programs and in quality patient care.

In the past three years we have seen a continuous increase in the numbers and types of medical devices being processed using ethylene oxide. This increase was a result of implementing universal blood and body fluid precautions because of acquired immunodeficiency syndrome (AIDS) and Hepatitis B.

Employee Education and Training

Ethylene oxide gas sterilization processes are generally carried out in the central service department of the health care facilities. Employees in the central service department receive education and training in the safe use of ethylene oxide. Work practices and training encompass:

- proper cleaning and decontamination of reusable medical devices;
- assembly and packaging techniques to achieve sterilization;
- cycle parameters of the equipment and signs to watch for proper operations;
- techniques and procedures to use subsequent to sterilization for aeration of the devices for safe use in patient care and employee safety;
- methods used in monitoring the cycle parameters for sterilization; and
- monitoring of the environment in achieving a safe workplace below the limits established by the Occupational Safety and Health Administration (OSHA) (Table I).

Table I. OSHA—Ethylene Oxide Exposure Limits

PEL	1 ppm	8-hour TWA
PEL "action level"	0.5 ppm	8-hour TWA
EL	5 ppm	15-minute TWA

PEL = Permissible Exposure Level
EL = Excursion Level
ppm = parts per million
TWA = Time Weighted Average

Processes in Achieving Sterilization

Manufacturers of medical devices intended for reuse in patient care are ultimately responsible for providing general guidelines outlining the safe use of ethylene oxide as a sterilant to the user. Manufacturers of sterilization equipment provide the user with operating instructions and general guidelines on the care and handling of devices undergoing the cycle parameters outlined for the specific equipment. The combination of manufacturer's instructions and the health care facility's experience with products and

equipment result in the development of policies and procedures to ensure quality patient care products and a safe work environment.

Cleaning and decontamination of reusable medical devices are carried out using processes that will render the device safe for employees to handle during assembly and packaging. These processes include thorough cleansing for removal of dried blood, pus, mucus, oil, crystals or other foreign matter. Decontamination procedures recommended by the manufacturer of the device and/or established written procedures are then followed to reduce the microbial load.

Assembly and packaging is carried out in an environment conducive to maintaining a relative humidity above 35% to prevent dehydration of the materials to be sterilized. These processes require written procedures on how to properly assemble the device and how to package the device to achieve sterilization and aeration (Table II).

Understanding ethylene oxide gas sterilization and subsequent aeration of the materials is a complex process in the health care facilities. Reusable medical devices (Table III) reprocessed in a health care setting cover a wide range — from simple to complex devices. Assuring compatibility of the device with the type of equipment in use is critical. The manufacturer's guidelines are needed to assist the user in making rational decisions and in providing a safe product for use in patient care. In some instances the supervisor of the central service department must conduct product testing to determine the effectiveness of the ethylene oxide process. This testing and other monitoring techniques of the process must be documented.

Health care facilities generally do not have the inventory or necessary equipment to utilize dedicated loads; instead they combine a variety of devices in the same load. Loading the sterilizer cart or basket is important for proper circulation and penetration of the sterilant.

Several designs of ethylene oxide sterilizers are manufactured to meet the needs of health care facilities. Depending upon the design, the equipment may operate above atmospheric pressure or at or below atmospheric pressure, as shown in Figure 1, Graph A and B.

Sterilization cycle parameters are generally established by the manufacturer in order to achieve a balance in time, temperature, ethylene oxide concentration, and humidity. They should not be altered by the user. Exposure time may vary from 1 3/4 hours (105 minutes) to as long as 12 hours. Operating temperatures range from 49°C (120°F) to 60°C (140°F) for warm cycles and 29°C (85°F) to 38°C (100°F) for cold cycles. Concentration of ethylene oxide gas ranges from 450 milligrams per liter (mg/L) of chamber space to 1,200 mg/L. The moisture content of the microorganisms themselves is extremely important to the lethal activity of ethylene oxide; therefore, humidity in the chamber during the sterilization process is maintained at 80% to 90%, which provides the load with 60% to 75% relative humidity. In order to assure the probability of sterility, biological monitoring of the cycle is recommended for each load processed using *Bacillus subtilis* var. *niger* contained within a challenge or routine test pack and strategically located within the chamber load.

Table II. Acceptability of Packaging Materials

Acceptable	Unacceptable
Peel pouches:	Packages that are made entirely of any of the following materials:
Spun-bonded olefin (Tyvek*)	
polyethylene-polyester laminate	Foils
Paper/polyethylene-polyester laminate	Cellophane
Paper/polypropylene-polyester laminate	Polyvinylchloride (PVC)
Polyethylene plastic bags (designed for: ethylene oxide packaging)	Polypropylene
	Polyesters (Mylar*)
Wraps:	
Woven textile	Polyamides (Nylon)
Nonwoven materials	Polyvinylidene chloride (e.g., Saran Wrap)
Paper (coated and uncoated)	
Rigid sterilization containers designed to be used in ethylene oxide systems/plastic trays with paper or Tyvek* lids	

* A registered trademark of E.I. DuPont de Nemours & Co.

Aeration requirements vary with product and equipment design, for both the sterilization equipment and aeration equipment. Technological advances in equipment design have brought about the use of the same equipment for both sterilization and aeration to take place in the same chamber. This technology reduces the risk associated with the transfer of the load and employee exposure. To assure a safe workplace environment in the use of ethylene oxide, health care facilities have implemented monitoring protocols to measure the residual levels within the environment. These protocols may include the use of diffusion badges or charcoal tubes for employee breathing zone samples, and infrared analyzers or gas chromatograph for area sampling. Some facilities use a combination of analytical methods.

Table III
Materials Appropriate for EO Gas Sterilization

Rigid and Flexible Fiber Optic Telescopic Instruments	Equipment	Instruments	Rubber Products	Plastic Products	Miscellaneous
Arthroscope	Anesthesia equipment	Battery-powered burr holder	Blood pressure air-valve	Airways **	Books **
Bronchoscope	Artificial kidney	Battery-powered cautery	Blood pressure bladder **	Catheter	Toys **
Cystoscope	Blood pressure monometer **	Battery-powered drill	Catheter *	Dilator	Filiform
Endoscope	Camera with film	Burr	Dilator *	Endotracheal tube	Bougie
Gastroscope	Diathermy equipment	Cautery pencil, probe	Drain *	Gloves	Stethoscope
Laparoscope	Electrical cord **	Dermatone	Endotracheal tube	Pacemaker	Sealed glass ampule (outside surface)
Mediastinoscope	Gauge **	Electrode	Face mask **	Heart valve	Thermometer (cold cycle)
Ophthalmoscope	Hair clipper **	Flexible fiber optic light cord	Heating pad **	Nebulizer	
Otoscope	Heart-lung equipment	Lighted retractor	Latex tubing *	Petri dish	
Pharyngoscope	Incubator	Microsurgical instruments	Red rubber tubing *	Test tube	
Proctoscope	Respiratory therapy equipment	Nerve stimulator pencil	Sheeting	Syringe	
Resectoscope	Vacuum regulator **	Tonometer	Surgical gloves *		
Sigmoidoscope		Urethral stone basket	Tourniquet **		
Thoracoscope					
Urethroscope					

* These devices are normally made of materials that will withstand the process of sterilization with saturated steam under pressure, although they are used in setups where other accessories need to be EO-sterilized.

** This material is normally not used as a sterile device but may need to undergo an EO sterilization cycle before reuse.

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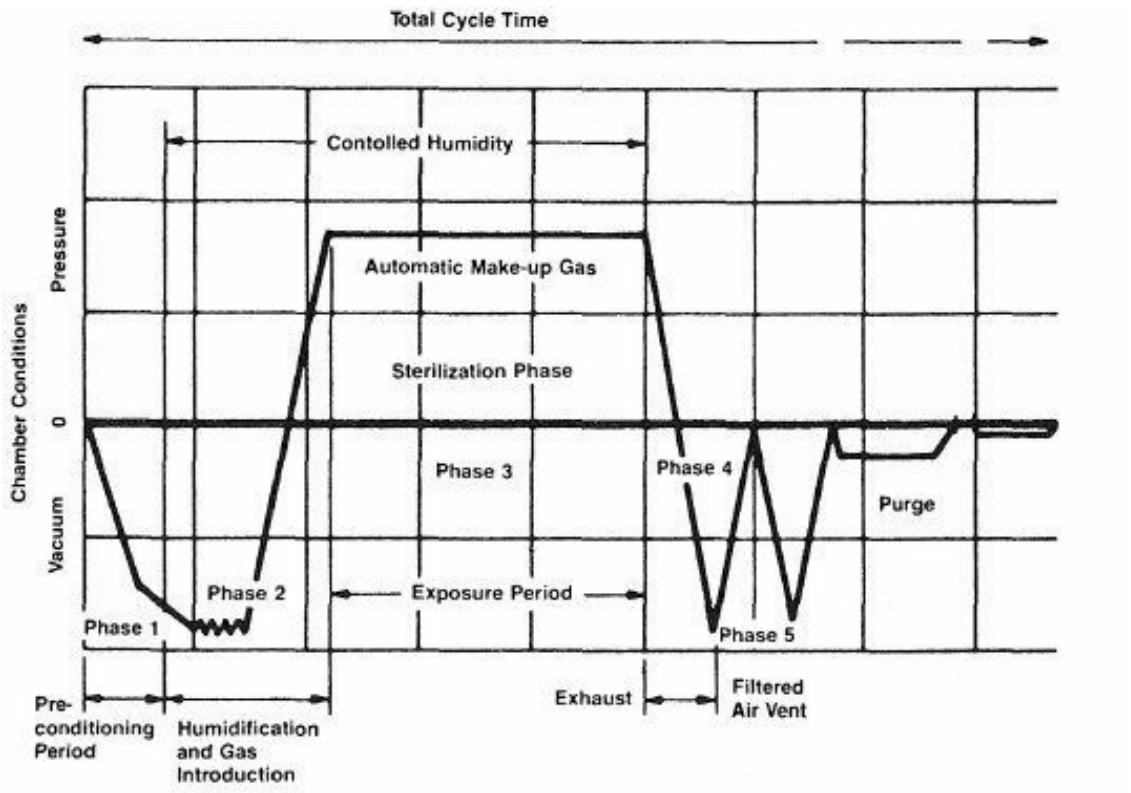
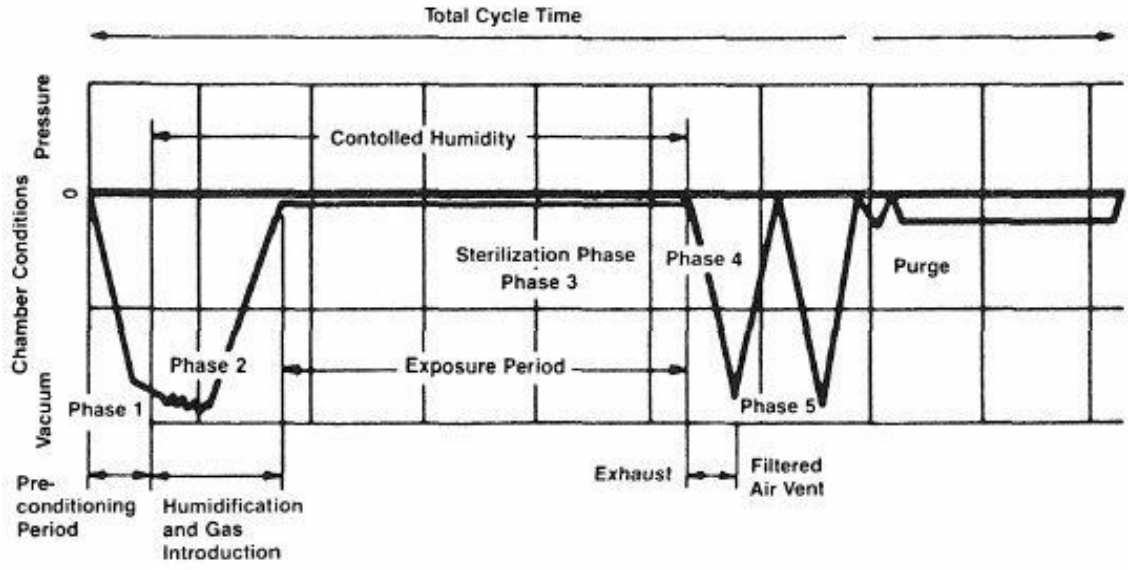


Graph A Cycle phases of EO Sterilizers that require above atmospheric pressure



Graph B Cycle phases of EO Sterilizers that operate at or below atmospheric pressure

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Figure 1. Ethylene Oxide (EO) Sterilizers Cycle Phases

Other Issues and Concerns

In today's healthcare environment we are confronted with issues surrounding the continued use of ethylene oxide:

— employee exposure and its acute and chronic effect, if not properly managed;
— chlorofluorocarbons (stratospheric ozone depleting chemical); and
— increased cost to operate.

At this point in time concerns exist that there is no substitute for the ethylene oxide gas sterilization process. There are some alternatives — such as hydrogen peroxide, gas plasma, and peracetic acid, but these are not a total replacement. Health care facilities are implementing procedures to utilize steam sterilization where applicable and to optimize the use of each ethylene oxide load.

Conclusion

Ethylene oxide gas sterilization is a vital part of quality patient care in the hospital setting and will continue to be until research and technology advances to provide a total replacement. Implementing good work practices, providing ongoing education and training, and continuously improving the processes will permit the continued safe use of ethylene oxide in our health care facilities.

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Session IV
Prevention and Control of Hospital Infection
Chairman: John F. Burke, M.D.
Harvard Medical School, U.S.A.

Current Principles for Prevention and Control of Hospital Infection

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Introduction

The control of hospital infection involves a chain of responsibility ranging from politicians who enact the legislation to the most junior nurse providing care for the individual patient. The whole system has recently been reviewed in the U.K. following the report "Public Health in England" of a Committee of Inquiry into the future development of the Public Health function (the Acheson Committee) chaired by the Chief Medical Officer (1). An essential component of this report is "Hospital Infection Control", a guide to the control of infection in hospitals prepared by a joint Department of Health and Social Security/Public Health Laboratory Service Working Group (4). Arrangements are now being made to implement the recommendations of these two official documents and to redress deficiencies. As far as local infection control in hospitals is concerned, the proposals reinforce current practices, but clarify responsibilities.

Administrative Arrangements

The Secretary of State of the Department of Health is *inter alia* ultimately responsible for hospital infection control. The chain of command passes to the Hospital Infection Control Doctor (HID) in two directions, one via the Chief Medical Officer and Regional and District Directors of Public Health and the other via Regional and District Health Authorities. The District General Manager (DGM) is responsible to the District Health Authority (DHA) for "setting up and maintaining effective hospital infection control" (4).

Two people have responsibility for infection control at the level of the District. One is the Consultant in Communicable Disease Control (CCDC), a new post established in accordance with the Acheson Report's recommendations and the other is the Hospital Infection Control Doctor (HID). The CCDC is responsible for infection control outside hospitals, while the HID is responsible for hospital infection control. The two are expected to liaise at all times, and the CCDC has the responsibility of producing formal reports on infection for the overall annual report of the Director of Public Health. The CCDC is also required to take overall responsibility for "major outbreaks" including such outbreaks affecting patients in hospitals. This requirement arose in response to two major outbreaks of nosocomial infection—one of food poisoning, the second of Legionnaire's Disease (2,3)—in which deficiencies in arrangement were identified. The difficulty in its application stems from the fact that HIDs are well-established and effective in most districts, whereas CCDCs are a new cadre of staff with, as yet, little experience of any kind of infection control, let

alone that in hospitals. It remains to be seen how this arrangement will work in practice. As a general principle, it seems advisable to avoid ambiguity in the chain of responsibility, but at the same time specific training and skills must be recognized.

Hospital Infection Control

In any hospital (or group of hospitals in a District) the District General Manager is responsible to the District Health Authority for establishing an Infection Control Committee and an Infection Control (IC) Team (Figure 1).

Members

Infection Control Doctor (HID)—Leader
Infection Control Nurse
(Consultant Microbiologist)
± District General Manager or representative

Role

Formulation and implementation of IC policies
Day-to-day advice on procedures
Assessment of risk of new procedures
Monitoring infection
Control of outbreaks
Education on infection control
Advice on allocation of resources

Figure 1. The Hospital Infection Control Team

The key member of this Infection Control Team is the Hospital Infection Control Doctor (HID) who is usually Chairman of the Committee and Leader of the Team. He/she is also usually a medically-qualified microbiologist, who has been trained in hospital infection control and has had that training assessed in the examinations of the Royal College of Pathologists. As a microbiologist, the HID supervises laboratory work, including that associated with infection control, and in addition offers advice on the management of patients. As IC Team leader, he/she liaises with a large number of bodies and individuals (see Figure 2) but, most importantly, works closely with the Infection Control Nurse (ICN) who is responsible to him/her. The ICN, with specialist skills and expert knowledge “on which the effectiveness of infection control will largely depend” (4), is the full-time member of the Team. The nurse must have the authority and status to carry out his/her duties which cover all the functions of the Team (see related paper by E. Jenner).

The Infection Control Committee (Figure 2) is broadly representative of all those with clinical or administrative responsibility in the Hospital. In addition to the members of the Infection Control Team, its core members include a Consultant Microbiologist (if the ICD is not a microbiologist); an Infectious Disease Physician (if they exist); an Occupational Health Physician, a General Physician and a Surgeon representing the medical staff; a senior nurse; the DPH and CCDC; and a representative of the DGM. The Committee usually has

powers of co-option as necessitated by local circumstances (4).

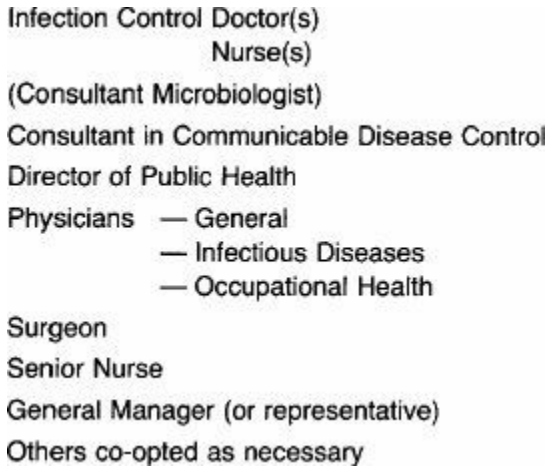

Figure 2. The Hospital Infection Control Team Committee

Outbreak Control

Despite the most efficient functioning of an Infection Control Team, outbreaks of infection continue to occur, and probably always will as microorganisms evolve and find new niches. This is sometimes not understood by those at higher levels in the chain of command who intend to set up an impregnable organizational barrier. Local teams will always have the duty of identifying outbreaks and acting to control them.

The procedures involved in outbreak control are now well recognized, although there is disagreement as to how each is to work in practice. The experienced ICD can economize considerably and solves problems daily without benefit of formal descriptive epidemiology or case-control studies.

Recognition

First, it is essential to recognize outbreaks at the earliest possible opportunity. This function is increasingly, though not nearly sufficiently, assisted by computers (5). Computers can collect and process clinical, epidemiological and microbiological information from the wards and departments of the hospital, making it possible to screen automatically for clusters of infection associated with a person or a place over a relevant time period. As far as I am aware, no complete hospital-wide system is currently in operation in the U.K., and much of the time of the ICD and ICN is used in manual data processing. Discussions on surveillance of infection led to conclusions in the pre-computer era that can no longer be accepted. For example, while it was clear that it was undesirable for a lone ICN to spend all available time collecting information on, for example, surgeon-specific infection rates, this does not mean that such information is of no value, but merely that time would be more valuable spent in taking action to prevent infection. Computers *can* make the information available and allow the Team to identify problems and act in their major preventative role. Unfortunately, computer systems in hospitals tend to develop in isolation and those who

commission them seldom see their epidemiological potential for automatic scanning of information from operating theater, ward, and laboratory systems. Surveillance thus remains inadequate in most U.K. hospitals, and is limited in many cases to an analysis of laboratory results in isolation.

Investigation

There is also discussion on the level of investigation required in a potential outbreak. HIDs have traditionally been trained as medical microbiologists by apprenticeship, and have not had formal training in epidemiology. Potential CCDCs are thought likely to be recruited from the profession of Community (now Public Health) Medicine where they will have been trained in epidemiological methods, but will have had minimal experience in applying their training to practical infection problems. Improvements in the training of both groups are planned.

The first requirement of an investigation is to confirm that an outbreak exists. A definition of its extent in time and space will follow, and help may be needed from outside the District. The ICD must decide whether to involve the CCDC (rather than inform him/her when the problem is resolved), the Public Health Laboratory Service (for technical assistance or advice), or the Communicable Disease Surveillance Center (a centralized national resource). He/she must also decide whether it is necessary to set up an Action Group which includes the IC Team augmented by clinicians and senior nurses, and sometimes the CCDC and representatives of CDSC.

Dissemination of Information

Whatever measures are instituted, it will be vital to keep all involved appropriately informed as the outbreak progresses. The arrangement for this may range from one or two telephone calls from the ICD or ICN, to the setting up of a press room manned by professionals in regular communication with the media and the Chief Medical Officer at the Department of Health.

Control

Specific control measures will depend on the circumstances and are the subject of many other contributions to this symposium as well as many publications, both general and specific during the past three decades (7, 8). The role of the IC Team is to ensure that clear instructions reach all involved, ranging from clinicians responsible for the care of infected or susceptible patients to those responsible for dissemination of information outside the hospital.

Report

Finally, the IC Team is required to report on its activities. It is unfortunate that this aspect

of the work of IC Teams has been neglected since it is fatally easy for those not directly involved to fail to perceive that anything is being done. It is much easier to see when things go wrong, however, and the Acheson Committee was set up in the wake of problems (2, 3) and largely in ignorance of the overwhelming success of the system.

Conclusion

Is Hospital Infection Control effective? The question is difficult to answer. Even if resources were available, it would still be extremely difficult to set up appropriate experiments to scientifically prove that epidemics are prevented or truncated by intervention. Too much of our infection control practice is still, therefore, empirical. The major source of support for the conclusion that our efforts are effective is the massive SENIC project undertaken in the U.S.A. (6). From this it was clear that hospitals with active infection control programs fared better than those without them—and we must be content with this conclusion. We are also asked whether Hospital Infection Control is cost effective. Both epidemics and their prevention cost money, and in purely financial terms, the difference is sometimes a narrow one. But hospital infection is undesirable in more purely human terms—to the doctor who sees his efforts undermined or thwarted and to the patient who suffers.

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Prophylactic Antibiotics in Obstetrics and Gynecology

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Introduction

The pathophysiology of soft-tissue infection following obstetrics and gynecology operations is well known. These post-operative infections are caused by the diverse bacterial flora of the vagina and endocervix. In the post-operative environment of the tissue destruction caused by clamps and the placement of foreign body sutures in the operative wound, these organisms overgrow and invade tissue. The response in the human to these multiple bacterial invaders mimics those reported by Bartlett and Gorbach in an animal model of a mixed bacterial intraperitoneal infection. In the animal, there is an early onset phase of infection that can be associated with a bacteremia. In humans, this is paralleled by women who become infected with an endomyometritis and an associated bacteremia after an emergency cesarean section. Following hysterectomy, a vaginal cuff infection or pelvic cellulitis can be seen early in the post-operative course. In the human, as in the animal model, there is also a late onset phase of infection in which abscess formation occurs and anaerobic bacteria seem to be the dominant bacterial pathogens. These events include uterus with micro-abscesses following cesarean section, but the best parallel to the animal model is the adnexal abscess following vaginal hysterectomy. This serious problem occurs late in the clinical course. Fortunately, the frequency of these early and late events can be reduced by the use of therapeutic antibiotics.

The prevention of these post-operative site infections by the use of antibiotics is based upon well established scientific principles. Dr. Burke evaluated the role of systemic antibiotics in preventing local infections caused by bacteria. He found that there was a measurable response after 24 hours to the injection of live and killed staphylococci at a skin site in animals. If he used intravenous penicillin effective against this strain of staphylococci, the timing of the antibiotic administration was very important in determining the response. If the penicillin was given before or at the time of the bacterial injection, it effectively decreased the extent of the host response (i.e., the area of induration was reduced). If penicillin was delayed three hours or more after the injection of bacteria, it was ineffective, i.e., the area of induration was the same as when no antibiotics had been given. This critical timing has been the underlying principle of all successful studies of antibiotic prophylaxis in obstetric and gynecologic operations. Improved post-operative results with prophylactic antibiotics have all involved the administration of these agents at the time of the operation.

Guidelines for Prophylactic Antibiotic Use in Obstetrics-Gynecology

There are a number of principles that can be applied to the use of prophylactic antibiotics in obstetric-gynecologic operations. If they fit a particular operation, a good case

can be made for the use of prophylactic antibiotics in that instance.

Guideline One: The Operation Should Have a Significant Risk of Post-Operative Site Infection

The most important aspect of this guideline is operative site infection. For the obstetrician-gynecologist, this means post-operative infections such as a vaginal cuff infection, pelvic cellulitis, adnexal abscess, abdominal wound infection following hysterectomy, or an endomyometritis following cesarean section. If the post-operative infection problem is an organ system away from the operative site, i.e., the lungs in post-operative pneumonia, prophylactic antibiotics will not be the solution to the problem.

The significant risk in this guideline includes the frequency and severity of the infection at the operative site. Infections frequently follow hysterectomy and cesarean sections, and they can be serious. This is an important clinical problem for obstetrician-gynecologists. In contrast, infections following pregnancy termination and reconstructive tubal surgery are much less common, but the outcome can be serious for the occasional patient who becomes infected because the resultant tubal damage can prevent future pregnancies.

Guideline Two: The Operation Should Be Associated With Endogenous Bacterial Contamination

One of the realities of any operation for an obstetrician-gynecologist is the bacterial contamination of the operative field by the endogenous bacteria of the vagina and endocervix. This is the source of the bacterial contamination in vaginal hysterectomy, the cesarean section in the patient in labor, and the woman undergoing pregnancy termination. Some novel ways to successfully reduce this contamination have included a hot conization of the cervix just prior to hysterectomy and antibiotic lavage of the uterine wound at the time of the cesarean section. In other gynecologic operations, unexpected bacterial contamination can occur. Recent studies have demonstrated the presence of *Chlamydia trachomatis* in the pelvic adhesions of women undergoing pelvic operations to restore fertility, with no gross evidence of inflammation. Clearly, endogenous bacterial soiling in one form or another exists in most obstetric-gynecologic operations. Careful pre-hysterectomy preparations of the vagina with an antiseptic solution can reduce the number of surface organisms, but it does not eliminate the problem. Bacteria can be recovered from the surface of the vagina at the end of the operation.

Guideline Three: The Prophylactic Antibiotic Should Have Laboratory Evidence of Effectiveness Against Some of the Contaminating Microorganisms

This is the most controversial of the guidelines. The controversy is related to the general success of prophylactic antibiotics in the most widely studied operations, vaginal hysterectomy and cesarean section. Prophylactic antibiotics work and there seems to be no difference depending upon the antibiotic used, i.e., penicillin, cephalosporin, tetracycline or

metronidazole. This seeming lack of difference is related to study design problems in the evaluations reported to date. Serious infections, such as the post-operative adnexal abscess, occur infrequently, and the small number of patients in many of the studies will not permit discrimination of differences in infrequently seen complications, a statistical problem of beta error. For example, all of the post-operative adnexal abscesses following vaginal hysterectomy reported to date have occurred in those patients who received prophylactic antibiotics with little activity against gram-negative anaerobic bacteria. There are similar concerns about the choice of antibiotics for prophylaxis in cesarean section. Antibiotics with gram-negative anaerobic activity have the best results. For pregnancy termination and reconstructive tubal surgery, the clinical concern is *Chlamydia trachomatis* and the best antibiotic to prevent infection with this organism is a tetracycline. Although there is a wide range of possibilities, a preferred list of antibiotics for prophylaxis can be made for each operative procedure.

Guideline Four: There Should Be Clinical Evidence of Effectiveness

This is the most important of all the guidelines. Unless prophylactic antibiotics prevent post-operative infection, there is no justification for their use. Table I lists the operative procedures in obstetrics-gynecology in which prophylactic antibiotics are used. There is a wide variety of response reported to date. These include unequivocal success in operations with high rates of post-operative infection, including vaginal hysterectomy, cesarean section for the patient in labor and a radical hysterectomy for a genital malignancy. There are also successful results with prophylaxis in pregnancy termination, which has a much lower rate of infection. There have been mixed results for the patient undergoing elective cesarean section and abdominal hysterectomy. None of these studies have shown an increase in the infection rate when prophylactic antibiotics have been employed, but some studies have shown no benefit.

Table I. Results with Prophylaxis

Operation	Results
Vaginal hysterectomy	Unequivocal success
Cesarean section—patient in labor	Unequivocal success
Radical hysterectomy	Unequivocal success
Pregnancy termination	Unequivocal success
Cesarean section—patient not in labor	Mixed results
Abdominal hysterectomy	Mixed results

Guideline Five: The Prophylactic Antibiotic Should Be Present in the Wound Some Time During the Operation

As noted in the introduction, the timing of administration of the prophylactic antibiotics is crucial for success. For most operations, the antibiotics should be administered just before the operation begins so that it will be present in tissue as pedicles are clamped, cut, and ligated with a foreign body suture. If the operation lasts more than three hours and a

prophylactic antibiotic with a short half-life has been employed, a second dose should be given intraoperatively. Antibiotics with a short half-life include ampicillin and cefoxitin. Those with a long half-life in which a second dose will not be necessary include cefazolin, cefoperazone, tetracycline, doxycycline, and metronidazole. There is a different clinical consideration with cesarean sections. To avoid the administration of antibiotics to the baby, the antibiotics should be given after the cord is clamped. Blood levels of antibiotics observed following maternal administration can make evaluation of the newborn difficult for the pediatrician, since it may mask early signs of neonatal sepsis.

Guideline Six: A Short Course of Antibiotic Prophylaxis Should Be Employed

This is one of the most important guidelines for the rational use of prophylactic antibiotics. A short course of prophylactic antibiotics has been shown to be just as effective as prolonged administration of antibiotics in vaginal hysterectomy and cesarean section. Utilizing a short course reduces the risk of adverse reaction to the antibiotic and lessens the risk of colonization of the patient with resistant organisms. If the patient becomes febrile after the use of prophylactic antibiotics, then a different antibiotic should be used for treatment, because the failure can be due to the presence of resistant organisms.

Guideline Seven: First Line Antibiotics Should Be Used For Prophylaxis If Results Are Superior

This guideline has recently been revised because of clinical observations. A number of studies show that antibiotics with gram-negative anaerobic bacterial coverage provide superior results in vaginal hysterectomy and the patient in labor undergoing cesarean section. Saving these agents for treatment makes no sense, particularly where so many good alternative antibiotics are available. In contrast, for the patient undergoing pregnancy termination or tubal reconstructive surgery, a tetracycline should be employed to cover *Chlamydia trachomatis*. This is a serious pathogen, which can produce irreversible tubal damage with minimal patient symptomatology post-operatively.

Guideline Eight: The Benefits of Antibiotic Prophylaxis Should Outweigh the Risks

This is the final concern in the physician's decision regarding administration. Currently, prophylactic antibiotics are clearly indicated for vaginal hysterectomy, cesarean section in the patient in labor, radial hysterectomy, and pregnancy termination. They are used on occasion for those patients requiring abdominal hysterectomy and for the patient needing tubal reconstructive surgery. For the patient who needs an elective cesarean section, more study is needed to confirm the appropriateness of prophylactic antibiotic use.

Conclusion

The above discussion represents the status of prophylactic antibiotics in obstetrics-

gynecology in 1989. Prophylactic antibiotic use has been a tremendous therapeutic advance for these agents have reduced the frequency and severity of post-operative infection. For vaginal hysterectomy, I favor the use of cefoxitin, cefotetan, or metronidazole for prophylaxis. In cesarean section, cefoxitin, cefotetan or mezlocillin give good results. For radical hysterectomy, either cefoxitin, cefotetan, mezlocillin or metronidazole is helpful. For pregnancy termination and reconstructive tubal surgery, doxycycline should be employed because of its effectiveness against *Chlamydia trachomatis*. For the elective cesarean section and abdominal hysterectomy, a first generation cephalosporin like cefazolin will probably suffice.

Infection Control in Intensive Care Settings

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Introduction

The life-saving technology of the modern intensive care unit (ICU) can be viewed as a double-edged sword. Invasive devices for monitoring of physiologic status and support of vital functions improve the survival rates of critically ill patients but, because they bypass normal host defense mechanisms, dramatically increase the risk of hospital-acquired infection. A recent study found that 28% of adult medical and surgical ICU patients developed at least one nosocomial infection (6), a substantially higher rate than patients in non-ICU areas of most hospitals. The mortality of nosocomial infection is also significant—for example, the mortality rate in patients who acquire gram-negative pneumonia while receiving mechanical ventilation may exceed 50% (5). This review will summarize current concepts of the epidemiology, pathogenesis, therapy and prevention of infections in intensive care unit patients.

Intensive Care Units

Approximately 10% of hospital beds in the United States are classified as providing intensive care: more than 95% of acute care hospitals have at least one ICU (1). Many larger hospitals have specialty ICUs to provide care directed at the needs of specific subsets of patients, such as premature neonates or those with burns, neurosurgical trauma, or myocardial infarction. All intensive care units are labor intensive (the ratio of nursing staff to patients in ICUs is usually close to 1:1) and staff members typically have frequent, repeated contact with patients.

In addition, ancillary service personnel (X-ray technologists, respiratory therapists, phlebotomists, etc.) may have multiple hands-on encounters with each patient per shift. This large number of contacts can facilitate transmission of infectious agents from staff to patient, between patients, or even from patient to staff member and underscores the need for washing hands between patients and observing barrier precautions when indicated.

Rates of ICU Infection

Rates and types of nosocomial infection in ICU patients differ strikingly depending upon the type of ICU studied. A preliminary report analyzing surveillance data collected between October, 1986 and May, 1988 at 39 hospitals participating in intensive care unit surveillance

under the National Nosocomial Infections Study or the U.S. Centers for Disease Control found a pooled infection rate for all types of ICUs of 24.4 infections per 1000 patient days (unpublished data). However, rates varied from a high of 47.1 infections per 1000 patient days in burn ICUs to a low of 16.5 infections per 1000 patient days in pediatric ICUs. There is also a wide variation in infection rates for each type of unit between institutions, which may reflect differences in patient populations. Other studies conducted in adult intensive care units with rates calculated as number of infections per 100 admissions have found rates of between 10.3% and 50.9%. It is difficult to calculate the excess cost of intensive care unit infections because of the confounding effect of the patient's underlying disease. The patients with the most severe underlying disease tend to have longer stays in ICUs. Nosocomial infection also tends to prolong hospital stay, but not all of this excess morbidity relates to the nosocomial infection itself (11). It seems reasonable to conclude, however, that intensive care unit infections contribute substantially to the $4 billion additional hospital charges generated by nosocomial infections in the U.S. alone (13).

Body sites involved by infections acquired in intensive care units vary according to the type of unit (Table I). When all infections were considered in the NNIS data previously cited, the most common site of infection was pneumonia (32.1%), followed by urinary tract infections (23.5%), blood stream infections related to intravascular devices (15.1%), surgical wound infection (8.9%), and miscellaneous other sites (20.4%). However, urinary tract infections constituted 34% of infections in neurosurgical ICUs versus 15% of the infections in pediatric ICUs. Blood stream infection secondary to catheters, by contrast, caused 27% of the pediatric ICU infections versus only 5% of the neurosurgical infections. Thus, the types of infection seen in different types of ICUs vary, probably reflecting underlying differences in the patient populations and the type of invasive devices used.

Table I. Distribution of Infections in ICU's by Major Site[1]

Type of Unit	Overall Infection Rate[2]	Percent of Infections by Body Site		
		Pneumonia	UTI	IV Catheter
Burn	47.1	31.1	17.8	21.1
Trauma	35.8	ND[3]	ND	ND
Surgical	35.0	34.9	21.2	14.1
Medical/Surgical	22.5	30.3	20.5	16.5
Medical	22.3	34.7	32.3	12.2
Respiratory	20.2	ND	ND	ND
Coronary	16.8	25.2	24.8	17.1
Neurosurgical	16.7	32.8	34.4	4.9
Pediatric	16.5	24.7	15.1	26.9
Overall	24.4	32.1	23.5	15.1

1 Adapted from Analysis of NNIS Surveillance Component Data, October 1986-May 1988 (see text)

2 Number of infections at all sites/total patient days × 1000

3 ND—data not given

Pathophysiologic Considerations in ICU Infections

The pathophysiology of intensive care unit infections involves a complex interaction

between the individual patient, his or her underlying disease, invasive devices, endogenous bacterial flora, and flora acquired exogenously from the environment or personnel of the ICU. Intensive care unit patients tend to be at extremes of age. Immunologic function may be immature in the premature neonate; cell-mediated immunity is well-known to wane with advancing age. The incidence of malignancy which may affect immune function primarily (e.g., lymphoma, leukemia) or secondarily (because of administration of cytotoxic chemotherapy or corticosteroids) also increases with age. Polymorphonuclear leukocytes, the first line of defense against most bacterial pathogens, may be reduced in number by the administration of chemotherapy. Lymphocyte function may be impaired by the effects of trauma or severe burns (15). Local mucosal defenses can also be dysfunctional in the elderly or severely ill patient. For example, pulmonary mucociliary clearance is diminished in the chronic smoker, predisposing to the development of lower respiratory infection. Numerous medications given in the hospital, particularly analgesics and sedative-hypnotics, may produce somnolence, increasing the likelihood of aspirating oropharyngeal secretions in the ICU patient who is not mechanically-ventilated. Even intubated patients may aspirate oropharyngeal or gastric contents around a cuffed endotracheal tube.

The predominance of gram-negative aerobic bacteria as etiologic agents of nosocomial pneumonia is believed to occur because the oropharyngeal flora of the hospitalized patient tends to shift from aerobic streptococci and other penicillin-sensitive organisms to the more antibiotic resistant gram-negatives of the fecal flora or environment. The process of gram-negative oropharyngeal colonization is clearly influenced by the administration of antimicrobial agents which destroy the susceptible oral flora, allowing overgrowth of resistant organisms to take place. However, colonization with gram-negatives can occur in the absence of antibiotic administration and appears to be more frequent in normal geriatric populations. One hypothesis is that conditions of stress cause degradation of fibronectin, a large molecular weight glycopeptide which coats the surface of the oral mucosa (26). Gram-positive organisms adhere well to this substance; gram-negatives, adhere poorly. In the absence of fibronectin, however, *Enterobacteriaceae* and other gram-negatives can adhere to the oral mucosa, initiating the process of gram-negative colonization.

The observation that surgical intensive care unit patients have less severe underlying chronic disease than medical ICU patients, yet have higher rates of infection, underscores the importance of devices and procedures as predisposing factors (6,21). We will briefly discuss the pathogenesis of the most common device-related infections in ICUs.

Ventilator-Associated Pneumonia

Pneumonia clearly occurs in patients not receiving mechanical ventilation, but the presence of an endotracheal tube increases the risk of pneumonia by fourfold (7). As discussed previously, the aspiration of oropharyngeal and/or gastric secretions into the tracheobronchial tree around the endotracheal tube is believed to be the initial step in a process which can culminate in pneumonia in the patient whose host defense mechanisms are insufficient to clear the bacteria in the aspirate. Local defenses, such as cough and the mucociliary escalator, are impeded by the presence of the foreign body. Inspired,

oxygenated air in the ventilator circuit must be warmed and humidified. Droplets of water ("condensate" or "rainout") accumulate in ventilator tubing and can support the growth of large numbers of bacteria (4). If proper care is not taken in positioning the patient, this material can reflux into the trachea, delivering a concentrate of bacteria directly to the lungs.

The presence of a nasogastric tube, used in mechanically-ventilated patients to prevent gastric dilatation, can increase the likelihood of aspiration by rendering the lower esophageal sphincter nonfunctional. It has been suggested that colonization of the stomach with gram-negative organisms may precede oropharyngeal colonization, at least in some patients (10). Craven et al. (5) found that treatment with cimetidine, an H2-receptor blocker which increases gastric pH, or antacids was a risk factor for the development of ventilator-associated pneumonia. These drugs are frequently given to ICU patients as prophylaxis against stress gastritis and hemorrhage. Increasing gastric pH by the use of antacids or H2 blockers appears to allow overgrowth of bacterial organisms in the normally sterile stomach, in some patients leading to retrograde colonization of the trachea, particularly in the presence of a nasogastric tube (10). The likely etiologic agents of ICU pneumonia are given in Table II.

Table II. Pathogens in Ventilator-Associated Pneumonia

Pathogens	Percent
Pseudomona aeruginosa	21.1
Staphylococcus aureus	16.9
Enterobacter spp	10.5
Serratia marcescens	6.6
Klebsiella pneumoniae	6.3
Hemophilus influenzae	5.5
Acinetobacter sp	4.3
Candida albicans	3.5
Escherichia coli	3.4
Proteus mirabilis	3.4

Adapted from Analysis of NNIS Surveillance Data (see text)

Infections Related to Intravascular Catheters

Intravascular catheters compromise host defenses by transecting the barriers of skin and subcutaneous tissue and providing a direct route into the bloodstream. The presence of the foreign body may diminish the bactericidal activity of neutrophils. Bacteria, particularly *Staphylococcus epidermidis*, also secrete an exopolysaccharide "slime" which can coat the device, facilitating adherence of the organisms and subsequent infection (9). Gram-positive cocci, particularly normal skin flora such as coagulase-negative staphylococci, are the major pathogens in intravenous catheter-related infection (Table III). Organisms may proliferate at the insertion site, along the course of the catheter, in the subcutaneous tunnel created by the line or in the wall of the catheterized vessel, creating septic thrombophlebitis. Diagnosis of infection may be difficult because inflammation may be absent at the insertion site. Maki and co-workers developed a semi-quantitative culture method (22) used by most hospitals

to culture intravascular catheters—the presence of greater than or equal to 15 colonies suggests actual colonization of the catheter rather than contamination by skin flora, but correlates poorly with the presence of systemic signs of catheter-related infection. Rarely, intravenous infusates are contaminated and produce bacteremia when administered. This should be suspected when uncommon species of bacteria, particularly aerobic gram-negatives, produce bacteremia in several patients without evidence of a primary site of infection (20).

Table III. Pathogens in Line-Associated Bloodstream Infections

Pathogens	Percent
Coag-neg staph	30.0
S. aureus	18.3
Strep D Ent	10.7
C. albicans	6.7
Enterobacter spp	6.1
P. aeruginosa	4.8
Other Strep spp	3.0
S. marcescens	2.2
Other *Candida* spp	2.2
Acinetobacter sp	2.0

Adapted from Analysis of NNIS Surveillance Component Data (see text)

Urinary Tract Infections

ICU patients require indwelling urinary catheters for many reasons — accurate measurement of urine flow, management of shock, control of incontinence secondary to altered consciousness, relief of urinary outflow obstruction, etc. A closed drainage system using plastic disposable drainage tubing and reservoir is used in modern hospitals. Urinary tract infection begins with irritation of bladder and urethral mucosa by the Foley catheter and its balloon. Garibaldi et al. (12) demonstrated that the major pathway of infection in the closed system is the extraluminal migration of bacteria in the periurethral space. The application of topical polymicrobial ointments to the urethral area has not been shown to decrease the frequency of urinary tract infections (13). In recent years, knowledge of the pathophysiology of catheter-related urinary tract infections has increased, but there have been no major advances in their prevention (17). The only practical way to diminish the frequency of these infections is to remove the catheter as soon as it is no longer necessary or to use intermittent or condom catheterization in the more stable patient. Common pathogens in ICU urinary tract infections are listed in Table IV.

Table IV. Pathogens Associated with Urinary Tract Infections

Pathogens	Percent
E. coli	17.4
C. albicans	15.2
P. aeruginosa	13.6
Strep D Ent	13.0
Enterobacter spp	6.6

K. pneumoniae	4.6
Other *Candida* spp	4.6
Other *Fungi*	4.1
P. mirabilis	3.6
Torulopsis spp	3.5

Adapted from Analysis of NNIS Surveillance Data (see text)

Miscellaneous ICU Infections

Many body sites other than those discussed in previous sections may become infected in the ICU patient. Nosocomial sinusitis, secondary to sinus obstruction by a nasotracheal or nasogastric tube has been recognized with increasing frequency (8, 16). Stasis of bile in the gall bladder may result in a calculous cholecystitis. Administration of broad spectrum antimicrobials which eliminate normal bacterial flora may allow for the overgrowth of *Clostridium difficile*, which causes a diarrheal syndrome of variable severity mediated by an exotoxin. This illness can produce significant morbidity in the severely ill, often marginally nourished ICU patient and can be transmitted nosocomially (23).

Diagnosis and Clinical Manifestations of ICU Infection

The clinical manifestations, diagnosis and therapy of ICU infections will be discussed only briefly. The diagnosis of these infections often relies upon a high index of suspicion, since some patients will not manifest fever or elevations of white blood cell count. Abnormalities of physiologic parameters measured by Swan-Ganz catheterization—e.g., low systemic vascular resistance with high cardiac output or so-called “septic parameters“—may be the only early clue to a significant nosocomial infection. Empiric antimicrobial therapy is frequently instituted before all diagnostic tests are obtained or results received because of the high morbidity associated with many of these infections. Initial antibiotic therapy must be broad enough in spectrum to cover the likely pathogens endemic to a specific unit or hospital. Support of vital functions, including blood pressure, respiration, acid-base and electrolyte metabolism, and nutrition is often crucial to the already ill ICU patient who develops an infection.

Transmission of Pathogens in ICUs

Multiple modes of transmission of bacterial, fungal, and viral pathogens exist in ICUs. Initial colonization with gram-negative organisms as described in previous sections, occurs from the patient’s endogenous gastrointestinal tract flora. Antibiotics play an important role in eliminating susceptible flora and allowing their replacement with more resistant bacterial organisms. The bacterial flora of an ICU may be endemic with rates of colonization by specific organisms dependent on length of hospital stay or epidemic. Multiple studies have demonstrated that the major route of transmission in both types of infections appears to be person to person on the hands of hospital personnel (21). Plasmids, circular pieces of DNA which may contain resistance genes to one or more antibiotics, can be transmitted between

bacterial species, usually in concert with the selection pressure of antibiotics resulting in epidemic outbreaks of antimicrobial resistance or prolonged endemic occurrences of resistance (24). Other mechanisms of resistance besides person to person spread may operate in ICUs, such as common source epidemics (usually due to contaminated materials), airborne droplet spread (e.g., nosocomial influenza or respiratory syncytial virus), or percutaneous (accidental needlestick injury to health care worker).

Prevention and Control of Nosocomial Infections

Meticulous hand washing between patient contacts remains the cornerstone of infection control in hospitals, particularly in ICUs because of the frequency of patient contact. Many studies have shown control of both epidemic and endemic infection with handwashing (18). Barrier precautions (masks, gown, gloves) are frequently used to control spread of resistant organisms and a recent study has suggested they reduce overall infection rates (14). Proper sterilization and disinfection of equipment and changing equipment at specified intervals (e.g., ventilator tubing, intravenous catheters, and arterial lines) appears to favorably influence colonization rates, which should lower rates of infection. Removing devices when they are no longer necessary continues to be paramount, but the future may hold better devices, for example those impregnated with antibiotics, which could be safely left in place for long periods of time (21).

Although the administration of prophylactic antibiotics to prevent nosocomial infection is not practiced in most American intensive care units, selective intestinal decontamination has become popular in European countries (2, 19, 25). Although definitive proof of the efficacy of this practice is lacking, preliminary trials suggest that rates of both endemic and epidemic infection with gram-negative organisms can be lowered. Further work should concentrate on identifying subsets of patients in whom this technique would accrue the most benefit.

Modern molecular immunology offers the potential for bolstering host defenses by actively immunizing against major nosocomial pathogens, by prophylactic passive immunization with high potency antibody preparations, and by use of immune modulators. Improved knowledge of factors mediating attachment of pathogens to host sites and the elaboration of microbial factors involved in pathogenesis offer other targets for preventive steps aimed at reducing colonization or inhibiting pathogenesis factors (without killing the microbe). The next major advancements in the prevention of ICU infections are more likely to occur in these areas than by the development of new antibiotics or new hospital infection control measures.

Therapy

In recent years the ability of pathogens to develop antimicrobial resistance has been matched by the addition of new and more effective antimicrobial drugs. The quinolones and the newer beta lactams have led the way but gains have also been noted in antifungal and antiviral therapy. The armamentarium of antimicrobial drugs is more powerful than ever before and yet the mortality of ICU-related infections remains high. The recent expansion of

knowledge regarding the role of monokines (e.g., tumor necrosis factor) and other mediators of “septic shock” give promise to new potential therapeutic measures to ameliorate the clinical consequences of bacteremia.

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Infection Prophylaxis After Successful Organ Transplantation[1,2]

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Despite marked improvements in the overall results of renal transplantation over the past three decades, infection remains an important cause of morbidity and mortality (20). Over a four-year period ending in 1981, during which 518 renal transplants were performed at the University of Minnesota Hospitals, 32% of the patients required hospitalization for treatment of infectious complications. Infections resulted in death in 6% of these patients. Cytomegalovirus (CMV) was clearly predominant among the various pathogens, causing 50% of infectious episodes; bacterial infections of the lower respiratory and urinary tracts, as well as soft tissue, skin, and bone infections in diabetic extremities, were also attended by significant morbidity and mortality. Fungal infections by *Aspergillus* and *Candida*, and to a lesser extent *Cryptococcus, Histoplasma*, and *Mucormycosis*, resulted in occasional serious posttransplant problems. Rare in this report, but frequently reported, are other transplantation-associated pathogens including *Nocardia asteroides*, (28) *Streptococcus pneumoniae*, (4) *Listeria monocytogenes*, (29) *Legionella*, (19) *mycobacteria*, (18) *Pneumocystis carinii*, (16) and *Toxoplasma gondii* (25).

A wide variety of approaches have been undertaken to prevent infectious complications after transplantation (Table I); however, there are few prospective blinded, randomized trials examining the efficacies of each of these approaches. This review briefly describes those methods of prophylaxis that have been proved to be effective, or may possibly be effective, in the prevention of infection in renal transplant recipients.

Prophylaxis Against Bacteria

One of the most extensively studied antibacterial prophylactic measures has been the routine use of TMP-SMZ after transplantation. TMP-SMZ has been shown to reduce infection rates in granulocytopenic leukemics (6) and other neutropenic oncology patients. Its use in solid organ transplantation remained empiric until Tolkoff-Rubin et al. (10) demonstrated in a randomized prospective trial that one double-strength TMP-SMZ tablet daily was associated with a fourfold decrease in the incidence of urinary tract infections. In a more recent double-blinded, randomized, prospective trial in 132 renal transplant recipients, Fox et al. (8) reported that two double-strength TMP-SMZ tablets (320/1,600 mg) daily:

- significantly reduced hospital days with fever;
- resulted in fewer bacterial infections after the removal of the bladder catheter; and
- reduced the incidence of infection after discharge from the hospital.

TABLE I. Antimicrobial Prophylaxis Protocols—University of Minnesota

	Proven Benefit	Controversial
Bacterial		
Urinary tract	TMP-SMZ (oral) (8, 33) Bladder irrigations (17)	—
Wound	Systemic antibiotics intraoperatively (15, 17, 31) Topical irrigations (26)	—
Bacteremia	TMP-SMZ (oral) (8) Pneumococcal vaccine for splenectomy patients (7)	—
Viral		
CMV	Donor selection (20) Blood product selection	Towne vaccine (2) Acyclovir (10,27) Interferon Hyperimmune globulin
HSV	Acyclovir (10, 11, 21, 27, 30)	—
VZV	Acyclovir (30) Vaccine	—
Hepatitis	Vaccine	—
Fungal		
Aspergillus	HEPA filter (20)	—
Candida	Nystatin	—

Abbreviations: TMP-SMZ, trimethoprim-sulfamethoxazole; HSV, herpes simplex virus; VZV varicella-zoster virus; HEPA, high-efficiency particulate air.

Furthermore, fewer urinary tract infections were observed in the treated group once the catheter was removed, and fewer bacteremic episodes occurred, including those due to Gram-negative enteric organisms, enterococci, and *Staphylococcus aureus*. There was no appreciable increase in colonization with TMP-SMZ-resistant organisms in the TMP-SMZ group. *Candida* colonization was less frequent in the TMP-SMZ group, probably reflecting less frequent broad-spectrum antibiotic usage in that group. Centers using TMP-SMZ have also enjoyed lower incidences of *P. carinii* infections, *Nocardia* infections and Listeria-related infections (8, 13, 20, 24).

Wound infection rates as high as 36% have been reported in the absence of systemic antibiotic prophylaxis at the time of kidney transplantation (15). Clearly, reduction in wound infections has been one of the factors responsible for the improvement in the results of transplantation (32). Although improvements in technique have had an important role in preventing wound infections, intraoperative systemic and topical antibiotics have been shown to reduce wound infection rates to approximately 1% (15, 17, 26, 31). The protective effects of intraoperative antibiotics, however, are lost when Penrose drains are left in the wound (15). The frequent finding of identical organisms in both infected urine and wounds suggest that the urinary tract may be an important source for nonstaphylococcal wound infection (17). To this end, bladder irrigation with antibiotic solution may reduce the incidence of wound infection, but a randomized study has not been carried out.

As mentioned above, bacteremic episodes have been reduced with oral TMP-SMZ. For patients undergoing splenectomy prior to transplantation, pneumococcal vaccination has also been demonstrated to reduce pneumococcal sepsis (17).

Prophylaxis Against Cytomegalovirus

Human cytomegalovirus remains the leading cause of infectious complications after renal transplantation (20). After renal transplantation at the University of Minnesota Hospital and Clinic (UMHS) during the year 1985, 50% of recipients shed CMV, while 18% demonstrated overt CMV disease (H. Balfour, personal communication, 1987). The substantial morbidity and mortality (20, 34) resulting from CMV infection, particularly in association with other pathogens, call for an effective means of preventing this posttransplant complication.

Three routes of CMV infection may occur after transplantation. A primary infection may occur in previously uninfected recipients after inoculation of virus, either from the graft (14) or from the transfusion of infected blood products in the peritrans-plant period (23). Reactivation may occur when a previously infected patient harboring latent virus is immunosuppressed after transplantation (1). The typical syndrome of fever, malaise, and arthralgia may occur associated with pneumonitis, encephalitis, hepatitis, gastrointestinal bleeding, retinitis, leukopenia, possible graft loss, and death (20). Reactivation infection is by far the most common form. More recently, restriction endonuclease analysis has demonstrated a route of infection whereby recipients with previous CMV infection experience superinfection with a different strain of CMV acquired from the graft or blood products (12).

The most effective means of preventing primary CMV infection is donor selection (34). Of 20 seronegative recipients receiving seropositive donor kidneys, 12 sero-converted within four months. Others have quoted 65% to 75% primary infection rates after seropositive donor kidney transplantations (3). Primary infections of seronegative recipients of seronegative donor organs are rare.

Active vaccination trials using the attenuated Towne strain demonstrated T and B cell responses to the vaccine in normal and uremic patients (22). The immune responsiveness to the vaccine persisted in most patients after transplantation (2). In the most recent update of the double-blinded randomized trial of the vaccine, benefit was seen only in previously seronegative patients who received a seropositive kidney after vaccination. Although there was no difference in the overall rate of infection (38% in vaccine recipients vs. 43% in placebo recipients) the severity of disease was less in the vaccine group, although not to a statistically significant extent. In 1983, Merck Sharp & Dohme discontinued support for these clinical trials, and this vaccine is no longer available. New vaccines developed by vascular closing are currently being studied (9).

Passive immunoprophylaxis using CMV hyperimmune globulin has been effectively used in bone marrow transplantation (5) but it has not been adequately tested in renal transplantation.

Chemoprophylaxis of CMV and other herpesvirus infections has been the focus of many recent investigations (10, 11, 21, 27, 30). It is clear that the incidence of HSV reactivation is suppressed by prophylactic oral acyclovir (11, 21, 27, 30). Similarly, VZV is also suppressed by oral, low-dose acyclovir therapy (21, 30). In one study, the incidence of CMV infection was not different in either group. However, Gluckman et al. found acyclovir effective in preventing CMV infections when given orally to bone marrow transplant patients

(10).

A preliminary double-blinded, placebo-controlled study at UMHC in 47 CMV seropositive bone marrow transplant candidates demonstrated that intravenous acyclovir at 500 mg/m^2/d for 35 days did not suppress reactivation; however, the time to reactivation was prolonged by acyclovir. No CMV disease was seen during the treatment period (H. Balfour, personal communication, 1987). Although low-dose oral acyclovir is capable of suppressing HSV and VZV reactivation, a different approach must be used to prevent CMV reactivation. An acyclovir derivative 9(2-hydroxy-1-[hydroxymethyl]ethoxymethyl)-guanine (ganciclovir, DHPG) is under investigation for the treatment and prophylaxis of CMV infection. DHPG has greater *in vitro* effect against wild strains of CMV. It is hoped that this agent may provide some protection against CMV infections.

Prophylaxis Against Fungi

The most common primary fungal disease at UMHC has been aspergillosis, which accounted for five of seven primary fungal infections in 518 consecutive transplant patients (20). The incidence of aspergillosis was dramatically reduced by the installation of HEPA filtration units at our institution.

In this series of 518 patients, primary *Candida* infection was never identified (20), but *Candida* infections were frequently found in association with polymicrobial infections. We currently prevent overgrowth of *Candida* in the oropharynx and the gastrointestinal tract with topical nystatin. Although not proved by randomized trials, a strong clinical impression exists that thrush or *Candida* esophagitis can be effectively prevented in this way.

Summary

All of the antimicrobial protocols currently used at UMHC are listed in Table II.

TABLE II. Antimicrobial Prophylaxis Regimens - University of Minnesota

Recipient	Donor
Evaluation	
Hepatitis profile	
VDRL + HIV	
Urine culture	
Voiding cystourethrogram	
Viral titer — CMV, VZV, HSV	
Throat culture — CMV, HSV	
Chest x-ray	Evaluation — cadaver donor
Upper gastrointestinal series	Systemic infection
Oral cholecystogram	Local infection involving organ
Dental evaluation	Urinalysis and culture
Gynecologic exam with viral cultures	Chest x-ray
Penicillin for splenectomized patients	VDRL
Heal all skin lesions	HIV
?Evaluation for TBC	Hepatitis screen
Perioperative prophylaxis	Evaluation — living donor
Clip (not shave) hair	Hepatitis screen
Bladder culture and 0.1% cephalothin or 0.25% neomycin	CMV titer
Intravenous antibiotics on induction of anesthesia	VDRL

— Cefamandole 2 g
Topical antibiotics in all irrigation solutions

Postoperative
Required
TMP-SMZ for life
Penicillin VK
For TLI and splenectomized patients
Children for life
Nystatin orally
3 month nondiabetics
For life, diabetics
Optional
Acyclovir
Hyperimmune CMV globulin

HIV
Urinalysis and culture
Chest x-ray
Intravenous Pyelogram

Intraoperative
Betadine scrub of surgical area
Systemic antibiotics on induction of anesthesia

Abbreviations: HIV, human immunodeficiency virus

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Microbiological Aspects of Nosocomial Infections in Obstetric Hospitals

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One of the most important aspects of the problem of nosocomial infections is a pyoseptic pathology in infants in the first month of life. Since the formation of a child's microbioceonosis (indigenous microflora) begins within the first hours of life, diseases caused by opportunistic pathogens are likely to be associated, at least in part, with the stay in obstetric hospitals. In this regard, our research sought to establish the importance of bacterial colonization of newborns in obstetric hospitals, and to study the role of hospital infection sources in the development of pyoseptic pathology in infants in the first month of life.

The study was conducted from 1985 to 1988 in Moscow obstetric hospitals. The average period of infants' stay in an obstetric hospital from birth was 5 to 6 days. Infants, their mothers, medical staff, and environmental objects were subjected to microbiological survey. Infants and mothers were examined on the first and fifth day following birth. Washes from environmental objects in the maternity and pediatric departments, as well as air samples from these locations, were taken. Furthermore, infection foci microflora, in the case of localized forms, and blood, in the case of sepsis or suspected sepsis, were studied for determination of etiologic structure of infectious diseases. Isolation and identification of microorganisms and study of their biological properties were conducted by accepted methods. Determination of gram-negative bacteria species was conducted with the help of API-20E; antibiotic sensitivity was determined with the help of ATB for gram-negative bacteria and staphylococci. Tripticase-soy agar with 5% sheep blood was used for streptococci B isolation.

At the conclusion of the study, 78 infants, 77 mothers, and 120 employees from the obstetric hospitals of the centralized type (where multiple infants were maintained in a nursery) and 91 infants, 54 mothers, and 150 employees from the obstetric hospitals of the decentralized type (where infants were kept with their mothers) were examined.

The analysis of morbidity with infectious diseases among infants born in the surveyed hospitals during the first month of life demonstrated the following findings. The incidences in centralized and decentralized hospitals were comparable—an average of 53.6 and 48.8 cases, respectively, per 1000 newborns. The infectious disease structure study demonstrated similarity in the distribution of various nosoforms. Pyoderms, conjunctivitis, omphalitis, enterocolitis, phlegmons, and abscesses were the most prevalent forms observed in both hospital types.

Taking into consideration that conjunctivitis and omphalitis are among the most prevalent

infections observed among newborns in this study, we paid special attention to the state of the umbilical wound and conjunctiva as loci most vulnerable to infection. As demonstrated by our studies, *Staphylococcus aureus* and representatives of enterobacteriaceae are the leading pathogens associated with these diseases. We compared the morbidity with omphalitis, conjunctivitis, and enterocolitis in infants colonized and not colonized with these bacteria in the appropriate loci on the fifth day of life. According to our data, morbidity with omphalitis in infants with *S. aureus* colonies on the surface of the umbilical wound was 43.2%; no omphalitis cases were registered in infants not colonized with this microorganism. Enterobacteria were noted in 57.1% of omphalitis cases; in 22.5% of omphalitis cases, these microorganisms were not detected on umbilical wounds. Conjunctivitis developed in 48.4% of infants colonized with *S. aureus* and in 10.5% of infants with non-colonized conjunctiva.

Thus, these results indicate that population of the umbilical stump or conjunctiva with *S. aureus* or representatives of the Enterobacteriaceae family during hospitalization following birth predispose an infant to the subsequent development of omphalitis and/or conjunctivitis, the most prevalent forms of infectious disease. It follows that permanent surveillance of bacterial colonization peculiarities allows both detection of infectious disease risk groups, and the timely conduct of necessary preventive measures for infection. One of the preventive anti-infectious disease measures in newborns is the introduction of cohabitation rooms in obstetric hospitals where mother and infant stay together. This limits the contact with hospital microflora.

We compared the processes of microbial colonization of newborns in hospitals with centralized and decentralized systems of service (Figure 1). The results of the microbiological study of nasopharyngeal mucosa, eye conjunctiva, and umbilical wounds in infants on the fifth day of life testify to the pronounced prevalence of *S. aureus* in all of these loci in infants from obstetric hospitals of the centralized nursery type (1.5-6 times higher), the quantitative characteristic of microbial presence being 2-3 orders of magnitude higher. The incidence and level of colonization of nasal mucosa with gram-negative bacilli were virtually identical in both groups. *Escherichia coli* on pharyngeal mucosa were found more than twice as seldom in infants from the decentralized or “mother-infant cohabitation” obstetric hospitals than in children from obstetric hospitals of the centralized nursery type. *Klebsiella pneumoniae*, on the other hand, were observed in 14.3% of cases from decentralized hospitals but in only 5% of all infants examined from centralized hospitals. Coagulase-negative staphylococci were found with the same frequency and in the same quantity in infants from both types of obstetric hospitals. Thus, pathological colonization is found both in an obstetric hospital where mother and infant are kept together and in an obstetric hospital of a centralized nursery type. Representatives of the Enterobacteriaceae family colonize primarily one or rarely two loci. *S. aureus* is characterized by a disseminated and intensive colonization—three or four loci.

Summing up the data on colonization of newborns, the advantages of the system where mothers and children stay together must be noted. In these cases, *S. aureus* circulation was lower, although the same pattern of results was not seen for other bacteria.

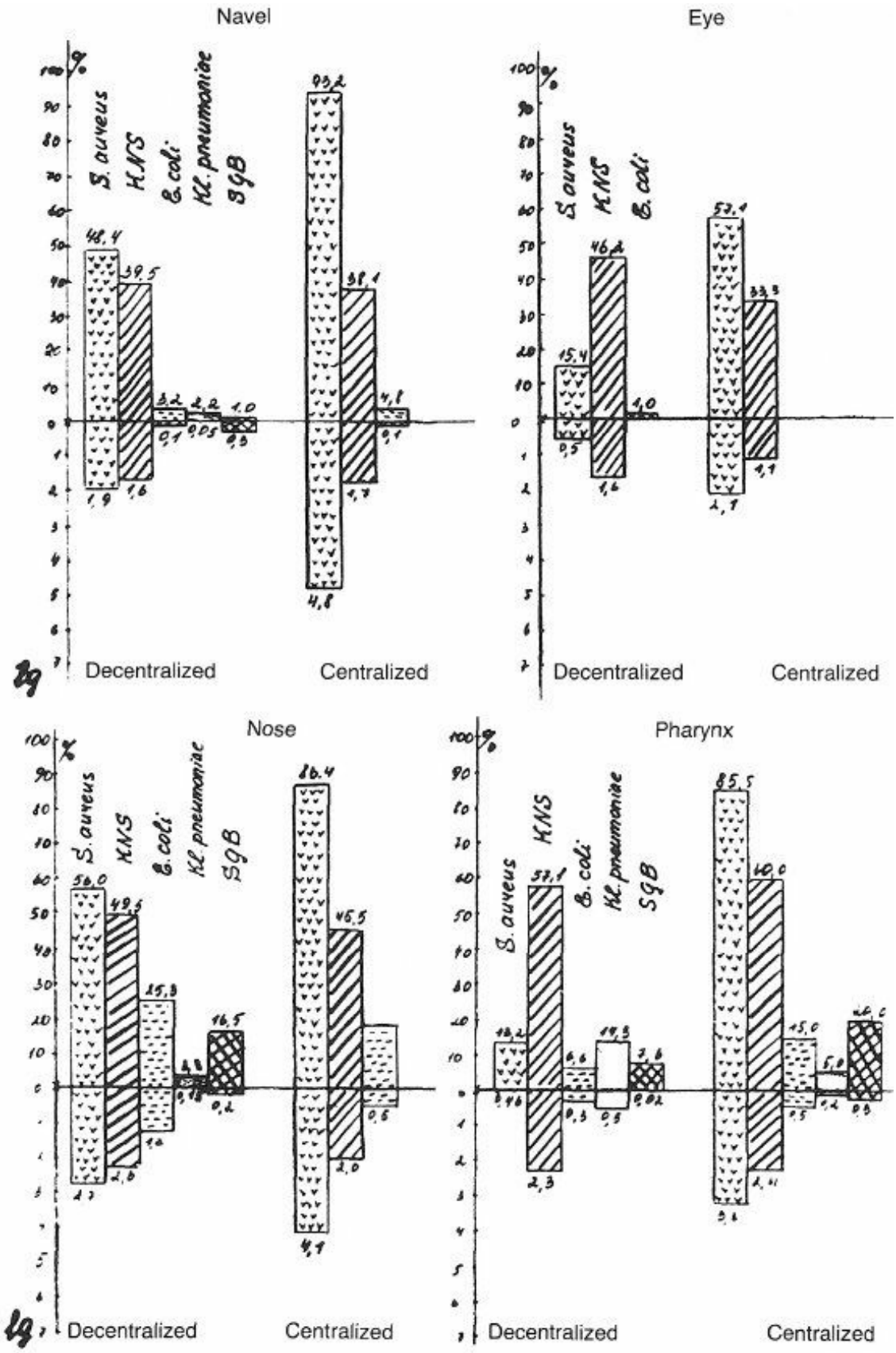

Figure 1. Microbial Contamination of Newborns in Centralized and Decentralized Hospitals

Microbiological study of all infectious disease cases developed in infants from both types of obstetric hospitals during the neonatal period made it possible to determine the infectious disease etiologic structure in each obstetric hospital. In reviewing the analyses of the etiologic structure on the whole, it must be noted that almost in half of the cases, gram-

negative bacteria of the Enterobacteriaceae family were isolated from the infection foci. *E. coli* (27.8%) was the most prevalent, while *K. pneumoniae* (15.6%) was observed almost two times less frequently and was isolated mainly from the intestine. Staphylococci were isolated from one-third of the patients, the predominant form being *S. aureus* (29.2%). This microorganism, more often than others, caused omphalitis, conjunctivitis, and profound lesions of the skin and subcutaneous fat. Coagulase-negative staphylococci were isolated in 8.3% of cases (massive presence in monoculture on conjunctiva, as well as in single cases of omphalitis, pyodermia, and sepsis). *Streptococcus faecalis, Pseudomonas aeruginosa, Candida albicans, Proteus*, and group B streptococci were only rarely isolated. Enterocolitis was primarily associated with gram-negative bacilli, found as a rule in a monoculture, while bifidobacteria and enterococci were not present or their number abruptly decreased.

When comparing the infectious disease etiologic structure with peculiarities of newborns' bacterial colonization characteristics of each of the obstetric hospitals under surveillance, one pays attention to the following. *E. coli* and *K. pneumoniae* were the leading infectious disease nosoforms; conjunctivitis and omphalitis were associated with these bacteria in more than half of the cases. A different pattern was seen for obstetric hospitals of the centralized type. *S. aureus* was the prevailing etiologic factor for these nosoforms. *S. aureus* was detected in virtually all infants from obstetric hospitals of the centralized type with these nosoforms and in three to four loci. In the "mother-infant" decentralized obstetric hospitals, *S. aureus* was detected in only 13.2%-56% of the examined infants with a lower (2-6 times) level of loci contamination. The conjunctiva colonization with representatives of enterobacteriaceae was not observed in the obstetric hospitals of the centralized type; conjunctivitis cases associated with these pathogens were not detected either.

The study of biological properties of the strains isolated upon discharge from the hospital and from purulent foci demonstrated their identity with respect to phagotype characteristics and sensitivity to antibiotics.

Thus, it was shown that infectious diseases among newborns have different etiologies, the leading roles belonging to *S. aureus* and representatives of enterobacteriaceae. The relationship between the infectious disease etiologic structure and the peculiarities of newborns' bacterial colonization was established. The identity of the bacterial strains' biological characteristics, as well as the early development of frequently observed nosoforms, testify to the importance of microorganism colonization in the development of pathological processes among newborns during a stay in obstetric hospitals.

Prevention of Infection in Contaminated and Uncontaminated Surgical Wounds

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Whenever a surgical wound discharges pus, it is not only a reflection on the surgeon, but also an added burden on the patient and on the costs of the health service. Although rare, if the infection extends deeper than the subcutaneous fat, it can be a disaster. These deep infections not only destroy the local tissues, but they can also cause severe illness, culminating in sepsis and even death.

The occurrence of infection was thought to be reflective of the number and virulence of contaminating bacteria versus the host resistance. Recent elucidation of the actions of the cytokines liberated from activated macrophages and other cells has altered our understanding, and we now consider infection to represent the balance between bacterial contamination plus host response versus host defenses.

Bacterial contamination of surgical wounds usually occurs in the operating room, resulting in a *primary* wound infection, but we must not overlook the fact that contamination is possible in the postoperative period if the wound does not remain dry. It is impossible to contaminate a dry closed surgical wound after the first few hours even if the dressing is deliberately contaminated with virulent bacteria (22). If, however, the wound discharges fluid, it becomes susceptible to bacterial invasion, which causes *secondary* infection. The distinction between primary (operating-room-acquired) and secondary (ward-acquired) infection is important because many of the principles of prevention do not apply to both types of infection. This is particularly true of prophylactic antibiotics, which do not play a part in the prevention of secondary infection.

There are five aspects to the prevention of infection in surgical wounds. They are:

— prevention of exogenous contamination;
— prevention of endogenous contamination;
— prevention of infection if contamination is inevitable;
— prevention of sepsis if infection is inevitable; and
— prevention of death if sepsis is inevitable.

Prevention of Exogenous Contamination in the Operating Room

Everything that comes in contact with the surgical wound must be as sterile as possible; above all, no virulent bacteria must be allowed to contaminate the wound. This means that measures must be taken to sterilize the instruments (including drapes, swabs, sutures, and any implanted material), the hands of the surgical team, the skin of the patient, and the air of the operating room.

Sterilization of Instruments

Instruments made of metal, cotton, and most synthetic materials such as nylon and other polyesters are most efficiently sterilized by the combination of heat and moisture which is produced in an autoclave. Modern autoclaves are guaranteed to render all these materials sterile, and modern packaging in paper ensures that they are delivered sterile to the operating room.

The sterilization of instruments that do not tolerate heat is a different matter. Many disposable instruments are packed in paper and then sterilized by ionizing irradiation. The outside of the paper will be contaminated in transit, but the contents remain sterile. Some of the more robust syringes and polyester prostheses can be resterilized by autoclaving. Resterilization by irradiation is not a practical proposition for two reasons. The first is logistical; it is not practicable for hospitals to have their own machinery. The second is physical; heat-sensitive materials can become brittle and unusable after repeated irradiation.

The alternatives for sterilization and resterilization of heat-intolerant instruments that are too costly to be discarded after a single use are exposure to ethylene oxide, activated glutaraldehyde, or low temperature glow-discharge plasma. The merits, defects, and dangers of these methods are described in other chapters in this book. What is not in doubt is that in all cases, the essential preliminary step before sterilization, particularly of flexible endoscopes, is meticulous decontamination. In simple words, an instrument must be clean before it can be sterilized.

Sterilization of Hands and Skin

It is impossible to remove all bacteria from the hands of the operating team or from the skin of the patient. The aim is to reduce their number and to eliminate any that are sufficiently virulent to cause wound infection.

Long before bacteria were recognized as the cause of infections, Ignac Semmelweis in Vienna showed that when accoucheurs dipped their hands in chloride of lime before attending women in labor, the incidence of puerperal (streptococcal) sepsis was significantly reduced—from 11.4% in 1846 to 1.3% in 1848 (16). William Halsted persuaded the Goodyear Rubber Company to produce surgical gloves to protect his operating room nurse from dermatitis caused by corrosive sublimate, not to prevent bacterial contamination of surgical wounds (18). Nowadays, it is customary for the members of an operating team to decontaminate their hands by washing in running water with detergent containing either povidone iodine or chlorhexidine, and then to immerse their hands in 70% alcohol or alcoholic chlorhexidine. Surgical gloves [which can be coated with the antiseptic cetylpyridinium chloride (13)] are worn for the protection of the surgeon, not of the patient.

The skin of the patient at the site of the surgical incision is decontaminated by application of an alcoholic solution of an iodophore or Chlorhexidine. Three additional measures have been advocated, only one which has proven value: the skin at the site of the incision should not be shaved on the day before operation (5). A measure of questionable value required

patients to bath with Chlorhexidine detergent before their operations and reduced the incidence of groin wound infection in clean vascular operations from 17.5% to 8% (3) and from 10% to 7% (8). These results could not be duplicated by Ayliffe et al. (1) in a study of 5,536 operations or by an international multicenter trial in 2,813 clean operations (4). In both these trials the control rates of wound infection were much lower than in the two trials that showed benefit from the bathing. The third additional measure, covering the skin at the site of incision with an adhesive plastic drape, either plain or incorporating an iodophore, has been shown not to be of any value (9).

Sterilization of Air

It was the belief that aerial bacterial contamination was an important cause of surgical wound infection that led Joseph Lister to use a carbolic acid spray during operations. This fell into disfavor when the importance of sterilization of instruments and skin was appreciated. From time to time, however, outbreaks of wound infections occurred that were traced to movements of contaminated air from adjoining wards into the operating room. These infections no longer occur since the universal adoption of plenum ventilation in operating rooms. The only aerial contamination that continues is caused by the shedding of skin squames that carry bacteria by the people moving about in the operating room. Three rules should always be observed: no one with an active skin infection (usually caused by *Staphylococcus aureus*) should be allowed to enter the operating room; as few people as possible should be in the operating room; and those who are there should move about as little as possible.

Even if these conditions are observed, however, settle plates or slit samplers will reveal aerial bacterial contamination (mainly by *S. epidermidis* and other resident skin flora) of about 160 colony forming units in each cubic meter of air (11). John Charnley was the pioneer in Britain of measures to reduce this hazard by vertical laminar flow ventilation and by the wearing of exhaust ventilated impervious clothing by everyone in the operating room. Laminar flow ventilation reduced both the number of bacteria in each cubic meter of air to six (11) and (in a multicenter trial in 8,055 operations to replace diseased joints by prostheses) the deep wound infection rate from 3.4% to 1.2% (10).

Prevention of Exogenous Contamination in the Wards

Closed surgical wounds that remain dry are immune to invasion by bacteria (22). It is the discharging wound that is in jeopardy. The classical example is a burn, but the same principles of prophylaxis apply to surgical wounds that discharge blood, serum, lymph, or any other fluid. Such wounds must be covered by sterile dressings and urgent steps must be taken to dry the wound.

Hands are the most important purveyors of bacteria from one ward patient to another, and it should be routine practice for all attendants to clean their hands after touching any patient. If there is no gross contamination, application of alcoholic Chlorhexidine is sufficient. Otherwise, the hands must first be washed with a Chlorhexidine or povidone iodine

detergent under running water and dried on a disposable paper towel before application of the alcoholic Chlorhexidine. In certain circumstances, particularly for patients whose immune system is compromised by disease or treatment, complete isolation of the patient in a transparent tent supplied with air through a High Efficiency Particulate Air (HEPA) filter is necessary to prevent exogenous contamination.

Prevention of Endogenous Contamination in the Operating Room

Whenever infected tissue or a hollow viscus is incised, it is likely that bacteria will be liberated to contaminate the wound even if there is no overt spillage. This is particularly true of colon operations, but applies to most abdominal and many non-abdominal operations. Apart from careful surgical technique that avoids gross contamination, two other steps should be taken. The viscus should be empty, and its bacterial burden should be reduced by appropriate use of antimicrobial substances.

The stomach, bladder, and colon can be emptied in most cases, and the choice of methods is wide. One way of emptying the colon that causes little discomfort to the patient is the prescription of mannitol 100 g in a liter of tap water to be drunk rapidly and followed by copious drinking of clear fluids (up to 3 liters). As for reduction of the bacterial burden at the site of operation, many options are open. The vagina and rectum can be washed out with aqueous povidone iodine and the colon can be rendered less contaminated by a short course (not more than 24 hours) of an oral aminoglycoside and either erythromycin base or metronidazole (14).

Prevention of Endogenous Contamination in the Postoperative Period

Surgical wounds can become contaminated after the operation by leakage from an internal suture line that allows infective material to gain access to the wound. Its prevention depends first and foremost on good surgical technique. The second possibility is that bacteria may be carried by the blood stream from a distant focus of infection. This is rare, but may explain some deep infections after total arthroplasty operations. It is, therefore, unwise to undertake clean operations in the presence of distant infections or to undertake a clean operation soon after a contaminated operation.

Prevention of Infection if Contamination is Inevitable

Prophylactic antibiotics, of which one preoperative dose and certainly not more than two postoperative doses should be given, are essential when endogenous contamination of the surgical wound is likely, or when exogenous contamination could result in disastrous infection such as during clean operations to insert prostheses. The choice of antibiotic must be dictated by the probable nature of the contaminating bacteria and by the results of random controlled clinical trials. The intravenous route of administration is most convenient and is under the direct control of the surgeon if the antibiotic is given immediately before induction of anesthesia. There are theoretical grounds for believing that antibiotics instilled

into the wound before closure might be more successful since the concentration of antibiotic is higher. A controlled clinical trial, however, failed to show any clinical benefit (7).

Preoperative intraincisional injection of an antibiotic was introduced by Taylor et al. (23). The cefoxitin solution was injected along the line of the proposed incision with a spinal needle. Four of 91 patients so treated developed wound infections, compared with 15 of 91 controls given no antibiotic. This technique was compared in a random controlled trial in 624 patients undergoing abdominal operations with the same dose of the same antibiotic (amoxycillin-clavulanate) given intravenously (17). The incidence of major wound infections was 3% in the intraincisional group compared with 5% in the intravenous group, and the total wound infection rates were 8% and 16%, respectively.

When heavy parietal contamination occurs during abdominal operations, (e.g., if pus is encountered), the skin should not be closed primarily, but the wound left open either to heal by granulation or to be closed five or six days later if it is clean.

Antiseptics have no place in the prevention of infection in contaminated wounds. This was recognized by Alexander Fleming in 1920 (6) and reiterated by Mont Reid, who, in 1936, wrote: “In order to worship at the shrine of Pare we must be rid of our bondage to advertising pharmaceutical concerns which delude our profession into the belief that the one essential aid to wound healing is the killing of germs with agents which they do not tell us are also killers of countless invisible delicate living cells” (19).

In that same paper, Reid wrote: “In the performance of identical operations and with presumably the same bacterial contamination, one surgeon may get an infected wound while the other will get primary healing”. This still holds true more than 50 years later. The additional factors that determine the transition from contamination to infection are partly technical—good surgical technique—and partly attributable to the patient. It is rare for clean operations in the upper half of the body to become infected, but not nearly so rare in the lower half. This is probably a reflection of the superiority of the blood supply to the head, neck, arms, and chest wall. Surgical wounds in fat young people are more likely to become infected than those in thin ones, and wound infections in old people are more likely to be major (15). Infections are more likely to occur in the wound of a patient whose immune system is compromised by disease or treatment, and in one whose tissues are scarred and relatively avascular as a result of previous radiotherapy.

Prevention of Sepsis if Infection is Inevitable

Sepsis is a clinical syndrome often, but not always, accompanied by bacteremia and usually associated with an uncontrolled focus of infection. The syndrome is characterized by fever, cardiovascular changes (increased cardiac output and reduced systemic vascular resistance in the early stages, followed by “shock”—low blood pressure, reduced cardiac output, and increased vascular resistance), and progressive failure of organ systems.

Sepsis is certainly precipitated by endotoxins and exotoxins, but there is increasing evidence that the effects are mediated by the host response to these toxins. The cytokine that has attracted most notice is tumor necrosis factor (cachectin), which is produced mainly by activated macrophages and which not only damages microvascular endothelium,

but is the principal activator of neutrophils. Neutrophils in turn release toxic oxygen radicals and proteolytic enzymes (12). These products cause disintegration of capillary endothelium, escape of fluid into the tissues, vascular thrombosis, and ischemia. The usual sequence of organ system failure is pulmonary (adult respiratory distress syndrome), cerebral (clouding of consciousness), renal, hepatic, gastrointestinal (bleeding and translocation of bacteria and endotoxin from the gut), and hematological (diffuse intravascular coagulation).

The prevention of sepsis depends on the intelligent use of antibiotics appropriate to the infection, the urgent search for, and elimination of, sites of infection (including high guillotine amputation if the sepsis originates in clostridial myonecrosis in the lower limb), and possibly —in the future—the use of monoclonal antibodies against tumor necrosis factor. High dose steroids are useless (2), and the place of standard or hyperimmune immunoglobulins has not been established.

Prevention of Death if Sepsis is Inevitable

The mortality of established sepsis is in the region of 70%. “In [septic] shock, the portal of death yawns hungrily, and to snatch back a life we must be appropriately aggressive and say instead that the patient is too sick not to be operated on” (24). Apart from the vital need to identify and operate on foci of infection, the treatment of sepsis is at present merely supportive: pulmonary ventilation to maintain a high oxygen tension in the arterial blood; intravenous fluids to maintain blood volume; inotropic agents, of which the most promising is enoximone; peritoneal or hemodialysis for uremia, possibly plasmapheresis; and possibly hemofiltration for hepatic failure. The regimen of selective decontamination of the digestive tract may reduce the incidence of nosocomial pneumonia, but it appears to have little effect on the death rate (21). The endorphin antagonist naloxone may have an effect on cardiac contractility, and infusion of naloxone appeared to improve the condition of eight patients with septic shock (20).

Conclusions

The prevention of infection in surgical wounds depends on three things: meticulous asepsis, skillful surgery, and intelligent use of antibiotics. We should aim for a zero rate in clean operations, and a percentage rate in single figures in contaminated operations.

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AIDS: A Global Perspective

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Based on the experiences from the first decade with AIDS, and on the predictions for the next decade, we are forced to conclude that the AIDS pandemic is still in its beginning. In spite of the high number of AIDS cases that have occurred during the 1980s, it is obvious that the number will be multiplied dramatically during the 1990s.

Table I shows the cumulative AIDS cases officially reported to the World Health Organization (WHO) as of August 31, 1989. About 180,000 cases have been reported from 152 of the 166 countries which are members of WHO. It is indeed remarkable how rapidly the AIDS virus has spread over the globe to so many countries on all continents.

The reported numbers of cases do not seem to correspond to the actual number. Studies have shown that there is considerable underreporting. The official figures answer to more than 80% of the cases in industrial countries compared to 10% or less of the cases in many developing countries. The reports should, therefore, mainly be considered as a sign of political commitment from the governments to acknowledge that cases of AIDS occur in their countries and that they are prepared to cooperate with WHO. The poor state of reporting in some of the most affected developing countries principally reflects the deficiencies of the health and communication infrastructure.

A list showing the incidences of reported AIDS cases per 100,000 population clearly points to the fact that the highest incidences are to be found in the Carribbean and in East and Central Africa, their incidences being much higher than in the USA (Table II). The USA has a higher incidence than in Europe. This list is based on the cases reported during one year, 1988. The differences between industrialized and developing countries are even greater when the underreporting is considered.

Table I. Cumulative Aids Cases Reported to WHO as of August 31, 1989

Africa	31,000	cases	from	48	countries
Americas	120,000	"	"	43	"
Asia	400	"	"	25	"
Europe	25,000	"	"	29	"
Oceania	1,500	"	"	7	"
	178,000	"	"	152	"

Table II. Incidence of Reported AIDS Cases/100,000 Population in 1988

Bermuda	49.1
Bahamas	38.0
Uganda	26.3
Burundi	22.3

USA	11.1
Switzerland	4.8
France	4.2
Denmark	2.4
Sweden	1.0

Table III shows the cumulative incidence rates per million population in some Western countries. Switzerland, France, and Denmark score high in Europe, though lower than the USA. In Europe the incidences are now rising in Italy and Spain due to the many AIDS cases among intravenous (IV) drug users. On an average, 28% of the European AIDS cases occur in IV drug users. In Spain and Italy that proportion is 61% and 66%, respectively. In contrast, in North West Europe more than 70% of the cases occur among homosexual/bisexual men. The increase of AIDS cases among homosexual/bisexual men has slowed, a 61% increase during March 1988 to March 1989, compared to a 131% increase among drug users. Current trends suggest the start of a leveling off in the total incidence in northern Europe; this is not apparent in other countries.

Table III. AIDS Cumulative Incidence Rates per Million Population

Switzerland	122
France	115
Denmark	77
Sweden	33
Eastern Europe	< 3
USA	373

Due to the obvious underreporting, WHO has estimated the actual number of the cumulative global total of AIDS cases. Among the estimated 500,000 AIDS cases, more than half are supposed to be in Africa (Table IV). Of the estimated 180,000 cases in the Americas, at least 100,000 are from the USA.

I will now turn to the number of HIV-infected persons, most of whom are healthy and have not yet developed symptoms of immunodeficiency. The global calculated number of HIV-infected persons is believed to be 5-10 million (Table V). As this estimate, uncertain as it is, has been used since 1987 we may by now be close to the higher level. However, if we use 5 million as a global total, 2.5 million are believed to live in Africa, 2 million in the Americas (some 1-1.5 million in USA), 0.5 million in Europe and only 100,000 in Asia.

Table IV. Actual (Estimated) Cumulative Global Total of AIDS Cases, July 1989

Africa	280,000
Americas	180,000
Asia	< 1,000
Europe	33,000
Oceania	2,000
approx.	500,000

Table V. HIV Infection Global (Estimated) Number of HIV-Infected Persons

Beginning of the 1980s	100,000
During the 1980s	5-10 million
If 5 million:	
Africa	2.5 “
Americas	2 “
Europe	0.5 “
Asia & Oceania	0.1 “

WHO has executed a study in an attempt to predict the development of the AIDS pandemic during the 1990s (Table VI). Fourteen eminent experts in epidemiology from various parts of the world were independently asked for their best opinion concerning the number of HIV infections and AIDS cases. To underline that these are estimates based on opinions, the exercise was called “The Delphi Study”. As seen in Table VI, about three times more infections are predicted to occur during the 1990s than occurred during the 1980s, which would mean a cumulative number in the magnitude of 20 million infected persons by the year 2000. Up to 40% of the new HIV infections were considered to be preventable by the intervention measures at hand today, that is public health measures, information campaigns, health promotion, control of blood products and blood transfusions, and so on.

Table VI. Prediction of HIV Infections

During the 1990s: About three times more (new) infections than during the 1980s; a cumulative number of *20 million infected persons* by the year *2000* (according to the “Delphi” Study). Up to 40% of the new HIV infections are considered preventable.

This forecast is based on the assumption of a continued slow transmission of the virus in Asia. However, as I will discuss, there are large vulnerable, high-risk populations in many densely populated areas in Asia, as on the Indian subcontinent. Should there be an extensive spread in such areas, the predicted figures may be much too low. The prophecy on the proportion of preventable HIV infections may also be over-optimistic.

The dynamics of the AIDS pandemic is illustrated in Table VII. During the first five years, 70,000 AIDS cases appeared; during the three-year-period 1986-88, 300,000 cases were calculated to have appeared, and for the three-year-period 1989 to the end of 1991, 700,000 new cases are expected, bringing the cumulative number to 1.1 million through 1991. The steep rise during the ‘80s is graphically illustrated in Figure 1.

Table VII. Number of AIDS Cases

1980-1985	70,000	new AIDS cases
1986-1988	300,000	“
1989-1991	700,000	“
End 91	1,100,000	cumulative number

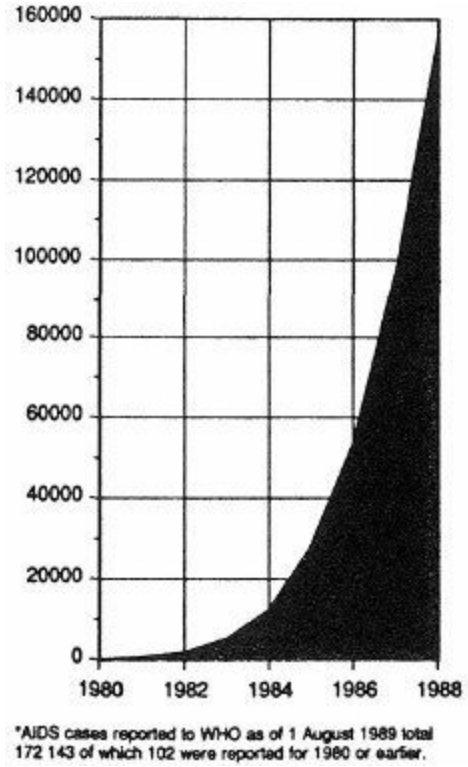

Figure 1. Cumulative Number of AIDS Cases Reported to WHO, 1979-1988*

During the 1990s, nine times more adults are thought to develop AIDS compared to the 1980s. Fifty percent of them are already HIV-infected and, therefore, not preventable. This would mean 6-8 million AIDS cases by the year 2000 (Table VIII).

Table VIII. Prediction Regarding AIDS Cases

During the 1990s: Nine times more adults estimated to develop AIDS compared to the 1980s; 50% of them already infected (therefore, not preventable).
Cumulative total by the year 2000: 6-8 million.

I would now like to talk more specifically about some current epidemiological trends (Figure 2).

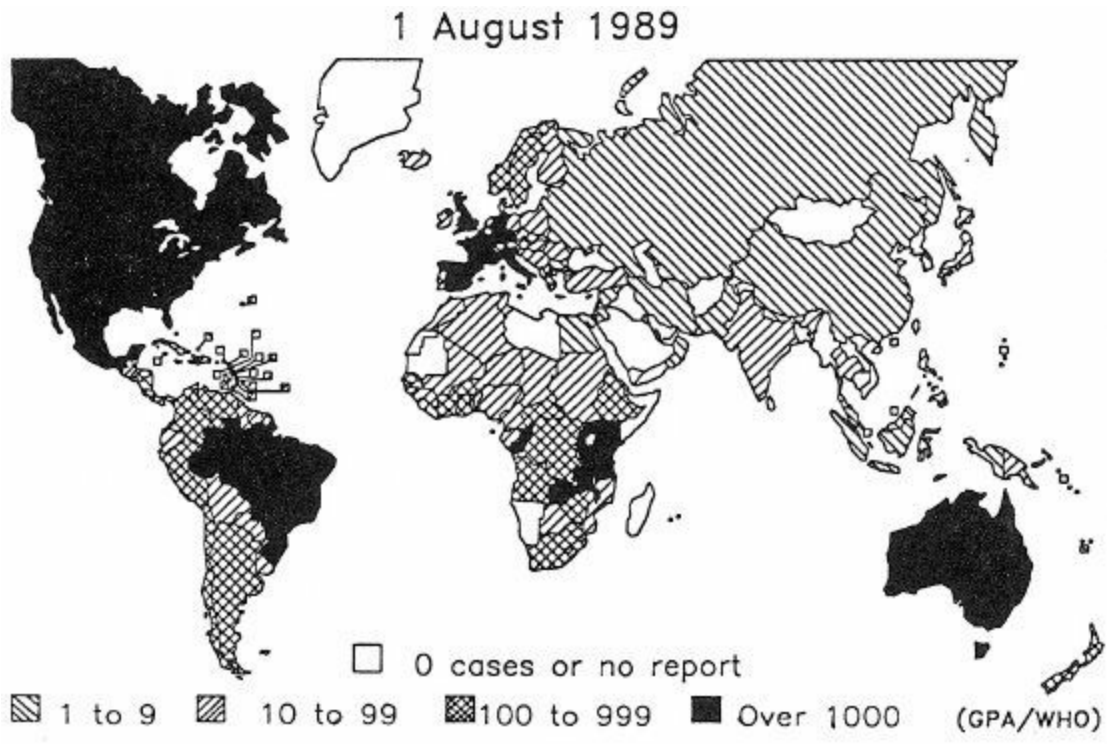

Figure 2. Reported AIDS Cases

Africa:

There is a continued rapid increase in many central and east African countries, especially in the nine countries which stand for two thirds of the AIDS cases. Examples: **Malawi** — survey among pregnant women revealed 3% seroprevalence in 1988 compared to 18% in 1989. **Ruanda** — in urban areas more that 20% seroprevalence is found and in rural areas, 2% seroprevalence. In the **Kagera** region of western **Tanzania** (west of Lake Victoria at the border to Uganda), 33% seroprevalence in the sexually-active age groups is reported in urban zones. The age specific prevalence for adults was highest in the age group 25-34 years (41%). In children, it was highest in the age group below one year of age (21%). Up to 80%-90% seroprevalence among female prostitutes has been reported from several studies in some African capitals, e.g., Nairobi, **Kenya** and Kinshasa, **Zaire.**

A widespread HIV-1 epidemic is underway throughout West Africa in addition to the HIV-2 epidemic already present in that part of Africa: e.g., Abidjan, **Ivory Coast** — the HIV-1 seroprevalence was 1% a few years ago, but has now increased to above 4% in the general population, and 25% of all hospital deaths are associated with AIDS. Over 30% of males 35-39 years of age admitted to the hospital are HIV-infected. In **Nigeria,** situated between the Central and West African foci, there is an increasing number of HIV-infected persons since last year, as studies among blood donors have shown. The 15 AIDS cases so far reported represent only the tip of the iceberg. The development in Nigeria is especially important as this country has the largest population in Africa.

There is a continued difference between urban and rural areas in Africa, but the difference is now less marked (e.g., 12% HIV infections in the adult population reported

from rural **Uganda**). Unfortunately, there is a rapid increase of pediatric cases in Africa due to the heterosexual spread, with an equal distribution of HIV infection in males and females. AIDS cases in children are even more underreported, as AIDS is difficult to identify and distinguish from other common diseases among young children such as parasitic diseases, tuberculosis, diarrheal diseases, meningitis, and malnutrition. In addition, a co-morbidity of AIDS with malaria and tuberculosis is a characteristic feature in Africa. Thanks to successful health care programs, infant mortality had been reduced in many African countries. Due to AIDS, this trend is again reversed. It should be noted that the HIV epidemic coincides with the deep economical crisis affecting the poor African countries which has led to a deterioration of the public health and educational systems.

South America:

Brazil: AIDS cases related to IV drug use have increased from 3% to 13% in one year. There is a continued increase due to a new urban epidemic of cocaine injections. Cocaine is affordable compared to heroin. Frequent injections are common, which increases the risk of contracting HIV infection through shared needles. It is remarkable that blood donation is still not under control. Therefore, HIV infections due to blood transfusion are still common. From the beginning, most AIDS cases in Brazil appeared among homosexual/bisexual men, but now more women are HIV-infected, meaning that there also are more HIV infections among newborns.

Asia:

Thailand: An explosive spread of HIV among heroin IV drug users is going on from a very low occurrence in 1987 to over 40% in 1989. An increased HIV seroprevalence in female prostitutes has been noted from < 1/1000 a couple of years ago to the percent level presently, 10%-20% in special areas. HIV infections are now reported from 70 of the 73 provinces in Thailand, and thus are no longer concentrated to Bangkok (Figure 3).

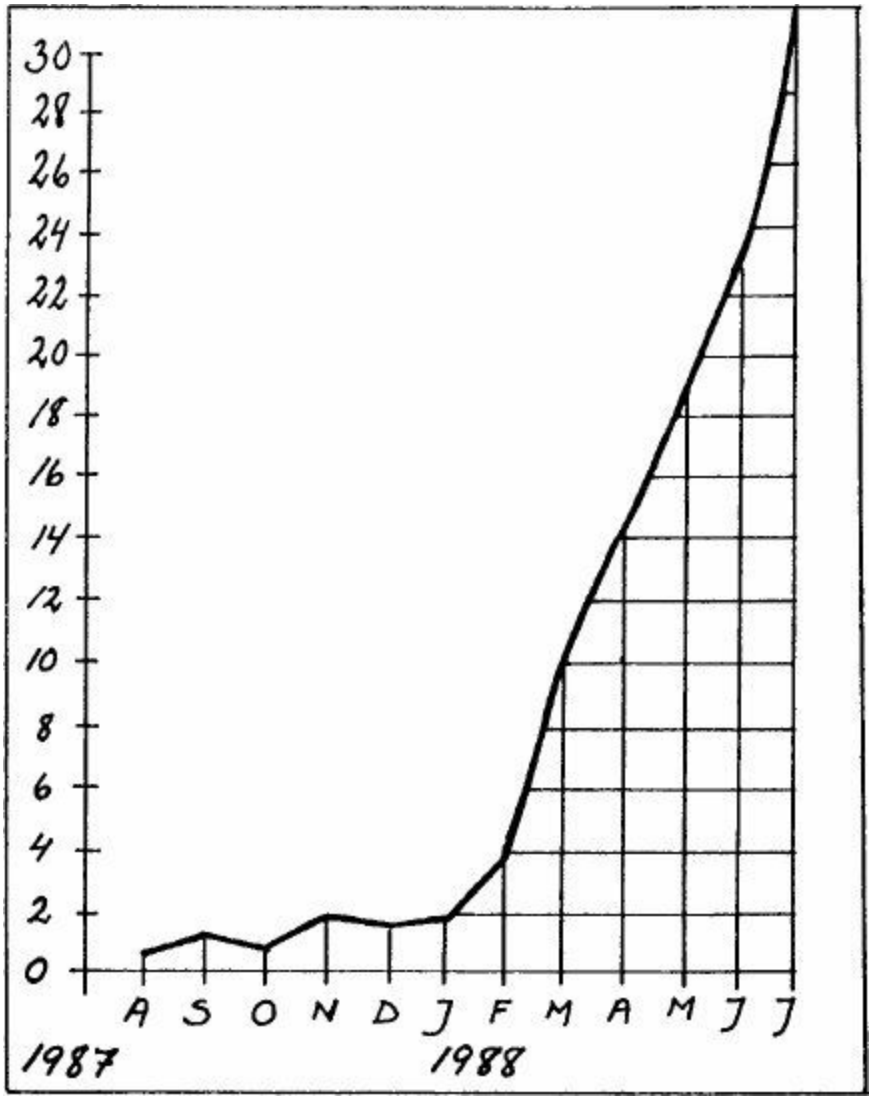

Figure 3. Percentage HIV-infected I.V.-Drug Users in Bangkok (August 1987-July 1988)

Pakistan: A rapid change of using opiate by IV injections, injection of heroin instead of traditional smoking of opium, has created a new pool of population which is vulnerable to HIV infection.

Burma: HIV infections occur currently among IV drug users in this comparatively isolated country, causing apprehensions about a development similar to that in Thailand.

India: HIV seroprevalence 3%-7% among female prostitutes in South-East India is reported; infected prostitutes also occur in Calcutta and Bombay. Each country seems to have a specific profile of its AIDS problem. The specific problems in India are HIV-infected blood-products and deficiencies of blood transfusion services.

Eastern Europe:

U.S.S.R.: The specific AIDS problems in the U.S.S.R. are related to the nosocomial pediatric infections which occurred in more than 90 children in several hospitals in **Elista** and **Volgograd** in 1988/89 due to the bad hygienic routines for IV-injections in these hospitals. The father of the index case was HIV-infected in Congo in the beginning of the 1980s. This shows that all societies need to be prepared for HIV infections, from the

tropical rain forests in Central Africa to the steppe of Kalmyckia. These incidents have pointed to the need for supply of sterile hospital equipment and improvement of sterilization and disinfection procedures plus strict adherence to regulations and guidelines to prevent hospital infections.

Western Industrialized Countries:

Drug use increases HIV infections, particularly in teenagers. Now, there is not only IV drug use, but also the crack epidemic. Due to the practice “Sex for drugs”, HIV is increasingly spread sexually among drug users. The “War against drugs” has not been successful so far. On the contrary, the global illegal drug situation is worse than ever. However, the annual seroincidence of HIV is decreasing in some centers (e.g., in New York City from 12%-15% yearly to 7%, and in Amsterdam from 12% to 3%). Increased proportion of HIV-infected is found in underpriviledged inner-city populations. The appearing dominance of HIV-infected in lower social-economic classes coincides with erosion of public health systems (e.g., in U.S.A.).

Changed behavior in middle class and upper middle class gay men has resulted in fewer HIV infections among them. However, no change is noted in newly recruited young gay men and in the lower economic and educational classes.

All Countries:

The inefficient control of other sexually transmitted diseases constitutes a specific problem as they have been shown to increase the rate of spread of HIV.

I would like to sum up the current trends of this extremely complex and heterogeneous epidemic. As stated in Table IX, the global epidemic remains dynamic and continues to expand. There is a dramatic increase in many already infected areas, along with an expansion of the geographical scope, which results in increasing public health and socio-economic impact. In addition, there is an increasing psychosocial risk for confrontation between population groups due to anxiety, fear, and competition for limited resources (e.g., medical care, as is already the case in New York City). There are many large population groups vulnerable to HIV infection due to their risk behavior. The existence of such high risk groups fuels the dynamics of the epidemic. IV-drug use is rapidly spreading to developing countries in Asia and Latin America. The situation regarding drug use has never been as bad as it is now.

Table IX. Conclusions

- Global epidemic remains dynamic, continues to expand
- Dramatic increase in many already infected areas
- Expansion of the geographical scope to new areas
- Increasing public health and socio-economic impact, concentration in underprivileged population groups
- Increasing psycho-social risk for confrontation between population groups due to anxiety, fear, and competition for limited resources (e.g., medical care)
- Continued or new risk behavior patterns, fueling the dynamics

We were overtaken by the AIDS epidemic and we ask ourselves what will come next? Will there be other infections that can cause serious epidemics? Yes, there might! In addition to HIV-1 and HIV-2, we have already other retroviruses, especially HTLV-1. HTLV-1 is associated with adult T-cell leukemia/lymphoma and a chronic neurological disease known as HAM/TSP, tropical spastic paraparesis. HTLV-1 is now spreading in many countries along the same routes as HIV, including blood transfusion and needle sharing among drug users.

The attitudes of the professionals in the health care settings also deserve to be mentioned. Even if the risk for occupational HIV infection in health care workers is low (less than 1% per parenteral exposure to infected blood), it has contracted a lot of attention and caused fear among the staff. There are reports of reluctance of doctors or medical students to see AIDS patients due to fear and aversion. The concern for AIDS is so emotionally charged and associated with feelings of shame and death agony that even the most experienced health care professional may react in an irrational way. This will require intense work to influence the attitudes of health care professionals to overcome the fears hidden in our unconscious. The fear and suspicion damages the bond between physician or nurse and patients; the contract between the “caregiver” and “caretaker” that is of paramount importance for counseling and treatment. As well put by a clinician at Cornell University in New York City: “Replacing the samaritanism of medicine has become a guarded, almost adversial mind-set.”

In spite of the gloomy prospects I have presented, there is hope! It is not all that dark! The antiviral drug zidovudine, marketed as Retrovir, better known as “AZT”, has prolonged and improved life for people with AIDS and has been used routinely for a couple of years. AZT works by inhibiting viral reserve transcriptase, thereby interrupting HIV replication in human cells. Quite recently, the U.S. National Institute of Health announced that AZT significantly slows progression of HIV infection in persons with early AIDS-related complexes or in persons who have not yet developed clinical symptoms, but have decreased levels of the essential type of lymphocytes specifically attacked by the virus, the T-cells. These findings are significant steps in the efforts to change AIDS from a terminal, untreatable disease to a chronic disease that can be managed for years before it ultimately becomes fatal, similar to diabetes. AZT given to AIDS patients has caused severe adverse reactions, but showed no more side reactions than placebo in a randomized, double-blind, controlled study when given to asymtomatic HIV-infected persons. Furthermore, a second drug related to AZT, dideoxysinosine (DDI) is now being tested in clinical trials and seems promising with low side effects, high efficiency, and a lower price than AZT. These possibilities to postpone the disease in HIV-infected persons will have far-reaching implications for AIDS prevention and control. According to my view, the recent results represent a turning point in the attitude to AI DS and to the intervention policies. The prolonged survival is not only due to the antiretroviral treatment, but also to improved prevention and treatment of the opportunistic infections, as for instance, *Pneumocystis carinii* — pneumonia, toxoplasmosis, candidiasis, and tuberculosis. The present status of AIDS treatment can be compared to the situation regarding treatment of leukemia in children in the 1960s and early 1970s. At that time, leukemia was considered inevitably

fatal. Now, we have learned how to handle the therapeutical armament, when to treat, for how long, which dosages and which combination of drugs, which has resulted in a cure for a considerable proportion of the leukemic children.

I will finish my presentation by pointing to the beneficial effect of the AIDS epidemic on research. The new knowledge and insights gained during research on AIDS will have tremendous cross-fertilizing and spin-off effects in other fields. For instance, the understanding of the etiology and pathogenesis of other diseases with involvement of the central nervous system such as multiple sclerosis and dementia; in cell biology and tumor diseases; in the chemotherapy of other viral infections; in entirely new approaches to immunoprophylaxis and chemotherapy.

I will just give one example of the last mentioned approach. HIV binds specifically to the CD4 receptor on T-cells, the lymphocytes which have a key role in our immune system. After binding, the virus infects and kills the cells. Now, the research on the interaction on the molecular level of the CD4 receptor with its ligand on the virus surface, the glucoprotein 120, has revealed that it is possible to block the HIV infection by synthesized CD4 peptide analogues supplied as decoys for the virus. The virus attacks the decoy instead of the human cells. This has now been taken a step further by production of chimeras, hybrids containing both the binding domain of CD4 and neutralizing antibodies through DNA recombinant technique, thus combining immunoprophylaxis and chemotherapy in one molecule. This represents an entirely new approach, a new way of thinking. The hybrid antibody-CD4 molecule is termed “immunoadhesion”. Whether immunoadhesion will work in practice we do not know yet, but clinical studies in combination with AZT, zidovudine, will start now, only six months after the initial publication — a remarkable speed!

Other examples of the expansion of science and new technology are the use of transgenic animals as, for instance, laboratory mice producing HIV, and the SCID (severe combined immunodeficient mice) mouse model carrying human T-lymphocytes and macrophages. The transgenic animals have been produced by injecting genetic material from HIV in the egg cells of the animals which will result in progenies capable of producing the AIDS-virus, a virus specific to human beings and a few other higher primates only. By the aid of such animal models, HIV and its interaction with human cells can be studied in a way that would otherwise be impossible.

From this point of view, it is only with luck that the AIDS pandemic did not hit mankind before we had all these marvelous new tools in molecular biology. This makes me optimistic regarding our possibilities to control AIDS in the long run! Homo sapiens have been seriously challenged by the cunning AIDS virus and now fight back!

AIDS: Guidelines for Safety in the Hospital and the Laboratory

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Dr. Burke, colleagues, and attendees of the International Kilmer Memorial Conference, I am delighted and honored to participate in this conference. The purpose of this presentation will be to point out potential modes of HIV transmission in the laboratory and hospital setting and to discuss Hepatitis B virus transmission as the basis for the precautions that have been developed for use in these settings. The primary blood-borne infections that this presentation will cover are Hepatitis B, Hepatitis non-A, non-B, and the human immunodeficiency virus or HIV.

Dr. Kallings has discussed the worldwide epidemiology of AIDS and has pointed out that in the United States the number of reported cases now exceeds 110,000. He further pointed out that HIV infection is primarily transmitted sexually, by intravenous drug abuse, or from infected mothers to newborn infants.

In the United States in the last several years, there has been concern about the transmission of HIV in health care settings such as laboratories, hospitals, and dialysis units. The Centers for Disease Control (CDC) has formulated and published a number of guidelines designed to prevent the transmission of HIV, and other blood-borne agents such as the Hepatitis B virus (HBV), in health care settings. In fact, since the epidemiology of HBV and HIV are similar, and the modes of infection transmission in health care settings appear to be similar, the scientific rationale for recommended precautions for HIV are based on the epidemiology and the known modes of transmission for HBV.

It has been known for many years that HBV is a significant occupational hazard of health care workers in the United States (5). The CDC has estimated the amount of morbidity and mortality from HBV infection among health care workers. Approximately five to six hundred health care workers are hospitalized annually due to HBV infection and approximately three to four hundred health care workers die annually because of complications of either chronic HBV infection due to cirrhosis or liver cancer, or to acute HBV infection.

Other CDC studies on the risk of HBV infection by duration of high-risk behavior show that almost 100% of a cohort of intravenous drug abusers demonstrated evidence of current or previous Hepatitis B virus infection after a period of 10 to 15 years of intravenous drug abuse. For a cohort of male homosexuals over the same period of time, approximately 70% of the group would show evidence of current or previous HBV infection. Two other cohorts, one that includes health care workers and the other, individuals who are heterosexuals and have multiple sexual exposures, have a risk of HBV infection that is significantly less than that for intravenous drug users and male homosexuals. Specifically, about 25% of health care workers would be positive for markers of Hepatitis B virus infection after 10 to 15 years. This represents about four to five times the risk over the

normal population.

There are a number of modes for transmitting blood-borne diseases and a special emphasis is placed on HBV. These modes can be listed in the order of transmission efficiency. The most efficient means of transmitting HBV or any other blood-borne infectious agent is direct inoculation through skin. A good example of this would be a blood transfusion with contaminated blood prior to the time the blood supply was screened for Hepatitis B surface antigen (HBsAg). Another example would be an accidental needlestick. A second mode of transmission is contamination of broken skin with blood or serum. The amount of circulating HBV per mL of blood is approximately 100 million to one billion. Consequently, even a small cut in the skin can allow for a risk of HBV transmission. The third mode of transmission is by blood contamination of mucosal surfaces. This includes sexual transmission as well as blood or serum contamination of the eyes, nose, or mouth. The fourth method of transmission is by means of environmental surfaces. This mode is operative for HBV but not for HIV. HBV is known to be more durable in the environment and because of it's initial high concentration, populations of HBV are able to survive longer on environmental surfaces. In certain health care settings such as dialysis units, this information is used for developing strategies for dialyzing HBV-infected patients. Dialysis is recommended to be in separate areas on separate dialysis machines if the patient is HBsAg positive.

There are also theoretical modes of transmission which should be mentioned. The first one is the fecal-oral route of transmission. Technically speaking, HBV is not transmitted by the fecal-oral route. HBV is inactivated in the stomach and in the intestinal tract and HBV cannot survive through the intestinal circuit in order to set up infection. However, blood that might be splashed into the oral cavity has been shown, in some cases, to result in infection. This is due to HBV being in high numbers and entering into the vascular system through abrasions and cuts in the oral cavity such as along the gumline. This is not fecal-oral transmission, but rather peri-oral transmission.

The other theoretical mode of transmission is airborne transmission. In the United States especially, this particular mode of transmission has been proposed by some not only for HBV but also for HIV. In fact, there is absolutely no epidemiologic or laboratory evidence that would support this view. There have been studies that have shown that it is extraordinarily difficult to produce viable airborne particles, in the size range of five to 10 microns, of agents that are suspended in fluids such as blood or serum. In fact, my group at CDC has performed experiments in this area and we have developed an air sampling procedure for HBsAg. Using this air sampling technique, we were unable to detect airborne HBsAg in settings that would be "worst case" conditions; a dental operatory where patients were HBsAg seropositive with HBsAg positive samples taken from saliva as well as from the gumline. Air samplers placed around the dentist and elsewhere in the dental operatory failed to detect airborne HBsAg. The same type of results were obtained in a hemodialysis unit that reprocessed hemodialyzers. Air samplers were placed in an unventilated area where the dialyzers were cleaned and taken apart, and no HBsAg positive samples were detected in spite of the fact that a high percentage of the patient population was HBsAg positive.

On the other hand, 12% to 15% of the environmental surfaces in the same areas were positive for HBsAg. These results, as well as the results of other investigations, indicate that airborne particles of blood-borne agents are very difficult to produce in the size range of 10 to 15 microns. Particles would have to be in this range to account for true airborne transmission. Large droplets of blood and blood splatter can occur and these droplets do not account for true airborne transmission, but rather transmission by direct droplet contact. These results are important when formulating strategies for prevention of transmission in health care settings. For example, if HBV were truly transmitted by the airborne route and existed in very small particle size, then masks, safety glasses, etc. would have to be of the same type that are used in maximum containment laboratories and would be very expensive. On the other hand, to prevent droplet contamination for health care workers, simple common sense use of safety glasses, surgical masks and common laboratory clothing are sufficient (6).

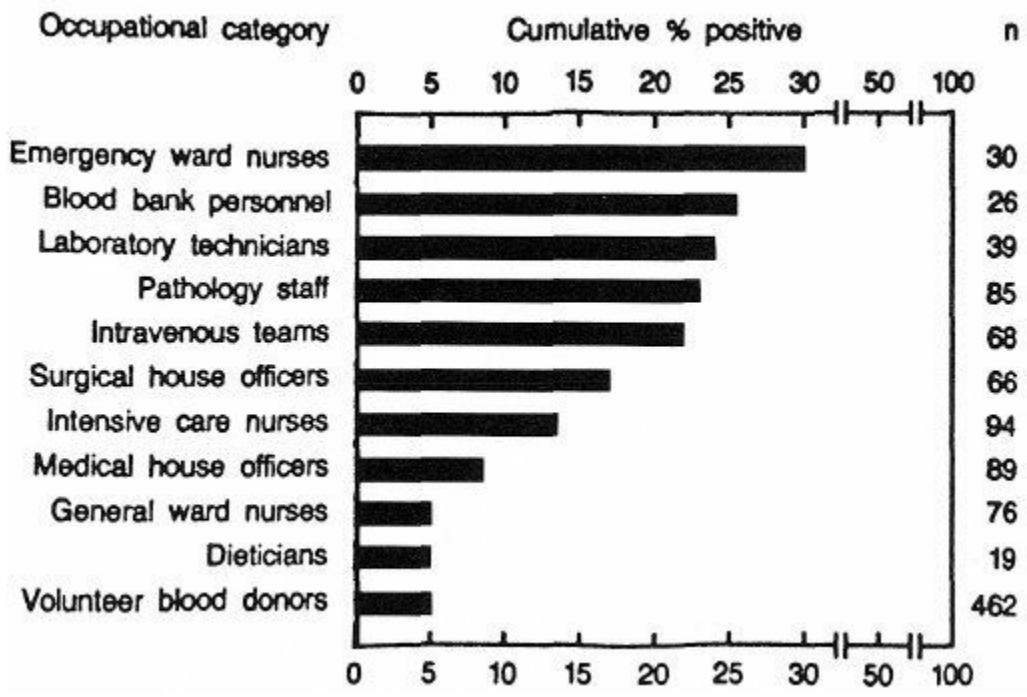

Figure 1. Occupational Categories and Risk of HIV

Figure 1 shows data from studies by Dienstag and Ryarr (4) who tested hospital workers for markers of HBV infection. Hospital employees who are most at risk of HBV infection included emergency ward nurses, blood bank personnel, laboratory technicians, pathology staff, intravenous teams, surgical house officers, and intensive care nurses. These are all examples of health care workers who have frequent contact with blood. The prevalence of HBV markers among hospital employees was most likely in those who had frequent contact with blood compared to those employees who had rare contact with blood. On the other hand, those employees who had rare contact with patients, in this particular study, had a higher percentage of HBV markers than those who had frequent contact with patients (Table I). Who were these employees? These were the laboratorians who had no contact with patients, but did have contact with the patients' blood. These studies and others emphasize that it is frequent contact with blood and related fluids such as serum and

plasma that constitute the risk of HBV infection. We assume that the same phenomenon occurs for other blood-borne agents, including HIV.

Table 1. Prevalence of HBV Markers Among 624 Hospital Employees by Blood and Patient Contact

Category	No. of Employees	Percent Positive
Blood Contact		
Rare	266	9%
Frequent	358	21%
Patient Contact		
Rare	175	21%
Frequent	449	14%

Adapted from Dienstag and Ryan (4)

In hemodialysis centers, the risk of HBV infection in patients and staff has been quite high, especially in the mid-1970s. It was during these years that the CDC made a number of recommendations involving the use of proper gowning and gloving; avoidance of needlestick injuries; and good environmental control in order to prevent the transmission of HBV from patients to staff members, from patients to patients, and from staff members to patients. Figure 2 shows that the incidence and prevalence of HBsAg positivity among patients decreased significantly over a 12-year period. The same phenomenon occurred with the incidence of infection among staff members where infection rates decreased significantly from 1976 to 1987. These decreases were the result of infection control practices and not the result of the use of the Hepatitis B vaccine which entered the United States market in 1983. Table 2 shows the effect of separation practices for HBsAg positive patients on the incidence of HBV infection in hemodialysis patients from 1976 to 1987. Patients who were infected with HBV and were dialyzed in a separate room with a dedicated machine had significantly lower infection rates, 3.9%, than those who were dialyzed in centers who did not use such separation practices (6.8%). This phenomenon holds true when the infection rates were high in 1976 and when they were lower in 1980, 1983, and 1987. I would also like to point out that if one simply looks horizontally at those centers that did not use separate rooms and machines, but only practiced the basic barrier precautions, which are now referred to as Universal Precautions, one can see that the infection rate also decreased, although at a lower rate than those centers that practiced separation. I believe this is the effect of Universal Precautions on the decrease of this particular blood-borne infection, HBV.

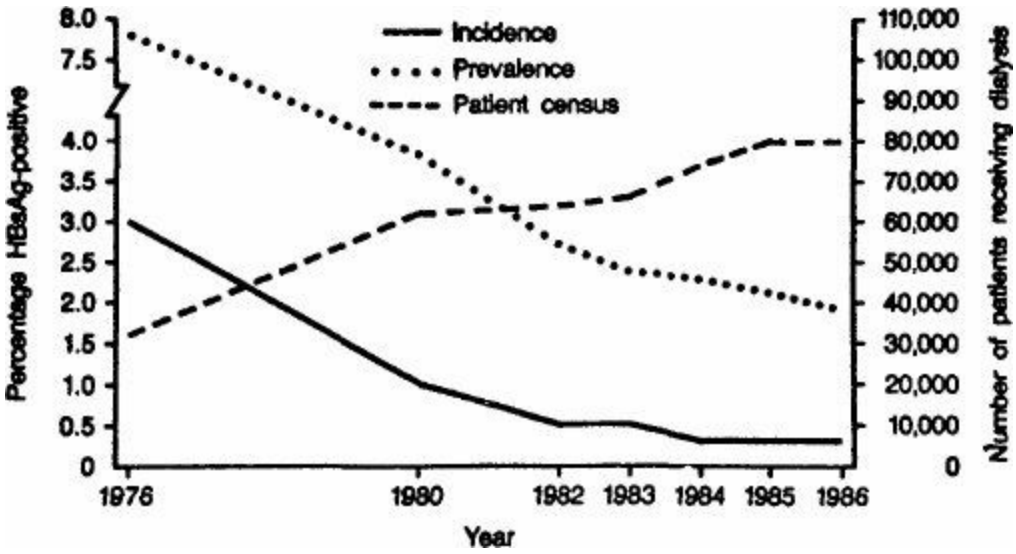

Figure 2. HBsAg Positivity in Patients Receiving Dialysis

Table 2. Effect of Separation Practices for HBsAg-Positive Patients on Incidence of HBsAg in Hemodialysis Patients 1976-1978, United States

Separation Practices	Incidence of HBsAg					
	1976	1980	1983	1986	1987	1988
Room and Machine	3.9%*	0.8%*	0.5%*	0.4%	0.3%*	0.2%
Machine Only	---	2.0%	0.9%	0.5%	0.5%	0.2%
None	6.8%	1.5%	1.1%	0.5%	1.7%	0.4%

Note: Only centers with ≥ HBsAg-positive patients are included
* $p < 0.01$; Room and Machine vs. Machine only or none

Basic barrier precautions have been used in dialysis centers for all patients since 1977. As they are applied today for the control of blood-borne infections, they are referred to as Universal Precautions. The origin of these Universal Precautions was primarily in health care settings such as dialysis units. These precautions involve the common sense use of gloves and protective clothing, such as laboratory gowns, and the use of face and eye protection when necessary. All staff members do not need this type of protection—only those who may come in contact with blood. Another infection control strategy is to avoid needlestick injuries. For example, the CDC recommends that needles should not be recapped by hand and that needles should be discarded whole into puncture resistant containers. In addition, good handwashing techniques should be used. Hands should be washed after changing gloves. Lastly, there should be good environmental control procedures — that is to say, good housekeeping, sanitization, disinfection, and sterilization procedures. I might add here that none of the protocols or procedures that deal with housekeeping, disinfection or sterilization need to be changed because of considerations for HBV or HIV.

These protocols are based on worst case conditions and they do not need to be changed in the instance where patients are known to have HBV or HIV infection.

There are several additional precautions that are used for HBsAg positive dialysis patients. This strategy is only used in dialysis units and only for HBV infection. These extra precautions include testing of patients and staff members routinely for HBsAg, dialyzing HBsAg positive patients in separate areas and on dedicated machines, and not including HBV-infected patients in dialyzer reuse programs.

As was mentioned above, HBV transmission, and especially efficacy of transmission, is used as a model for other blood-borne agents such as HIV. Because the efficiency of HBV transmission in health care settings is so much higher than HIV, those precautions that are designed to prevent HBV transmission are likely more than adequate to prevent HIV transmission.

Dr. Kallings has discussed the epidemiology of HIV infection on a world-wide basis and pointed out that HIV is transmitted by three major modes: sexual transmission, blood exposure including needle sharing and occupational needlesticks, and transmission of HIV from infected mothers to newborn infants. These are the only modes of transmission known. The scientific literature and/or meetings have reported that fluids from which HIV has been isolated include blood, semen, vaginal secretions, breast milk, saliva, tears, amniotic fluids, and urine. This list will undoubtedly become longer. But the simple presence of a pathogen, such as HIV, in any body fluid does not mean that the particular fluid is associated with disease transmission. Based on epidemiologic evidence, it is known that of these fluids, only blood, semen, vaginal secretions, and probably breast milk are involved in HIV transmission in the community. In the health care setting, in hospitals and laboratories, it is only blood that has been incriminated in HIV transmission. With respect to Universal Precautions, those fluids that come under this patient management infection control strategy include blood, because it is the most hazardous fluid in health care settings; vaginal secretions and semen may be important in particular specialized laboratories; and other fluids that have not been incriminated in HIV transmission, but theoretically could be—such as cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, amniotic fluid, and fluid with visible blood. Under Universal Precautions, feces, nasal secretions, sputum, sweat, tears, urine, and vomitus would not be included. For these types of body fluids, standard infection control strategies that have been used for many years in hospitals and in laboratories would apply.

There is a large difference in the concentration of infectious virus in blood between HBV and HIV. For HBV, there are approximately one hundred million infectious circulating virions per mL of blood compared to HIV, where the range is approximately one to 1000 per mL of blood. HBV has a high concentration in blood; HIV does not. Both viruses are not resistant to heat or to chemical germicides. An environmental route of transmission has been demonstrated for HBV but not for HIV. This is most likely due to the difference in concentration of the viruses as well as the durability of HBV and the sensitivity of HIV in the environment (1-3, 7).

The general reasons for the use of Universal Precautions include: prevention of the spread of infection from patient to patient; protection of patients from infection carried by the health care worker; and protection of health care workers from infection carried by the patient. I would like to make two additional points. First, the use of Universal Precautions

has no effect on the definitions of infectious waste. Secondly, the use of Universal Precautions has no effect on the standard strategies for biological safety in laboratories. In other words, there is nothing extra to do nor do protocols have to be changed because of the use of Universal Precautions.

In summary, I make the following three statements. Procedures adequate for preventing HBV transmission are more than adequate for preventing HIV transmission. Second, barrier precautions in biological safety procedures are most important in preventing blood-borne infections in health care facilities and laboratories. Third, extraordinary measures are not needed to prevent HBV or HIV transmission. I would also like to point out that basic barrier precautions — that is to say, Universal Precautions—are sufficient to prevent HIV transmission in hospitals and laboratories, and common disinfection and sterilization procedures are adequate.

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Role of Surveillance in the Prevention of Surgical Wound Infections: Data Collection Analysis and Use

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Introduction

The mission of the Centers for Disease Control (CDC) is prevention of unnecessary disease, disability, and premature death caused by infectious diseases, chronic diseases, injuries, and controllable risk factors such as smoking, high blood pressure, and substance abuse. The “tools of prevention” used by CDC staff include surveillance, epidemiologic investigation, etiologic agent identification and characterization, prevention strategy development and evaluation, and technology transfer.

With that background, let me now turn to the problem of nosocomial or hospital-acquired infections in the United States today. The objectives of this paper are to describe the magnitude of the problem of nosocomial infections in the United States, provide a definition of surveillance applicable to public health and disease prevention, review some of the evidence for the importance of surveillance in the prevention of surgical wound infections (SWI) and illustrate some of the ways in which surveillance data on surgical wound infections are used in hospitals participating in the National Nosocomial Infection Surveillance System in the United States.

Nosocomial Infections in the United States

Between 5% and 6% of patients admitted to acute care hospitals in the United States develop a nosocomial infection (3, 10). Since approximately 35 million patients are admitted to our acute care hospitals each year, nearly two million infections occur annually. On the average, a nosocomial infection prolongs hospital stay by approximately four days (12, 13) resulting in approximately eight million extra hospital days annually. Studies suggest that approximately one percent of nosocomial infections cause the death of the patient (17), yielding an estimate of approximately 20,000 deaths each year due to nosocomial infections. Nosocomial infections are estimated to result in 3.5-4 billion dollars in direct patient care cost each year.

Four major nosocomial infections account for approximately 80% of all nosocomial infections (17). Urinary tract infections are the most common followed by surgical wound infections, pneumonia, and bloodstream infections. Surgical wound infections account for 25%-30% of all nosocomial infections. However, because these infections prolong hospital stay by an average of approximately seven days (12, 13), surgical wound infections account for over 50% of all extra hospital days due to nosocomial infections in our country.

Surveillance as a Disease Prevention Tool

Surveillance, as used in public health and disease prevention, may be defined as the systematic, active, ongoing collection of data on the occurrence and distribution of disease in a population, and the events or conditions that increase or decrease the risk of disease. In addition, the definition of surveillance includes the timely analysis, interpretation, and dissemination of data for use in disease prevention (23, 24). Surveillance is an essential component of a program designed to prevent surgical wound infections.

The objectives of nosocomial infection surveillance are to determine the magnitude of the disease problem, monitor trends, identify patient risk groups, detect outbreaks of infection, compare infection experiences with those of other similar institutions, and satisfy the requirements of accrediting agencies in our country.

CDC has actively promoted the concept of disease surveillance since the 1950s (14). With respect to nosocomial infections, emphasis was placed during the 1960s on the development and evaluation of methodologies for comprehensive hospital-wide surveillance and adoption of these methodologies by hospitals throughout the country. During the 1970s, the national surveillance of nosocomial infections was implemented and the importance of surveillance in individual hospitals was evaluated. National surveillance was maintained and alternative strategies were developed and evaluated during the 1980s.

A number of problems are encountered when doing surveillance. First, surveillance is resource intensive, requiring considerable commitment on the part of hospital personnel, and surveillance programs often lack specific objectives. The appropriate denominator data for use in calculating specific nosocomial infection rates are often difficult to obtain. Measures of patient risk are often inadequate. Finally, infection control programs in many hospitals lack data analysis capability.

The challenge for those committed to the practice of surveillance is to overcome these problems, to develop more meaningful nosocomial infection rates to permit identification of infection control priorities, and to assess progress toward specific infection control objectives.

Role of Surveillance in the Prevention of Surgical Wound Infections

With that background, let me now focus on the role of surveillance in the prevention of surgical wound infections. A system for the classification of surgical wounds was published in 1964 in the report of a National Research Council study conducted in the United States (20). Wounds were classified as clean, clean-contaminated, contaminated, or dirty. Observed infection rates in patients having clean wounds defined as non-traumatic uninfected wounds not involving entry into the respiratory, gastrointestinal, or genito-urinary tract, had an overall infection rate of 3.3%. Patients with clean-contaminated wounds, i.e., wounds involving entry into the respiratory, gastrointestinal, or genito-urinary tract without unusual contamination, had an infection rate of 10.8%. Patients with contaminated wounds (wounds involving recent open trauma, a major break in sterile technique or acute inflammation), had an overall infection rate of 16.3%. Finally, patients with dirty wounds,

i.e., old wounds involving perforated viscera or an abscess, had an infection rate of 28.6% (20).

Risk factors for surgical wound infection include the wound classification, the urgency of the procedure (whether or not emergency surgery was required), the duration of the procedure, and host susceptibility as reflected by age, underlying disease, and nutritional status.

A landmark study in the history of surgical wound infection prevention was published by Cruse and Foord in 1973 (2). They reported the results of a five year prospective study of 23,649 wounds. The overall infection rate was 4.8% and the clean wound infection rate was 1.8%. The authors observed a decrease in the clean wound infection rate over time with dissemination of infection rate data to surgeons as part of their comprehensive program for the prevention of surgical wound infections. Other investigators have subsequently reported similar experiences (1, 19).

The Study of the Efficacy of Nosocomial Infection Control (SENIC Project) was conducted from 1974 to 1983 by the Hospital Infections Program in the Center for Infectious Diseases at CDC to evaluate the effectiveness of nationwide nosocomial infection prevention and control programs. This retrospective study focused on the four major nosocomial infections, including surgical wound infections. Several important findings emerged from the assessment of surgical wound infections (8). The SWI rate was 2.9% for clean wounds, 3.9% for clean-contaminated wounds, 8.5% for contaminated wounds, and 12.8% for dirty wounds. Clean wounds accounted for 55% of procedures but only 39% of infections. Clean-contaminated wounds accounted for 36% of procedures and 35% of infections. Contaminated wounds accounted for 2% of the procedures and 4% of the infections. Finally, dirty wounds accounted for 7% of the procedures and 22% of all surgical wound infections.

As part of the SENIC Project, a surgical patient risk index was developed. The elements of this risk index were duration of operation greater than two hours, wound class III or IV (i.e., contaminated or dirty), three or more discharge diagnoses, and an operative procedure involving the abdomen. For each patient undergoing an operative procedure, one point was assigned for the presence of each of these elements. Within each wound class, the surgical wound infection rate increased within the categories of the risk index. For example, for clean wounds, the overall infection rate was 3%; however the rate increased from 1% for the lowest risk patients with a clean wound to 16% for the highest risk patients with a clean wound. Similarly, the rate increased from 7% for the lowest risk patients with a dirty wound to 27% for the highest risk patients (8).

The “bottom line” of the SENIC Project with respect to surgical wound infections was that 35% of those infections that would otherwise occur were found to be preventable by a well-organized infection control program that included both surveillance and control activities, a hospital epidemiologist, and the reporting of surgical wound infection rates to surgeons (9). In addition, the risk index offered a useful alternative to the traditional classification based solely on wound contamination (8). These results have important implications for nosocomial infection control programs (16).

Use of Surgical Wound Infection Surveillance Data

I will now turn to a brief discussion of current national surveillance data in the United States on surgical wound infections. These data are derived from the National Nosocomial Infection Surveillance System (NNIS) which was organized in 1970 by the Hospital Infections Program at CDC. Participation is voluntary and members include approximately 100 acute care hospitals throughout the United States. Hospitals agree to use uniform definitions of nosocomial infections developed by CDC.

The goals of NNIS are to provide timely data from a representative sample of acute care hospitals in the United States to permit valid national estimates of the magnitude of the problem of nosocomial infections, recognition of trends and outbreaks, and comparison of infection experience of a hospital with that of other hospitals with similar patient populations. A second goal is to assist hospitals in developing more efficient and effective methods of surveillance and analysis.

From 1970 through September 1986, participating hospitals conducted hospital-wide surveillance and in October 1986, four additional surveillance options (surveillance components) were introduced. One of these focuses on surgical patients. The other three components include patients in intensive care units, infants in high risk nurseries, and patients located throughout the hospital.

Central to the practice of surveillance is the need for use of uniform definitions of diseases of interest (6, 25). As an example, the definition used by NNIS hospitals for incisional surgical wound infections is as follows: onset within 30 days of the operative procedure of a process involving the skin, subcutaneous tissue, or muscle which is characterized by purulent drainage from the incision or drain, *or* by isolation of an organism from fluid from a wound closed primarily or by opening of a wound by a surgeon, unless cultures are negative, *or* by a physician diagnosis of a surgical wound infection (6).

To facilitate data analysis in participating hospitals and reporting of data to CDC, the Hospital Infections Program developed a software package called the Interactive Data Entry and Analysis System (IDEAS) for use on microcomputers (15). In the surgical patient component, a surgical patient risk index based on the SENIC Project risk index is used. Elements of the NNIS surgical patient risk index include duration of operation of greater than two hours, a contaminated or dirty wound, and an American Society of Anesthesiologists patient classification of 3, 4 or 5 which is a measure the patient's clinical condition and serves as an indication of host susceptibility to infection (18). As in the SENIC Project risk index, one point is assigned for the presence of each element of the risk index. The NNIS surgical patient risk index has subsequently been modified to make the duration of surgery break-point specific for the type of surgical procedure.

During 1987 to 1989, data from surgical wound infections were reported by NNIS hospitals using the surgical patient surveillance component. During this time, 50% of operations involved clean wounds, 39% involved clean-contaminated wounds, 8% involved contaminated wounds, and 3% involved dirty wounds (Figure 1). The infection rate increased from 1.4% for clean wounds to 2.6% for clean-contaminated wounds, to 2.8% for contaminated wounds, to 6% for dirty wounds.

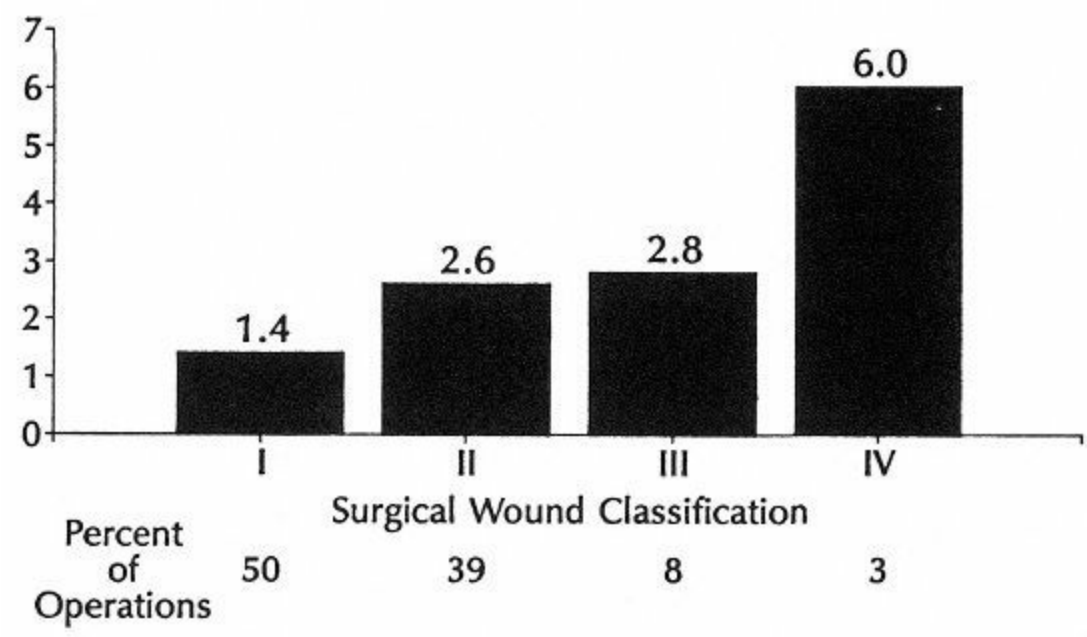

Figure 1. Surgical Wound Infection Rates By Surgical Wound Classification NNIS Hospitals, 1987-89

Using the NNIS risk index, 47% of operations involved patients with none of the elements of the risk index (Figure 2). Thirty-four percent of operations involved patients with one element, 18% involved patients with two elements, and 1% involved patients with all three elements. The infection rate increased from 1.2% in patients with none of the elements of the risk index to 17.8% in patients with all three elements.

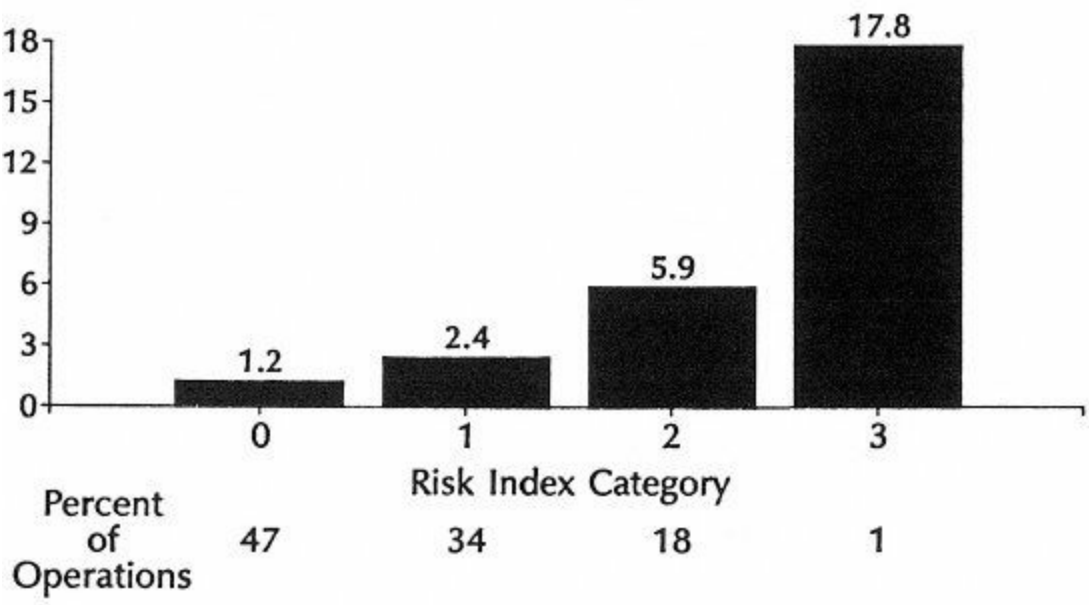

Figure 2. Surgical Wound Infection Rates Within Categories of the Surgical Patient Risk Index

The risk index effectively stratifies patients in all four wound classes (Figure 3). In the upper left, the data on clean or Class I wounds indicate that the surgical wound infection rate increased from 0.8% in patients with none of the elements of the risk index to 2.0% in patients with one element, to 4.2% in patients with two elements of the risk index. A similar trend with increasing wound infection rate with increasing NNIS risk index scores is

apparent for clean-contaminated wounds, contaminated wounds, and dirty wounds.

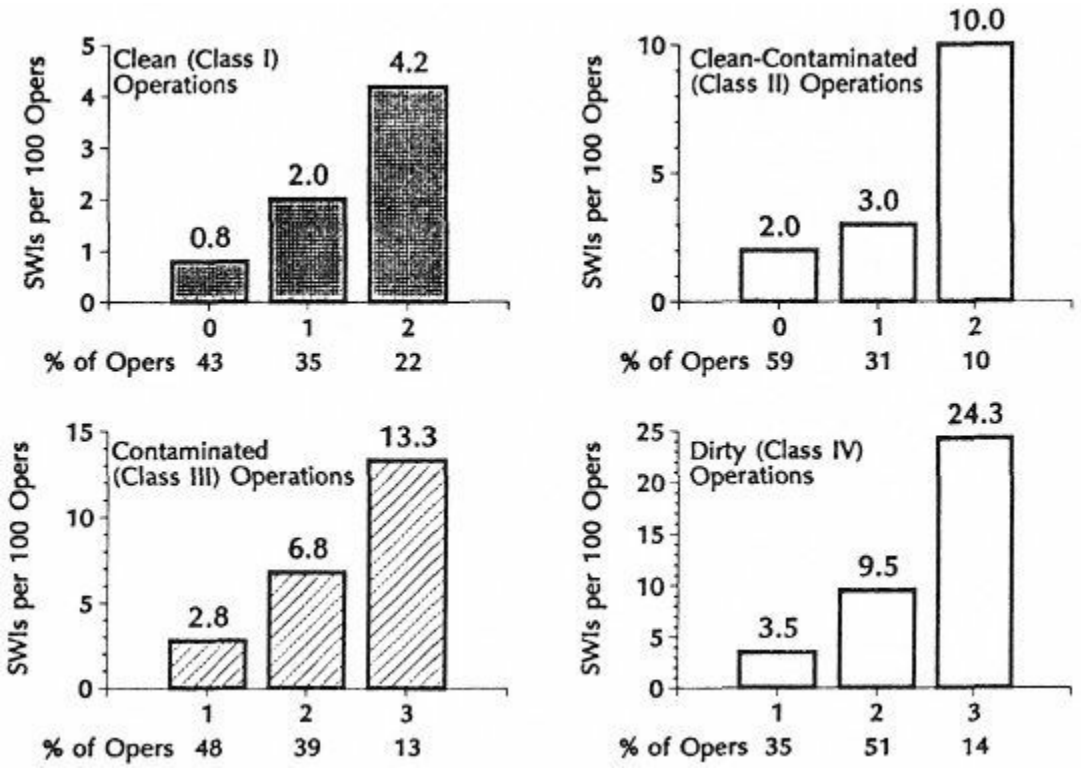

Figure 3. Surgical Wound Infection Rates By Surgical Patient Risk Index

Infection rates vary dramatically by specific type of operative procedure (Figure 4). NNIS data on surgical wound infection rates for several common procedures performed from 1987 through early 1989 indicated that the lowest observed rates were in the range of 1% to 2% following herniorraphy, vaginal hysterectomy, and cholecystectomy. The highest rates in the range of 5% to 7% were observed following gastric and colonic surgery. Further analyses of these data using the risk index are in progress.

Prevention of Surgical Wound Infections

Beginning in 1981, the Hospital Infections Program at CDC has published a series of guidelines for the prevention and control of nosocomial infections in acute care hospitals in the United States. These guidelines address prevention of the four major nosocomial infections including surgical wound infections (4, 21, 22, 27), isolation precautions (7), infection control and hospital personnel (26), and handwashing and environmental control issues (5).

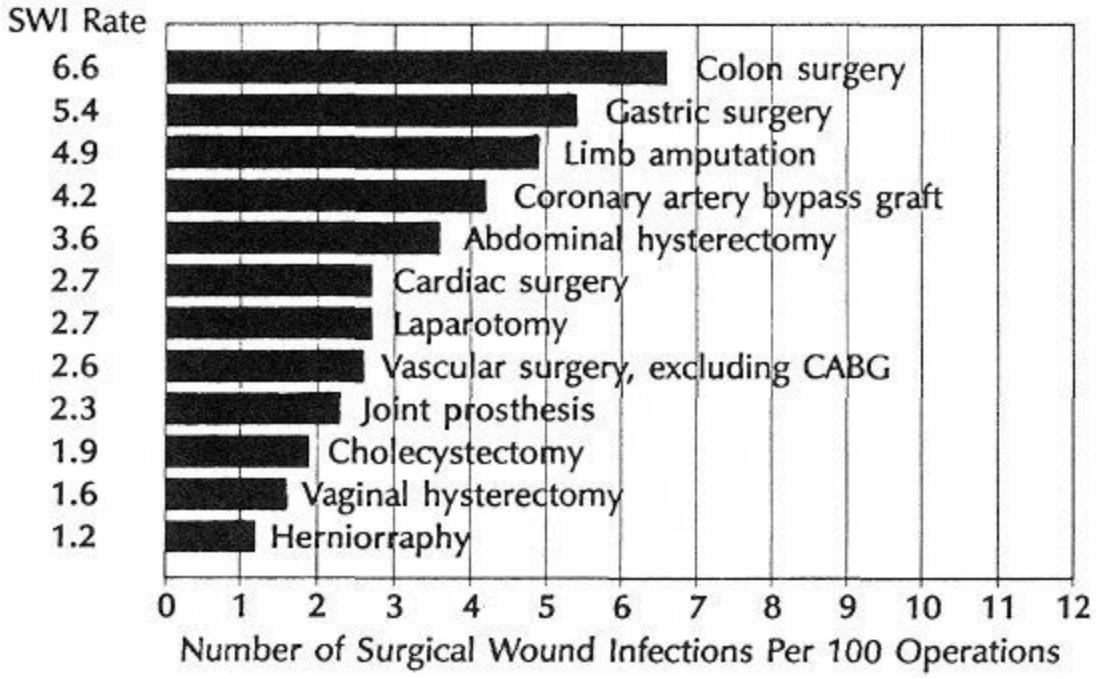

Figure 4. Surgical Wound Infection Rates By Operative Procedure Category

Current CDC recommendations for the prevention of surgical wound infections include performance of prospective surgical wound infection surveillance, calculation of wound class-specific, surgeon-specific, and procedure-specific surgical wound infection rates, and reporting of these infection rates to surgeons on a regular basis (4).

Conclusion

In summary, in addition to proper surgical technique, strategies for the prevention of surgical wound infections involve a multiplicity of approaches which include appropriate sterilization and disinfection procedures as emphasized during this conference, use of antiseptics and barrier precautions, avoidance of preoperative shaving of the patient, shortening of the preoperative hospital stay, treatment of remote infection prior to surgery, appropriate use of prophylactic antimicrobials, and surveillance and feedback of surgical wound infection rates stratified by patient risk to surgeons in a timely manner.

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Hepatitis B

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Introduction

Hepatitis B is one of the most important unconquered infectious diseases of mankind. The treatment provided is usually only supportive in nature. The potentially severe consequences of acute or chronic infection with hepatitis B and sequelae of cirrhosis and primary hepatocellular carcinoma (PHC), substantiate the need for preventive measures to interrupt the spread of hepatitis B virus (HBV) within the identified high-risk groups.

Hepatitis B virus is a DNA-containing virus that mainly replicates in the liver cells of man and some higher primates. The viral coat protein contains the hepatitis B surface antigen (HBsAg); the core includes a capsid protein, bearing the core antigen (HBcAg) and the e-antigen (HBeAg).

Epidemiology

It has been estimated that there are 250 million chronic asymptomatic carriers—about 6% of the world population. Variations in the prevalence of HBV markers exist in different geographic locations as evidenced by various epidemiological studies, with the prevalence highest in Africa, Asia, the Middle East, and Southern and Eastern Europe. Isolated hot spots exist in more temperate areas such as Greenland. The low prevalence of HBV markers in Western Europe and the United States does not mean that the problems there are relatively minor. In the Netherlands with a population of 14 million people, 750 hepatitis B cases are reported each year. Only about one-third of the cases have clinical symptoms however, and only one-third of these are reported. The true incidence, therefore, is much higher and should be estimated at 7,500 cases per year or 50 cases per 100,000 people.

In temperate climates, parenteral transmission of HBV predominates. In developing countries, perinatal transmission is the suspected primary route, although other possible vehicles include insects, tattooing, and acupuncture. Not everyone in the community is at the same risk of the disease.

Medical Personnel

Hepatitis B is well-recognized as an occupational disease among medical personnel who come in frequent contact with blood and blood products. Inconspicuous exposures and subclinical infection render it unlikely that post-exposure immunoglobulin prophylaxis contributes significantly to reducing the incidence of hepatitis B.

Perinatal Transmission

The HBeAg status of the mother as well as whether or not the mother has an acute infection during the third trimester, determines the risk of the child becoming a chronic HBV carrier. A combination of hepatitis B immune globulin (HBIG) and hepatitis B vaccine administered immediately after birth is highly effective in preventing perinatal transmission of the HBsAg carrier state.

Kidney Patients

Hemodialysis and renal transplant patients develop hepatitis B more frequently and react differently to infection than normal, healthy volunteers. A large proportion become asymptomatic chronic carriers of HBsAg, with 90% of this group remaining HBeAg-positive and infectious. These patients certainly form a major source of hospital infection. The result is that 30% of the personnel of a hemodialysis unit have serologic evidence of past infection, and 60% of the family contacts have evidence of HBV exposure (HBsAg or antibodies to hepatitis B [anti-HBs]).

Sexual Habits and Drugs

Sexual contact plays a role in the transmission of HBV. There is a high rate of HBV markers among spouses of asymptomatic chronic carriers of HBsAg. A high rate of HBV markers is also found in highly promiscuous populations, patients with venereal disease, and male homosexuals. In this respect it is important to note that hepatitis B is much more infectious than human immunodeficiency virus (HIV). The rate of clinical infection with HBV is not affected by HIV, but the risk of becoming a HBV carrier is increased by up to 90% if the patient is already an HIV carrier.

Clinical Facts

Ninety-five percent of individuals infected at infancy become chronic carriers, putting them at risk of developing chronic hepatitis, cirrhosis, and PHC. Less than 10% of the individuals infected with hepatitis B during adulthood become chronic carriers; however, clinically apparent infection is more common.

Consequences of hepatitis B infection depend on age at onset and range from asymptomatic carrier state to fulminant disease, with the real danger arising from the many subclinical cases. Acute hepatitis B has a 1% mortality rate, and 10% of the patients with acute disease progress to the chronic state. Acute cases usually recover without sequelae but chronic infection can lead to cirrhosis and PHC.

Treatment of hepatitis B can consist of a double attack on the virus by interferon and acyclovir. Interferon alone is ineffective, mainly because it does not affect DNA replication. Interferon plus acyclovir is an effective treatment for chronic hepatitis B, with transfer to virus latency in 33% of cases and cure to HBsAg-positive in 7%.

A remarkable correlation exists between HBsAg carrier state and the incidence of PHC, particularly in China, Asia, and sub-Saharan Africa. In some locales with endemic HBV, PHC

is the most common neoplasm in young men, with an annual incidence which is up to 100 times higher than in the West.

A superinfection can occur with delta agent which is a viral agent that requires concurrent HBV synthesis for its own replication. Delta agent represents a major threat to populations already at risk of hepatitis B infection since the superinfection is a proven cause of frequently fatal hepatitis. Histopathologic findings suggest that delta agent exerts a direct hepatotoxic effect. The routes of transmission of the agent are similar to those of HBV. Polytransfused patients, male homosexuals, and parenteral drug addicts are at high risk of delta agent superinfection due to the high rate of HBV exposure in these groups. Although delta agent has a world-wide distribution, epidemiologically, Southern Italy has the highest percentage, and it is present in 50% of its HBsAg-positive population.

Prevention

Prevention of hepatitis B can be provided by universal blood and body fluid precautions, and germicides, vaccines, and immunoglobulins.

Universal Precautions

Universal precautions for the prevention of transmission of HBV and other blood-borne pathogens in health-care settings have been published by the Centers for Disease Control. Universal precautions are intended to prevent parenteral, mucous membrane, and non-intact skin exposures to HBV. Blood is the most important source of HBV, and therefore, control efforts focus on preventing exposures to blood. Universal precautions also apply to semen, vaginal secretions, and other fluids such as cerebro-spinal fluid, synovial, pleural, and peritoneal fluid. Feces, sputum, and urine are not important unless they contain visible blood. Examples of universal precautions are gloves, gowns, masks, and protective eyewear. However, aseptic procedures which protect skin exposures cannot prevent penetrating injuries due to contaminated needles and other sharp instruments. General guidelines to minimize the risk of nosocomial transmission of HBV are: prevention of injuries when using needles and sharp instruments, use of protective barriers to prevent exposure to blood, application of the five guidelines for gloves, and immediate and thorough washing of hands and other skin surfaces that are contaminated with blood, etc. The likelihood of infection after skin exposure to blood containing HBV depends on the concentration of virus, the presence of skin lesions, the duration of contact, and the immune status of the health-care worker.

Staff infected with HBV should be allowed to continue to work, provided that their standards of hygiene are correct.

Germicides

Heating, sodium hypochlorite, ethanol, and glutaraldehyde are well-known effective germicidal effects. A general condition for viral germicides is that one should not be dealing

with a gross interfering organic load. The most important part of any disinfecting scheme is adequate precleaning of surfaces before conducting the actual germicidal step.

Heating destroys all viruses at temperatures commonly used for killing vegetative bacteria. As proven by direct infectivity testing, HBV in plasma is inactivated by heating at 98°C for two minutes.

Sodium hypochlorite is a broadly active and inexpensive virucidal agent used for general sanitation purposes. It is highly susceptible to the inhibitory effect of organic material. Ethanol 80% for two minutes at a temperature of 11°C is effective in killing HBV as shown by direct transmission tests in primates. Glutaraldehyde in low concentrations (1%) is effective for HBV inactivation within five minutes at 24°C. Cidex® (glutaraldehyde 2%, pH 7.5 to 8.5) has no corrosive properties and is advised for surgical and optical instruments (endoscopes) in contrast to povidone-iodine 1% and ethanol 30% in water (Betadyne®).

Other inactivating virucidal agents include beta-propiolactone, ethylene oxide, ultraviolet light, and ionizing radiation.

Vaccines and Immunoglobulins

Immunization with HBV vaccine is recommended as an important adjunct to sterilization procedures and universal precautions. It should be highly recommended by the hospital advisory boards to risk groups, e.g., sterilization units, dialysis, and operating room staff. In the Netherlands, free HBV vaccination is offered to hospital staff on a voluntary basis. A vaccination program has also been started which focuses on male homosexuals, prostitutes, infants of HBV-positive mothers, sexual partners of HBV-positive patients, and the medical profession. Since the initiation of this program, the number of new cases has decreased. However, this may also be due to a concomitant change in lifestyle of male homosexuals and the establishment of needle exchange schemes set up to prevent the spread of HIV infection in drug abusers.

A plasma-derived vaccine (PDV) and a yeast-derived recombinant vaccine (YDV) are available. The supply of PDV is limited by the amount of HBsAg-plasma available for the production of vaccine. To produce YDV, the fragment of HBV-DNA that codes for HBsAg is transplanted from the virus to the yeast cells which then assemble amino acids in the sequence programmed by the HBV-DNA. This results in identical HBsAg molecules since they are duplicated from the same gene. Antibody titres are similar for both vaccines.

The aim of vaccination is to reach a long-term anti-HBs level above the protective level. Hypothetically, humoral antibodies protect as long as they remain above a level of 10 ratio units; immunological memory protects if infection occurs within two years of the humoral antibody dipping below this level. Using a 0-1-6 month schedule, a booster injection will not be required for most subjects until four to five years following vaccination. A booster shot every seven years would save the optimum number of cases without the necessity of antibody re-testing. When rapid immunization is required and a 0-1-2 month schedule for YDV is employed, a booster dose is recommended at month 12, resulting in a predictive antibody level above protective plasma titre levels for six to seven years.

It is clear that multiple, scheduled vaccination activities including booster injection, offer a

severe logistic problem with travelling populations in rural tropical areas. Although hepatitis B can be eradicated just as smallpox was, the problems are enormous and include organization, cost, and vaccine acceptability. The main cost problem is that an intramuscular injection is needed and that the jet-gun method is discouraged because of possible transfer of blood with repeated injections. Vaccine-acceptability differs world-wide and is low in Africa, e.g., 20% for polio and just over 20% for measles. Accordingly, there is a high prevalence of HBV markers.

In the areas of low prevalence, vaccination priorities must be divided into preexposure and post-exposure cases. Pre-exposure vaccination is advised to healthcare personnel, dialysis patients, patients and staff in institutions, drug addicts, male homosexuals, prostitutes, and people working on rescue teams. Post-exposure vaccination plus immunoglobulin is supplied after accidental inoculations, to newborns from HBsAg-positive mothers, and after sexual contact with HBV patients and carriers.

Children are a specific problem. If an infant is exposed to HBV, it is an exception rather than the rule for the child to develop clinical hepatitis and jaundice, and then progress to recovery. Most infected infants have a subclinical infective course which progresses to chronic hepatitis, cirrhosis, and PHC.

Eighty to 90% of unvaccinated babies from HBsAg-positive mothers develop chronic hepatitis B. Once the mother is known to be a carrier, a single dose of HBIG, administered as soon as possible after birth, protects 50% of the babies and three doses protect 75%. Vaccine alone is as effective as HBIG alone, but repeated HBIG with full vaccination provides 95% protection.

There is a need to test every pregnant woman routinely as part of prenatal service and to give the appropriate therapy to the newborn. This will prevent much disability and economic suffering in the poorer parts of the world where infections are endemic. Even by routine vaccination of women in high-risk groups, perinatal transmission of HBV and congenitally acquired hepatitis B, arising in the 20 to 40 year age group, can, and should be, prevented.

Nosocomial Legionellosis

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Introduction

In the 14 years since Legionnaires' disease was first described in 1977, the genus Legionellaceae has grown fast, and now comprises 25 species and 46 sero-variants. Among legionella, *Legionella pneumophila* serogroup 1 is by far the most commonly found species causing pneumonia. Infections caused by any legionella species are called legionellosis or legionella infections, while the name Legionnaires' disease is restricted to infections caused by *L. pneumophila* serogroup 1.

Legionella bacteria are ubiquitous organisms found in fresh water lakes and rivers and of late, have also been found in ground waters. The growth temperature for legionella covers a wide range, 20 to 55°C, optimum 35°C, but they can survive at temperatures of 4 to 65°C. The growth is promoted by the presence of bluegreen algae and amoeba. The pathogenicity of these environmental bacteria has been the focus of considerable investigation. A number of toxins and enzymes have been described, some of which play a role in the pathophysiology of legionella infections. Sometimes heavy exposure to legionella does not cause disease, but to date, no single virulence factor has been reported which would distinguish the virulent strains from harmless isolates.

Legionella infections usually present as a pneumonic illness with a distinct onset which is clinically indistinguishable from broncho-pneumonias caused by other bacteria that are pathogenic in the respiratory tract, such as pneumococci and mycoplasma. Legionellosis can also cause symptoms in other organ systems, pontiac fever is a nonpneumonic febrile illness caused by a legionella species.

Epidemiology

It is difficult to estimate the true incidence of legionella infections, as the grounds for reporting vary greatly. As a result of prospective and retrospective studies, between 0% and 29% of community-acquired pneumonias have been reported to be caused by legionella. In part, this variation reflects a true difference, as legionellosis varies over time and within geographical areas. The proportion of community-acquired pneumonias caused by legionella is typically cited as approximately 2% and reports should be based on active surveillance. In the absence of a well-established active surveillance system, there is considerable underreporting. Although there is an observed difference in geographical distribution, legionella bacteria, and legionellosis, have been reported from all continents, and they seem to be present in all countries once they are looked for.

Nosocomial Legionellosis

The proportion of nosocomial pneumonias caused by legionella varies widely. The number of cases is often underestimated, as cases are often identified only as a result of active surveillance. The fact that legionella have been isolated from the water supply system in a hospital, does not necessarily mean that there are cases, but they must be looked for. In Pittsburgh, 30% of nosocomial pneumonias were identified as legionella infections until effective control measures were undertaken. From 1975-85, 421 cases encompassing 15 outbreaks in the U.S. and Europe were reported in the literature. A number of outbreaks have occurred in hospitals as a consequence of events happening to the water supply system. The largest outbreak so far, 174 cases during three years, occurred in a Veterans Administration hospital in Los Angeles. The major part of this outbreak followed a “pressure shock” in the domestic water system.

Clinical features are very similar for nosocomial and community-acquired legionella infections. Nosocomial disease tends, however, to be more severe and the mortality rate is higher; 30%-50% and higher, as compared to 5%-20% for community- acquired cases.

Nosocomial pneumonias in general are more difficult to manage than are community-acquired pneumonias, as the spectrum of etiologic agents to be considered are wider in the nosocomial situation. Hospitalized patients rapidly become colonized with gram-negative bacteria in the nasopharynx, from where the pathogen may be aspirated. In the case of legionella infections, there has probably been an inhalation of a contaminated serosal.

The Susceptible Patient

Legionella infections are more frequent among males over 50 years; other predisposing factors include smoking and alcohol consumption. Risk factors that are of special importance in nosocomial legionellosis are immunosuppression (comprising a 9-fold risk), and surgery under general anaesthesia. In patients under immunosuppression, the local pulmonary defense mechanisms are impaired, while general anaesthesia and surgery reduce the cough reflex, resulting in a decreased clearance of potential pathogens from the mucous membranes in the respiratory tract. It is noteworthy that patients undergoing transplantation surgery are at higher risk to contract legionellosis than are patients with only immunosuppression. This may be because they are subject to two major risk factors, immunosuppression and surgery. Chronic diseases such as organic cardiac disease, pulmonary disease, renal disease, and diabetes are also risk factors.

Diagnosis

The usual therapy for nosocomial pneumonias caused by gram-negative bacteria or staphylococci is not applicable to legionella infections. Therefore, it is of utmost importance to make the correct diagnosis at an early stage. Legionella should always be considered as the etiology of nosocomial pneumonia, even if legionella infections are not known to have occurred previously. Invasive techniques, such as bronchial lavage, brush biopsy, or transtracheal aspirates, are justified in order to obtain adequate samples for isolation and

diagnosis analysis methods. If such samples cannot be obtained, any respiratory secretion, including sputum, should be cultured. Legionella infections should be considered as a differential diagnosis in the early phase of a nosocomial pneumonia and not only as a second or last alternative.

To determine the etiology, a culture is preferred as the most specific and sensitive diagnostic method. Other methods utilized for legionella detection are immunofluorescent staining of secretions (DFA); antigen detection in urine; and most commonly encountered, antibody detection DNA hybridization and polymerase chain reaction (PCR), but these methods need further evaluation before they should be used routinely.

Multiplication of Legionella

Legionella bacteria quite commonly colonize water distribution systems from the fresh water sources. The colonization, as such, is probably harmless, as the number of bacteria is usually quite low. It is when the bacteria start to multiply within the plumbing system that they become a hazard to the susceptible patient. Multiplication has been shown to take place particularly in the calorifiers, but has also been found to occur in hot water cylinders or storage tanks and in the piping system itself, because rubber and some other fitting materials support the growth of legionella. Other sources reported are nebulizers, room humidifiers, shower heads, shower tubings, cooling towers, and evaporative condensers. Excavations have also been associated with outbreaks, but it is unclear whether the bacteria are disseminated directly from the soil or via other sources. Although multiplication most commonly takes place in the water distribution system within a building, the mains supply itself, especially if it is connected with storage tanks or cisterns, must not be discounted as a potential source of contamination. The most important promoting factor for growth of legionella is the temperature. Sediment, sludge, and the subsequent presence of algae in stagnant water, act as growth promoters (Table I).

Table I. Growth Habitats for Legionella in Hospitals

Heating Cylinders
Water Storage Tanks (Hot)
Mixer Tanks
Fittings of the Plumbing System
Shower Heads
Water Tap Aerators
Water Faucets
Respiratory Therapy Equipment
Nebulizers
Humidifiers
Cooling Towers
Whirlpools

Transmission

The transmission of bacteria to man is presumed to take place via a contaminated aerosol, although there is only circumstantial evidence that this is actually the case. Such an

aerosol may be generated by cooling towers, evaporative condensers, humidifiers, respiratory therapy equipment (nebulizers), showers and water faucets, and whirlpools and physiotherapy equipment used at health resorts and spas. Alternate routes of transmission, e.g., aspiration, have been discussed on the basis of case reports. These alternatives may have a greater significance in the hospital situation. In one report, the risk of acquiring legionellosis was found to be higher if a patient was receiving steroids and antacids. These findings would seem to indicate that after ingestion, legionella survive better in a neutral gastric pH and may be subsequently aspirated. The use of nebulizers in these patients may, however, have been a confounding factor. There is one report of a possible parenteral transmission of *L. pneumophila*.

Outbreak Investigation

The epidemiological definition of an outbreak is when the observed rate of disease exceeds the expected rate of that disease. Legionella bacteria are from an exogenous source; they have not been found to be part of the commencal flora of man, and there is no evidence so far of carrier stages. Therefore, the expected rate of legionella infections in the hospital environment is zero, and for even one case, the source should be investigated.

A strict case definition is crucial in any epidemiological investigation. As legionellosis cannot be clinically distinguished from pneumonias of other etiology, the diagnosis has been confirmed by microbiological examination. However, at the early stage of an investigation of a legionella outbreak, cases may be defined merely on clinical findings in order to save time. Case searching should be started, which is easily done if active clinical surveillance by an infection control team is done on an ongoing basis. Surveillance is needed in the first place to recognize pneumonias, and therefore, is part of a general surveillance.

If no general surveillance is maintained, cases should be identified among immunosuppressed patients by retrospective investigation of serum samples, taken for other reasons, or among post-mortem lung tissue specimens.

For investigational purposes, as well as for the individual patient care, it is important to have isolates from the patient in order to confirm the link between source and patient. There are often several legionella serogroups as well as several species isolated from one source. By modern biotechnology methods, such as the use of monoclonal antibodies and restriction fragment length analysis, the tools are available to subtype bacteria and make sure that isolates are identical.

Environmental investigation should take place as soon as there is reasonable evidence that a legionella case is diagnosed. The water supply system, especially the hot water system, and the cooling water system should be checked for obvious amplifying sources. Building service engineers are important participants on the outbreak investigation team. Samples should be collected from the water mains supply, storage tanks, heating cylinders, taps (especially hot water taps), and shower heads. Nebulizers and humidifiers should be sampled if there is any association with such devices, and the pools and showers of the physiotherapy department must not be forgotten. Attempts should be made to isolate legionella bacteria from the environmental samples. If there is a large number of samples,

some of the newer methods such as DNA hybridization or PCR might be used for screening purposes.

Control Measures

The immediate control of an outbreak can be achieved by high level shock chlorination (50 mg/L) and/or by raising the hot water temperature. High temperature is probably the most important measure. A temperature of 55°C, even at the outlets must be verified. Ultimately, it is most important to eliminate possible sites of multiplication. Storage tanks have to be disconnected if they cannot be operated in a safe manner. The construction of heating cylinders (calorifiers) should be checked and altered in order to eliminate stagnant luke warm water at the bottom of the cylinder and dead legs of the plumbing system should be cut off. The drift from a cooling tower or an evaporative condenser must not have an opportunity to enter the fresh air intake of a building.

Good Engineering Practice

All these control measures can be described as “good engineering practice” and are not restricted to the prevention of legionellosis. Maintenance, in order to keep the water distribution and air conditioning systems free from multiplying legionella, is crucial. Such maintenance can be called “good routine housekeeping” and includes keeping heating cylinders free from sludge and sediment and checking the temperature at appropriate sites (e.g., at the intake from the mains supply, at the outlet, at the bottom of heating cylinders, and at the tapwater outlet). It also includes cleaning cooling towers at regular intervals. The use of biocides has been discussed extensively; however, there is no general agreement on their use, and they certainly cannot replace mechanical cleaning. It should be mandatory that the staff who are responsible for maintenance is educated and kept informed and records should be kept of maintenance procedures.

Effect of Control Measures

Some examples of the effect of control measures are given below; one is from a hospital in Brussels. The hospital water distribution system was investigated over a period of three years, during which time high numbers, in the order of 10^{-6} cfu/L of legionella bacteria, were found. A disinfection cycle of 6 mg/L free chlorine failed to eradicate the bacteria, and 15 cases of legionella infections occurred during the following three years. Increasing the temperature in hot water cylinders was effective in eradicating the bacteria locally, but the mixer tanks, where hot and cold water was mixed to achieve 45°C, remained a reservoir. The mixer tanks were disconnected and a temperature of 60°C was maintained in the heating cylinders, in connection with an accelerated flow rate in the hot water system. These efforts proved to be effective, and no further cases occurred (Figure 1).

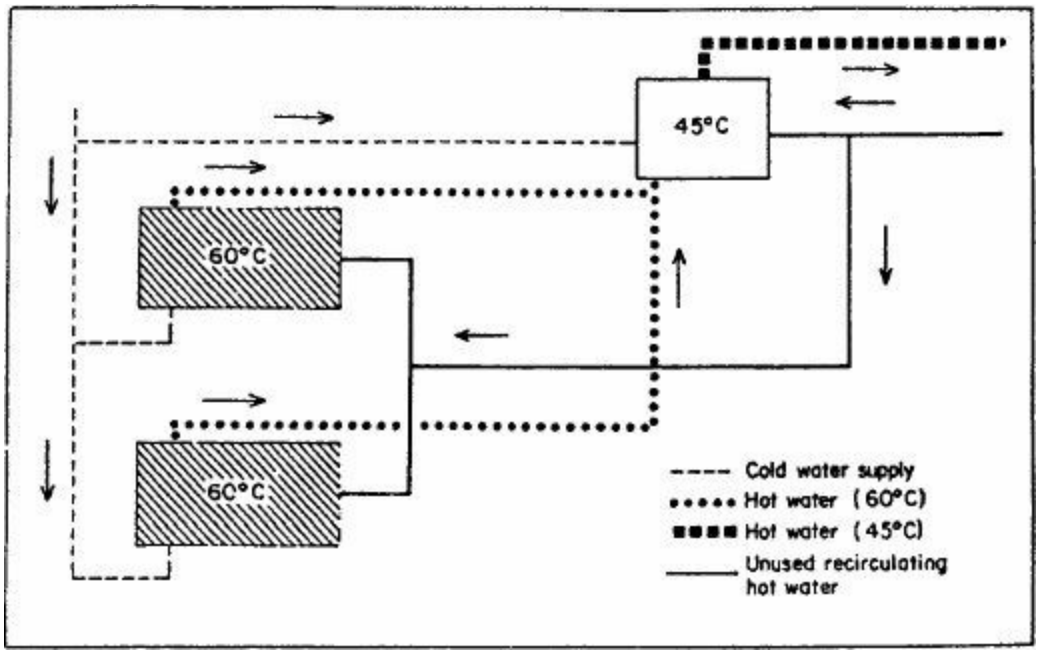

Figure 1. Flow Chart for a Hospital Water System Showing How Hot and Cold Water are Mixed in a Tank to a Temperature that will Support the Growth of Legionella

Adapted from Ezzedine, 1989

Another example is from Huddinge Hospital, south of Stockholm, where three cases of legionella infections occurred in kidney transplant patients over a period of 18 months. The causative agent, *L. pneumophila* serogroup 6, was isolated from shower tubings in the transplant ward. After hot water treatment and installation of an ultra violet filter at the hot water inlet to the ward, legionella could still be isolated, and two more cases occurred. Hot water treatment in combination with hyperchlorination, a change of shower tubings, and an improvement in the maintenance of the UV filter, diminished the amount of bacteria and no more cases have been identified in the following six years (Figure 2). Some other wards, e.g., the children's leucemic ward, have been similarly disinfected. Legionella can still easily be isolated from other wards, despite extensive case searches, however, there have been no cases in any of the wards besides the transplant clinic. No multiplication site, other than the shower tubings, was found (Figure 3).

A general investigation of all hospitals for the presence of legionella is not recommended. An investigation in England revealed that a majority of large buildings, such as hotels and hospitals, harbour legionella in their water distribution systems. However, there have been few cases of legionella infections associated with these buildings. It is not possible to eradicate legionella totally from the mains supply, but since the small number of bacteria in the incoming cold water are probably harmless to man, it is imperative to maintain the bacteria at these small numbers, e.g., to prevent multiplication.

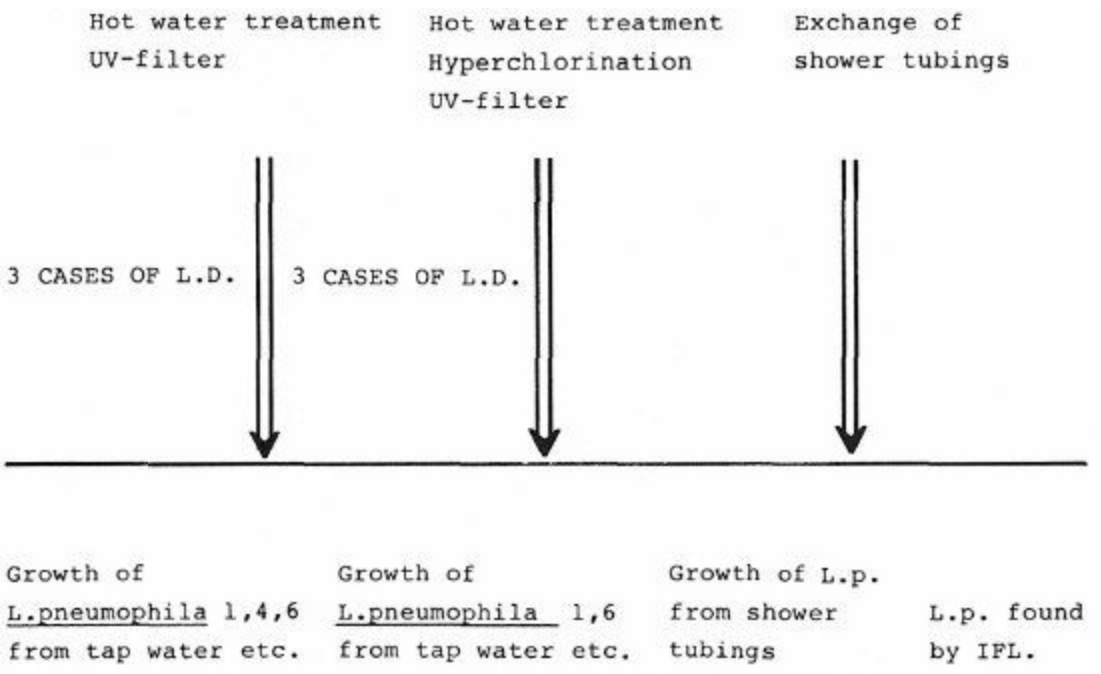

Figure 2. Decontamination Measures in Order to Prevent the Growth of Legionella in the Transplant Unit in Huddinge Hospital, Sweden

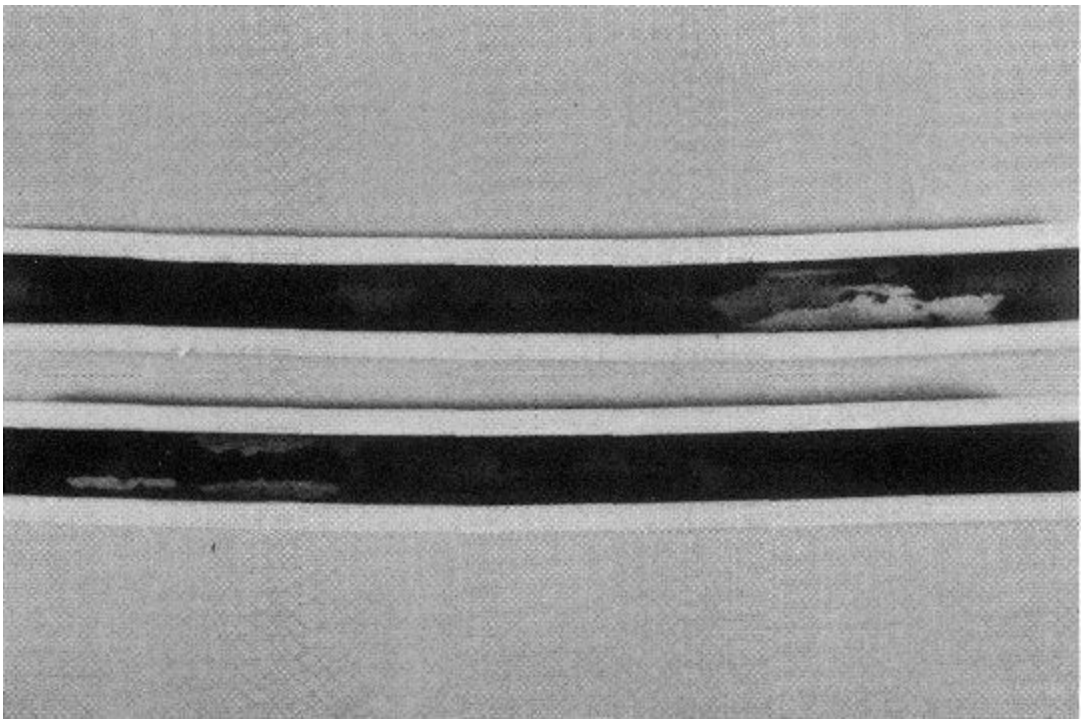

Figure 3. Inside of a Plastic Shower Tubing after Two Months of Use at Which Time Legionella was Isolated

Summary

There are five parts, or links, in the chain of causation of legionellosis:

— the environmental reservoir;

— the site of amplification;
— the dissemination route;
— the virulence of the microorganism; and,
— the susceptible host (see Table 2).

Table II. Chain of Causation Leading to Legionella Infections

Ubiquitous Legionella Bacteria
Fresh Water Lakes
Rivers
Ground Waters
Sludge
Soil (?)
Multiplication
Heaters
Storage Tanks
Shower Heads
Faucets
Humidifiers
Cooling Towers
Others
Transmission (Aerosol From):
Showers
Faucets
Whirlpools
Ingestion (?)
Others (?)
Virulent Organism
Susceptible Host

Control measures can be implemented with only some of these links. Clearly, there is nothing to be done about the environment, especially now that legionella have been found in ground waters. At present, not enough is known about virulence factors in the bacteria to recommend any precautions. An understanding of these factors has grown, but more research is still needed. As for the susceptible host, some risk factors can clearly be identified. Persons with these risk factors, e.g., kidney transplant patients, should be protected from exposure to the infectious agent. Sometimes, the disease strikes an apparently healthy person without notice and with a fatal outcome. Obviously, there are host risk factors for acquiring legionellosis that are not yet known. The knowledge of how to enhance specific or unspecific immunity in persons at risk is not at hand yet.

Amplification of bacteria in the water distribution system can and should be prevented. Some general recommendations on how to achieve these are given above. The dissemination of the infected aerosol can, at least to some extent, also be prevented.

Guidelines

In some countries, such as the U.K., the Netherlands, Germany, Denmark, and Australia, there are guidelines on the prevention of legionellosis available that are applicable for both construction and maintenance. WHO has four reports addressing the topic, the most recent one, from 1990, deals with surveillance as well as prevention: Epidemiology, Prevention and Control of Legionellosis: Memorandum from a WHO Meeting.

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The Role of the Nurse in the Prevention and Control of Hospital Infection

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Introduction

The prevention and control of hospital infection is an intrinsic part of the nursing practice. Nevertheless, for over 30 years, there has been a recognized need for nurses to specialize in this field (3). In England, it is now recommended that each Health Authority should appoint at least one Infection Control Nurse (ICN) (1).

The ICN of today is a Clinical Nurse Specialist. S/he is a member of a multidisciplinary team which is responsible for the monitoring, surveillance, and investigation of infections and for advising on preventative and control measures.

S/he is the only member of the Infection Control Team (ICT) whose entire time is devoted to infection control. The ICN's role consists of four main activities: surveillance, control, teaching, and research. It is within the context of a team approach that each of these activities will be described.

Surveillance

In order to prevent and control hospital-acquired (nosocomial) infections, surveillance activities must be focused on:

— the patient and his environment.
— the staff and their practices.

Patients and staff congregate in wards and departments, and the majority of nosocomial infections result from procedures and practices carried out on patients by staff.

Clearly, not all infections are preventable, but the SENIC (Study of the Efficacy of Nosocomial Infection Control) project demonstrated that a highly efficient surveillance and control system could possibly reduce the infection rate by one-third (4). Therefore, wherever there are patients and staff, there is a need to develop a surveillance program. Failure to do so imposes an unnecessary burden on the finances of the Health Service and also exposes many patients to pain and distress which could be prevented.

Surveillance activities are therefore undertaken for several reasons:

— to measure the incidence or prevalence of hospital infection(s).
— to identify infection or colonized patients who pose a risk of cross-infection.
— to observe whether infection control policies and procedures are being practiced as recommended.

Surveys of Infection

Surveys of infection are necessary because, in the absence of credible figures concerning the frequency of hospital infection and knowledge of the types of infections which are most common, attempts at control are likely to be ill-founded. As we do not have the manpower required to conduct incidence surveys of infection, occasional prevalence surveys of infection are conducted.

In the autumn of 1987, such a survey was carried out in the Health Authority in which I work (6). Five hundred and sixty-one patients in three hospitals were included in the sample. The results showed that 27.9% were infected, of whom about half had acquired their infections in the hospital. Overall, lower respiratory tract infections were the most prevalent but over half of these were already present on admission to hospital. Of the infections acquired as a result of hospitalization, urinary tract infection was the most common (26.8%) and there was an association between bladder catheterization and urinary tract infection. Lower respiratory tract infections accounted for 21.6% of all hospital-acquired infections, surgical wound infections, 15.5%, medical wound infections (i.e., decubiti and varicose ulcers), 10.3%, gastrointestinal infection, 9.2%, and skin infections, 5.1%.

Although the general pattern and distribution of infection is similar to that in the National Prevalence Survey conducted in 1980 (7), the overall prevalence rates of infections by percent of patients surveyed are considerably higher.

The results of this survey clearly highlighted where future educational effort should be directed.

Laboratory Surveillance

The ICN should, if possible, visit the microbiology laboratory twice a day to scrutinize all positive laboratory reports that have been drawn to his/her attention by the Infection Control Doctor. After consideration of the clinical and microbiological data, the ICN decides whether a follow-up visit to the wards is required, either to obtain further information or to offer infection control advice. By using nursing expertise, s/he imparts appropriate information to ward staff and so influences direct patient care. The influence is exerted in a variety of ways but mainly by informal interaction with nurses and doctors about specific patient-care practices. Experience shows that it is a clinically oriented infection control team, in constant liaison with the laboratory but spending most of its time on the wards, that is best equipped to prevent or control cross-infection.

Record Keeping Systems

Some form of record keeping system is required to monitor infections and plot epidemiological trends of hospital bacteria. Most laboratories now have computerized record keeping systems, but for those that have not, a card index system and a wall mounted epidemiology board can be just as effective, though somewhat more laborious to update.

A card index system can be used to register all pathogens from all sites. Under ward headings, the date, name of patient, site of specimen, culture, antibiogram, and any relevant comments are recorded. This is a cumulative record keeping system. Hence, if one particular organism is causing sporadic infections, the problem will be clearly highlighted.

An epidemiology board reflects the current state of the hospital from the infection control viewpoint. It is a visual display board situated in the Infection Control Doctor's Office. It shows the whereabouts throughout the hospital both of clinically infected patients and of those who are colonized with organisms about which there is concern for epidemiological reasons. It is cheap and simple to use. Information is communicated from one member of the ICT to another by means of cards and colored markers.

Under the appropriate ward heading, a card is generated on any patient who is of concern to the ICT. Each patient card is flagged with the appropriate markers which are color coded for various hospital pathogens. If, therefore, two or three markers of the same color are placed under one ward, this indicates that "clustering" is occurring and further investigation is required. However, "clustering" or evidence of an endemic organism will only be obvious on the epidemiology board if the isolates are recovered contemporaneously, because the markers are updated daily by the ICN after she has scrutinized the positive laboratory reports. The cards are brought up to day by the appropriate member of the ICT following a visit to the patient. The ICN can, therefore, see at a glance where her attentions should be focused.

Clinical Surveillance

There is absolutely no point in having well-thought-out infection control policies and procedures if no one has the responsibility for seeing that the recommendations are being practiced correctly. The ICN is in an ideal position to observe and assess the adequacy of clinical procedures involving any risk of cross-infection. The ICN can undertake clinical secondments on a cross-section of wards and departments on a full-time basis for several days or weeks. The main aims of one such clinical attachment (the details of which have been published elsewhere, [5] were:

i) to determine whether infection control measures, as taught in the Department of Nurse Education, were being practiced in the wards, e.g., maintenance of a closed system of urinary drainage.
ii) to determine, by observation, whether the recommended infection control policies had been implemented correctly, e.g., safe disposal of clinical waste.
iii) to identify problems that posed particular hazards with regard to infection control, e.g., the use of glutaraldehyde in the absence of adequate ventilation systems.
iv) to gain insight into various difficulties encountered by ward staff that could have hindered the prevention of cross-infection, e.g., lack of planned, preventative maintenance programs for bed-pan washing machines; the refusal of some domestic staff to clean isolation cubicles for fear of contagion.

On completion of clinical secondments like this, areas of conflict between recommended practice and actual practice can be discussed and resolved. In order to minimize the occurrence of such conflict, the ICN is a co-opted member of the Clinical Nurse Practice Committee.

It must be stressed that it is not only the clinical practices of nurses that are observed. Those of our medial colleagues are too. Deviations from recommended practice are pointed

out to the offenders. Reinforcement of suggestions or advice given can be effected by follow-up visits to the ward to identify and help solve any problems that are preventing the implementation of such advice.

Control

The second function of the ICN is the control of infection of which the two key aspects are:

— a role in the control of an outbreak, and
— giving advice on policies, procedures, materials, and techniques for the control of infection.

Control of an Outbreak of Infection

The purpose of one of the surveillance activities already described is to enable early recognition of an outbreak. Clinical surveillance and daily examination of laboratory reports are useful in detecting outbreaks or potential hazards. Clinical surveillance may highlight an outbreak before it is detected in the laboratory. For example, an explosive outbreak of hospital-acquired food poisoning will probably manifest itself at the ward level before it is evident via laboratory reports.

If the ICN is known to ward staff, they are more likely to alert the ICN to such an occurrence spontaneously. Conversely, if the ICN is not constantly out and about on the wards, s/he may not be told, and by the time the outbreak is recognized via laboratory reports, it may be too late to obtain food samples for culture from the implicated meal. This highlights the importance of the ICN's role in the clinical setting. On the other hand, some outbreaks of infection such as those caused by methicillin resistant *Staphylococcus aureus*, will be more readily identified in the laboratory first. The methods of control will be determined by the mode of transmission of the organism causing the outbreak.

Advising on Policies, Procedures, and Techniques to Control Infection

The ability of the ICN to assist in formulating policies and procedures to prevent and control infection, is based on a sound knowledge of epidemiological and microbiological principles. Examples of such policies include:

— isolation precautions for the care of infected patients which are determined by the mode in which the organism is spread;
— protective isolation precautions for the care of those who are immunocompromised and therefore highly susceptible to infection;
— cleaning, disinfection, and sterilization;
— guidelines for wound management; and
— aseptic techniques for the care of urinary and intravenous catheters.

All infection control policies must be simple and practical if there is to be a high level of compliance. They must also be easy to read and understand and readily accessible to all

staff. For ease of reference, they should be compiled in a manual and updated at regular intervals.

Aspects of infection control which relate to staff health include:

— encouraging Hepatitis B vaccination.
— providing gamma globulin to those at risk of Hepatitis B following needle stick injury.

Teaching

The teaching role of the ICN is not entirely discrete from surveillance and control because the ICN is constantly teaching by giving advice and demonstrating technique in normal ward activities.

Education of all hospital staff is a vital and continuing part of the role of the ICN because the more widely her philosophies are understood, the more effective she becomes. In the Parkside Health Authority, the ICT has cast its net very wide in the pursuit of audiences.

Broadly, we cover nurses in training, nurses undertaking post-registration courses in various specialties, other professionals including medical students and physiotherapists, and ancillary staff such as domestics and catering staff. All are taught basic hygiene measures such as handwashing technique, which is first demonstrated and then carried out by the students. They also receive lectures on topics relevant to their area of specialization and level of understanding. We believe that we are relatively unusual in providing lectures on infection control for medical students in their third year of training (first clinical year). Attendance is, however, optional. Emmerson and Ridgway (2) point out that “such is the nature of the overcrowded medical undergraduate curriculum that few students would pay more than lip service to such efforts (the teaching of the principles and practice of aseptic procedure), unless they are formally examined on aseptic practice in the final qualifying exam”. This raises the question, “Why aren’t they examined in aseptic techniques in the same way that nurses are”?

Research

The fourth activity of the ICN is research. It involves asking appropriate questions, investigating changes in clinical practice, studying reports, and acting accordingly on the findings.

My current research is focused on the prevention of sensory deprivation in patients who are confined to single-room accommodations for isolation nursing. I have been concerned about the adverse reactions I have observed in patients cared for in this way for a long time. It is argued that if nurses were educated to understand the principles of effective infection control, they would be more prepared to interact with infected patients both in a professional and social capacity. Furthermore, if nurses were taught the skills of assessment, they would be able to identify those patients most at risk of adverse reactions to isolation, and make real attempts to minimize those risks.

As a result, I am designing a study to develop teaching materials for nurses on:

— assessment of patients at risk of sensory deprivation, and
— the principles of effective infection control.

Conclusion

In conclusion, the role of the ICN has been described as that of a clinical nurse specialist.

The ICN is a specialist nurse member of a clinically orientated, multi-disciplinary team, providing specialist knowledge of, and expertise in, all aspects of surveillance, prevention, and control of infection. The main objective of the role is to reduce preventable infection to the lowest possible level by the use of practical, cost-effective techniques.

Teaching and implementation of infection control measures on the wards are vital functions of the role.

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DISCUSSION

Prevention and Control of Hospital Infection

Question for Dr. Favero, Centers for Disease Control, U.S.A.

What first aid measures would you recommend for a needle stick from injection following drawing blood from a high-risk patient? Should fluid material be aspirated as is done when administering first aid techniques for snake bites?

Answer by Dr. Favero, Centers for Disease Control, U.S.A.

I'd like to answer the last part of that question first. I am not aware of any protocols or procedures for snake bites that are applicable in the case of hepatitis B infection or HIV. In the case of snake bites, there are actually two things to worry about. In the case of hepatitis B, it really depends on whether a person is immunized or not. If a person is immunized, then there is no need for urgent measures. If a person is not immunized, the recommendation would be to immunize simultaneously with the vaccine. In the case of HIV, there is information which supports the use of a short prophylactic course of AZT after a needle stick involving a person with HIV infection.

Question for Prof. Brummelkamp, University of Amsterdam, The Netherlands

I think we can all agree that vaccination for hepatitis B is important. What measures would you suggest for an institution or community to use in order to increase the number of people who will voluntarily accept vaccination?

Answer by Prof. Brummelkamp, University of Amsterdam, The Netherlands

Presently, I think the only way to influence voluntary vaccination is through the education of those people potentially involved.

Question for Dr. Phillips, UMDS, St. Thomas' Hospital, U.K.

What are the policies and procedures at St. Thomas' with respect to infection control through disinfection and sterilization of instruments, especially endoscopes?

Answer by Dr. Phillips, UMDS, St. Thomas' Hospital, U.K.

I don't think there is anything unusual in what we do. We try to follow the

recommendations of others. For endoscopes, sterilization is performed largely with glutaraldehyde, particularly for sterilization required between the cases being handled in an endoscopy suite. Although there is increased controversy regarding glutaraldehyde, and other sterilization methods may be used, there is nothing as fast as glutaraldehyde which is why it is often used for endoscopes.

Question by Dr. Burke, Harvard Medical School, U.S.A.

What is your sterilization time?

Answer by Dr. Phillips, UMDS, St. Thomas' Hospital, U.K.

Unfortunately we can't afford enough endoscopes to allow sterilization to be continued for the full length of time that we've defined here. I suspect that the glutaraldehyde sterilization achieved is actually a disinfection process.

Question by Dr. Burke, Harvard Medical School, U.S.A.

Do you use a flush-through system?

Answer by Dr. Phillips, UMDS, St. Thomas' Hospital, U.K.

Yes, there is usually a flush system in a vertical container, and there is a certain dwell time there.

Question for Dr. Gardner, State University of New York at Stony Brook, U.S.A.

In ICU patients during the last decade, there has been an increase in infections caused by coagulase negative staphylococci. Some of these strains are resistant to two or three of the second and third generation cephalosporins. Do you use cephalosporins in coagulase negative staphylococcal infections, or do you recommend other antibiotics?

Answer by Dr. Gardner, State University of New York at Stony Brook, U.S.A.

I think that in the United States, the majority of *staphylococcus epidermidis* isolates are resistant to the beta-lactam drugs. I alluded to this briefly when I mentioned the resurgence of the use of vancomycin as the drug of choice not only for the mezlocillin-resistant *staphylococcus aureus* but for *S. epidermidis*. That is becoming a major problem. We used to consider *S. epidermidis* mostly a saprophyte, but clearly, in the age of prosthetic devices, it has emerged as a high level pathogen.

Question for Dr. L. Kallings, Ministry of Health and Social Affairs, Sweden

Do you have a comment on hepatitis B vaccination?

Answer by Dr. L. (Kallings, Ministry of Health and Social Affairs, Sweden

Yes, I would like to take this opportunity to mention hepatitis B vaccination. Since we tend to be very ethnocentric, these comments are especially appropriate since there are at least three Chinese colleagues with us. When we talk about hepatitis B vaccination, we are very often referring to what is going on in the Western countries. I am very impressed, however, by the mass hepatitis B vaccination program in China. I think it is either 8 or 13 million people that have been vaccinated with plasma-derived hepatitis B vaccine. I think that program is only the beginning of mass vaccination, in addition to the EBI program, of which China is one of the countries involved. There are several other countries that are contemplating the addition of hepatitis B vaccination to the expanded program of childhood vaccination.

Question for Dr. I. Kallings, National Bacteriological Laboratory, Sweden

Have there been any cases of legionella in dental practice, i.e., with the use of drills and the airdriven devices that make aerosols. This would seem to be a fertile source of aerosolization of legionella. Are there any reports of this or is this not a problem?

Answer from Dr. I. Kallings, National Bacteriological Laboratory, Sweden

This area has been studied, but I am not aware of any documented cases.

Comment by Dr. Burke, Harvard Medical School, U.S.A.

Dr. Pollock, there are several related questions concerning Chlorhexidine for which you raised an issue. Chlorhexidine is an agent used in the disinfection of instruments and hands. What is your opinion of its efficiency, should it be used in shower treatment for burned patients, and should all surgeons jump into a Chlorhexidine shower before they jump into the operating room?

Answer by Dr. Pollock, Scarborough Hospital, U.K.

There is no evidence that a patient benefits when the surgeon jumps into a Chlorhexidine shower before operating. Rather the reverse may be true. It is probably better for the surgeon not to take a shower, because after a shower, one tends to shed more skin scales. Chlorhexidine has not been shown to be particularly effective as a treatment for burns. Curiously enough, and I am afraid I don't know why, the most effective local application is silver in the form of silver nitrate or silver sulphadiazine. I would also like to inject a comment about the question of stimulating the immune response. I am not convinced that that is what we should be looking to do. I think that by stimulating the immune response we may be doing a great deal of harm. What we should really want to do is to modulate the immune response to the patient's benefit.

Question for Dr. L. Kallings, Ministry of Health and Social Affairs, Sweden

Please comment on the situation in the U.S.S.R. where AIDS was spread within the hospital environment.

Answer by Dr. L. Kallings, Ministry of Health and Social Affairs, Sweden

I will make a comment, but perhaps some of our Soviet Union colleagues would care to answer as well. In Volgograd at least, the nurses were not following the regulations or guidelines. According to the information that I received from my Russian colleagues, each child was infected due to malpractice. The infants had severe underlying diseases that had brought them to the intensive care units. Due to their severe diseases, they had indwelling subclavian venous catheters and were given injections through these catheters. The nurses used the same syringe from one child to the next to administer treatment and to withdraw blood samples. This is a very similar situation to drug users sharing needles. It is no wonder that this was an effective way to transmit the HIV infection. A shortage of syringes may have helped to perpetuate this unfortunate practice. As long as one follows the usual guidelines, there should be no reason for transmission of cross infection in the U.S.S.R. As Ms. Jenner discussed already, there is a constant need to inform and educate and then to follow precautions.

Question for Dr. Hughes, Centers for Disease Control, U.S.A.

Are current biological barriers considered acceptable in terms of virus permeability or is there a need to upgrade latex, for instance, as a barrier in order to avoid contamination? Are the barriers that exist now suitable to prevent the hepatitis B virus from going through the barrier?

Answer by Dr. Hughes, Centers for Disease Control, U.S.A.

This question comes up quite a bit in the United States, especially with the increasing usage of gloves. Let me just point out two basic things. If one speaks of a surgeon, a surgeon wears gloves for two reasons. Gloves are worn both to protect the patient by preventing direct exposure of the surgical site to the surgeon's microorganisms and to prevent the patient's blood from infecting the surgeon. For all other glove-wearing professionals, the gloves are used solely for protection of the wearer. Therefore, these gloves do not have to be sterile and they do not have to made of latex. I think that as long as the glove grossly protects the skin from blood, it makes an adequate barrier protection. There are some disposable gloves for the laboratory that come in a sheet, in which the gloves are punched out. For that purpose, those gloves are fine, although they would never pass the test designed for a condom, where one fills it with water to see if it leaks. The point is that a micro-pinpoint in such a glove doesn't prevent adequate protection.

Question for Dr. Ledger, New York Hospital, U.S.A.

What are the disadvantages to the use of prophylactic antibiotics?

Answer by Dr. Ledger, New York Hospital, U.S.A.

I think there are two. The first is the concern that, because these antibiotics are available and are used by surgeons, there is the potential for surgical techniques to become lax. This is something that has to be guarded against. The other concern about the widespread use of prophylactic antibiotics deals with the proliferation of resistant organisms, because one antibiotic is being used over and over again. I don't think that if the antibiotics are used for a short period of time (typically one dose) that there is any good evidence that a problem exists. At least no problem has been reported.

Question for Dr. Gardner, State University of New York at Stony Brook, U.S.A.

Would you please comment on the use of immunoglobulins for the diagnosis and treatment of surgical infections?

Answer by Dr. Gardner, State University of New York at Stony Brook, U.S.A.

There is a great hope that immunoglobulins will have a larger impact then they have already. In my talk this morning, I covered three or four areas that might make a difference. One would be in developing a common antigen that could be used to mount an antibody in a patient who is at risk for the gram-negative bacteria. Another would be to develop a battery of passive immunoglobulins which could be used for the passive immunization of people who are ill. And the third would be to develop antibodies, as has been done experimentally, to modulate the mediators of infection, such as tumor necrosis factor or interleukin-1. These are all areas for investigation. The area that has been looked at the most has been the use of the J5 antiserum, but after some initial positive reports, we are now beginning to see some negative ones. There is one situation in which it is generally accepted that gammaglobulins have been helpful, and that is in the newborn, premature infant who has inadequate levels of IGG. This is a situation where gammaglobulins have clearly been beneficial. Although the current uses are not well established, this should be considered an important area to investigate.

Session V
Radiation Sterilization
Chairman: C.M. Herring
Johnson & Johnson, U.S.A.

Introduction Radiation Sterilization

C. M. Herring

Johnson & Johnson, U.S.A.

Radiation sterilization is still considered a fairly new process although its origins obviously go back to the discovery of atomic energy in the 1890s. In 1896, it was first reported that X-rays could kill microorganisms. This new source of energy was exciting and was put to wide and often indiscriminate use for diagnostic, therapeutic and cosmetic purposes which resulted in many over exposures.

During the 1930s and 1940s, more powerful X-ray machines were developed along with high-energy electron accelerators which were used for both medical and industrial purposes. The development of high power radio frequency generators for radar applications during World War II provided the breakthrough needed for the expansion of accelerator technology and led to the first commercial radiation sterilization of medical products. In 1956, Ethicon, Inc., a Johnson & Johnson company, together with High Voltage Engineering, built a high-energy accelerator in Somerville, New Jersey, for the sterilization of surgical sutures. This accelerator operated until 1964, when it was replaced with a Cobalt 60 irradiator. Accelerators have been widely used in a number of industrial processes but have captured only a small share of the medical products sterilization market compared to gamma irradiators.

Gamma processing can trace its origins to December 2, 1942 at the University of Chicago, where a reactor designed by Enrico Fermi achieved the first selfsustained chain reaction and brought the world into the age of nuclear energy. The development of nuclear power reactors provided a way to economically produce high specific activity radioisotopes — of particular interest to us, Cobalt 60.

Gamma irradiation on an industrial scale was pioneered by the English and French Atomic Energy Agencies. In 1960, the first two industrial gamma irradiators were put into service, one in Australia and the other in France. In the nearly 30 years since that modest beginning, the use of gamma irradiators for sterilization has grown tremendously. There are now approximately 145 gamma irradiators worldwide with a combined source strength in excess of 135 MCi. Much of the growth in the use of ionizing radiation for the benefit of mankind has been a result of the promotional and support activities of the International Atomic Energy Agency headquartered in Vienna.

During this morning's session, we will hear presentations by Mr. Stepanov and Mr. Saylor describing new developments in radiation sterilization equipment and how they may impact the way we use radiation to sterilize medical products in the future.

One of the primary considerations in the application of radiation sterilization to medical products is the compatibility of the materials in the products and packages with ionizing radiation. All materials exposed to ionizing radiation are affected to some extent depending on the absorbed dose. This effect can range from inconsequential at doses much higher

than needed for sterilization, to unacceptable alterations of materials at doses below those needed to assure product sterility. In the early days of radiation sterilization a lack of alternatives for noncompatible materials kept some products from being sterilized in this manner. Today many more alternative polymers and polymers specifically stabilized for use in radiation sterilization are available which make it possible to redesign many products for radiation compatibility.

Dr. Ishigaki will present some very interesting information on radiation effects in some polymers used in medical products.

The key to any successful sterilization process is the ability of that process to destroy the microbial challenge presented on the products and packaging. Microorganisms exhibit a wide range of resistances to sterilization processes and radiation is no exception. The radioresistance of microbes and the impact of this on the minimum sterilizing dose requirement have been a subject of on-going interest since the work of Drs. Artandi and Van Winkle in the late 1950s. Their studies established 25 KGy as an effective sterilizing dose for sutures, and this has evolved into a widely accepted sterilizing dose for a variety of products in many countries. However, there are advocates of both higher (i.e., Scandinavian countries) and lower doses (i.e., United States).

Presentations by Ms. Rakitskaya and Dr. Whitby on the subject of radioresistance and dose setting will provide us with some current thoughts on sterilizing dose requirements.

Radioresistance of Microorganisms: Theoretical and Practical Aspects

G.A. Rakitskaya, I.I. Samoilenko, Ye.P. Pavlov, N.V. Ramkova, S.V. Zykova and V.V. Saribekian

All-Union Scientific Research Institute of Medical Polymers, Moscow, U.S.S.R.

Radioresistance refers to the ability of microorganisms to resist ionizing radiation. We shall consider three aspects of radioresistance: the nature of radioresistance; the study of radioresistance on microorganism-contaminants from various products sterilized by ionizing radiation; and a possible modification of radioresistance using different physical and chemical factors.

Nature of Radioresistance

The study of the nature and mechanisms of radioresistance is of interest both from the view-point of the general biological problem, that is, the nature of cell viability under extreme conditions, and for applied purposes in radiation biotechnology.

On the living creatures' hierarchical ladder, microorganisms occupy a high place with respect to radioresistance. The main lethal doses for microorganisms exceed those for other representatives of the living world by 2-3 orders.

But there is a considerable difference in radioresistance among microorganisms. Years of studies on the effects of ionizing irradiation on microorganisms has established a hierarchy in the affinity of organisms to radiation. Sporeforming microorganisms have proved to be the most resistant, particularly their spores; then in descending order of radioresistance, yeast, mold, fungi, viruses, and bacteria (Figure 1). Moreover, as a rule, gram-positive microorganisms are more radioresistant than gram-negative ones. Discovering the reasons for the considerable variation in radioresistance is one of the pressing problems in microbiology.

A group of non-sporeforming, pigmented micrococci is among the most radioresistant microorganisms. Superradioresistance of these bacteria as well as the peculiarities of their cellular structure organization served as the basis for distinguishing *Deinococcea* in a separate family in 1981.

Of five species included in the *Deinococcus* genus, *Micrococcus* (*Deinococcus*) *radiodurans* is the best studied. This microorganism has been obtained from canned meat exposed to radiation sterilization. We have noted several major factors conditioning the superradioresistance of this gram-positive *tetracoccus* (Table I). Besides a peptidoglycan layer, at least four other layers comprise the cellular wall of *M. radiodurans*: intermediate, outer membrane, electronic-thin zone, and the superficial hexagonal protein system. The superficial system of *M. radiodurans* is formed by protein molecules; it contains less than 1% carbohydrates and no lipids. The cells are notably resistant to lysosomal action (i.e., lysis resistant). There is not consensus regarding the connection between the observed peculiarities in the cellular wall organization of *M. radiodurans* and its radioresistance.

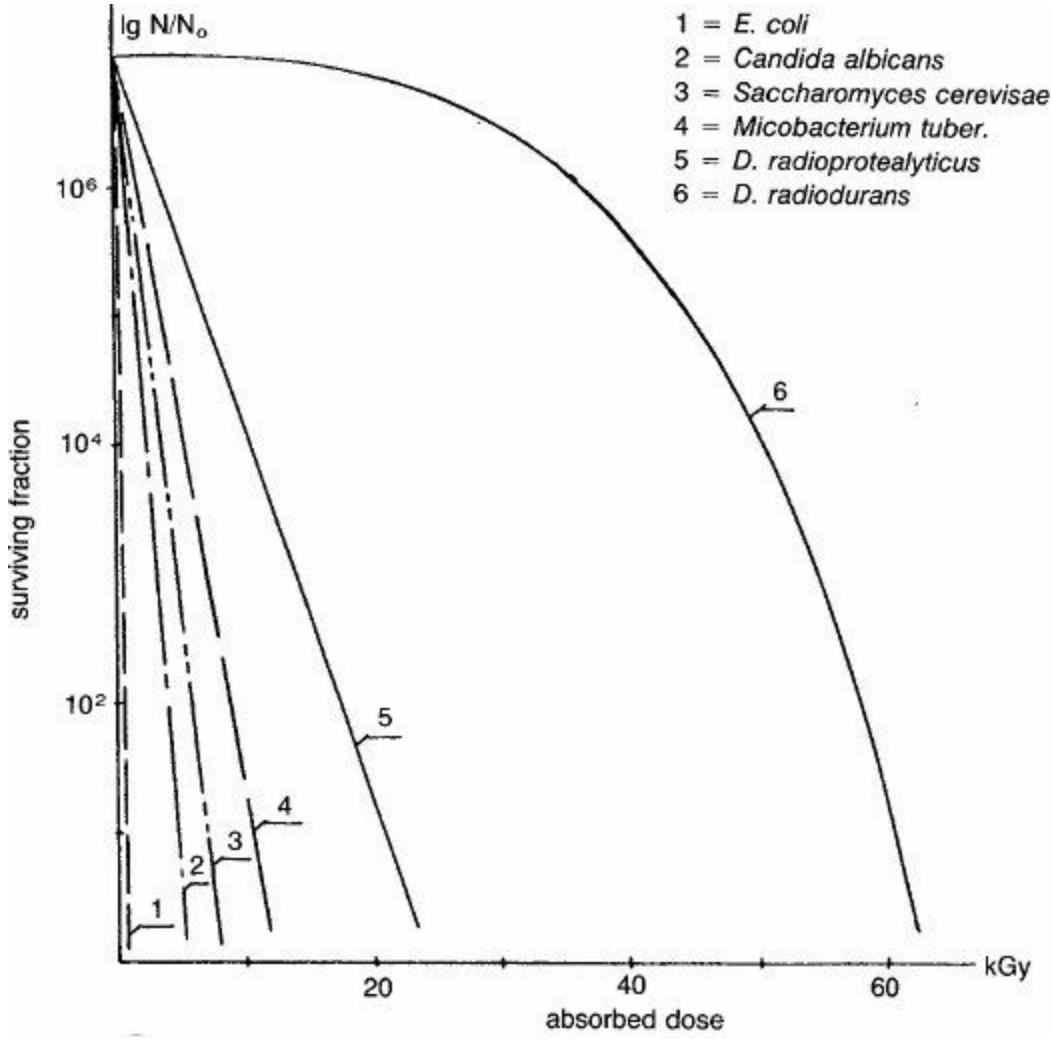

Figure 1. Dependence Dose-Effects

Table I. Factors Determining the Highest Radiation Resistance of *D. Radiodurans*

Cell Wall Structure
Membrane Structure
DNA Structure
High Level of Endogenous Protector's Content
Redundancy of Genetic Information
Multiplicity of DNA-Membrane Binding Sites
High Activity of Antioxidative and Antiradical Systems
Balance of Processes of Degradation and Resynthesis of Genetic Material
Highly Efficient Faultless System of Repair of DNA Damages

Despite this lack of consensus, some scientists have proposed a connection between the unusual radioresistance of *M. radiodurans* and the peculiarities of its cellular wall structure, in particular, with the hexagonally packaged protein particle monolayer. It is noteworthy that in the layer outside the peptidoglycan site of *M. radiodurans*, no usual bacterial phospholipids, such as phosphatidylethanolamine, phosphadylserine, phosphadylcholine, and phosphadylinosine have been found.

Attempts have been made to correlate the radioresistance of micrococci with the

presence of carotenoid pigments. However, a release of pigmentless mutants of *M. radiodurans* which have a radioresistance similar to that of the wild type, along with the demonstration of pigmented radiosensitive strains, have made it possible to infer that pigments do not play any appreciable role in radioresistance of bacteria.

The question of DNA structure and its amount in *M. radiodurans* was studied in detail. Despite thorough investigations, the data can not account for the radioresistance of micrococci. For example, a high amount of GC-pairs (67%) was shown, but a number of radioresistant bacteria (pseudomonads) have an analogous nucleotide DNA composition. Radioresistance of bacteria was not shown to be connected with the value of the proportion: DNA/protein.

Reparation of DNA impairments and reparative systems of *M. radiodurans* were thoroughly studied. Two processes resulting in a reparation of DNA impairment were shown to occur following exposure of micrococci cells to gamma-radiation. The first type of reparation is suppressed by chloramphenicol in the case when the antibiotic is added immediately after radiation exposure. The second type of reparation process is not sensitive to chloramphenicol, but is completely suppressed when the bacteria are exposed to radiation in medium with EDTA. Reparation of this type occurs without a thymidine inclusion in DNA and is probably made of polynucleotideligase. EDTA effects are of an irreversible nature. Evidently, during the incubation with EDTA, the enzyme activity results in single-stranded breaks turning into gaps.

Nalidixic acid in a concentration of 5 mcg/mL for 30 min suppresses by 70% DNA synthesis in *M. radiodurans* cells growing exponentially; however, further suppression of the replication is not seen even with 100-fold higher concentrations. According to some scientists, DNA synthesis in micrococci consists of two components, only one of which is sensitive to nalidixic acid.

Single-stranded breaks in the DNA induced in bacteria exposed to a dose of 2 kGy following a 5-hour incubation under growth conditions, are repaired (Figure 2). Their number is somewhat higher at exposure to the air (this applies to double-strand breaks, too), than in a nitrogen atmosphere.

The results of the analysis of the thymine radiolysis products in *M. radiodurans* suggest that during the first 30 minutes, up to 5% of damaged thymine is removed from the bacteria's DNA. After 20 minutes of incubation, the cells are free from the impaired DNA. The first phase of this process is supposed to be connected with DNA degradation by exonuclease, without endonuclease, and radioresistance of *M. radiodurans* is connected, to a great extent, with its ability to remove damaged thymine quickly from cells. Only a part of DNA impairments is shown to be involved in the degradation; 75% of single-stranded breaks in the DNA are repaired within the first 5 minutes of incubation at the exposure to the dose of 2 kGy. To stop the degradation, it is necessary to synthesize protein *de novo;* excessive degradation is lethal.

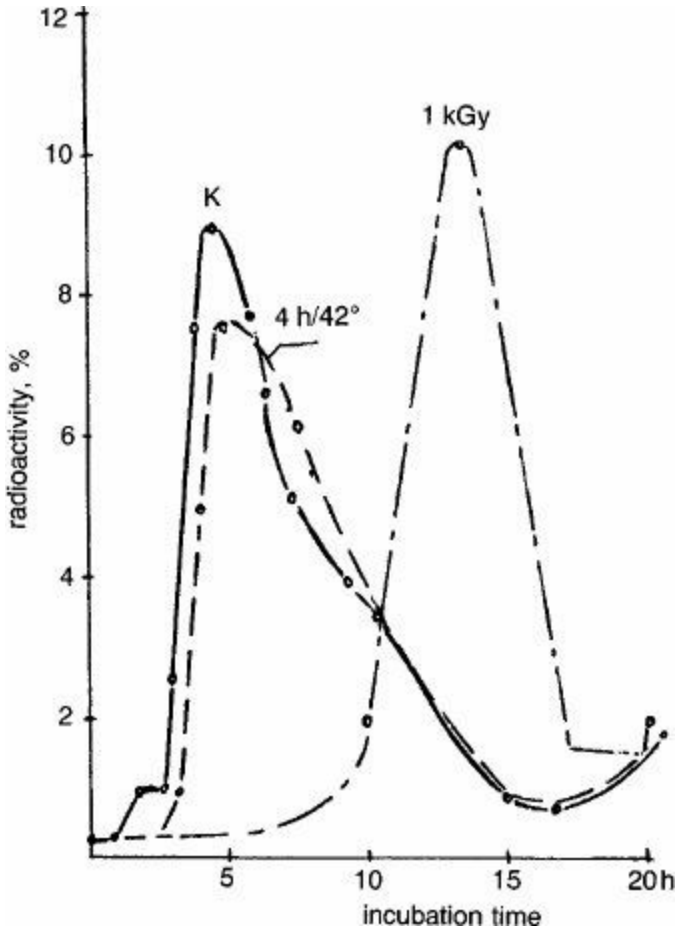

Figure 2. Influence of Postradiation Incubation on Reparation of SSB DNA of *D. Radiodurans*

The high resistance of *M. radiodurans* to ionizing radiation may largely be accounted for by its ability to repair double-strand breaks in the DNA. If the DNA molecular mass in intact cells is 3.8×10^8 dalton, after exposure to the dose of 2.2 kGy, it is reduced to 1.8×10^8 dalton because of double-strand break formation. Postradiation incubation in the nutrient medium restores the DNA molecular mass to its initial value within 180 minutes. The mean time of restoring double-strand breaks is 52 minutes during which time their number is reduced by 67%.

In the presence of 200 mcg/mL chloramphenicol, 100 mcg/mL tetracycline, or 1 mcg/mL actinomycin D, double-strand breaks are not restored; thus *de novo* protein and/or RNA synthesis is necessary. Perhaps these syntheses are a “primary target” of ionizing radiation in *M. radiodurans*.

M. radiodurans has also been shown to have a more effective system of super-quick reparation of single-stranded breaks in the DNA than does *Escherichia coli* which belittles the probability of subsequent formation of double-strand breaks. The majority of conclusions concerning DNA reparation systems was formed from the study of radiosensitive mutants with defective (as a rule) reparation systems (Figure 3).

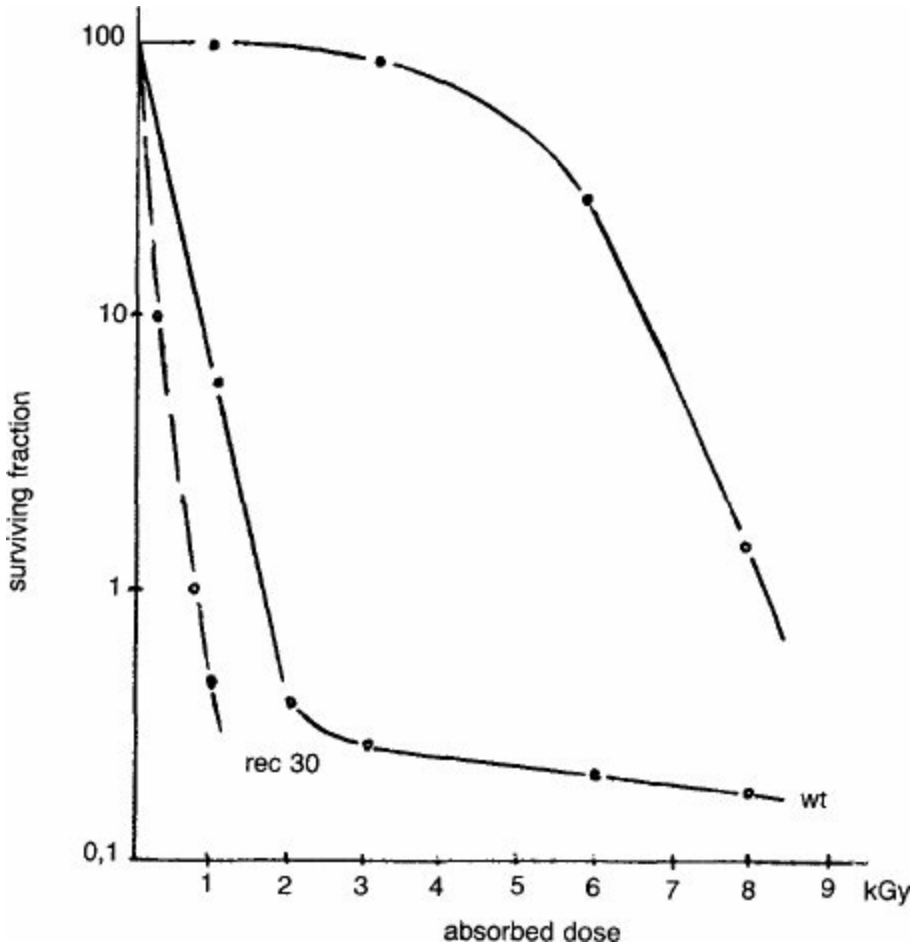

Figure 3. Experimental Survival Date for *D. Radiodurans* Strains wt. and rec 30. Postradiation Incubation During 4 hours, 42°C

Of special interest to this discussion is the observed disturbance in the coordination of DNA degradation processes and restoring synthetic processes in cells. Analysis of the data on radiosensitivity obtained from mutants and the wild type of *M. radiodurans* has led to the inference that the superradioresistance of wild type cells may be explained, to a great extent, by the fact that they have not developed reactions of exonuclease activity stimulation. Hence, in the exposed micrococci cells, there is no disturbance in the equilibrium of endonuclease and polymerase activities of reparation enzymes which determine a high efficacy of the DNA structural impairment reparation. The disconnection of rates of degradation and resynthesis of DNA processes in radiosensitive mutants results in a sharp decrease in radioresistance of these bacteria.

The analysis of mutagenic and lethal effects of a number of mutagens of *M. radiodurans*, compared with *E. coli*, shows that micrococci are 55 times as resistant to gamma-radiation, and 33 times as resistant to ultra-violet radiation. The high resistance of *micrococcus* to a lethal effect of agents inducing DNA impairments, repaired by es-system, is evidence that *M. radiodurans* has a highly effective recombination reparation mechanism. Mutagenic factors such as UV- and gamma-radiation or treatment by mitomycin C, are absolutely not mutagenic for *M. radiodurans*. Thus the micrococcus' reparation systems are not only highly effective, but they are more faultless as well.

Radioresistance of Microorganism Contaminants from Radiation-Sterilized Products

As we have already noted, the problem of microorganisms' resistance to ionizing radiation is of not only general biological importance, but also has an applied aspect, in particular, when ionizing radiation is used as a sterilization agent. In this aspect, it becomes of great importance to study the radioresistance of microorganism-contaminants obtained from products subjected to radiation sterilization.

The exponential nature of microorganisms' death make it theoretically impossible to reach their complete elimination in the system. In practice, it is generally acceptable to consider a product sterile (it is more correct to speak about a high microbial purity or the microbial safety of the product) when the probability of contamination does not exceed 10^{-6}. In contamination of the product by cells of one type i, with the initial number of cells (Ni):

$$N_i \text{ final} = 10^{-6} \text{ and } D_{st.} = D_{10} [1g(N_i)o + 6]$$

With low-resistant microorganisms, D_{10} is < 1 kGy (typical of the most microorganism-contaminants) even under conditions of a high initial contamination (N_0 approximately 10^3-10^4 cells per object) and $D_{st.}$ is ≤ 10 kGy. For radioresistant cultures, D_{10} is equal to 2-3 kGy or higher (with N_0 approximately 10^3) and $D_{st.}$ is 20-30 kGy.

Thus, the dose required for a reliable sterilization is initially determined by the radioresistance of the contaminants, and only slightly depends on the number of cells. For example, a 100-fold change in N_i, from 10^4 to 10^2, results in only a 20% reduction in $D_{st.}$

The microflora under study consists of cells with different radiosensitivities. Here it is noteworthy that a sterilizing dose is determined by the radioresistance of the most resistant forms. The results of studies on thousands of microorganism strains obtained in different industries, has shown that the total number of such cells is not large, i.e., only approximately 2%-3% of the total number of all obtained microorganisms; therefore, in determining a sterilizing dose, we should also proceed from the likelihood of finding radioresistant microorganism forms.

The composition of microorganisms contaminating different industries and different products is diverse and changes with season and geography location. The composition also depends on the products manufactured at a particular site.

We examined manufacturers of various medical products—both polymer- and other material-based.

Production appears to be contaminated by both vegetative and sporeforming forms of microorganisms. In some manufacturers, the proportion between these two types was 45% and 54%, respectively. For other manufacturers, depending on the season, this proportion was 12% to 16% and 65% to 73% in winter and spring, respectively, and 35% to 37% and 60% in summer and autumn, respectively. Production of some products is contaminated only by sporeforming microorganisms.

The study of the microfloral composition provided us with a preliminary estimation of its radioresistance. The definition of microorganisms' radioresistance was undertaken for three

purposes: to establish a sterilizing dose for products which are to be sterilized by radiation; to prove (or refute) the appropriateness of a 25 kGy dose for sterilization of highly contaminated productions (this primarily applies to the production of materials such as cotton and gauze); and to study the dynamics of microorganism radioresistance at enterprises manufacturing radiosterilized products depending on when radiation sterilization was initiated at the site, the season, and the geographical location, as well as to isolate highly radioresistant strains for the purpose of using them as test-microorganisms in evaluating modifications of the sterilizing regime.

For a decade, studies have been carried out on the radioresistance of microorganisms isolated at manufacturers of radiopharmaceuticals or utilizing radiosterilization during production. Separation of microorganisms from the industrial microflora and from the products was performed 2-4 times a year, by the method of random sampling in different geographic zones of the country. The essence of our technique is as follows.

Wash-offs from the products (or from sponges used for washing off the surfaces of the equipment, walls, floor, etc.) were filtered through membrane filters, dried, exposed to a dose of 1, 2, 3, 5 and 10 kGy and then placed in the solid nutrient medium (beef-extract or agar of Hottinger [pH: 7.0-7.2]). After incubation at 32°C for no fewer than 10 days, the growing colonies were resown on slant agar in test-tubes. For these microorganisms, the index D_{10} was determined by a generally accepted technique or by a peroxide sensitivity technique. It is well-recognized that direct determination of radioresistance by index D_{10} in industrial conditions meets with certain difficulties. Therefore, we studied the suitability of an indirect estimation method of microorganisms' radioresistance using a disk technique for determining microorganisms' sensitivity to hydrogen peroxide.

A correlation has been shown between a microorganisms' sensitivity to gamma-radiation and hydrogen peroxide exposure (Table II). In these studies, we used microorganism strains obtained from manufacturers of medical products as well as laboratory strains with a known index D_{10}. The essence of the peroxide sensitivity technique is as follows: 0.01 mL of hydrogen peroxide solution (0.3%, 1% and 3%) was dropped on each of the filter paper experimental disks with the diameter of 8 mm (Watmann, 3 MM) after their sterilization in a drying oven. The wash-off from the 18-hour culture under test was diluted by a 0.15 solution of NaCl to a concentration of 10^7 microbial cells in 1 mL, and 0.6 mL of the obtained suspension was distributed evenly on the surface of the solid nutrient medium in a Petri dish. After the dishes were dried, the disks were put in and hydrogen peroxide was dropped on the disks in the above concentrations. The dishes were kept one hour at room temperature and then put into a thermostat and incubated at 39°C within 18 hours. The diameter of the zone of the microorganism culture growth retardation around the disk was determined.

Application of the peroxide sensitivity technique favored the study of a large number of microorganisms by the "radioresistance" index. Comparison of the data on peroxide sensitivity and D_{10} index allowed us to classify the studied microorganisms into several groups (Table III). The majority of microorganisms are represented by non-radioresistant forms, whose D_{10} index did not exceed 0.5-0.6 kGy (up to 80%-90%). The remaining 10%

primarily comprise the group of medium-resistant microorganisms, with a D_{10} index up to 1.5 kGy. In only about 2% of microorganisms did the D_{10} index exceed 2 kGy. Not a single microorganism with a D_{10} index exceeding 3.2 kGy was found; with that index only solitary cells occurred. As seen in Table III, the growth retardation zone for the group of microorganisms with a certain D_{10} index has a rather fixed size.

Table II. Radioresistance (Index D_{10}) and Sensitivity to Hydrogen Peroxide (H_2O_2)

Microorganism	D_{10} (KGy)	Concentration of H_2O_2		
		3%	1%	0.3%
		Zone Delay of Growth ± 1-2 (mm)		
D. radiodurans	6.20	4	to border of disk	to border of disk
D. radioproteolyticus	3.10	5	2	to border of disk
Steptococcus faecium	2.90	4	to border of disk	to border of disk
B. subtilis 228	0.23	13	6	3
S. aureus 73	0.20	16	9	4
E. coli AB 1157	0.15	17	11	4
E. coli rec. A	0.07	26	21	16
E. coli 1257	0.16	25	16	12
Mycobacterium tuberculosis	1.80	8	5	3
$H_{37}Ba$	1.80	8	5	3
Academia	0.80	11	7	5
413 p	1.80	9	6	5
M. bovis valle	0.80	12	9	7
Saccharomyces cerevisae	1.34	9	8	5
Trichophyton rubrum	1.60	8	7	6
Microsporum gypseous	0.54	16	11	8
B. anthracis 96	1.60	9	7	6

Table III. Radioresistance from Index D_{10} and Sensibility to H_2O_2

Microorganism	Contents[a] (%)	D_{10} (kGy)	Concentration of H_2O_2		
			3%	1%	0.3%
			Zone Delay of Growth ± 1-2 (mm)		
Group 1	90-80	0.05-0.5	30-15	18-10	12-8
Group 2	12-8	0.75-1.5	12-9	8-6	4-3
Group 3	3-2	2.00-3.0	5-4	3-2	to border of disk

Our data on determination of radioresistance of microorganisms separated from the products and industrial microflora suggest that:

— Radioresistant microorganisms have not been abundant.
— Radioresistance of the same forms of microorganisms does not depend either on a season of the year or a geographic zone.
— “General radioresistance” of the microflora depends mainly on whether a larger or smaller number of sporeforming microorganisms is present in the microflora.
— Throughout the study we have not detected any increase in the percentage of radioresistant forms.

In all cases, when the dose of 25 kGy was used in production sterilization, it provided a coefficient of sterility assurance equal to 10^6. In some cases when the raw material is highly contaminated (over 10^3) and its decontamination is hampered for some reasons, the radioresistance index in the microorganism-contaminants may be used as a criterion for selecting a radiation dose.

Here, even with an initial contamination of up to 10^4 microbial cells per product, a radiation dose of 18 kGy provides sterility if the percentage of the represented radioresistant microflora is small. However, the coefficient of assurance decreases.

When production is only slightly contaminated, a determination of a low percentage content of radioresistant microorganism forms makes it possible to consider the question of lowering the sterilizing dose to 17-18 kGy while at the same time maintaining a sterility assurance coefficient of 10^6.

In practice, however, there are situations when a lowering of the sterilizing dose is impossible. Conversely, in some instances, the standard radiation dose cannot be used because it results in a disturbance of the physical, chemical, or functional properties of the products undergoing radiation sterilization.

Modification of the Radioresistance of Microorganisms

One of the causes of a high radioresistance in certain microorganisms is the presence of an effective DNA reparation system. Hence, a direct way to modify radioresistance and thus, lower the microorganisms' viability, is to suppress the DNA reparation system. However, a considerable obstacle exists with respect to this approach: there is yet no clear understanding of a community of enzyme systems repairing DNA impairments.

The problem of radioresistance modification is very close to the problem of synergism, that is, a non-additive, collective effect of several factors on the microbial cell. For example, warming of microorganisms' radioresistant cells at non-lethal temperatures (55°C) before their exposure to 0.25-2.0 kGy was established to result in an increase in the number of single DNA breaks and, hence, in significant increase in cell deaths (Figure 4, Table IV). The bactericidal effect observed is higher than just a sum of the effects of each factor in isolation. It is noteworthy that the synergistic nature of the higher bactericidal effect of thermoradiation impairment was less evident or completely absent in strains with different defects of reparation systems.

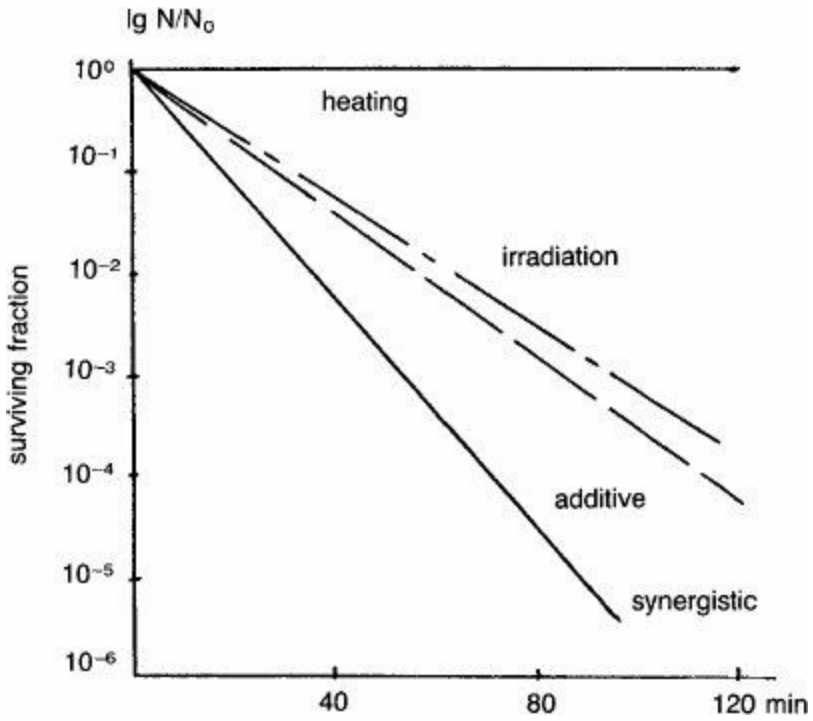

Figure 4. Thermoradiation Effect (*B. subtilis* GSY 228 — Spores)

Table IV. Quantity of SSB DNA Radioresistance Microorganisms by Radiation and Thermoradiation Effect

Microorganisms		Dose (kGy)				
		250	500	1000	1500	2000
Str. faecium ($A_2$1)	without heating	1.5	2.1	5.4	9.7	12.5
	heating 55°/11 min	3.7	4.8	7.7	10.8	12.9
M. radiophilus	without heating	1.3	2.2	4.5	8.2	10.3
(NCTC 10785)	heating 55°/6.5 min	4.0	5.8	7.8	10.1	11.2
M. radioproteolyticus	without heating	0.8	1.6	4.0	6.2	9.2
(CCM 2703)	heating 55°/5 min	4.1	6.0	8.3	9.8	10.7

Reunification of single DNA breaks is connected with a high activity of nucleotide ligase, and the cell spores reunite up to 90% of the single breaks as early as during the exposure. Upon exposure to irradiation or, for example, to temperature, in addition to thermoinactivation of a number of enzymes, there is also an increase in the number of unreparable impairments of the cellular genetic material.

Both magnetic field and hydrogen peroxide were used to modify the radiosensitivity of microorganisms. The level of post-radiation DNA degradation in microorganisms exposed to magnetic radiation appeared to be higher than in the control exposed cultures. The effectiveness of the reparation processes in microorganisms following an exposure to ionizing radiation and a magnetic field was lowered, which might be one of the causes of a stronger effect of ionizing radiation. The bactericidal effect of exposure to both gamma-rays and a magnetic field together increases substantially when the period of post-radiation exposure in the magnetic field is prolonged to 18-24 hours.

In a number of cases, a significant change in microbial cell viability may be attained if several factors are applied, each of which taken separately has only a slight effect on this value; i.e., ultra-violet irradiation, warming, and hydrogen peroxide. For example, in an experiment using spores of *B. subtilis*, the bactericidal effect was reinforced 2000 times. In our experiments, a synergistic effect was seen on microorganisms' mortality by the collective effects of ionizing irradiation, hyperthermia, micro-concentrations of chloride or lime, hydrogen peroxide, detergents, and a number of other antibacterial preparations.

In considering the leading mechanisms of synergetic effects, we see the principal role as being a disturbance in the DNA reparation processes. Complicated, unreparable impairments of the genome are formed under conditions which inhibit enzyme complexes, providing stability of the cellular genetic information. In addition, a joint effect of exposure to radiation and other bactericidal factors leads to a disturbance in other systems of cells ensuring its resistance to ionizing radiation; such as its membrane and systems providing cells with energy. Lastly, disturbance in the balance of DNA and protein syntheses with a reduction in the efficiency of the cellular restoration process, play a significant role in the observed synergistic effects on microbial cell deaths.

Application of the described effects in the creation of new technologies of radiation sterilization is, in certain cases, already a reality while others are still awaiting implementation and introduction in practical radiation biotechnology.

Radiation Effects on Polymeric Materials

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Introduction

Sterilization of medical supplies is one of the biggest areas in which radiation is effectively used, and this use has been rapidly growing the world over. Ethylene oxide is widely used and accepted for industrial sterilization of medical supplies. However, ethylene oxide is well known to be toxic, carcinogenic, and to have mutagenic effects on living organisms. It has been pointed out that the use of ethylene oxide for sterilization purposes is disfavored because of its toxicity for personnel working at the sterilization site and for patients who use the ethylene oxide-sterilized products. In comparison, the occupational safety of workers at the radiation process can be effectively and easily controlled, and no traces of radioactivity are introduced in irradiated products. The biggest advantage of radiation over other methods is that products can be sterilized after packaging, thus avoiding problems of recontamination. The main products sterilized by irradiation are so-called disposable medical products designed for one-time use, for example, syringes, catheters, and blood transfusion sets. These products consist mainly of polymer materials such as polyethylene, polypropylene, polyvinyl chloride, and so on, as shown in Table I. Some of these polymers are known to deteriorate with ionizing radiation. Thus, the durability of radiation sterilized materials is one of the most important matters to be examined for the industrial application of radiation sterilization.

Radiation Effects on Polymers

When a polymer is subjected to irradiation by ionizing radiation, such as gamma rays and accelerated electron beams, various effects can be expected as shown in Table II. These effects lead to crosslinking or degradation, depending on the polymer structure and the irradiation conditions. Polymers are classified into two groups as shown in Table III. However, both crosslinking and chain scission occur simultaneously when a polymer is subjected to the irradiation of ionizing radiation. If crosslinking is the predominant effect, the polymer is classified into a crosslinking type. Whether crosslinking or main-chain scission is the predominant outcome is largely dependent upon the irradiation conditions of the polymer. Although polypropylene is classified as a crosslinking type (Table III), it is well-known that some medical supplies made of polypropylene deteriorate with gamma rays irradiation or during storage after irradiation.

Table I. Materials for Disposable Medical Supplies

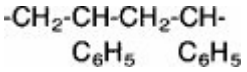
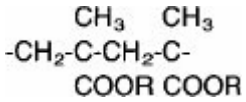
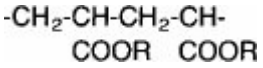
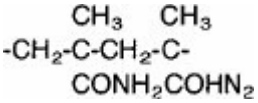
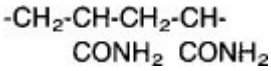
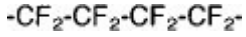
Medical Supplies	Materials
Syringe	Polypropylene, Polystyrene, Polyethylene
	Rubber, Glass
Blood Transfusion Set	Polyvinyl chloride, Polypropylene, Rubber, Polyamides
Blood Shunt	Polyvinyl chloride, Polypropylene, Polyamides
Nutrition Tube	Polyvinyl chloride, Polypropylene, Rubber
Dializer (Artificial Kidney)	Polypropylene, Polyvinyl chloride,
	Polyethylene, Polystyrene, Rubber, Cellulose
Surgical Suture	Silk, Collagen, Polypropylene, Polyamides, Polyesters

Table II. Effects of Radiation on Polymers

Formation of radicals
Formation of hydrogen and low molecular weight hydrocarbon
Formation of C-C bonds between molecules (crosslinking)
Increase of unsaturation
Breakdown of cyrstalline structure
Coloration
Oxidation

Table III. Classification of Polymers

Crosslinking type		Degradation type	
Polyethylene	$-CH_2-CH_2-CH_2-CH_2-$	Polyisobutylene	$-CH_2-\underset{CH_3}{\overset{CH_3}{C}}-CH_2-\underset{CH_3}{\overset{CH_3}{C}}-$
Polypropylene	$-CH_2-\underset{CH_3}{CH}-CH_2-\underset{CH_3}{CH}-$		
Poly(vinyl chloride)	$-CH_2-\underset{Cl}{CH}-CH_2-\underset{Cl}{CH}-$	Polyα-methylstyrene	$-CH_2-\underset{C_6H_5}{\overset{CH_3}{C}}-CH_2-\underset{C_6H_5}{\overset{CH_3}{C}}-$

Polystyrene		Polymethacrylates	
Polyacrylate		Polymethacrylamide	
Polycrylamide		Polytetrafluoroethylene	

Radiation Durability of Polypropylene

Figure 1 shows the elongation of irradiated polypropylene as a function of dosage. In this study, we used the value of elongation at break as one of the indicators to evaluate the deterioration of the materials. It can be seen that elongations of polypropylene decrease with increasing irradiation dose, and gamma ray irradiation over 5 Mrad causes a rapid decrease. Figure 2 shows the deterioration of the irradiated polypropylene during storage. The effects of radiation remain evident even after irradiation, and degradation proceeds during storage. As shown in Figure 2, elongation of polypropylene irradiated with 5 Mrad gamma rays decreases steeply during the three months after irradiation.

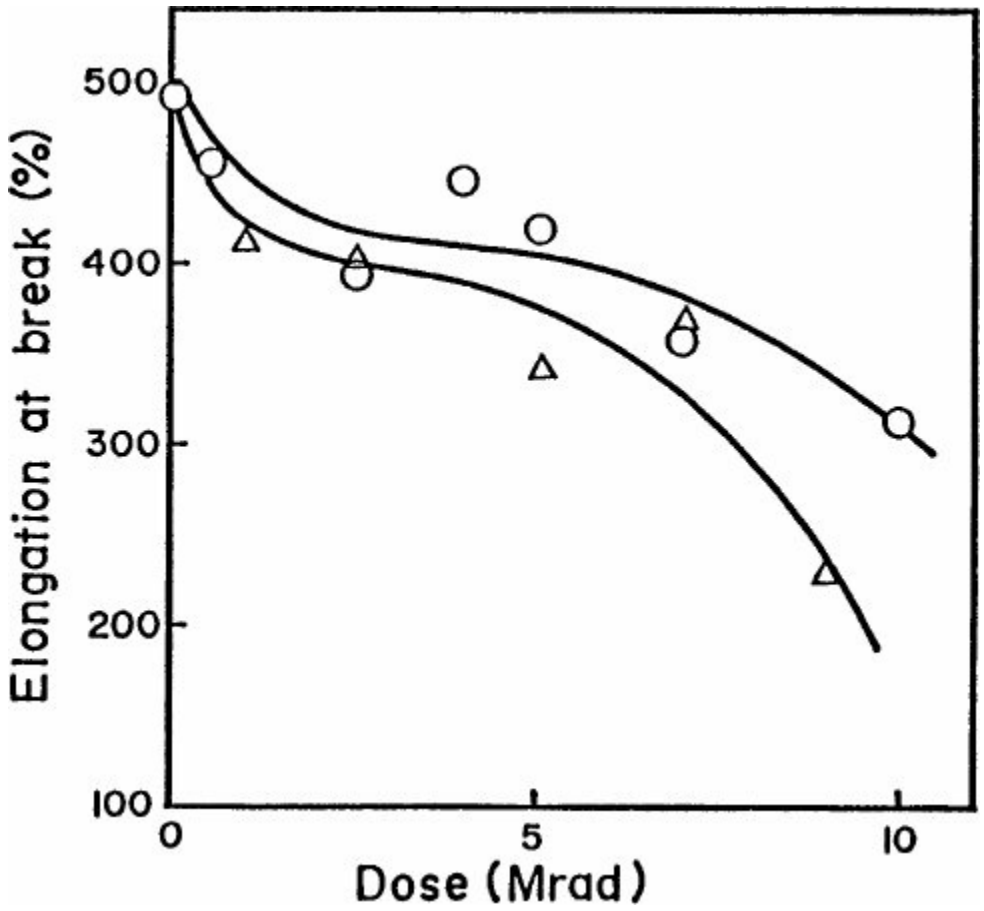


Figure 1. Elongation at Break of Irradiated Copolypropylene with 6% Ethylene

(Δ) Gamma Rays Irradiation, Dose Rate 1×10^6 rad/hr

(○) Electron Beam Irradiation, Dose Rate 0.143×10^6 rad/s

Figure 3 plots the relationships between elongation at break and thickness of polypropylene irradiated with electron beams and gamma rays while Figure 4 shows the molecular weight of irradiated polypropylene as a function of film thickness. These results indicate that surface area can be easily deteriorated upon irradiation with gamma rays and electron beams. Films thinner than 200 μm have an extremely small elongation and a lower molecular weight compared to a thicker one. These results also suggest that in the thicker film, degradation occurs heterogeneously even though the film is irradiated homogeneously.

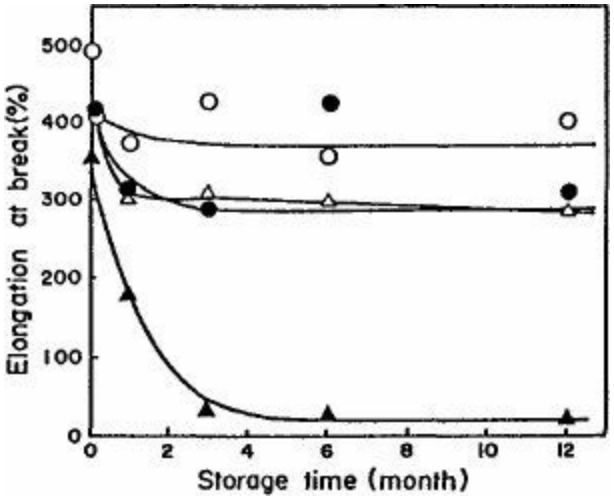


Figure 2. Degradation Time During Storage of Irradiated Copolypropylene

(○) Electron Beam, 2.5 Mrad
(●) Gamma Rays, 2.5 Mrad
(Δ) Electron Beam, 5.0 Mrad
(▲) Gamma Rays, 5.0 Mrad

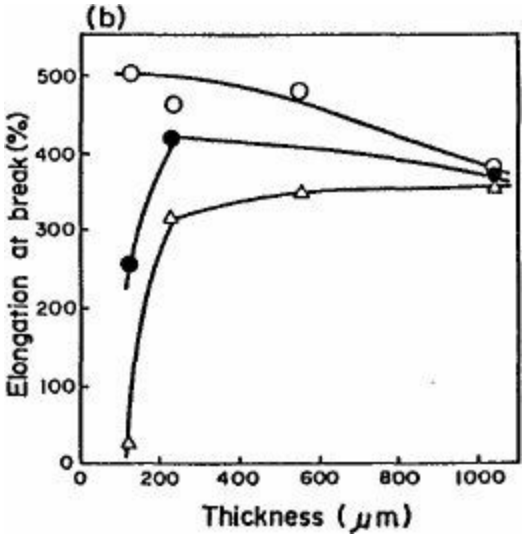


Figure 3. Relationship Between the Elongation at Break and Thickness in Polypropylene Irradiated with Electron Beam and Gamma Rays

(○) Unirradiation (control)

(Δ) Gamma Rays, 1×10^6 rad/hr

(●) Electron Beam, 1.43×10^5 rad/s

Total Dose, 5.0 Mrad

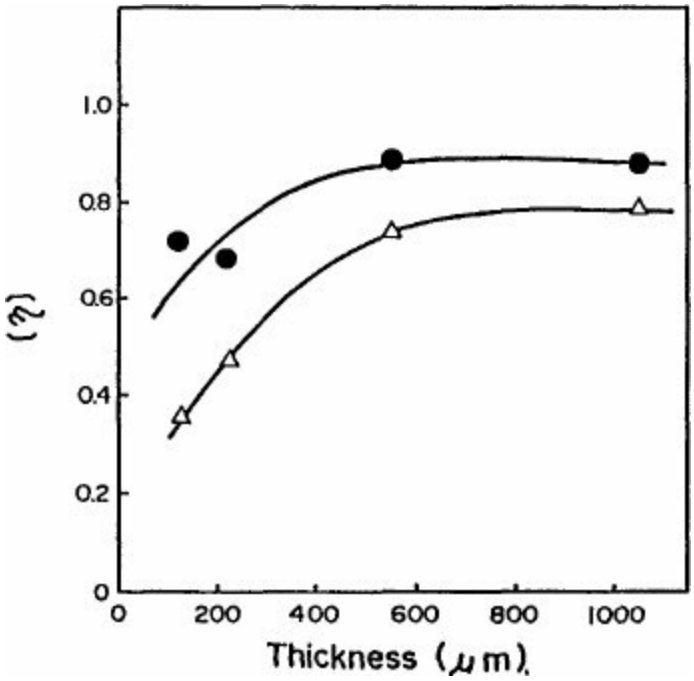


Figure 4. Molecular Weight of Polypropylene Irradiated with Electron Beam and Gamma Rays

(Δ) Gamma Rays, 1×10^6 rad/hr

(●) Electron Beam, 1.43×10^5 rad/s

Total Dose, 5.0 Mrad

It was found that degradation during both irradiation and storage was very small with electron beams relative to gamma rays.

In order to study the relationship between radiation deterioration and oxidation of polypropylene, radiation oxidation was evaluated by measuring the chemiluminescence of the irradiated polypropylene. When a polymer is subjected to irradiation of ionizing radiation in air, oxidation occurs as shown in Table IV. Polypropylene produces radicals upon irradiation in air according to eq. (1) and the radicals react with oxygen to form peroxyradicals through eq. (2). In eq. (3), the peroxyradicals draw out hydrogen from polymer to convert into hydroperoxide. This hydroperoxide decomposes into RO· and HO· as seen in eq. (4). In eqs. (5) and (6), RO· and HO· radicals draw out hydrogens from the polymer to form radicals R. These radicals, formed through eqs. (3), (5), and (6), react with oxygen and the oxidation of polymer proceeds with repetition of these reactions. This process is called autoxidation.

The oxidation of a polymer can be evaluated by measuring chemiluminescence. The principle of chemiluminescence (CL) is shown in Table V. CL is emitted when excited ketone formed in the recombination of the peroxyradical (ROO·) in polymer converts to ketones of the ground state (eqs. (3) and (4)). Figure 5 shows the intensities of CL of irradiated polypropylene as a function of irradiation dose. It can be seen that CL increases with increasing dose and CL from gamma rays-irradiated polypropylene are larger than that from electron beams. This finding is consistent with the results regarding elongation discussed above.

Table IV. Radiation-Induced Oxidation

Irradiation

RH ⟶ R· (1)

Oxidation

R· + O_2 ⟶ ROO· (2)

ROO· + RH ⟶ ROOH + R· (3)

ROOH ⟶ RO· + ·OH (4)

RO· + RH ⟶ ROH + R· (5)

HO· + RH ⟶ H_2O + R· (6)

Table V. Chemiluminescence

RH ⟶ R·

R· + O_2 ⟶ ROO·

2R00· ⟶ R-C-R* + ROH + O_2

‖

O

↓

R-C-R + hν

‖

O

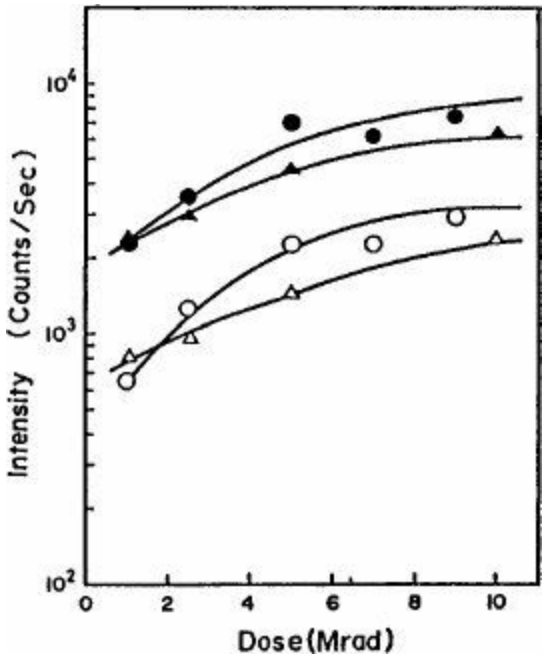


Figure 5. Comparison of Chemiluminescence in Copolypropylene Irradiated with Gamma Ray and Electron Beam

Electron Beam, (Δ) 80°C; (▲) 100°C

Gamma Ray, (○) 80°C; (●) 100°C

It is well-known that oxidation of polymers proceeds gradually from the surface to the inner part, because oxygen diffuses from the surface into the inner part. By measuring the CL of films, the degree of oxidation and depth of oxidation layer in irradiated polypropylene were determined.

The effect of dose rate on the CL intensity of films with various thicknesses is shown in Figure 6. It can be seen that a break-point appears clearly at any dose rate, and that the

thickness at the break-points varies with the dose rate, i.e., increases with decreasing dose rate. From these results it can be reasonably concluded that the degree of oxidation is less in the deeper regions than in the surface area. Thus, the break-point corresponds to the depth of the oxidative layer from the surface of the polymer and the polymer at the layer deeper than the break-point is hardly oxidized.

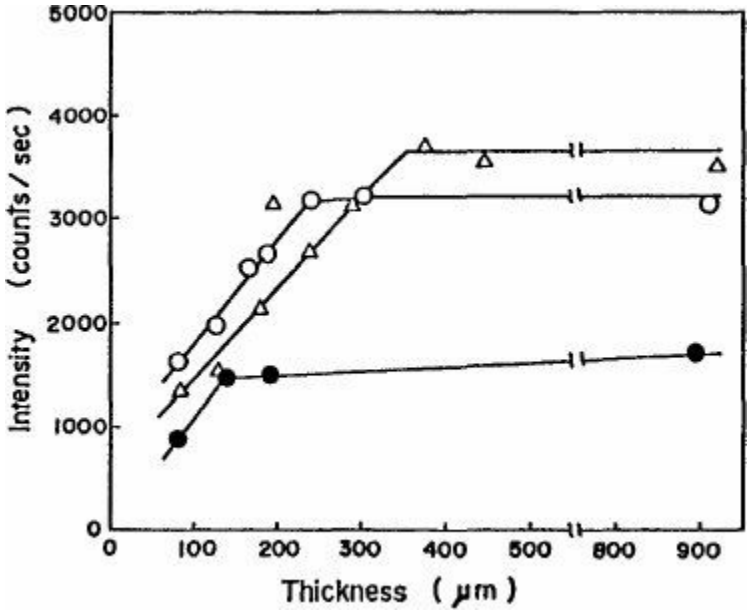


Figure 6. Effect of Dose Rate on Chemilumescence of Gamma-Irradiated Polypropylene

(●) 1×10^6 rad/hr, (○) 2×10^5/hr

(Δ) 5×10^4 rad/hr

Total Dose, 5.0 Mrad

The profile of the oxidative layer for a film of 1 mm thickness irradiated at different dose rates of gamma rays is shown in Figure 7. From these results it can be seen that oxidation occurs more easily around the surface than inside the polymer film. The degree of oxidation and depth of oxidative layer are larger at a lower dose rate than at a higher one. This is attributed to the diffusion rate of oxygen to react with the active site formed during irradiation, because the lower the dose rate, the longer the irradiation time.

Figure 8 shows the depth and degree of oxidative layer obtained for polypropylene film irradiated with electron beams. The CL profile is similarly U-shaped (see Figure 7). However, its intensity, i.e., degree of oxidation at the surface, is only one-third that obtained with gamma rays.

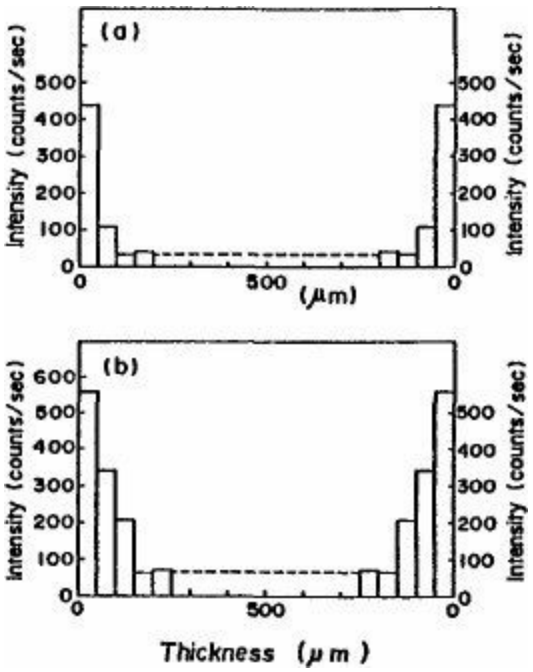


Figure 7. Profile of the Oxidative Layer of Gamma-Irradiated Polypropylene

(a) 1×10^6 rad/hr, (b) 2×10^5/hr,
Total Dose, 5.0 Mrad

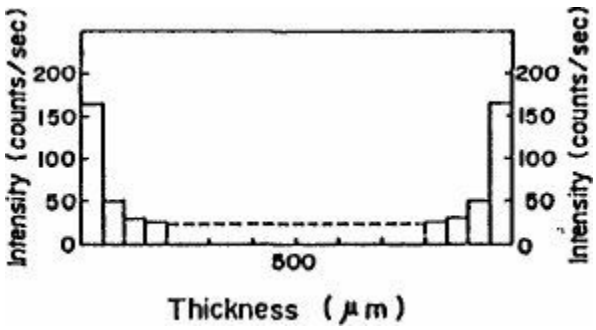


Figure 8. Profile of the Oxidative Layer of the Polypropylene Irradiated with Electron Beam Dose Rate, 1.43×10^5 rad/s, Total Dose, 5.0 Mrad

From these results, the degree of oxidation was found to be very high at the surface area of the film where oxygen can diffuse during irradiation, and was also found to decrease sharply with increasing depth from the surface. In the case of electron beam irradiation, oxygen cannot diffuse so rapidly from the surface area to react with radicals formed in the interior portion, since irradiation is carried out at a very high dose rate in a short time relative to gamma ray irradiation.

The surfaces of irradiated homopolypropylene sutures were compared with those of unirradiated sutures. The knotted part of some irradiated sutures were finely split. This fine split is believed to be caused by oxidative degradation that occurred either during irradiation or during storage after irradiation. Table VI shows the relationship between the number of split sutures and the storage period after irradiation with various doses with gamma rays and electron beams. It was found that copolypropylene (CPP) sutures were more stable

than homopolypropylene (PP) sutures, and that the degradation of the sutures irradiated with electron beams was smaller than that of gamma ray-irradiated ones. This finding is in good agreement with those obtained with film samples. The CPP used here is a copolypropylene which was developed as a raw material for disposable medical products and contains 6% ethylene unit.

Table VI. Number of Split Samples in Ten Sutures on Surgical Tied During Storage

(a) Homopolypropylene

Storage Time (month)	Gamma-ray Irradiation		Electron Beam Irradiation	
	2.5 Mrad	5.0 Mrad	2.5 Mrad	5.0 Mrad
0	0	7	0	1
1.5	1	10	0	10
3	1	–	0	–
4	3	–	1	–
7	3	–	4	–

(b) Copolypropylene

Storage Time (month)	Gamma-ray Irradiation		Electron Beam Irradiation	
	2.5 Mrad	5.0 Mrad	2.5 Mrad	5.0 Mrad
0	0	0	0	0
1.5	0	0	0	0
4	0	5	0	0
6	0	6	0	0

Figure 9 shows the relationship between elongation at break and storage time after irradiation for a copolymer of propylene containing 6% ethylene unit (CPP) in comparison with a homopolymer. CPP was found to have a higher durability during storage and to retain more than 60% of the initial elongation at break, even after 12 months storage. In order to elucidate the effect of the ethylene unit on the radiation durability of polypropylene, a dynamic mechanical analysis of PP and CPP were carried out using a torsion pendulum-type apparatus (Resca RD 1100), as shown in Figure 10. The curve of mechanical loss (logarithmic decrement) as a function of temperature consists of three parts, α, β, and γ. The highest temperature part, α, is a sign of the crystallinity of the sample polymer, i.e., the higher the α peak, the higher the crystallinity. The region of β indicates the amorphous fraction of polymer; an increase in the β peak indicates a decrease in the crystallinity. The low temperature part, γ, is due to the amorphous phase. The higher the γ peak, the lower the crystallinity. Shear modulus, G, is known to decrease with decreasing crystallinity. From Figure 10, it can be seen that crystallinity of CPP is lower than that of PP, because CPP has a lower α peak and higher β peak as compared with those of PP. This result suggests

that polypropylene with a lower crystallinity has a higher radiation stability.

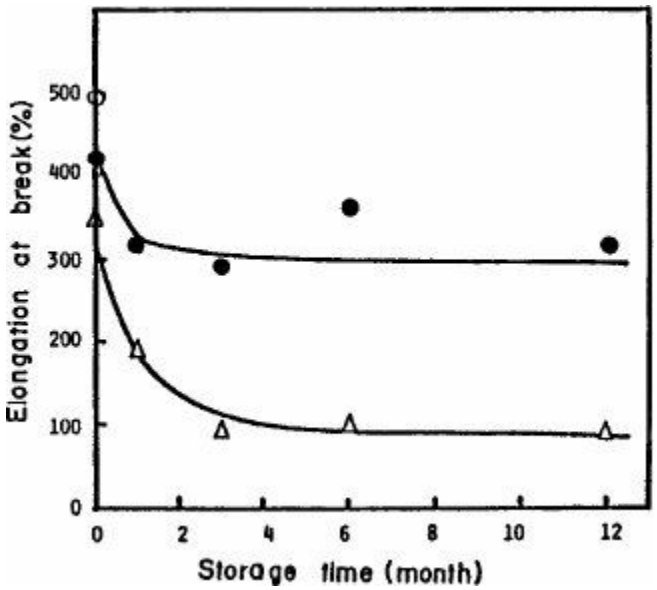


Figure 9. Degradation During Storage of Gamma-Irradiated Samples

(Δ) Homopolypropylene

(●) Random Copolymer of Polypropylene (94%) with Ethylene (6%)

Dose, 2.5 Mrad

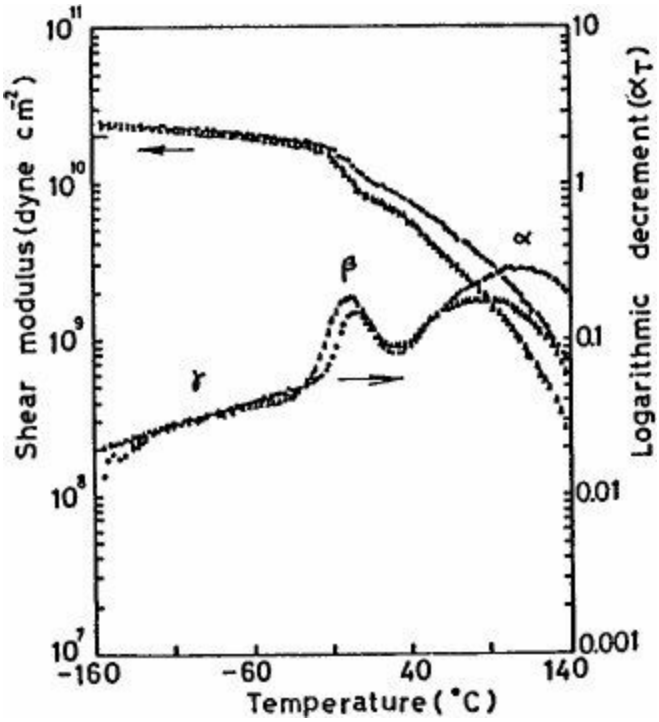


Figure 10. Dynamic Viscoelastic Properties of Homopolymer and Copolymer in Polypropylene

(●) Homopolymer

(▲) Copolymer

In order to clarify the effect of the crystallinity on the durability of the irradiated PP, the mechanical properties were studied by using PP films quenched at various temperatures. Figure 11 shows the relationship between elongation at break and dose. The elongation of

unquenched (slowly cooled) PP decreased remarkably with increasing irradiation dose, and its value at 2.5 Mrad (sterilization dose) reached nearly zero. On the other hand, the quenched samples were more stable against irradiation and were found to be almost constant up to 2.5 Mrad. The durabilities of these samples during storage after irradiation are shown as a function of storage time in Figure 12. In any samples, the degradation was observed only at early stages of storage (up to one month); thereafter, the elongation of the quenched samples did not decrease so greatly. It can be concluded that the durability of the irradiated PP is largely dependent upon its morphology (affected by conditions of molding), and that quenching is one of the methods which can prevent radiation degradation of polypropylene.

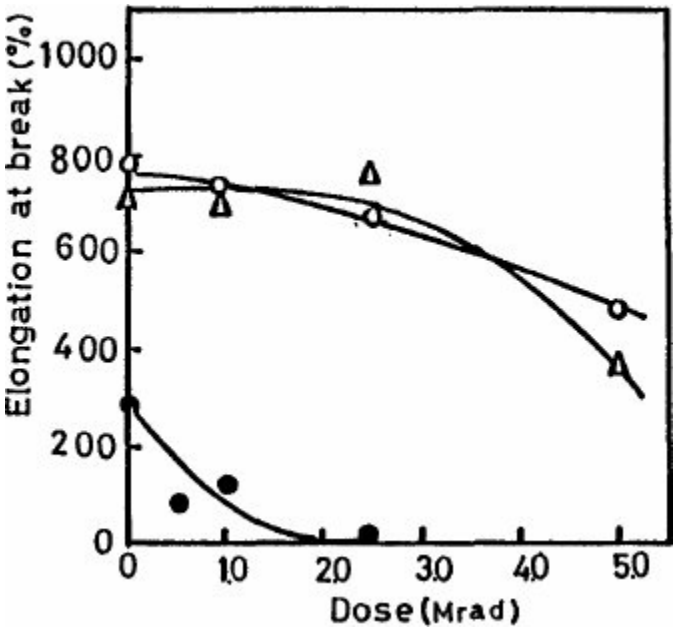


Figure 11. Elongation at Break During Irradiation of Quenched Polypropylene

(○) Quenched PP at −60°C

(Δ) Quenched PP in cold water

(●) Cooled PP gradually at room temperature

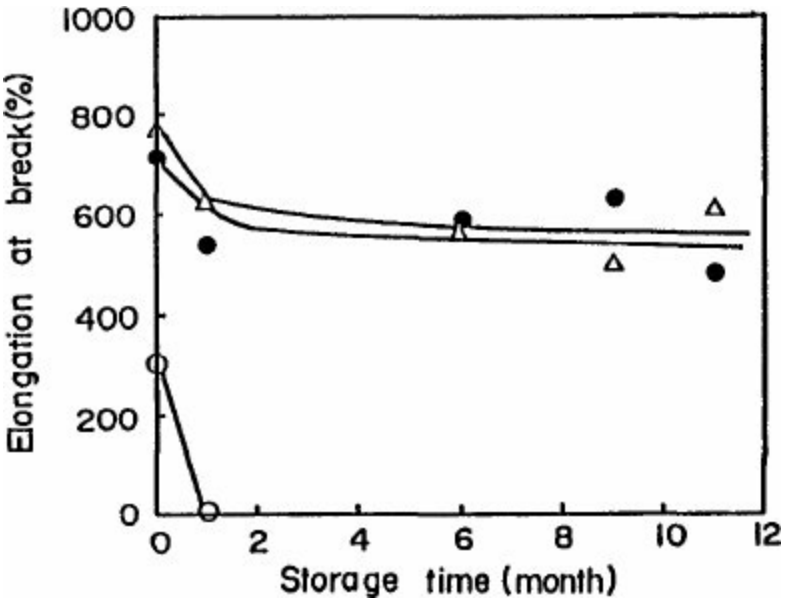


Figure 12. Degradation During Storage after Irradiation of PP Prepared by Various Cooling Rates

(Δ) Quenched PP at −60°C
(●) Quenched PP in cold water
(○) Cooled gradually at room temperature
Dose, 2.5 Mrad

The effects of the molecular weight of PP on radiation stability were also studied. Figure 13 shows the degradation during irradiation for various molecular weights. Lower molecular weight PP degrades markedly with increasing dose, and the elongation at break of the lowest molecular weight PP disappears entirely at a dose of 5.0 Mrad. However, the decrease in elongation during irradiation is much smaller in high molecular weight PP. Higher molecular weight PPs irradiated at 5.0 Mrad maintain approximately 70% elongation of the original value. The durability of various molecular weight polypropylenes during storage after irradiation is shown in Figure 14. In the case of 2.5 Mrad irradiation, degradation during storage of the higher molecular weight samples hardly occurs, while in the lowest molecular weight PP, the elongation at break sharply decreases with storage time and approaches 0% at six months. At a higher dose, 5.0 Mrad (as shown in Figure 14-b), only the highest molecular weight PP was found to degrade minimally during storage. It can be concluded that a higher molecular weight PP suffers a smaller degradation effect both upon irradiation and during storage after irradiation.

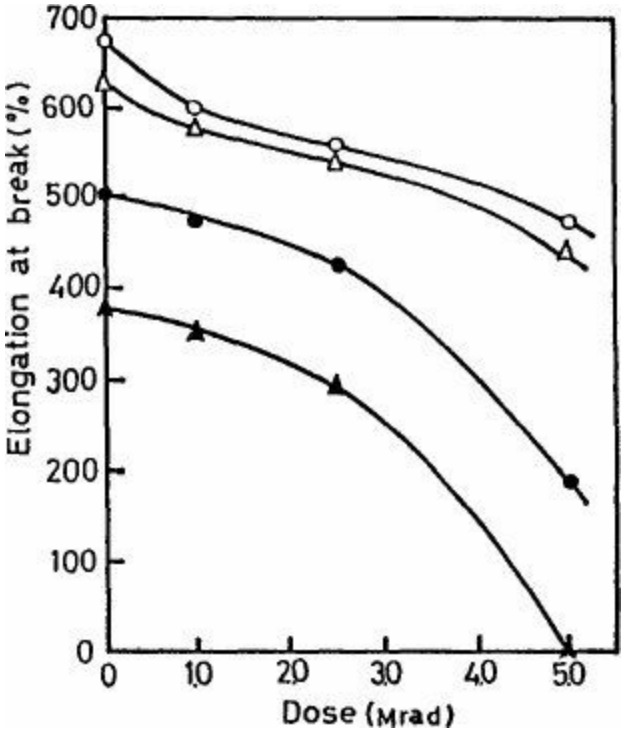


Figure 13. Effect of Molecular Weight in Polypropylene on Degradation During Irradiation

(○) 4.63×10^4

(●) 2.71×10^4

(Δ) 3.22×10^4

(▲) 2.11×10^4

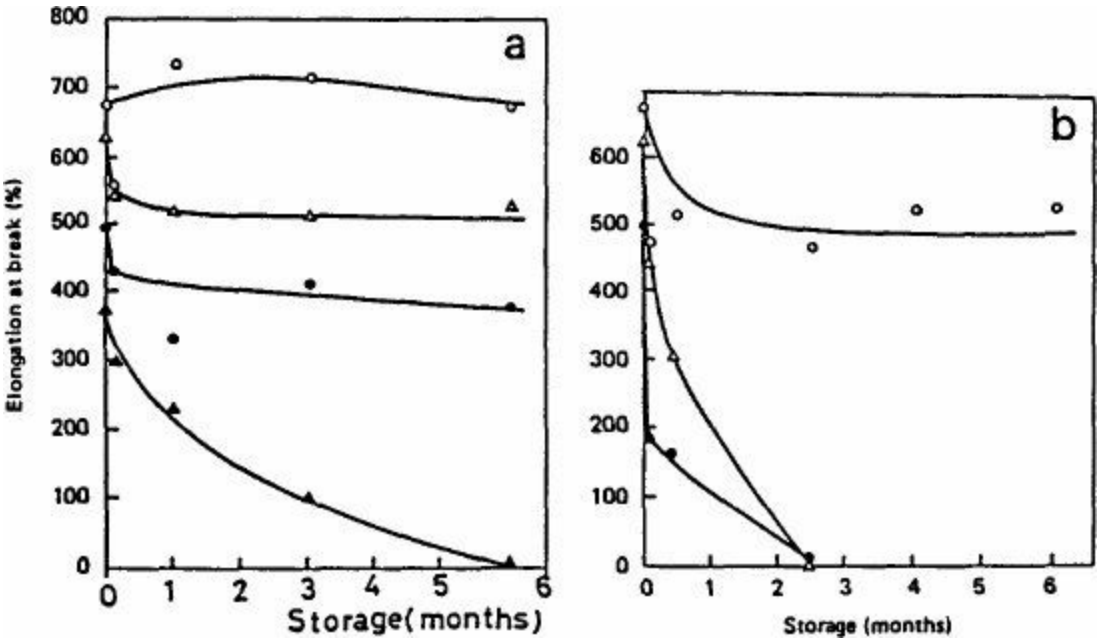


Figure 14. Stability During Storage of 2.5 Mrad (a) and 5.0 Mrad (b) Irradiated Polypropylene

(○) 4.63×10^4

(●) 2.71×10^4

(Δ) 3.22×10^4

(▲) 2.11×10^4

Conclusions

The results described above can be summarized as follows:

1. Radiation degradation of polypropylene occurs not only during irradiation, but also during storage after irradiation.

2. Radiation degradation of polypropylene is higher upon gamma-ray irradiation than upon electron beam irradiation from accelerator. Radiation degradation depends largely on dose rate, i.e., the higher the dose rate, the smaller the degradation.

3. Radiation degradation of polypropylene can be attributed mainly to the oxidation of polymer caused by oxygen which is dissolved in it and diffuses from polymer surface.

4. A copolymer of polypropylene containing a small amount of ethylene unit (CPP containing 6% ethylene) is more stable against radiation both during irradiation and storage after irradiation in comparison with homopolymer of propylene (PP). Degree of crystallinity and size of crystallite may affect the radiation durability of polymer. CPP was found to have a lower crystallinity compared with PP by an analysis of dynamic viscoelastic properties. The larger the amorphous regions, the smaller the radiation degradation.

5. Quenched polypropylene with a lower crystallinity has a higher radiation durability relative to unquenched polypropylene.

6. The higher the molecular weight of polypropylene, the smaller the radiation degradation both during irradiation and storage after irradiation.

7. Chemiluminescence analysis gives useful information about the oxidation of polypropylene caused by radiation. Relative and quantitative analysis of oxidation is possible for irradiated polypropylene.

Industrial Plants for Medical Product Sterilization

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Today there is a trend toward widespread application of radiation techniques and processing in virtually every branch of the national economy (i.e., industry, medicine, agriculture). Considerable importance has been attached to the radiation sterilization of medical products. There are more than 130 radiation technological plants intended for sterilization of sutures, polymer-based medical devices, instruments, bandages, transfusion systems, plastic bags, surgical gloves, and other medical products (3, 5-7).

Advantages of radiation over traditional sterilization methods such as gas and vapor have contributed to the development of this method (3, 5-7). In particular, radiation sterilization guarantees high sterility of treated products and homogeneous treatment of products already packaged, and the ability to sterilize thermolabile materials. To utilize the process of radiation sterilization in an industrial setting, flow-production lines have been organized. These are characterized by a high-level of mechanization and automation of industrial operations.

In designing national industrial sterilization plants, the objectives are to construct radiation technological complexes which provide a large flow-production capability, high radiation utilization, reliable systems, simple service, and the possibility to include plants in an industrial technology line. Great attention has been paid to the unification of the major units of the plant, realization of the module design concept, and the utilization of microprocessors in automating operations, safety interlocks, and plant monitoring (2, 3).

The national as well as the worldwide experience in the design and operation of plants made it possible to construct industrial high-efficiency sterilization plants using radionuclide radiation sources and electron accelerators. Among the first industrial sterilization plants were the sterilization facilities that had been operating in Leningrad for several years at the “Lenmedpolimer” factory, in Kazan at a surgical suture manufacturing factory, and in some medical organizations in our country (7). After these experimental plants, the first industrial technological gamma-plants were designed, such as “Sterilizatsiya-III”. It was designed at VNIIRT and adopted by industry in 1976 at the factory of medical polymers in the town of Belgorod-Dnestrovsk and at the factory “Lenmedpolimer” in Leningrad. These plants are intended for sterilization of blood transfusion systems. They consist of the irradiation chamber with the plane irradiator, dry storage (Figure 1), shielding labyrinth for transfer of the treated production with the aid of suspended conveyor, the preparation production room with the control panel and product loading-unloading mechanisms, and the chamber containing the radiation rods assembly. Treatment is carried out by way of discrete transfer of the product in the conveyor cages along the irradiator plane. To provide for treatment uniformity, the product is rotated 180° when it passes from one side of the irradiator to the other. The boxes are moved vertically at the loading-unloading areas. Full sterilization is

accomplished during a two-stage irradiation cycle: at the first stage, the product is irradiated on the upper story of the conveyor cage, while at the second stage, it is irradiated on the lower story. The safety of the operation is provided by triple interlocking: mechanical, electrical, and by the dose rate. Subsequent development of radiation technological plants was concerned with unification of the design, details, and mechanisms of the plants. There are: radiation equipment, operation and monitoring systems, transfer systems, building complex, and the complex of auxiliary equipment. Therefore, the plant is divided as if it were in “blocks” which form the entire unit. Every block of the complex is perfected, and thus, the blocks can be organized, depending upon customer needs, into the necessary manufacturing complex.

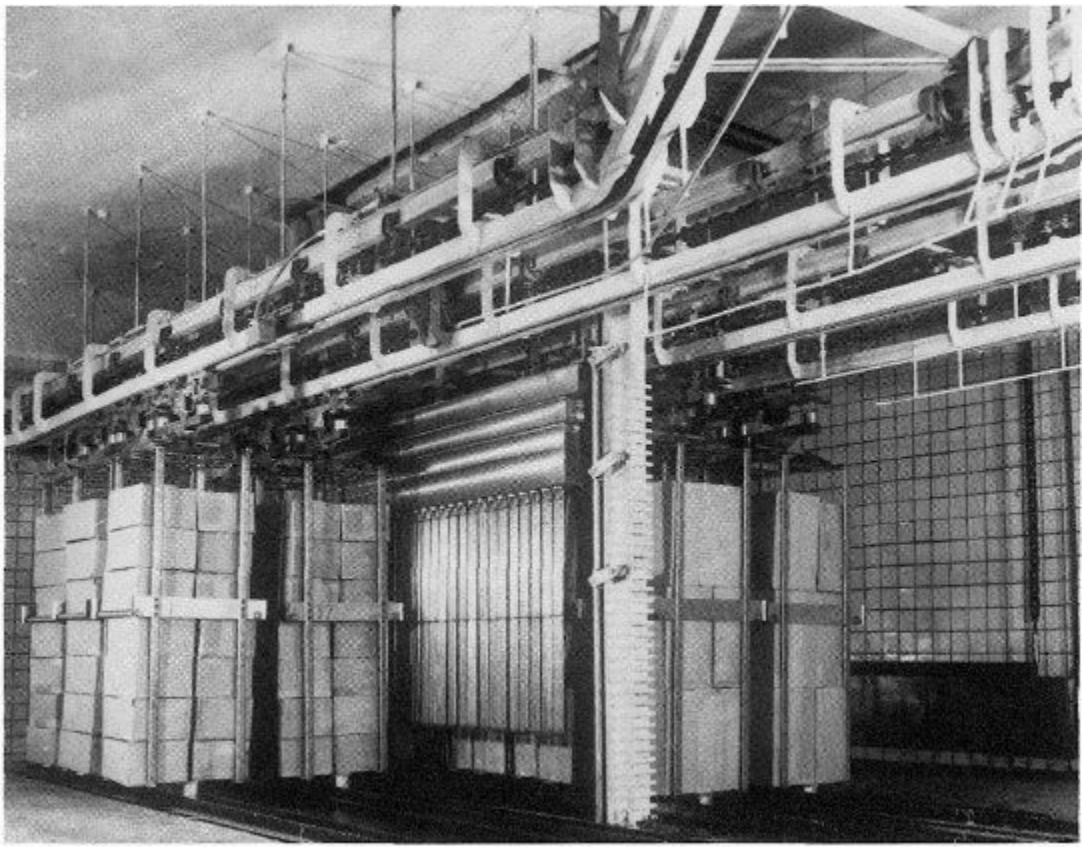

Figure 1. The Industrial Units for the Sterilization of Medical Products

The sterilization plant for pressed bandages has been designed and is widely used. The irradiator activity for the fixed throughput must be 350 TBq (950 kCi).The irradiator consists of six blocks intended for the cobalt-60 source arrangement.

According to this concept, several gamma-plants were designed and adopted by industry. One of these plants was the plant for sterilization of the new bacteriological fertilizer, rhyzotorphyne. It was put into operation at the factory of the fodder biomicyne in Neswizh (Byelorussia SSR). Rhyzotorphyne is produced by sowing microorganisms on a feed substance, consisting primarily of peat. The peat must be sterilized because it possesses a rich microflora that suppresses the development of injected wholesome microorganisms. The small addition of rhyzotorphyne (< 200 g/hectars) to legumes seeds before sowing rises the yield capacity of the seeds by 15%-20%.

In 1983, the industrial gamma-plant for the sterilization of obstetrical products, designed

in accord with a Finnish contract, was put into operation. To confirm the radiation physics parameters of the plant, and to determine the optimal dwell-time of cages on each irradiation position, technological dosimetry was carried out in accord with IAEA regulations (4). The measurement of absorbed doses in the irradiated object was performed with the aid of solid dosimeters ("Red Perspex Dosimeter" type). Calibration of dosimeters was carried out by the Atomic Energy Commission of Great Britain. Calculated technical characteristics of the plant were confirmed by the results of the technological dosimetry.

When designing a new industrial plant, or when modernizing an existing facility, great attention is paid to the ecological impact of the plant, the safety of the operation both to the workers and to the environment, and the reliability of the component systems. Concern for each of these factors should prevent accidents. In this regard, specialists of VNIIRT take their efforts for regular fulfillment of preventative and regulatory procedures. These procedures provide for mandatory operation of the plant using the following safety details: the irradiator safety frame which prevents damage to the irradiator from objects being irradiated; irradiator protective screen which prevents objects from getting into the irradiator transfer zone; and the irradiator transfer mechanism by use of rigid rods attached in the irradiator chamber ceiling. The institute worked out documentation about these units for every plant and organized their manufacturing for all operating and designed plants.

A review of dry vs. water irradiator storage systems failed to reveal any essential advantages of one system over the other. Each of these systems has several advantages. We prefer the dry type as it is more compact, reliable, and has a simpler design. It does not require a cumbersome water purification system, automatic monitoring of the water level in the pool, and the existence of germ reservoirs. However, to satisfy demands from customers who prefer water storage systems, we designed a multipurpose plant with a water storage facility. Figure 2 shows the irradiator from this plant. Depending upon the production treatment mode and the necessary radiation dose, the required number of radiation sources can be transferred into the irradiation position by the set program. The plant can sterilize both medical and food products.

A survey of national and foreign industrial use of radiation technology shows that in large radiation processes, electron accelerators predominate. When powerful and reliable electron accelerators (with the energy of 8-10 MeV) appeared, plants were designed for the industrial sterilization of medical products by electron accelerated beams. The energy of these accelerators does not exceed more than 10 MeV because of the danger of induced radiation appearing in the treated materials. This energy is sufficient to treat medical production packages of 30-40 cm in width, when its density is 0.1 g/ccm.

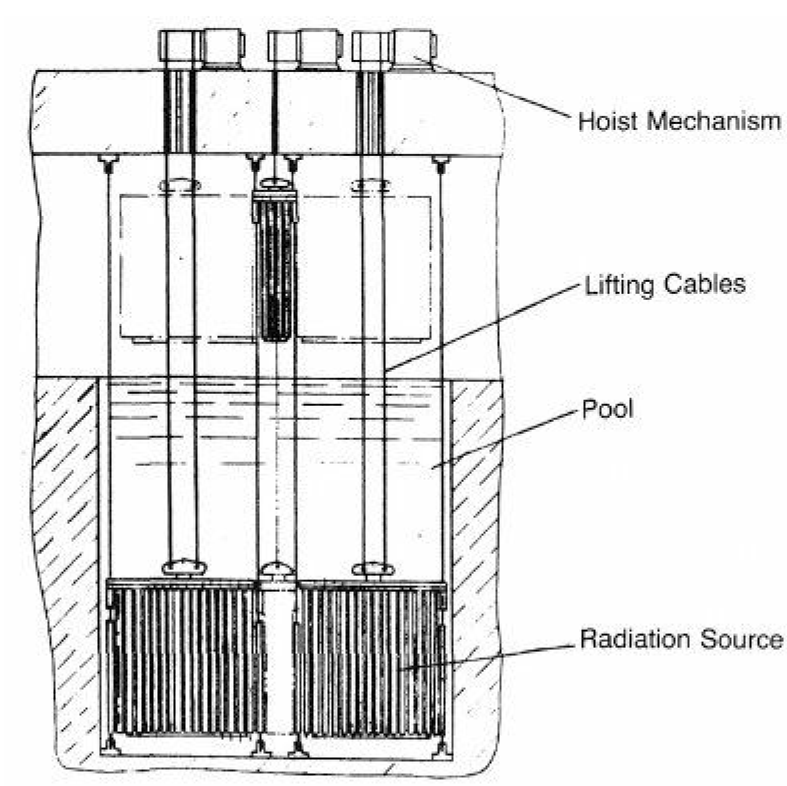

Figure 2. Water Storage of Radiation Source

In foreign countries (e.g., Denmark, France, Great Britain), plants for radiation sterilization with electron accelerators appeared at the end of the 1960s and the beginning of the 1970s. In our country, the first and, to date, only plant of this type—"Sterilizatsiya II"—has been in operation from 1976 at the "Syntez" complex in the town of Kurgan. The plant is housed in a separate building and consists of two independent technological lines. Each of them has a linear electron accelerator, transport system, operating and safety interlock systems. The nominal output of one line is about 300 kg/h (when the nominal absorbed dose is 25 kGy [2.5 Mrad]).

Ten years of operating experience with "Sterilizatsiya II" shows the expediency of using electron accelerators for industrial radiation sterilization. However, the output of this plant is no longer sufficient. Moreover, the plant does not meet current industrial guidelines. Therefore, VNIIRT has designed a new plant that will be housed in the old building. We calculated that the output of the new plant will exceed "Sterilizatsiya II" by 2-2.5 times. New mechanization and operation systems will be used in the new plant. The use of a new system of ultra-high frequency supply also rises the reliability of the electron accelerator.

We plan to use Bremsstrahlung electron accelerators, with the energy of 4-5 MeV and a beam power up to 300 KW for radiation sterilization. Experiments carried out in VNIIRT have shown that the output of electron accelerator plants is comparable with that of a gamma-plant with cobalt-60 source and activity of 5.5 GBq. However, to put into operation the industrial Bremsstrahlung plant, experiments are needed with an electron accelerator designed with the energy of 4-5 MeV and the beam capacity up to 300 KW because the use of the present accelerators is economically unprofitable. Also, experiments with a reliable target complex that gives the maximum Bremsstrahlung yield and the product

transfer mechanisms in the Bremsstrahlung field, are needed.

Concerning the question of providing the plants with radiation sources, one must note that the further escalation of radiation capacity escalation will be limited by cobalt-60 supply. For this reason, CS-137 may be used in these plants if its price will be three to five times lower.

After analyzing the design and operation experience of industrial radiation plants for the sterilization of different products, the following conclusions were drawn:

— Methods were worked out and programs were mastered for the calculation of the optimal radiation technological plant (RTP) sterilization parameters.
— The unified complex of RTP with a plane irradiator was designed. The set of unified irradiators was manufactured.
— Installed industrial gamma-plants and the plant with the electron accelerator for the different production sterilization made it possible to answer important questions.

Further improvements and increases in the efficiency of RTP will depend upon solving the following questions: designing sample industrial module type plants and standard complexes using unified details and systems; the designing of plants with multirow and multilevel transfer systems; the full mechanization and automation of the processes (including production storing with automatic loading) using microprocessors; industrial encapsulation of cobalt-60 and CS-137 sources and corresponding transport requirements; and designing a national accelerator on the 4-5 MeV energy and beam capacity up to 300 KW (for Bremsstrahlung).

Solving these problems will greatly contribute to the process of equipping the industry with up-to-date technology and hence, increasing the development efficiency of different branches of the national economy.

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Developments in Radiation Equipment Including the Application of Machine-Generated X-rays to Medical Product Sterilization

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Introduction

The irradiation of medical products by ionizing radiation has long been considered a functional means of providing sterility to a packaged device (2). Since the pioneering efforts of Artandi and co-workers at Ethicon, Inc. in the 1950s, the use of electron accelerators and radioisotope-based irradiation systems has evolved into a significant commercial process. The purpose of this article is to highlight the trends in radiation equipment design and evaluation as viewed during the period 1985-1990.

Isotope-Based Irradiators

Irradiation of medical products through the use of the radioisotope Co-60 (cobalt-60) continues to be the predominant mechanism for radiation sterilization of disposables. This is due to the economics, simplicity and reliability associated with the process.

Improvements in gamma irradiator design (3) and operation (10) are noted in the literature. The general trend in irradiator construction corresponds to increased processing power, increased utilization of power through product-overlapping-source configurations and an increased emphasis on the use of material handling equipment and the irradiation of palletized product, resulting in lower labor requirements and operating costs.

From a theoretical perspective, little has changed since Brynjolfsson's fundamental work describing the design and operation of Co-60 irradiation systems (5). Figure 1 depicts the standard premise utilized in processing medical devices. The reduction of dose rate as a function of distance from the radiation source, coupled with the absorption of radiation by the bulk material (in this case, shipping containers filled with shelf-packs of medical disposables), produces a depth-dose profile for a defined bulk density and radioisotope loading configuration. Rotating the absorbing materials 180° in the radiation field produces the desired dose (two-sided irradiation). Indeed, the simplicity of this process owes its nature to the essentially wave-like behavior of the 1 MeV (million electron volt) photon.

Future developments in gamma process control and equipment design will involve the use of computational-intensive methods for calculating radioisotope loading configurations and irradiator processing parameters (9, 15, 17, 28), leading to improved energy utilization and process uniformity. The current factors limiting the use of computer simulation techniques at the facility level are the cost of high-performance microcomputers, related data storage devices and the availability of computer codes designed to run on these machines.

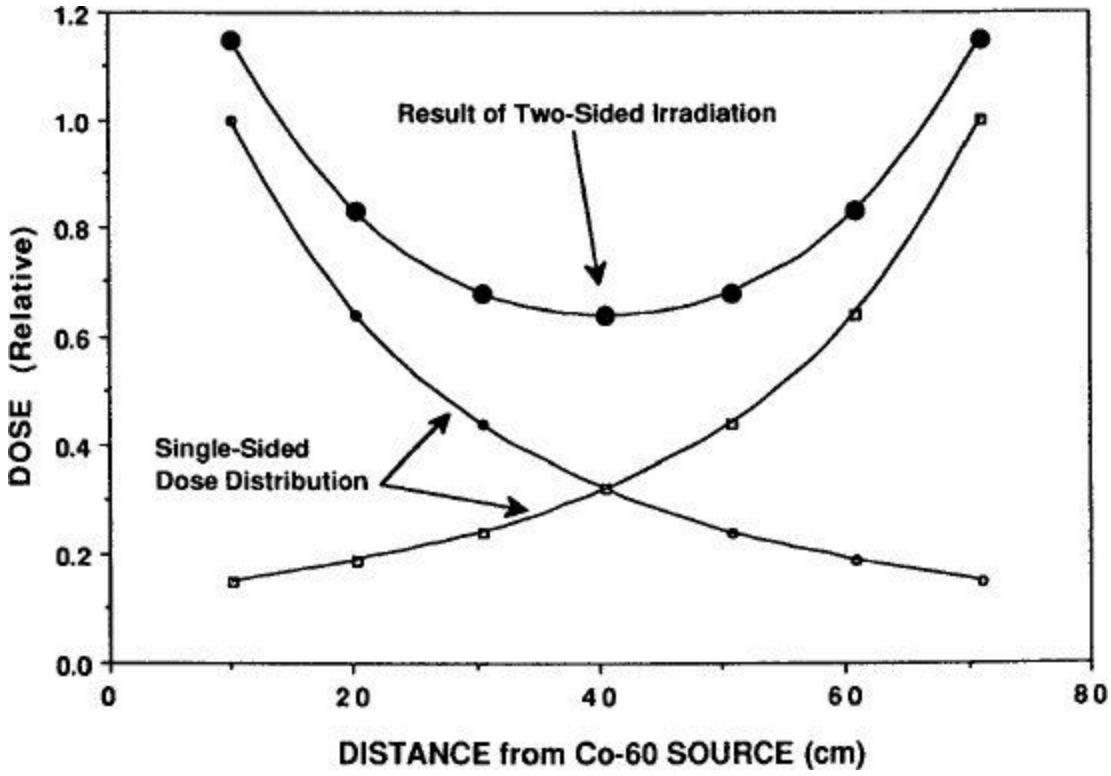


Figure 1. Gamma Irradiation of Medical Products

Electron Accelerators

Given that the principal mechanism of radiation sterilization lies in the interaction of an energetic electron with a microorganism, devices that produce directed streams of high energy electrons are of interest since they can provide high levels of processing power, high levels of energy utilization (> 50%) and, to a degree, user-definable penetration characteristics. The last half-decade has seen a near doubling of the power available, from commercial 10 MeV linear accelerators (linacs) to 30 kwatt, and a respectable rise in the output available, from 3-5 MeV DC accelerators to a current value of 200 kwatt.

The wave-like properties of accelerated electrons permit the use of depth-dose curves for the estimation of dose uniformity (ratio of maximum dose to minimum dose) and processing parameters, and in many cases, the penetration capabilities of the beam can be adjusted by changing the kinetic energy of the electron via simple modification of an acceleration parameter. Although the penetration of accelerated electrons in the range of 0.25 to 12 MeV is limited relative to a 1 MeV photon, medical product units such as individual devices, shelf-packs or single shipping containers can be irradiated as separate entities on a high speed conveyor. A simple schematic of this type of sterilization process is depicted in Figure 2.

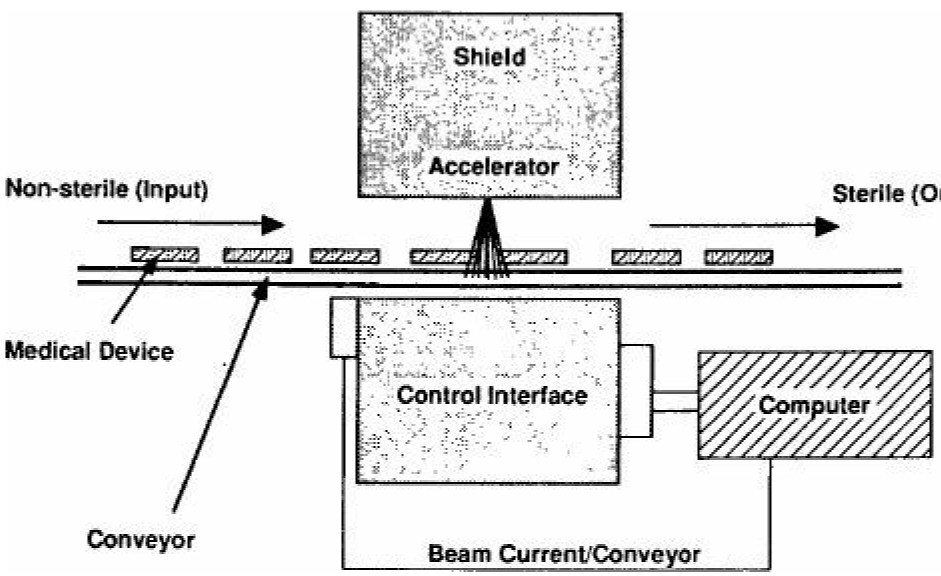


Figure 2. Irradiation via Electron Accelerator

While the perturbation of a photon field by medical devices acting as bulk absorbers can be estimated using relatively simple mathematical methods, and readily verified using conventional dosimetric techniques, dose distributions of accelerated electrons in medical products can be far more complicated to quantify. High energy electrons possess wave-like properties and exhibit predictable depth-dose profiles in homogeneous absorbers; however, they also possess significant rest mass and charge. As a consequence of these physical properties, small changes in the material composition of the medical device, the orientation of a device within a shelf-pack, the orientation of the neighboring devices, and the orientation of the product unit with respect to the electron beam, can alter dose deposition within the product regardless of the level of machine control available. Re-packaging of the

devices may be required in order to adapt the product to the electron beam sterilization process.

Literature exists to help guide the potential end-user of this technology through the preliminary evaluation of the process (1, 4, 13, 29, 36). The fact that equipment manufacturers continue to increase the power, flexibility and reliability of their accelerators leads one to the conclusion that growth and development in this sector merits continued study (18, 30, 32, 35).

Dosimetry

The measurement of absorbed dose is of fundamental importance in developing quality assurance and control programs for radiation sterilization techniques. Precise measurement of absorbed dose can aid the irradiator operator in improving his Co-60 source loading configuration, or can assist the sterilization technician in designing an optimum placement pattern of medical devices prior to irradiation by an electron accelerator. Much attention has been devoted to this subject in the literature (14, 16, 19-21, 24, 25), and the current efforts of organizations such as NIST, AAMI, ASTM, and IAEA in providing conferences, workshops, standards, and guidelines for gamma and electron beam processing are noteworthy.

While dose mapping is found to be a rather simple matter when working with a well-characterized radioisotope source, those individuals who have had the opportunity to perform conventional mapping exercises on medical devices irradiated by accelerated electrons (4 to 10 MeV) can attest to the wide degree of variability exhibited by dose measurements within a device and its associated container. Often, this leads one to question the ability of the process to perform its required goal. In many cases, this doubt can be attributed to a lack of dosimetry data spanning the dynamic dose range generated in a packaged medical product.

The author's approach to attacking this problem is to employ conventional scanning mechanisms as a means of collecting 8-bit (gray scale) images on radiochromic film (GafChromic DM-1260 [23, 33]) placed in strategic locations within the medical device package. Similar approaches employing image analysis to examine dose have recently appeared in the literature (11, 22, 34). Current radiochromic film technology has the capability of producing 10^6 discrete dose points per mm^2. While this volume of data is beyond the storage and manipulation capabilities of most analytical equipment, helium-neon laser and conventional light source densitometric techniques are available for the evaluation of dose-point density matrices on the order of 100 per mm^2.

Figure 3 displays the basic functions required to perform quantitative analysis of high dose-point density image media. In addition to image filtering, equalization, and other enhancement functions, inversion of the image aids the viewer in determining the specific regions of interest. Tracing objects (isodose zones or contours) present in the image reduces the dose distributions to sections, each section attributable to a range of doses (gray scale values). A 2-D or 3-D plot of measured value versus coordinate often helps the viewer identify regions of interest within a given image.

An example of the application of these image analysis elements to the evaluation of the effects of heterogeneous density distributions and nearest-neighbor interactions appears in Figure 4. In this experiment, a sheet of dosimetry film monitors the attenuation of a 10 MeV electron beam by a shelf-pack containing groups of packaged medical disposables (organic materials, low bulk density) irradiated in a two-sided manner. The dark portions of the image denote sections of the shelf-pack where attenuation of the beam can be attributed to packaging material and air, while the lighter sections denote attenuation of the beam due to electron interactions with the disposable devices. Figure 5 outlines the isodose regions

associated with the image (2-D contours) and inverse of image (3-D contours), identifying the regions of interest that should be pursued during process qualification.

Just as conventional radiographic techniques present the trained eye with the "condition" of our teeth, lungs, bones, etc., image analysis applied to radiochromic film media can produce a comprehensive picture of dose distributions in medical devices irradiated with electrons. This information can be applied to generate worst-case processing scenarios for products and thus identify the dose zone(s) associated with sterility (minimum dose) and maximum material degradation (maximum dose).

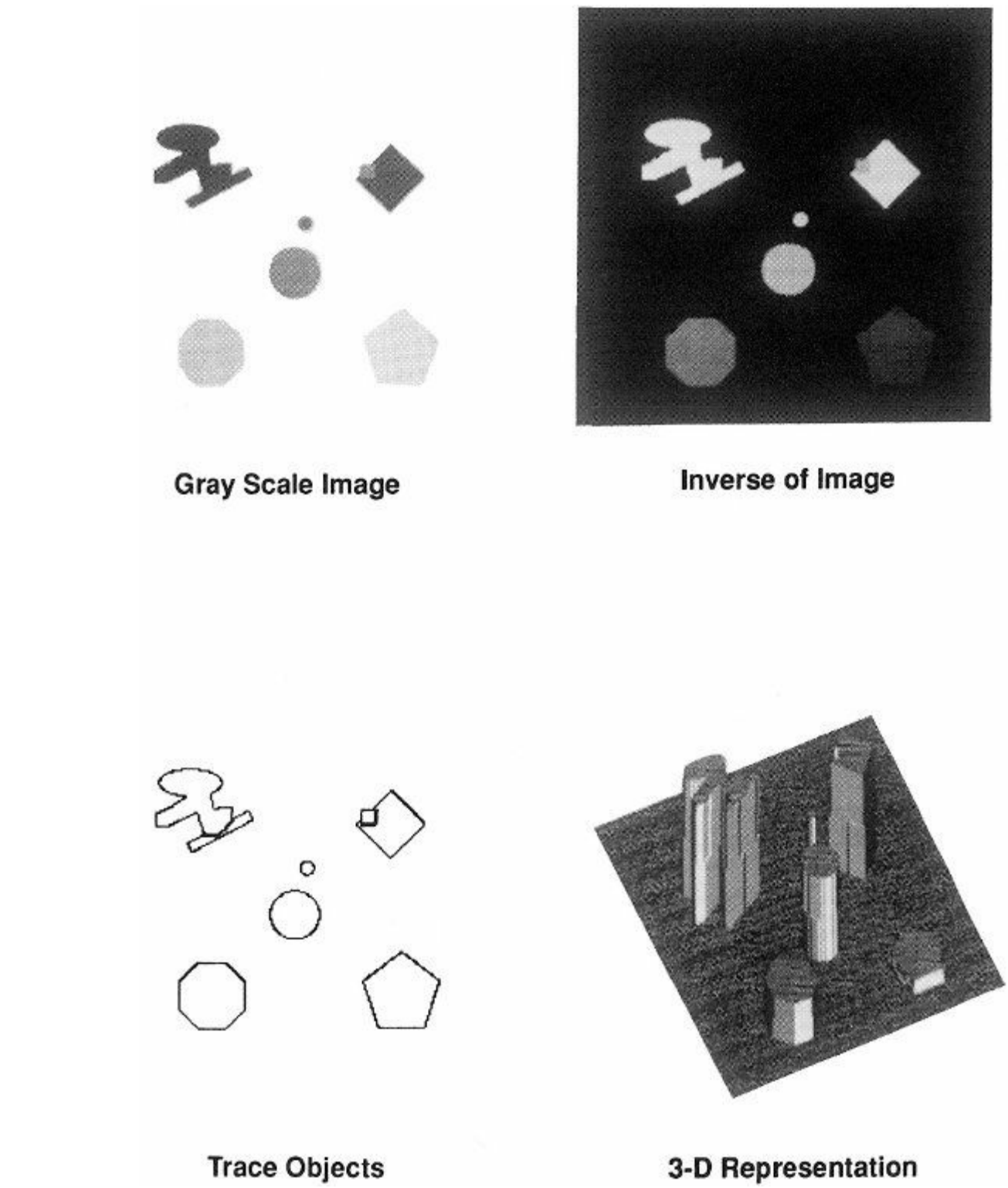
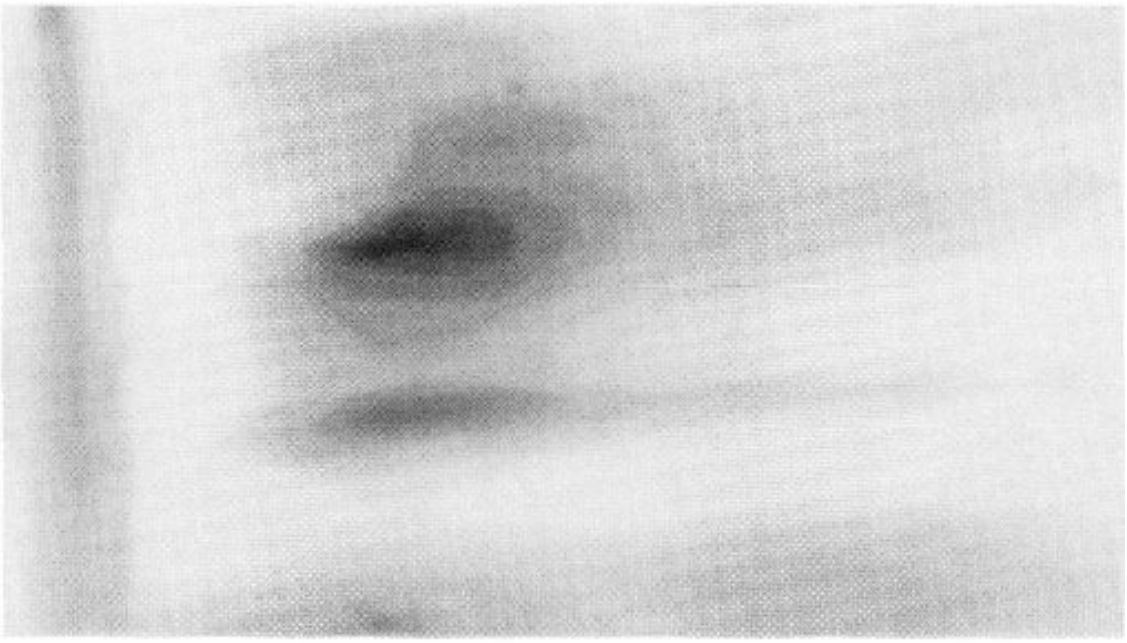


Figure 3. Elements of Gray Scale Image Analysis

Image of Irradiated Shelf-packs



Inverse Image

Figure 4. Gafchromic Response to 10 MeV Electrons

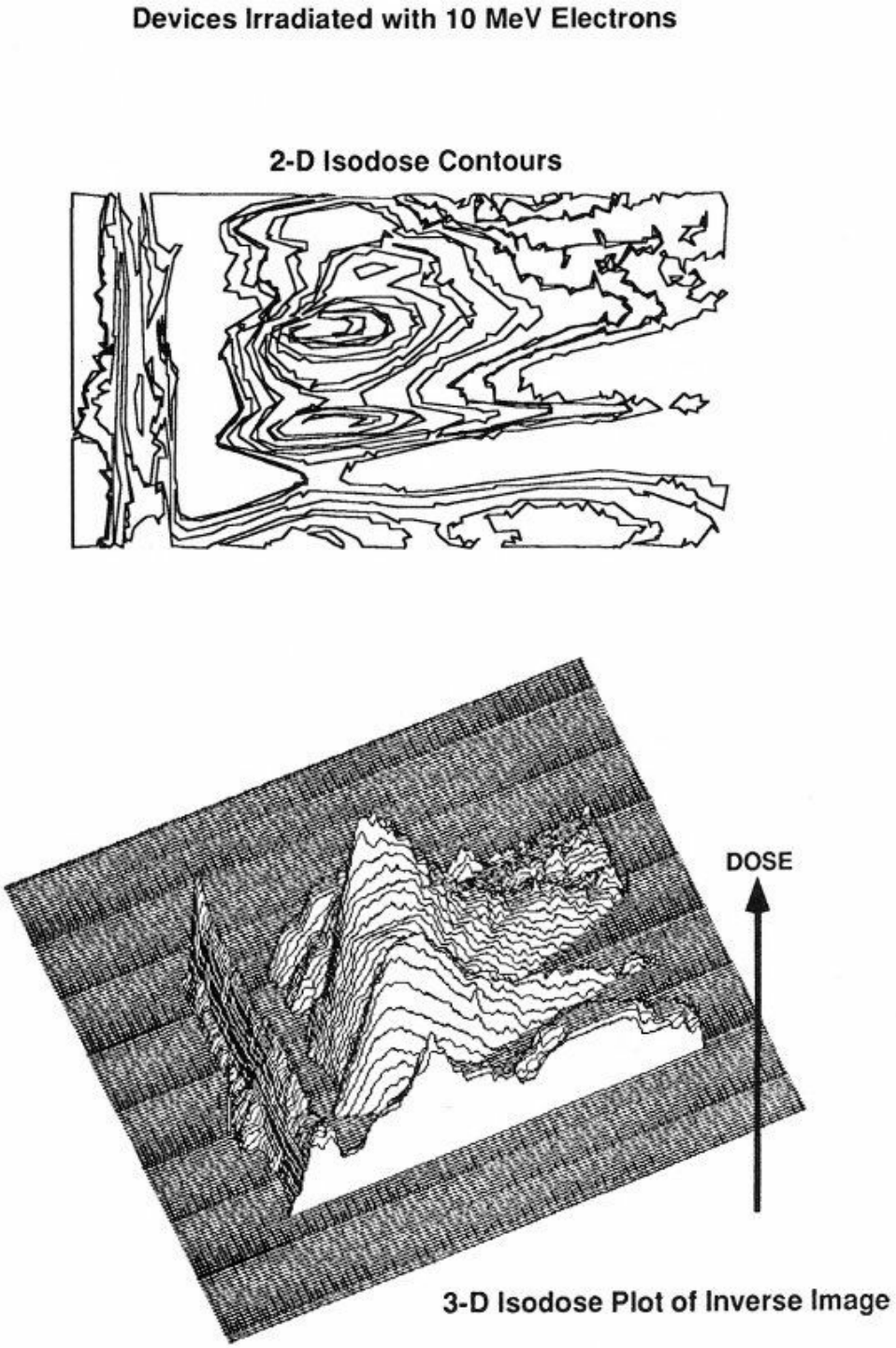


Figure 5. Gray Scale Dosimetry Results

Machine-Generated X-rays

It has always been considered desirable to have access to a machine source of photons with energies on the order of 1 MeV. Such a device, based on current accelerator technologies, would provide controllable power and energy deposition characteristics beyond the existing capabilities of Co-60 irradiators, in addition to providing a direct source of both photons and electrons.

Evaluation of the theoretical feasibility of producing X-rays (bremsstrahlung, “brem”) via electron bombardment of a high Z target, as well as economic comparisons of Co-60 and machine sources of ionizing radiation are well documented (6, 8, 12, 13, 26, 27, 37). Questions regarding penetration capabilities, the effects associated with the use of complex emitted photon energy spectra (Figure 6) and the operating efficiency of a machine-source of X-rays have been answered through both calculation and measurement.

Efforts by RDI (Radiation Dynamics Inc.) and SHI (Sumitomo Heavy Industries) have produced a functional 200 kwatt, 5 MeV Dynamitron electron accelerator (Figure 7), 300 kwatt X-ray target and target cooling system that has been evaluated (7, 31). Monte Carlo calculations have been performed in order to quantify selected aspects of the energy deposition process.

Figure 8 depicts the basic experimental set-up of uniform absorbers (phantoms) that pass below the scanned X-ray (brem) spot. Gafchromic dosimetry film was calibrated on-site against radiochromic optical wave guide dosimeters (Far West Technology Inc.) in order to build the effects of irradiation temperature, spectrum, and relative humidity into the film dosimetry system calibration curve (Figure 9). The measurement of variability associated post-irradiation color development was also evaluated (Figure 10).

Figure 11 represents an image of the X-ray spot produced by a stationary 5 MeV electron beam impacting on the X-ray target after the brem spot had traversed 15 cm of air and passed through approximately 5.5 cm of a water-equivalent material. Comparison of this image to the image produced on the external surface of the cooled X-ray target demonstrates the highly focused nature of bremsstrahlung emission, which can now be perceived as a quasi-point source whose intensity is a distinct function of the angle of observation versus the impact angle of the electron beam on the target.

Utilizing dosimeter films and moving absorbers, the depth-dose characteristics of a scanned X-ray spot have been evaluated. The high degree of correlation between the dose values calculated for this experiment via Monte Carlo techniques and the actual energy deposited as measured by single film dosimeters is exhibited in Figure 12.

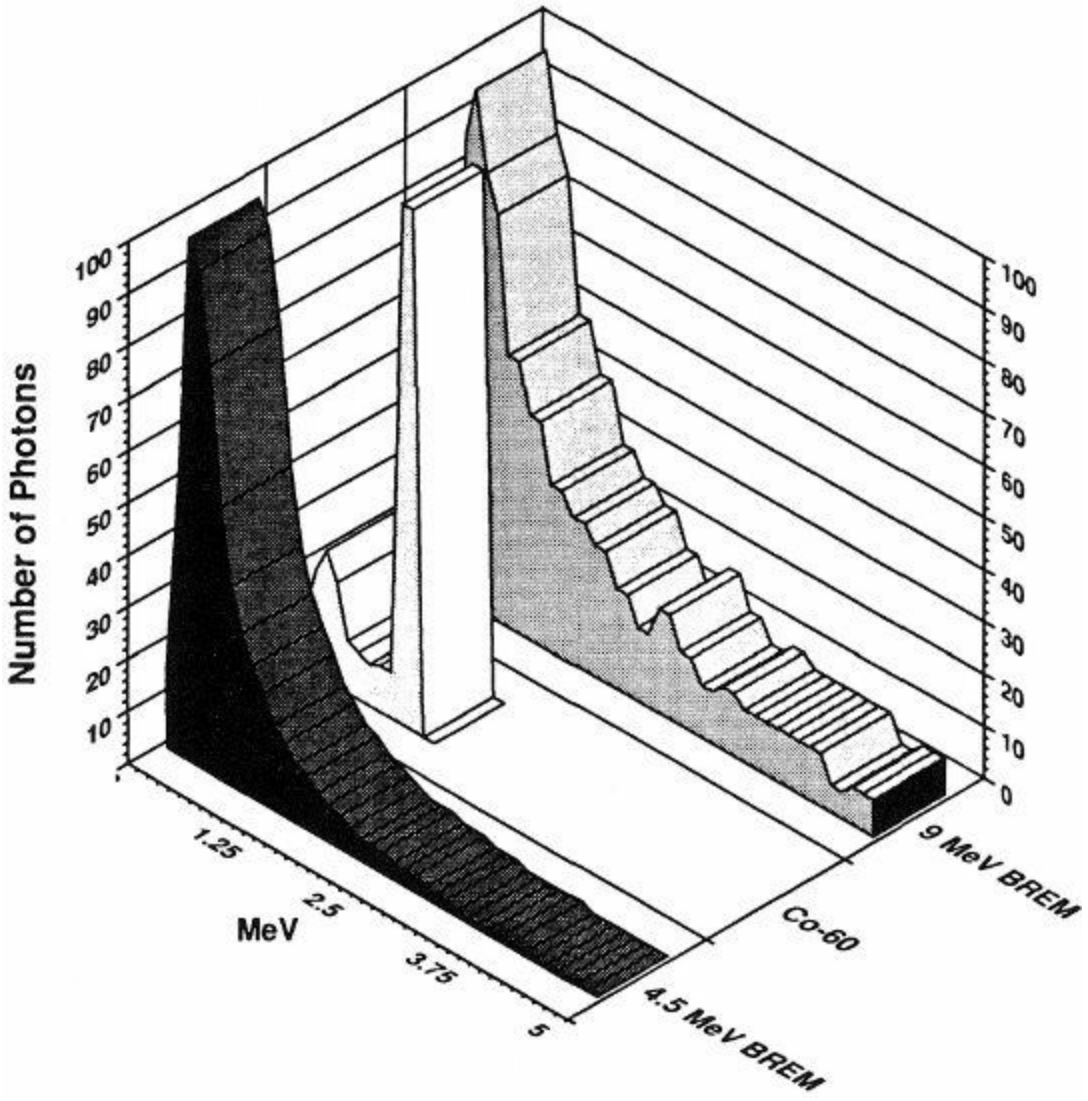


Figure 6. Photon Energy Distributions

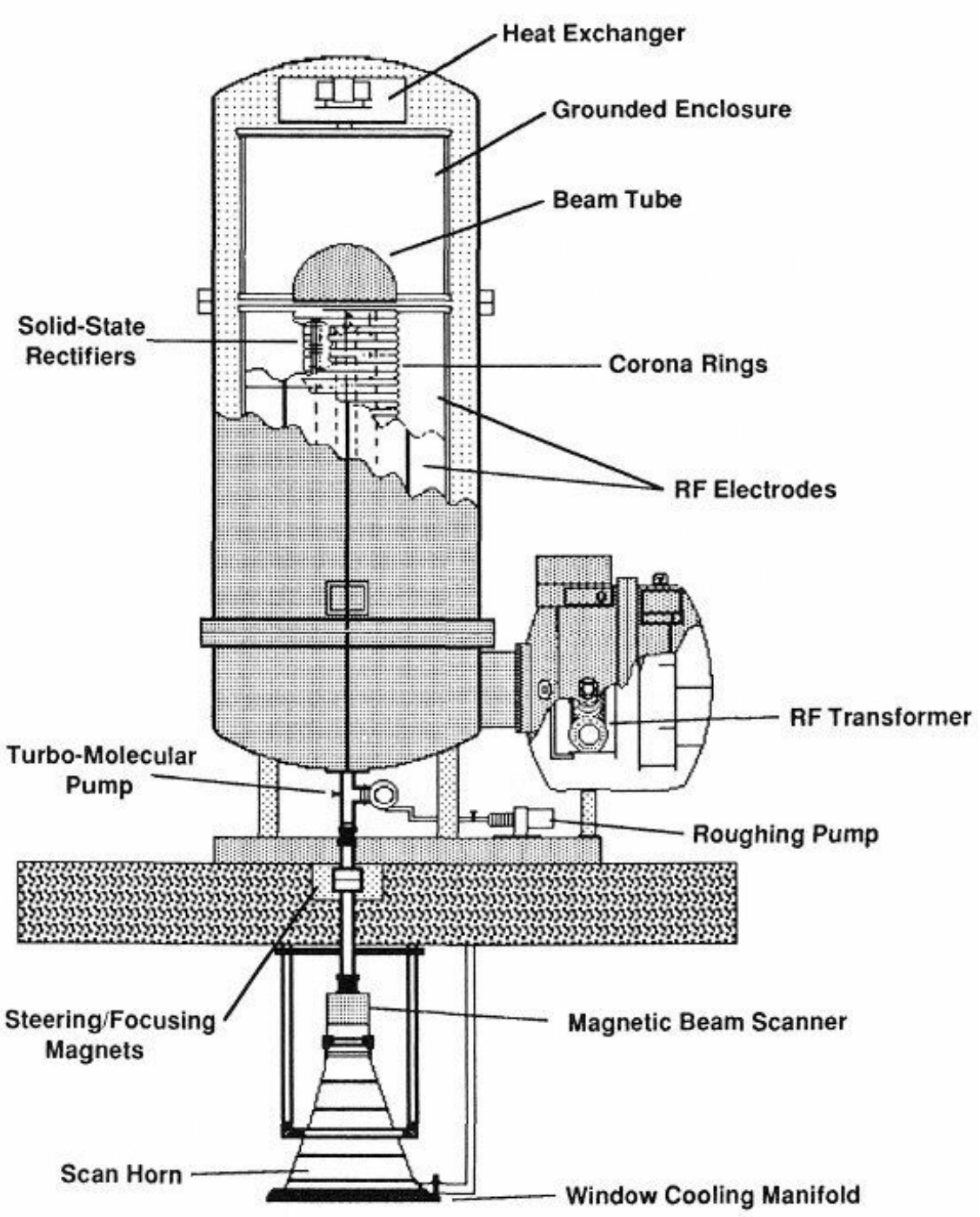


Figure 7. DC Electron Accelerator (RDI Dynamitron [5 MeV, 200 kwatt])

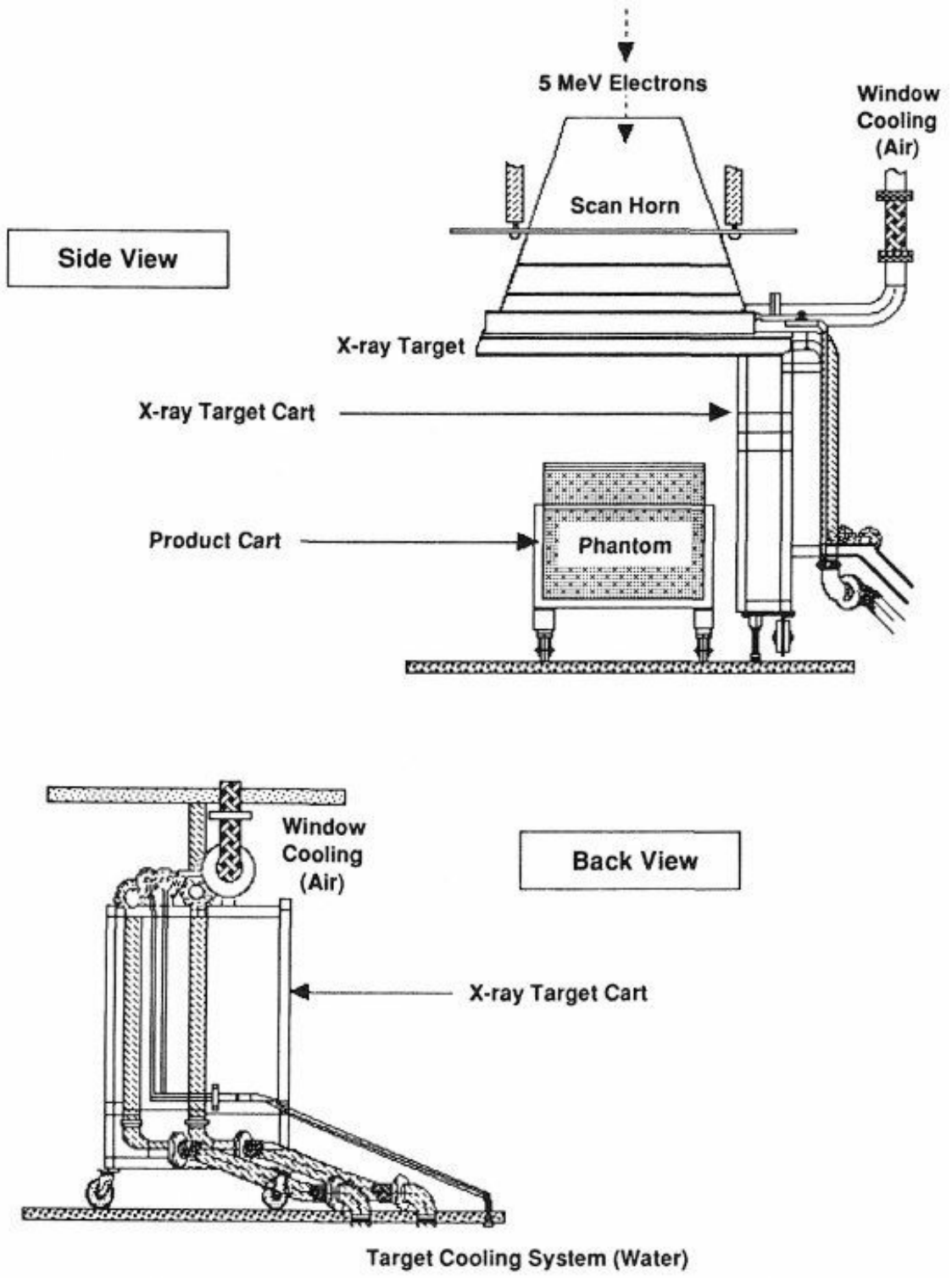


Figure 8. X-ray Target Experiment

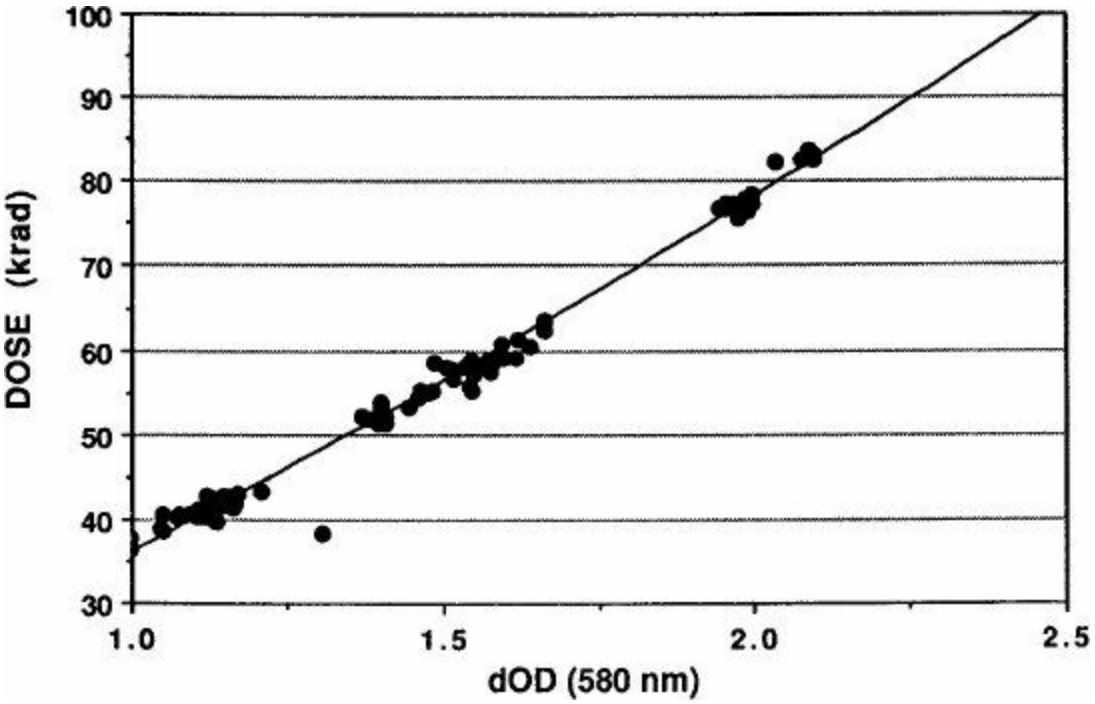


Figure 9. Gafchromic Response Function

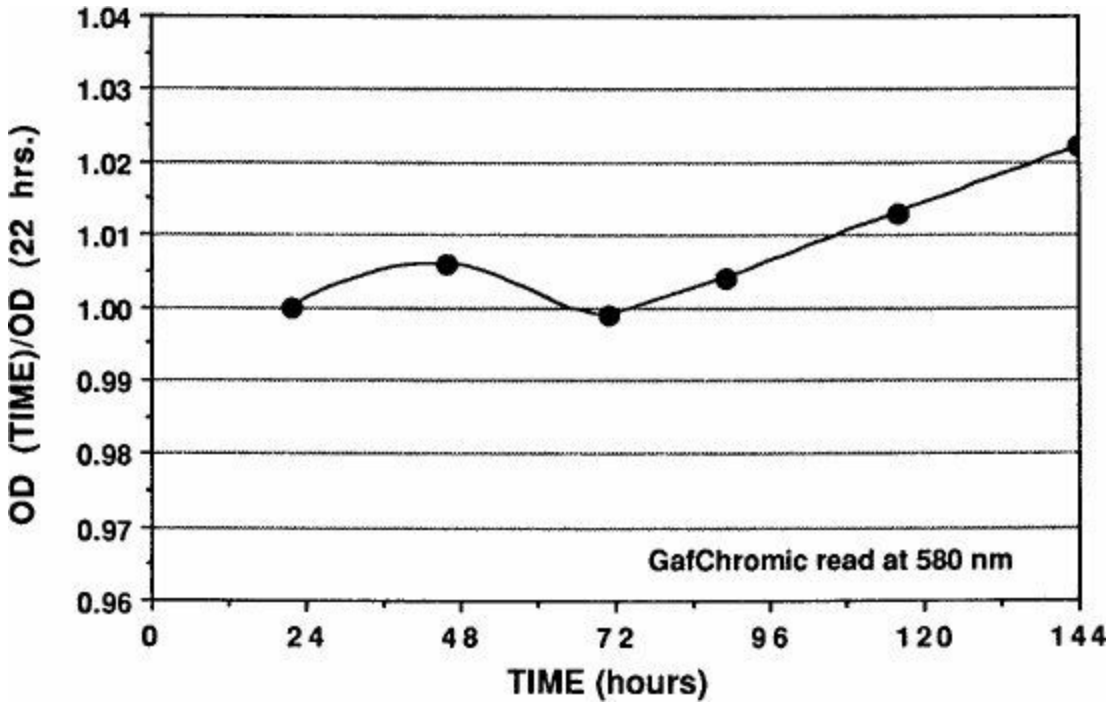
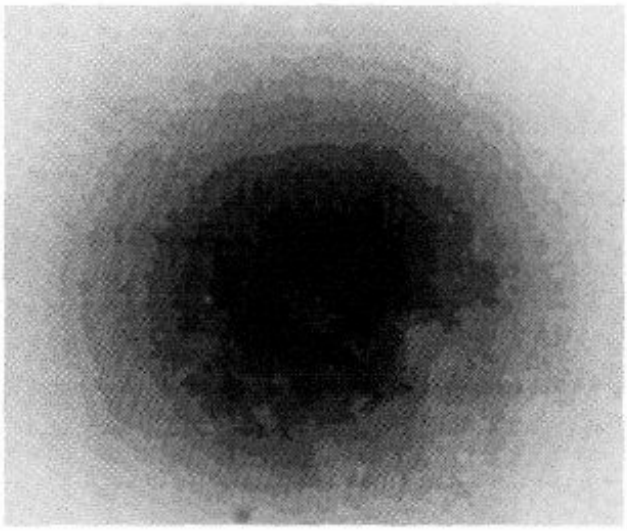


Figure 10. Gafchromic: Post-Irradiation Development



Enhanced image of the dose deposited in a piece of film by a 5 MeV X-ray "point source".

Trace of the spot image.

Note the circular nature of the isodose curves associated with the brem spot.

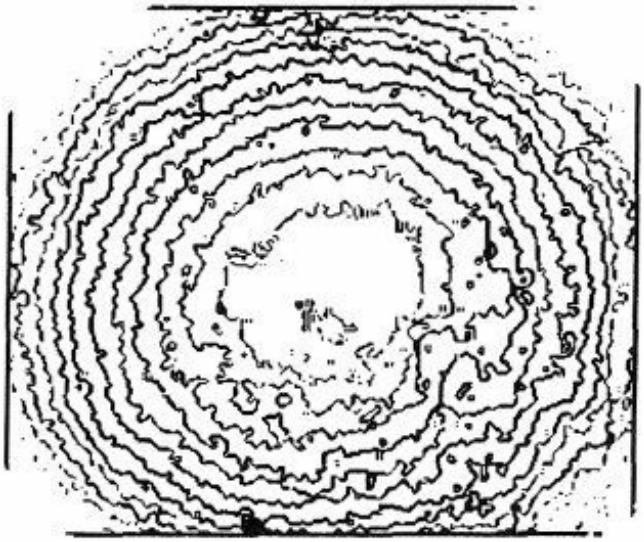


Figure 11. Gray Scale Analysis of a Brem Spot

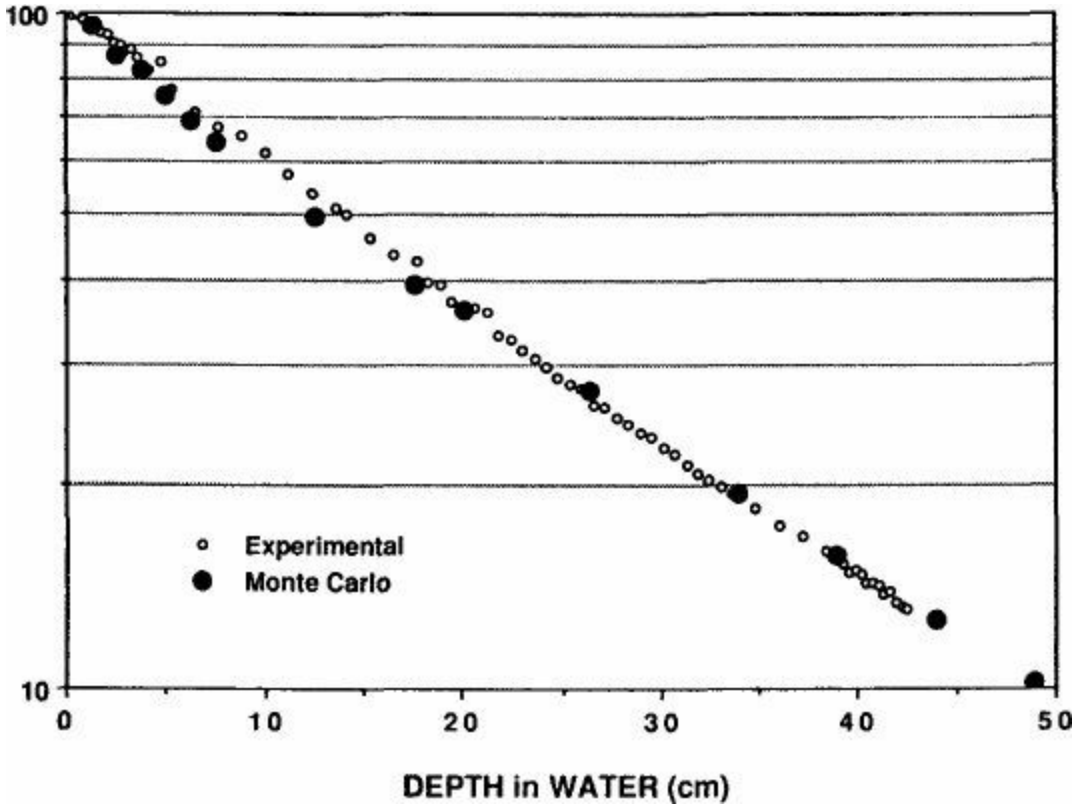


Figure 12. Depth-Dose: 5 MeV Brem

Film measurements made on moving absorbers of homogeneous bulk density provide further evidence of the degree of penetration available using a scanned 5 MeV brem spot. The results shown in Figure 13 exhibit a high degree of correlation between exponential fits and single film dosimeter measurements for a given absorber. Comparison of this figure to the results displayed in Figure 12 leads one to the conclusion that multiple attenuation coefficients will be required in order to produce an accurate model for the interaction of the scanned X-ray spot source with product, since the entrance dose will be a function of the quantity of low energy photons and scattered electrons present in a given accelerator/target environment.

Preliminary results obtained from these experiments and published results of experiments performed by RDI et al. allow one to estimate the potential improvement in dose uniformity obtained when employing the 5 MeV X-ray machine source as a processing mechanism relative to a Co-60 irradiation system. The results of such a calculation are depicted in Figure 14 and are accurate to 10%. Expectation of a 15%-25% improvement in process dose uniformity for the X-ray irradiator relative to the Co-60 irradiator is consistent with values calculated using published theoretical and experimental databases and makes sense, given the number of X-ray photons present with energies greater than 1 MeV.

Designing an irradiator requires a thorough understanding of the energy deposition characteristics associated with the chosen form of radiation, as well as an understanding of the directional nature of the emitted radiation. Figure 15 depicts a 2-D design for both isotope and machine-generated radiation sources. Although the majority of Co-60 irradiation mechanisms have been explored, significant efforts in the areas of physics and engineering will be required in order to determine optimum product flow and mechanism design for use

with X-ray sources. In any case, the use of product-overlapping-source designs will be necessary in order to maximize bremsstrahlung power utilization.

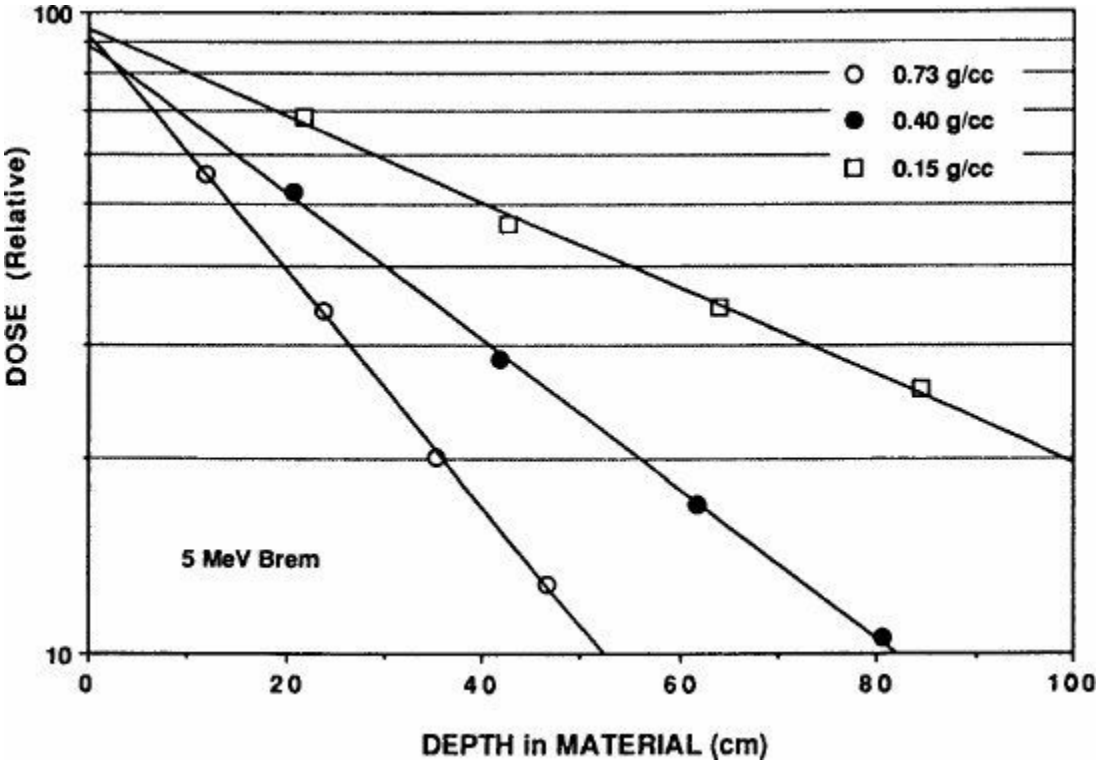


Figure 13. Depth-Dose: Unrestricted Origin

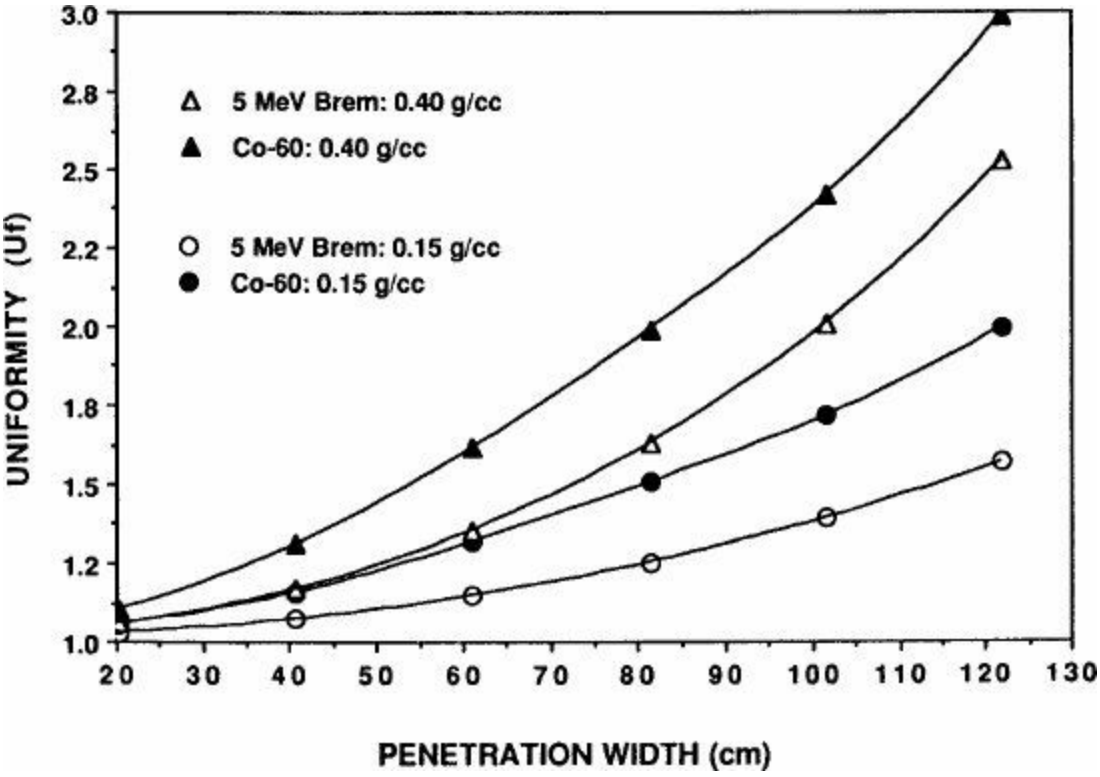


Figure 14. Dose Uniformity: Brem vs. Co-60

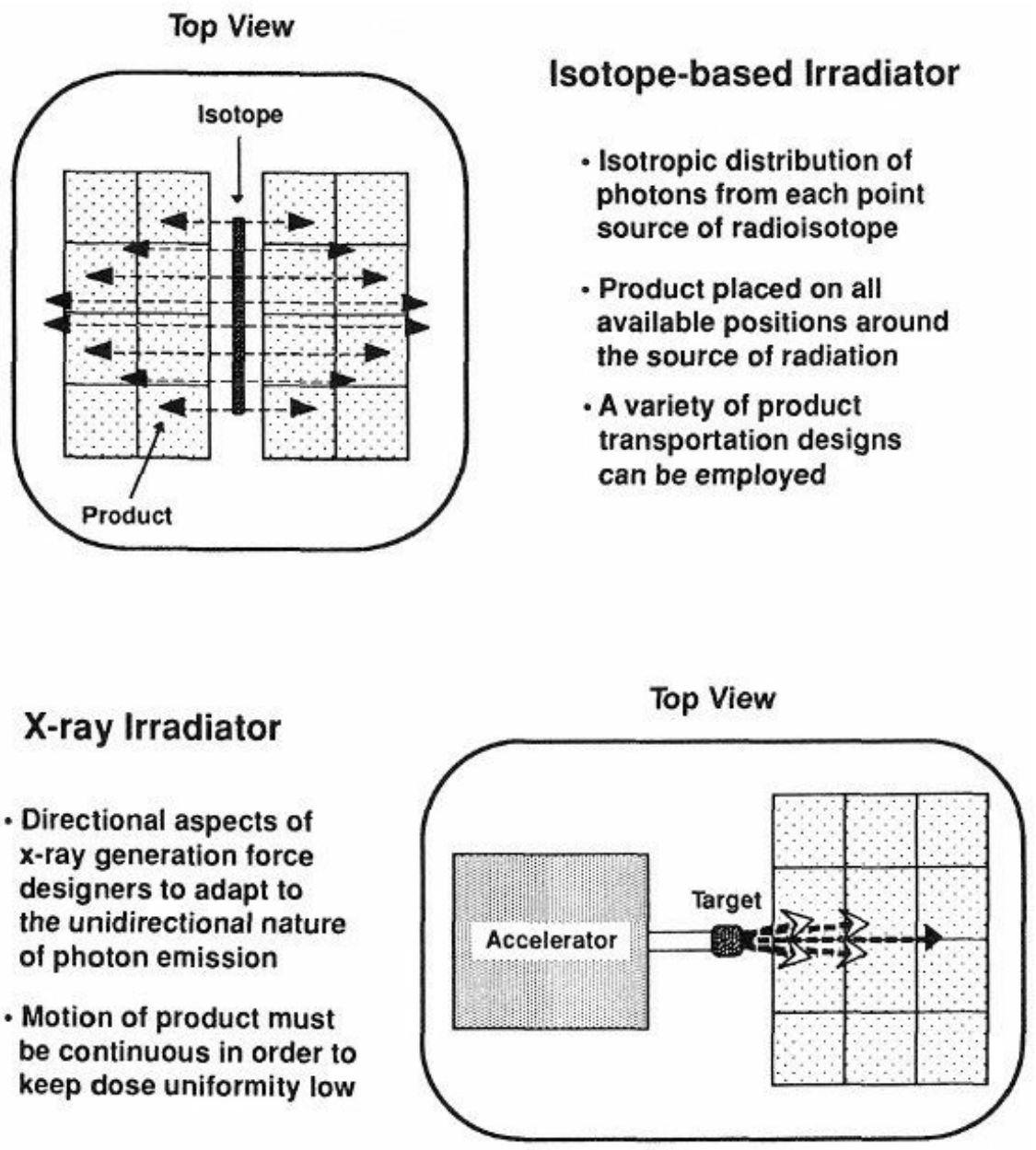


Figure 15. Irradiator Design Considerations

Summary

The use of ionizing radiation to sterilize medical devices will remain a major commercial treatment method. While the technologies associated with radioisotope-based irradiators have matured, complementary processes utilizing accelerated electrons and/or machine-generated X-rays will continue to be refined in an effort to compete for a share of the available market. This fact, coupled with economic concerns regarding isotope availability and price, regulatory issues involving isotope transportation and disposal, as well as the demand for processing power inherent in the commercialization of food irradiation, will continue to drive the development of machine-source technologies.

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Resistance of Microorganisms to Radiation and Experiences with Dose Setting

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Ionizing radiation is an acceptable and reliable method of sterilizing disposable medical devices which has been available for 30 years. Microbial life is susceptible to the lethal effects of radiation but there is a substantial degree of variability in the susceptibility of microbial species to radiation.

Susceptibility of Microbial Populations to Ionizing Radiations

The radiation survival curves for microbes can be plotted and analyzed to arrive at a D_{10} value for any microbe under investigation, which will express the susceptibility of the strain to radiation. D_{10} is that dose of radiation that will reduce a microbial population by one log, or 90%. D_{10} can be readily calculated using the Stumbo equation:

$$D_{10} = \frac{\text{Radiation Dose}}{\text{Log A-Log B}}$$

where Log A is the initial microbial population and Log B, the population remaining at the dose where the observation is made. Dose response curves may theoretically be of three kinds (20) when plotted semilogarithmically to make feasible calculations of the D_{10}. The Type A curve, where death proceeds continuously from the initiating exposure, represents a simple exponential relationship. Type C, which displays a shoulder effect, occurs importantly in some of the more resistant microbes. Here, if a line is drawn between two observed points, it will always overestimate the D_{10} value when produced beyond the point of observation. Curves of Type B, if they occur with individual microbial species, would be of concern because here, the true D_{10} value will be underestimated when produced. Curves of Type B have not really been demonstrated in individual strains but occur in hypothetical curves generated from mixed populations such as those that might occur on devices (5, 13). It is important to bear in mind that some species are intrinsically very resistant to the process. Some have been shown to display a pronounced shoulder effect, and it is also possible, by selection, to find mutants having a greater resistance to the lethal effects of radiation than the parent strain.

Because of the nature of the sterilization process, it is never possible to reach a point where there is not a theoretical chance of a survivor, although if the process is carried on long enough, one can reach a point where the probability of an item containing a living organism is infinitesimally small. In practice, a standard that not more than one item in a million (sterility assurance level 10^{-6}) might contain a living organism has been accepted internationally. The main difficulty is in proving that this standard has been achieved.

In forms of sterilization other than radiation, it has been customary to use biological indicators of a microbial species having a standard and known resistance to the process, and to provide evidence to satisfy this 10^{-6} arbitrary sterility standard on the basis of overkill. Nonetheless, records of aspects of the cycle which are recordable are a necessary adjunct in order to document control of the process, and cycles that do not meet specification for time or temperature achieved will be rejected.

I think it is fair to say that, in the early days of radiation sterilization some of the same thinking went on, and a search was made for microorganisms that would pose a challenge to the system (3). Of these, in particular, *Streptococcus faecium, Micrococcus radiodurans* and *Bacillus sphaericus* CIA posed a serious challenge. If we were to require that any of these be used as a biological indicator to control the radiation sterilization process, we would be compelled to use much larger doses of radiation than we currently think are necessary. The basis of Christensen and Sehested's (3) proposals to use *Str. faecium* was

that there was a sufficiently high probability that this organism would be found on the hands of workers manufacturing devices, and thus that it might contaminate the device itself. There is now general agreement that biological indicators have no place in routine control of radiation sterilization. Control is better achieved by the use of dose mapping, timers, and dosimeters. However, well-characterized biological indicator strains might still find a role in experimental studies; for example, in comparative studies of the effectiveness of different types of ionizing radiation since their high degree of radiation resistance might make minor differences between electron beam sterilization and gamma radiation processes easier to detect.

In practice, a minimum dose of 2.5 Mrad or 25 KiloGray (kGy) has been accepted in most countries as a satisfactory dose for the sterilization of medical devices, provided the bioburden does not exceed an agreed maximum.

In radiation sterilization, since the basic premise of a single overkill cycle cannot be countenanced and, in particular, since a major limitation of the process is radiation damage to articles, it is important that excessive doses be avoided. If a dose of 25 kGy is applied universally to all articles with a bioburden below an arbitrary maximum, those articles with a very low microbial load will be processed to a much higher standard of sterility than those with a bioburden closer to the maximum acceptable.

Flexible Dosing Procedures and the Adequacy of Sterilization

Is it possible to devise some way of satisfying our needs for product safety and yet allowing flexibility in the doses used dependent upon the concentration of the initial microbial population?

Tallentire (21) advocated the use of sterility tests applied to devices subjected to incremental doses of radiation as a means of validating sterilization cycles. He developed cogent arguments to support the concept but his long and intricate publication (22) did not come up with a way of calculating dose when mixed microbial populations occurred on articles. When I heard Tallentire speak on this subject, I was immediately impressed how logical and efficient it was to perform sterility tests on partly processed articles as a means of obtaining information on the kinetics of the sterilization process. When I became involved with the AAMI Working Group, I wrote a review suggesting that this would offer the best hope of substantiating flexible dosing. We were also encouraged by the late Ron Campbell of Canada to explore different standards of sterility (MSI), later to be incorporated into AAMI proposals as SAL.

There is plenty of documentation that even after quite low doses of radiation, articles will pass sterility tests (16, 25). The items reported by Ley et al. (16) probably had a very low initial microbial population, but White (25) reported on the effects of low doses of radiation on cotton wool balls which, at that time, would certainly have had a high initial microbial population. In initial attempts to adapt such observations to the determination of radiation sterilization dose and to the ongoing control of process, some wished to obtain the sterilization dose by using straight line extrapolation from the line obtained by plotting log initial bioburdens to log microbial survivors at the test dose. This process is likely to lead to underdosing as resistant members of the population would be more likely to be among the late survivors of the process.

Reports of resistance of natural bioburden, in general, do not indicate high levels of microbial resistance to the process (1, 24). However, it has been possible to demonstrate microorganisms from the environment or personnel associated with manufacturing with high levels of resistance (2, 4, 14). Because of the importance of radiation-resistant microbes, even in small numbers, Whitby and Gelda (23) specifically looked at the radiation resistance of survivors cultured from positive sterility tests, samples initially irradiated with 4 or 6 kGy. They believed that this sample should allow an enrichment of the radiation resistant component of the bioburden. The observed radiation resistance of the microbes so obtained was used as a model population to calculate the theoretical dose necessary to reduce that population to the point that one surviving organism would occur every 10^6 items, or the 10^{-6} SAL. A total of 397 isolates were examined and radiation resistance was determined by two methods—that of Czernianski and Stolarczyk (4), where microbial suspensions were dried on glass before irradiated and also microbial suspensions dried on fabric. While they reported two isolates among the 397 tested with a D_{10} of 4 kGy or greater, the highest D_{10} found was 4.2 kGy. The population in which the testing was carried out by the method of Czernianski and Stolarczyk (4) formed Population C of the AAMI method and was used to generate the tables for method B1 and was among those used in the simulations evaluating

method B2 (6). Whitby and Gelda (23) reported, as others have, that the method used to prepare the microbial suspension is influenced by the D_{10} values obtained. In particular, if suspensions were dried on cellulose, they were less resistant. D_{10} values obtained from cellulose-dried organisms were the basis for AAMI Population B.

The AAMI methods were developed to provide a means to extrapolate from the point where the microbial population become extinguished, as far as the sterility testing could confirm, to the point where the 10^{-6} SAL assurance level would be reached. Population C was considered to pose a serious challenge to the system but the proposals were tested in a computer simulation of the sterilization process which allowed microbes of any chosen resistance to be included in the model. It also allowed examination of microorganisms displaying Type B shoulder sterilization kinetics.

The AAMI document proposed four methods of determining the sterilization dose and I will now briefly review them. The methods are all based on attempts to obtain information of the behavior of the population when exposed to radiation that will yield a 10^{-2} sterility assurance level and then applying a dose-setting multiplier (DS) to extrapolate to a 10^{-6} sterility assurance level.

Method B1 requires the bioburden numbers to be known and a sterility test is then performed on 100 samples irradiated at a specified dose. If the result of the irradiation of 100 samples is satisfactory, the sterilization dose is calculated using a table. The table employs an extrapolation factor that directly increases in value as the bioburden numbers increase, and is based on allowing a 25 kGy sterilization dose with a bioburden of 1,000/device. We are currently in the process of revising the AAMI Guidelines and will replace the tables with equations which will allow calculation of the necessary sterilization dose for any bioburden figure. Additionally, we are looking at reducing the number of samples to be irradiated for verification and audit to 50 from the 100 we currently require.

Method B2 does not require bioburden information, though I believe manufacturers would be wise to have such information for quality assurance purposes. Because bioburden information is not required, it is necessary to irradiate more product samples than for B1 in order to characterize the resistance of the microbial population over that portion of the radiation sterilization process where microbial numbers can be counted. Currently, Method B2 requires irradiation of 20 samples from three separate lots, in incremental steps of 2 kGy up to 18 kGy; from the results obtained, a dose at which to irradiate 100 samples is calculated. If the results are satisfactory, an appropriate sterilization dose can then be determined. Methods B1 and B2, and also a modified method B2 that can be used for ultraclean items, are published in the AAMI guidelines.

Method B2 gives more information about the sensitivity of the microbial population to the lethal effects of radiation and allows us to draw a more detailed line relating the radiation doses necessary to reduce this population from an average of 3 microorganisms/device to 0.01/device. The need to calculate the dimensions of this window with some precision lies behind the correction factors for augmentors and the correction for the number of observed positives in the section where 100 samples are irradiated. The extrapolation factor (DS) employed becomes progressively greater as the width of this window increases, and it is

certainly possible for the method to give results that will demand a higher sterilization dose than 25 kGy.

We are also reviewing the B2 procedure in our general revision and again are likely to suggest that only half the number of samples we now propose be used in the future. This will be 10 samples from three lots at each incremental dose and 50 samples for Experiment 2 when the 10 sterility assurance level is investigated.

Both methods contain provisions for ongoing audit, which is recommended be conducted every three months unless bioburden data indicate that a more frequent audit may be required. The audit procedure is really a verification of the 10^{-2} SAL and is conducted at the dose required by the validated cycle. A weakness of the audit procedure is that, if the audit fails, the dose is adjusted upward and can never be brought down again unless the whole dose-setting procedure is repeated. Since audit will always fail when two or more positives are obtained and a 10^{-6} sterility assurance level is required, it is necessary to have confidence in the ability of the test laboratory to perform sterility tests reliably.

I will not discuss AAMI dose-setting methods B3 and B4 except to say that they were included because some devices do not lend themselves readily to methods B1 or B2. Methods B3 and B4 have not been widely used and will almost certainly not be included in the revised guidelines.

Last year, a questionnaire was sent to all device manufacturers on AAMI's list asking them to supply some information on their experience with the AAMI radiation sterilization dose-setting methods or other dose-setting methods if they were used. By no means did all those receiving the questionnaire respond. However, we received replies relating to 92 products. Joyce Hansen of Becton-Dickinson, Franklin Lakes, N.J., and I have been analyzing the results and expect to publish the information we have extracted from the returns shortly; however, I will review some of the results here.

The B1 and B2 methods were used by equal numbers of respondents; two respondents reported using method B4 and 18 respondents were using other methods for dose-setting. The matter of greatest interest is the experience with audit among those using AAMI methods for dose-setting. Audit is carried out on 100 samples, so we have results from 727 separate audits which have been carried out at doses ranging from 1-11 kGy. The results include 18 (2.5%) audit failures; positive tests overall were 0.55% of the samples tested. They clearly established the ability of North American laboratories to perform sterility tests with little problem from false positives.

It appears that many of the users have been conforming to the AAMI Guidelines when deciding what sterilization dose to employ although I can identify one or two exceptions. The survey shows that, at least among the responders, the current AAMI methods can be performed and yield sterilization doses that are below 25 kGy.

Methods of flexible dosing must be based on the premise that microbial numbers on different products vary. This can be handled by setting arbitrary doses which will be increased to match greater microbial numbers in the bioburden. Alternatively, we can experimentally determine at what point in the radiation sterilization cycle is the natural bioburden reduced to a certain level, and then use some extrapolation factor to calculate the additional dose of radiation necessary to achieve the desired sterilization standard. The

second method is what we have chosen for AAMI, although method B1 does incorporate some aspects of the first approach by specifying the actual test radiation dose to be employed for the 100-sample test on the basis of bioburden numbers. Others have also used this approach (10, 15), although clearly the credit for suggesting this approach must go to Tallentire (1972). The difficult part of the process is the extrapolation factor. In special circumstances, i.e., for devices with a very low and limited bioburden, it may be possible to be device-specific about the extrapolation. Doolan and colleagues (9) described such a situation for hypodermic needles and validated a sterilization dose of 7.5 kGy.

Thus far, I have talked about radiation sterilization generically as it might be applied to both gamma rays and to electron beams. However, the AAMI Guidelines, as published, relate only to gamma radiation. We are still in the process of preparing similar guidelines for electron beam sterilizers.

Equivalence of Gamma Rays and Electron Beams

There is some reason to believe that electron beam radiation, in certain circumstances, may be less effective as a sterilant than Cobalt 60. The reason for this has been attributed to oxygen depletion at the very high dose rates employed. However, it seems to me that the likelihood of there being a measurable difference would depend on whether the microbial cells were held under anoxic conditions in the post-irradiation period. There are circumstances where this might be so: where bacteria are coated with organic matter, as in Dr. Christensen's biological indicators, or at times when organisms are dried on hard surfaces from a proteinaceous solution, or where potent oxygen scavengers are included in the material for the purpose of lessening radiation damage to that material. Dose-setting procedures test the behavior of the natural bioburden in the sterilization process. If there is an effect, it will merely cause the procedure to indicate that a higher sterilization dose is required.

Nonetheless, we need to know whether or not gamma radiation and electron beam radiation are or are not comparable. If they are comparable, it would be much easier to carry out subprocess dosing of samples using a gamma source rather than electron beam. Comparative microbial resistance should be carried out using some of the well-characterized resistant microorganisms and hopefully the question will then be answered.

Microbial Contamination of the Product

In my opinion, it would behoove the device industry to take all reasonable steps to minimize microbial contamination of the device at the time of manufacture and to try to reduce the bioburden. The use of a laminar flow hood and controlled rooms would also go some way to reducing bioburden, since resistant organisms have certainly been shown to exist in the environment.

Microbiology Technique

When initially qualifying products for radiation sterilization, we should make sure that we are using the process with the highest efficiency of recovery. It is unrealistic to demand that a diversity of culture media be used at all times, but evidence should be produced that we are testing for the survival of microorganisms that are most likely to survive. If there is doubt about what recovery medium to use, the suggestion of Doolan et al. (8) of scoring net positives is a good one. There is evidence that soybean-casein digest broth is more efficient overall as a recovery medium, but this would not be the case for anaerobes (18). Sterility tests require an adequate time for incubation. The AAMI Guidelines call for 14 days and I think that, despite the inconvenience of waiting, this time cannot be reduced. Sterility testing must be carried out in properly equipped laboratories. Tallentire talks of a 1/1000 false positive rate as the limit of possibility; in AAMI, we have used 1/100 to establish a 10^{-2} standard. Yet when the British Public Health Laboratory Service was investigating contaminated dressings, they had an overall positive rate in controls of 26.8% (17).

If we are still concerned with the possibility that we are overlooking small numbers of radiation-resistant organisms, it would seem worthwhile to collect all positive cultures obtained from the audit procedure and determine their radiation resistance. The audits I have reported had an overall number of 402 positive cultures from 72,700 sterility tests. Radiation resistance studies on such isolates would surely reveal some truly resistant organisms should such organisms pose a real problem. Similarly, we might look at clinical records and cases to see if there have been infections with unusual organisms that might call into question the sterility of devices.

Overall, it seems to me there is good reason to believe that testing devices in the sterilization process, and then using an extrapolation formula based on the now fairly extensive experience of the microbial population, allows us to adopt a flexible dosing system with reasonable assurance that desired standards are met.

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Session VI
Sterilization by Heat and Chemicals
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Introduction Sterilization by Heat and Chemicals

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Most of the technical presentations thus far have dealt with the details of the science and technology of sterilization. My introductory comments will be an overview concerning the "body of sterilization knowledge" and I will address four problem areas.

Problem Area 1

To Carry Out an Efficient and Effective Sterilization Program There Must be Both Scientific and Technologic Input

The input of both scientists, who are seekers of knowledge, and engineers, who are problem solvers, must be considered. The microbiologist working in the sterilization area carries out research directed toward developing an understanding of microbial death rates, developing microbial-destruction data for specific microorganisms in specific products and processing systems, and determining the number and characteristics of microbes on and in products to be preserved. There must also be a corresponding input from the engineer or technologist. To them falls the job of designing and developing processing equipment; designing the sterilization process; developing standards; establishing equipment and process operating parameters; and validating the equipment and the process.

The scientist and the engineer must work together to solve sterilization problems. Since their respective outlooks are quite different, there must be a compromise as they work toward a common goal. The scientist, working on the sterilization process team, must recognize that his view of the world may be different from the engineer's. The engineer, whose goal is to solve production problems, must recognize the need for the scientist who will provide him with basic data. There must be a mutual appreciation and, of course, mutual respect between the two. The scientist is trained to search for truth and to report the detailed results that are found. The engineer must take these data and work them into a model that is usable over a wide range of conditions. A model and calculation system must be simple and meaningful in order to be usable. The engineer may choose models that appear to be gross simplifications of the biological system which may cause the microbiologists to feel that scientific compromises are being made. Before concluding that science is being compromised, we should note that it is absolutely necessary that the final system be relatively simple in concept and use; otherwise, the system will not be used, and all effort will be for naught.

Problem Area 2

A Model of the Sterilization Process Must be Available In Order to Design and Carry Out

Efficient Sterilization Processes

The sterilization process design model must meet requirements that may be quite different from those of a model used to correlate experimental data. Engineering models must be sufficiently simple to be widely usable, accurately represent or fit the scientific data on which they are based (probably to the 95% level), and must include a provision for a safety factor so sterilization processes will give a safe result, even when the fit of the model to the scientific data is poor.

We know that the semilogarithmic model, $F_T = D_T (\log N_o - \log N_F)$, does not accurately fit all experimental microbial thermal-destruction data but, to date, we know of no other model that fits all experimental data. (F_T is the equivalent minutes at temperature T, instant heat up and cool, of the sterilization process). A review of the literature on the wet-heat destruction of microorganisms, while showing that microbial-destruction curves of almost all shapes are possible, indicates that in about a third of the heat-destruction tests, the data form an approximate straight-line, semilogarithmic survivor curve. In another third, the data form a curve where the initial portion either shows a much lower destruction rate (concave downward) or a much larger destruction rate (concave upward) followed by constant-rate, straight-line destruction. The appropriate parameters, such as the initial number (N_o) (on a unit basis), and time until a 90% reduction in the microbial population (D_T) (the cotangent of the straight line) of the model, can be adjusted so that the model represents a worst-case condition (i.e., greatest degree of safety) based on actual microbial-destruction data. Fortunately, the endpoint term (N_F) of the semilogarithmic model seems to have been designed to fit with the idea that the endpoint of the sterilization process should be a numerical specification. If the endpoint specification for spoilage by mesophilic spore-forming organisms is one nonsterile unit in 10^6 units, then on a unit basis, the N_F value will be $1/10^6$ or 10^{-6}. Therefore, the semilogarithmic model not only acts to relate the microbial-destruction variables but also aids in realistically describing the endpoint of the process.

Problem Area 3

Keeping the System Simple

The basic principles of the sterilization unit operation are relatively simple aspects of microbiology, engineering, and mathematics; yet when put together and used to carry out the sterilization operation, they often seem very complex. We can conjecture that this occurs because concepts from several disciplines are integrated and thus, the final operation appears complex.

Since sterilization science, in its basic form, can appear confusing, the system must be kept as simple as possible so that it can and will be used by persons working in the pharmaceutical, health care, medical products, and food industries. When analytical procedures are simplified, overall accuracy is often lost. The accuracy level of sterilization must be maintained; any compromise must be in the direction of having a lower level of surviving microorganisms, i.e., a safer product.

Problem Area 4

Maintenance and Expansion of the Sterilization Process Body of Knowledge

The sterilization area has a body of knowledge that includes data on the mechanism of death of resistant microorganisms, the effect of lethal agents on microorganisms, the design of sterilization processes using models that relate lethal effect with microbial death, the design and construction of equipment for bringing the lethal agent in contact with the product, and procedures for validating and monitoring the delivery of the sterilization process to the product. Not only is there a unique body of knowledge, but the same body of knowledge that has been developed for the sterilization of medical products is applicable for any object that needs sterilizing. In the U.S., these areas can be grouped as follows:

- Pharmaceutical Industry
  Injectable Drugs and Nutrient Solutions
- Medical Devices
  Diagnostic Equipment, Implants, and Supplies
- Health Care
  Sterile Supplies in the Hospital, Medical, Dental and Veterinary Professions
- Food Industry
  Heat Processed Food in Hermetic Containers

It is important that we work to insure that sterilization knowledge, including new developments, moves quickly across all industries that produce sterilized products. The objectives of the sterilization process are the preservation of biological material against microbial degradation or spoilage, including contamination of the recipient in the case of toxic foods, injectable drugs, implantable objects, and sterile health care supplies. The job of maintaining a body of knowledge and producing sterile products is not easy. In baseball language, we have two strikes against us since the sterilization operation is often perceived as a service or adjunct operation to another activity such as surgery, critical care, or the manufacturing of medical devices, pharmaceuticals, or food products. Although sterilization is often viewed as only second in importance to the primary technology, if sterilization is compromised, the primary activity fails.

Not everyone views sterilization to be of secondary importance. Governmental health care regulatory agencies consider sterilization to be of Number 1 importance and support the area, as do progressive companies such as Johnson & Johnson. Johnson & Johnson has nurtured sterilization science and technology through this and other conferences as well as through the publication and distribution of the proceedings of these conferences. All of us working in the field of sterilization thank Johnson & Johnson for their public support of the sterilization area.

It is my basic belief that through the dissemination of information regarding sterilization, fewer public health problems due to inadequate sterilization will occur. No one in our industry benefits from a public health problem due to a sterilization process failure.

With these few introductory words, let us proceed to the reports dealing with specific

aspects of sterilization.

Low Temperature Steam Formaldehyde

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Introduction

It is recognized that certain medical products and medical devices need to be sterile in order to protect the health of patients. A variety of methods of sterilization are available: high pressure steam, dry heat, irradiation, gaseous or liquid chemicals, and filtration. However, no single method is universally applicable to all medical products and devices, and the choice of method to be used depends upon the properties of the product which is to be sterilized.

The choice of method for the sterilization of medical devices or surgical products within a hospital is limited. Clearly filtration is not possible, and irradiation is a large-scale industrial process which is not available within hospitals themselves. The limitations of liquid chemicals as sterilants means that they are used only as a last resort for complex pieces of equipment which require rapid turn-a-round times, for example, endoscopes. Thus, high pressure steam, dry heat, and gaseous chemicals remain the options for sterilization within hospitals.

High pressure steam is the method of choice for sterilization. A range of accepted time-temperature combinations are available for steam sterilization, and it is generally recognized that the highest temperature compatible with the equipment to be sterilized should be selected. Reliable automated high vacuum sterilizers are well established. The time-temperature combinations for dry heat sterilization are also well documented, although the total cycle time for sterilization with dry heat is extremely long. Both steam and dry heat sterilization subject equipment to high temperatures. Some equipment may not be compatible with such conditions. Some materials will melt, deform or decompose; furthermore, the fabrication of some items will not accommodate exposure to the conditions. For example, materials may expand differentially when heated leading to breakage of one or more components. There is a need, therefore, for an available lower temperature method of sterilization.

Heat-labile items may be sterilized using gaseous methods. Two such methods are in common use, utilizing either ethylene oxide gas or a combination of gaseous formaldehyde with low temperature steam (LTSF). Low temperature steam is saturated steam at sub-atmospheric pressure and a temperature between 70°C and 80°C.

The History of the LTSF Process

The bactericidal properties of formaldehyde were first reported by Lowe (10). Esmark (2) reported that heavily wrapped spores of anthrax were killed by the action of formaldehyde at 70°C with the addition of steam at sub-atmospheric pressure. Similar work in the United States between 1897 and 1899 reported by Sprague (13) described the effective use of formaldehyde in a vacuum chamber.

It is not clear why this early work was not followed up and the process was not developed further. Formaldehyde was widely used as a fumigant in air at atmospheric pressure, occasionally using elevated temperatures. In 1939, Nordgren (11) extensively reviewed previous work and undertook a comprehensive survey of such methods, their efficacy and limitations. In the 1960s, Alder et al. (1) again reported the successful use of the combination of gaseous formaldehyde and low temperature steam. Steam jacketed vessels were used and the air was removed from the chamber by evacuation. Formaldehyde was admitted, followed by steam, to produce a temperature of 80°C. Subsequent work described various modifications to the process and different concentrations of formaldehyde in conjunction with various temperatures were used. Further development aimed at modifying the operating cycle to improve the distribution of gas within the chamber and to improve cycle reliability was undertaken (Hurrell et al.; 7). This led to the development of sterilization cycles which are widely used today and these will be described later.

Parameters of LTSF

Low temperature steam formaldehyde is a complex multifactoral process. There are a number of variables within the process which will affect its efficacy, including the temperature; pressure and concentration of formaldehyde within the chamber; the distribution of formaldehyde throughout the chamber; and the ability of the gas to penetrate the load. All of these factors need to be controlled (Hoxey et al., 4). The efficacy of gaseous formaldehyde is affected by humidity (12). While nominally the LTSF process works at 100% relative humidity, this is dependent upon the quality of the steam which is provided as a service to the sterilizer. The provision of either superheated or wet steam will therefore also affect the efficacy of the process.

Changes in temperature and concentration of formaldehyde gas will affect the rate of microbial inactivation. For example, at a constant temperature of 80°C, decreasing the formaldehyde concentration from 27 mg/L to 6 mg/L reduces the rate of microbial inactivation. Conversely, at a constant formaldehyde concentration of 14 mg/L, a temperature reduction from 80°C to 70°C produces little change in the rate of inactivation but a further decrease in temperature to 65°C produces a marked slowing in the rate of inactivation. This suggests that the optimal temperature for an LTSF process is between 70°C and 80°C with equivalent pressures of 0.25-0.475 bar (4).

The effects of the individual parameters of LTSF in isolation and as a part of the LTSF process itself have been compared (5). These comparisons have shown that moist heat at 80°C took over a month to decrease the viable count of spores of *Bacillus stearothermophilus* NCIB 8224 spores by several log cycles. Neither exposure to low temperature steam at 80°C nor formaldehyde (8 mg/L) in aqueous solution at 80°C produced any marked decrease in viable spore count over a period of 5-8 hours. LTSF, however, produced considerable inactivation over a period of minutes. As this sporicidal activity cannot be related to the effects of any individual parameter in isolation, it suggests considerable synergism exists between the effects of low temperature steam and gaseous formaldehyde.

Formaldehyde concentrations ranging from 3-100 mg/L have been reported, but a temperature of 73°C at a pressure of 0.35 bar with a concentration of formaldehyde between 8 and 15 mg/L are now widely used in the U.K.

Comparison of LTSF with Other Sterilization Processes

Table I compares the rate of inactivation of spores of *Bacillus subtilis* var. *niger* when exposed to dry heat, steam, ethylene oxide and LTSF. This table shows that LTSF produces a rate of inactivation comparable to dry heat sterilization, but was faster than ethylene oxide for this particular organism. Table II illustrates the same data for the inactivation of *B. Stearothermophilus* spores. It can clearly be seen that these spores are more resistant to the LTSF process (6).

Table I. Comparison of Process Lethality for *Bacillus subtilis* var. *niger* spores

Agent	Temperature (°C)	Concentration (mg/L)	Relative Humidity (%)	D_{10} (min)
Dry Heat	160	–	0	1.5
Steam	121	–	100	0.7
EO	55	500	50	6.6
LTSF	73	14	100	1.4

Adapted from Hurrell (7)

Table II. Comparison of Process Lethality for *Bacillus stearothermophilus* spores

Agent	Temperature (°C)	Concentration (mg/L)	Relative Humidity (%)	D_{10} (min)
Dry Heat	160	–	0	0.4
Steam	121	–	100	2.2
EO	55	500	50	2.7
LTSF	73	14	100	4.0

Adapted from Hurrell (7)

LTSF Sterilization Cycles

An LTSF sterilization cycle operating at 73°C is widely used in the U.K. The cycle consists of evacuation followed by a number of pulses in which low temperature steam and formaldehyde are admitted to the sterilizer. These pulses are followed by a sterilization-hold phase after which there is a gas removal and drying stage. A single pulse consists of a vacuum being drawn and gaseous formaldehyde being admitted at the bottom of the sterilizer. This is followed by a holding period during which the formaldehyde diffuses through the load, after which steam is admitted to the required temperature. The process is then repeated. A complete cycle frequently includes 20 such pulses.

Gas is removed from the sterilizer chamber and load by repeated alternate evacuation and flushing with steam or air. The majority of sterilizers use an air flushing system. Although steam flushing has been reported as giving more rapid elusion of residual formaldehyde, it may require an extended vacuum drying stage to subsequently dry the load.

Monitoring of LTSF

Validation of an LTSF machine includes both physical and microbiological testing. Physical testing investigates the integrity of the sterilizer chamber and physical ability of the machine to remove air from the chamber and load, maintain the required temperature by admission of steam, and produce a uniform temperature throughout the load. Once the correct physical function of the machine has been established, the distribution of formaldehyde within the chamber can be estimated. This is usually performed by using chemical indicator papers or may utilize chemical analysis of samples from the chamber. Should a lack of homogeneity be found, it is best corrected before proceeding to microbiological qualification studies using biological indicators.

For any process for which microbiological monitoring is required, it is vital that standardized biological monitors are used. Spores of *B. stearothermophilus* NCTC 10003 have been used for a number of years in the U.K. as a biological indicator for LTSF. Microbiological monitoring consists of two parts: first, an assessment of chamber homogeneity and second, a determination of gaseous penetration. To monitor the homogeneity of the chamber atmosphere, 27 biological monitors are suspended within the sterilizer, suspended from strings and distributed evenly throughout an empty chamber. In addition, the adequacy of penetration is assessed using a Line-Pickerill helex (9). Two helises are in fact used in each test. These devices are made of a length of stainless steel tubing sufficient to give a 2000:1 length to bore ratio. At one end of the helex is a small capsule into which a biological indicator can be placed. This device is considered to be more difficult to penetrate than any device processed within a hospital sterile supply department and therefore provides an efficient and reliable means of monitoring the performance of sterilizer. Upon completion of the sterilization cycle the biological indicators from the chamber space and from the Line-Pickerill helises are cultured under defined conditions. For a machine to be accepted, there must be no recovery of the test organisms from three replicate cycles.

For routine monitoring of sterilizer performance, the physical record of temperature and pressure against time must be compared to the master temperature and pressure record produced during the validation studies. In addition, a biological indicator is included within a single Line-Pickerill helix in each production run.

Limitations of the LTSF Process

LTSF is a complex process that requires the attention of skilled personnel if it is to be operated satisfactorily. It should not be used to process items which can be satisfactorily processed in a steam sterilizer or for the re-processing of items intended for single use.

There are some devices which cannot be processed through the system because the material is not compatible either with the temperature or with the pressure variations which occur during the cycle. When selecting a sterilization process, its limitations must be compared with the available alternative methods of sterilization; therefore, it is useful to compare LTSF with ethylene oxide.

The process variables for both LTSF and ethylene oxide are essentially similar; however, there are a number of differences between the two processes. With ethylene oxide sterilization, it is well documented that water content can affect the resistance of bacterial endospores exposed to the process. There is frequently a need for products to be humidified prior to admission to an ethylene oxide sterilizer. Expensive humidification prior to processing is not a requirement for LTSF sterilization.

In addition, ethylene oxide is explosive and flammable. Formaldehyde is neither flammable nor explosive under the conditions at which it is used in LTSF, and there is no need for the expensive handling precautions which are necessary when dealing with ethylene oxide. Like ethylene oxide, formaldehyde is toxic. It is a known mutagen as well as a suspected carcinogen, although the carcinogenic potential remains unproven. LTSF sterilizers in operation in the U.K. have no difficulty in maintaining the operator exposure below the current recommended exposure level. Formaldehyde has an offensive penetrating odor which is readily detectable at a concentration as low as two parts per million, whereas ethylene oxide is difficult to smell even at a concentration of 200 parts per million. A number of studies have reported the residual concentration of formaldehyde in a variety of materials at the end of the sterilization cycle without further aeration. Using an aqueous extraction method, concentrations of 4-8 parts per million formaldehyde were found in polyethylene. Similar studies on ethylene oxide-sterilized materials using an ethylene oxide concentration of 500 mg/L at 55°C, showed concentrations of 1700-1800 parts per million ethylene oxide before aeration (3).

Low Temperature Steam Disinfeciton

In addition to the requirement for items to be sterile, there is also a need for certain products to be disinfected between use on individual patients in order to minimize the risk of cross-infection. Methods of disinfection rely on the use of either chemicals or moist heat. Heat disinfection methods can use low temperature steam (steam at a temperature between 70°C and 80°C at sub-atmospheric pressure without the addition of formaldehyde) or an automatic washing process with a rinse cycle operating at elevated temperatures.

The majority of LTSF sterilizers also offer the option of a low temperature steam disinfection cycle. Such a disinfection system has considerable advantages, including efficacy against a range of vegetative organisms. The process can be physically monitored and records of the physical conditions can be maintained. Furthermore, there are no toxic residuals within the products and the effect of the presence of organic or other residual matter is less than that with many chemical methods. Low temperature steam will penetrate porous materials well and offers a facility for processing wrapped goods and producing a dry load at the end of the process.

The Use of LTSF

Examples of products which are sterilized by low temperature steam formaldehyde or disinfected by low temperature steam alone are provided in Table III.

Table III. Samples of Items Sterilized by LTSF or Disinfected by LTS

LTSF Sterilization	LTS Disinfection
Telescopes	Endotracheal Tubes
Electrodes	Ventilator Tubes
Nephrostomy Tubes	Anaesthetic Circuits
Oesophageal Bougies	Oriscope Specula
Fibre-optic Leads	
Laparoscopes	

Conclusion

Low temperature steam formaldehyde sterilization is a reliable method of sterilization for heat-sensitive items which compares favourably with, and has a number of advantages over, ethylene oxide sterilization, particularly for hospital use.

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Dry Heat Sterilization

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Introduction

Dry heat is a sterilizing medium with penetrating properties for most materials. It is often the medium of choice for heat stable items that cannot be sterilized with steam. Examples are materials or objects that will not tolerate moisture or are impervious to steam. Dry heat may be used to sterilize non-aqueous liquids or semi-solids such as glycerin, oils, petroleum jelly, and waxes; certain powdered drug components or medicaments such as talc and sulphonamides; and glassware and stainless steel equipment. Unlike steam, dry heat is not corrosive for stainless steel and it does not etch glass surfaces, so it is ideal for the sterilization of sharp instruments.

Materials that are candidates for dry heat sterilization must be able to withstand high temperatures. Dry heat processes typically operate at temperatures between 140° and 170°C, but temperatures as high as 400°C are sometimes utilized. The advantage of temperatures above 170°C is that bacterial endotoxins (pyrogens), as well as bacterial spores, are effectively destroyed. Although dry heat sterilization as presently practiced is not well suited for terminal sterilization of packaged pharmaceutical products or medical devices, it is widely used to sterilize components and equipment used in aseptic manufacturing operations. High speed, continuous sterilization of glass containers for pharmaceutical dosage forms is one example of the efficient use of this sterilization method. A special application worth mentioning is the regeneration and depyrogenation of sintered activated charcoal (SAC) filters used to depyrogenate pharmaceutical solutions (12).

Heat Transfer Mechanisms

The three mechanisms of heat transfer in dry heat sterilization are conduction, convection, and infrared (IR) radiation. Heat penetration of materials via conduction is dependent in part upon heat availability in the surrounding medium. Air is the chief heat transfer medium used in dry heat sterilization, but it has a low specific heat and poor thermal conductivity properties. Approximately six times as much air is required to transfer a given amount of heat to an object as is required for steam at the same temperature (3). Due to the low heat capacity and heat transfer rate of air, gravity convection oven sterilizers have many serious drawbacks; i.e., heating is slow, long sterilization times at high temperatures are required, maintaining a uniform heating medium and reproducible cycles is difficult, and charring of materials can occur. Heat transfer can be made more efficient by forced convection or by moving the air stream. Most large industrial dry heat sterilizers use some form of forced air system to facilitate heat convection and cooling.

For many years, IR has been the conventional heat transfer mechanism used in the pharmaceutical industry to treat glassware in continuous belt (tunnel) sterilizers. IR will heat surfaces directly exposed to it, and heat is then disseminated from the heated objects via radiation, conduction, and turbulent air flow. In a similar manner, glass ampules have been sterilized by a brief, direct exposure to a gas flame. However, the demand for process quality improvement in pharmaceutical manufacturing has led to the development of laminar air flow, forced convection ovens, and tunnels for treating glassware. The adaptation of laminar air flow (LAF) systems to continuous high temperature sterilization/depyrogenation equipment has resulted in improved heat transfer, more uniform heat distribution, lower process times and temperatures, and better control of foreign particulate matter contamination.

Another form of dry heat sterilization is incineration, however, its use is restricted because of the destructive nature of the process. Decontamination of air or sterilization of air for use in fermentors and disposal of contaminated materials are examples of incineration processes.

Time/Temperature Relationships

Heat sterilization is a function of the integrated effects of time and temperature and is also true for the destruction of bacterial endotoxins. Time/temperature relationships that have been suggested for hot air sterilization range from overnight at 121°C to 60 minutes at 170°C (11). These exposure times do not include the time required for the material being treated to come to temperature. These parameters are suggested values and cycle development studies should be conducted to determine specific time/temperature conditions for each type of material treated.

Dry heat inactivation of microorganisms is a first-order reaction attributed to an oxidation process. The spores of aerobic, sporeforming bacilli (e.g., *Bacillus subtilis* var. *niger*) are generally used as biological indicators (BIs) to demonstrate the efficacy of dry heat sterilization processes. The dry heat resistance decimal reduction value at 170°C ($D_{170°C}$ value), or time required to reduce a spore population by 90% at this temperature, has been reported to be approximately 8 seconds for *B. subtilis* var. *niger* on glass (10). The Z value, or number of degrees of temperature required to cause a ten-fold change in the D value is approximately 20°C. As in the case of moist heat sterilization, thermal process analysis is based on the assumption that lethal effects at different temperatures over an entire heating cycle are additive. The key to the analysis of time/temperature data is the lethal rate (L):

$$\text{Log} = \text{log-1}\ \frac{T_o - T_b}{Z} = 10\ \frac{T_o - T_b}{Z}$$

where: T_o = exposure temperature

T_b = the process reference temperature, 170°C

Z = 20°C

During the analysis of a heating cycle, the come-up, holding, and cooling periods are evaluated in terms of the process holding (reference) temperature to determine the cycle F value:

$$F = \Delta t(L_1 + L_2 + L_3 \ldots L_{n-1})$$

or

$$F = \Delta t(\Sigma\ {}_{10}T_o - T_b/Z)$$

When the reference temperature is 170°C, the F value is referred to as F_H. Measurement of this physical parameter can predict the biological outcome of the process. For example, an F_H of 18 minutes would indicate that 6 logs of spores (N_o) having a D_{170}°C value of 1.0 minute would be reduced to zero and 12 logs below, for a probability of survival (N_s) of 10^{-2}; e.g.,

$$\text{Log}_{10}N_s = \text{Log}_{10}N_o - F_H/D_{170°C} = 6 - 18/1 = -12$$

Depyrogenation

Pyrogens are substances which cause fever in animals and lipopolysaccharides (LPS) from the outer cell membranes of gram-negative bacteria are the responsible agents. Until recently, standard conditions for dry heat depyrogenation in the pharmaceutical industry have been exposure to 250°C for 30 minutes. This recommendation was based on the work of Welch et al. (17) on the removal of pyrogens from penicillin. However, depyrogenation cycles may be determined empirically using an *in vitro*, inexpensive, quantitative assay method for bacterial endotoxins. This is accomplished by direct inoculation of articles with known amounts of purified endotoxin and measurement of the amount remaining following exposure to dry heat. A 3 log cycle reduction of endotoxin is considered adequate, and this level of depyrogenation assures a microbial survival probability much lower than 10^{-12} (13).

Tsuji and colleagues (14, 15) were the first to investigate the dry heat inactivation kinetics of purified endotoxin derived from various gram-negative bacteria. They utilized a heating apparatus that permitted rapid insertion and withdrawal of LPS-seeded aluminum planchets at carefully controlled temperatures much like a BIER vessel is used to evaluate steam sterilization BIs. At temperatures of 170°, 210°, and 250°C, the inactivation kinetics of endotoxin derived from *Escherichia coli, Salmonella typhosa, Serratia marcescens*, and *Pseudomonas aeruginosa* were very similar. In contrast to spore inactivation which is first-order, endotoxin inactivation was found to be non-linear and second order, and could only be described in terms of two first-order equations. The dry heat resistance of purified LPS from *E. coli* was also shown to be twice as great as native (whole cell) endotoxin, and 100 times greater than spores of *B. xerothermodurans*.

The authors manipulated their data mathematically to permit the estimation of D and Z values for *E. coli* endotoxin destruction. Thus, it was proposed that the mathematical expression for F value used in estimating spore inactivation, can also be applied to estimate endotoxin destruction during a dry heat cycle by using Z = 47.6°C. In this case, the $F_{250°C}$ value is determined and used to calculate the number of logarithmic cycles (L_{Dec}) of endotoxin reduction.

It was reported that endotoxin destruction at low process temperatures is not increased merely by extending the heat exposure time (i.e., is not necessarily additive) as in the case of spores. Thus, it was claimed that temperatures in the vicinity of 170°C which effectively destroy spores are relatively ineffective for endotoxin.

Akers et al. (2) confirmed the findings of Tsuji and Harrison (14) that inactivation of purified endotoxin follows second-order kinetics. They inoculated 50 mL glass vials with 10 ng of *E. coli* endotoxin in 0.2 mL of water and exposed the wet vials in a commercial dry-heat sterilizer oven to temperatures of 175°, 210°, and 250°C. These studies were aimed at determining the $F_{170°C}$ value requirements (at Z = 54°C) for validation of dry-heat sterilization/depyrogenation cycles. The LAL assay was used to quantitate endotoxin reduction, and the results were compared with corresponding $F_{170°C}$ values measured in containers probed with thermocouples. The results showed an approximate linear relationship between exposure temperature and the log of the $F_{170°C}$ values. However, the exposure times required for a 2 log cycle reduction of endotoxin greatly exceeded those

predicted by Tsuji and Harrison. It is possible that the different experimental approaches used by the two research groups account in part for these discrepancies. More research is currently being conducted in this area by Ludwig and Avis (9).

Sterilization Cycle Development

Both dry and moist heat sterilization cycles in the pharmaceutical industry are usually tailored specifically to the material being treated. This is done out of concern for product integrity, stability, and sterility, as well as for economic reasons. Sterilization cycle times and temperatures are adjusted to prevent underprocessing or overheating which may result in an unacceptable product. The objective is to sterilize materials to an acceptably low probability level of contamination without causing thermal degradation.

Two basic approaches are recognized for developing effective sterilization cycles; the bioburden and the overkill approaches. The bioburden approach takes into account the number and relative heat resistance of the bioburden, and may be related with a modest safety factor to a measurable spore log reduction of a bioindicator population. The objective is to reduce the bioburden population to a probability of survival of at least 10^{-6}. On the other hand, a microbial survival probability of 10^{-12} is considered overkill for heat sterilization processes. Thus, the overkill approach is highly conservative. It may be based on the actual or theoretical inactivation of a specified number of spores having relatively high dry heat resistance, such as those of *B. subtilis* var. *niger*, with no attempt being made to relate the challenge population to the bioburden.

Recently, a third approach has been suggested for dry heat processes which is based on pyroburden (endotoxin loading). Thus, USP XXII states that a dry heat treatment for heat stable items such as glassware should be able to inactivate 3 logs of endotoxin (13). According to Tsuji and Lewis (15), minimal conditions for inactivation of 3 logs of *E. coli* endotoxin are exposure to 170°C for approximately 3.3 hours (Akers > 66 hours). This amount of heat will inactivate approximately 1485 logs of *B. subtilis* var. *niger* spores having a $D_{170°C}$ value of 8 seconds. The “pyroburden” approach in dry heat sterilization is actually an extremely conservative overkill sterilization approach.

Equipment Considerations

Although sterilization by dry heat is a relatively simple process, the equipment can be complex. Forced-convection batch type ovens and continuous belt tunnels are the most common dry heat sterilizers used in the pharmaceutical industry. Small table top, rapid heat transfer sterilizes are now available that can process dental or surgical instruments in 6 to 20 minutes by exposure to a rapidly moving hot air stream at 190° to 210°C. An example of another application is the sterilization-in-place (SIP) of large pieces of manufacturing equipment that cannot be pressurized with steam or conveniently disassembled for sterilization in an autoclave. Critical operating elements associated with dry heat sterilizers include flame or electric heaters, electric fans, baffles and dampers, high efficiency particulate air (HEPA) filters, door interlocks, temperature sensors, cycle controllers and timers, electric motors, and moving belts. In addition, microprocessors and programmable logic controllers (PLCs) are increasingly being used to monitor, control, and document sterilization cycles.

Industry experience has shown that it is not enough to merely rely on information obtained from a sterilizer's sensor/controller/recorder system to indicate proper performance. Standard industry practice is to validate both equipment performance and sterilization procedures based on an experimental plan or protocol, and to show prospectively that a sterilization process will do what it purports to do.

Validation and GMP

The “validation life cycle” concept that has developed in recent years has played an important role in improving and optimizing the use of dry heat sterilizers in the pharmaceutical industry. The life cycle approach to validation involves:

— qualifying the initial installation and performance of each unit and sub-unit on a sterilizer (including instrument calibration);
— proving scientifically that the sterilizer will operate reliably and consistently at all operating ranges;
— conducting cycle development studies;
— accurately monitoring and documenting routine sterilization operations; and
— tracking all mechanical or procedural changes that may have an impact on performance over the entire life of the sterilizer.

This approach is consistent with contemporary statistical process control principles and focuses on the quality of the process or the ability to diagnose problems when they occur and to make appropriate process improvements.

There are also some important good manufacturing practice (GMP) concerns that must be taken into account with dry heat sterilization processes such as the preparation of articles for heat treatment, the condition of the interior of a sterilizer, and the quality of the air supplied to forced convection systems.

Pre-sterilization activities such as washing and rinsing of articles are aimed at minimizing contamination. Microorganisms, endotoxin, and foreign particulate matter can be restricted through proper cleaning techniques and the use of appropriate protective coverings. Foreign matter contamination is a special concern because of the potential for charring at high temperatures. For this reason, preparation of materials prior to sterilization is normally conducted in controlled environment areas under HEPA filtered, laminar air flow conditions. The final rinse of product components or product contact surfaces in manufacturing equipment should be with filtered high purity water such as water for injection. Finally, after articles have been washed and prepared they should be sterilized as quickly as possible to prevent recontamination.

All dry heat sterilizers should be thoroughly cleaned out periodically. Particulates tend to accumulate in corners and ducts, especially exhaust ducts, and beneath the belt in tunnel sterilizers.

The air supplied to the forced convection system should be drawn from conditioned air inside the temperature and humidity controlled building. This air must pass through non-fiber shredding HEPA filters that have been shown to be integral (i.e., via a dioctylthalate, DOP, challenge). Since the air in the cooling zone of a tunnel sterilizer never sees temperature that would be lethal to bacterial spores, the interior of this zone must initially be sterilized by some other means such as exposure to a gaseous vapor sterilant (e.g., formaldehyde).

Laminar Air Flow (LAF) Tunnel Sterilizers

Continuous-belt dry heat sterilizers of glassware became popular in the pharmaceutical industry in the 1960s. Although it is more complex than static-batch processing, there are some significant advantages to be gained from continuous processing. For example, the glassware is loaded onto a bottle washer and is not handled again during the process. After the glassware is washed and rinsed with purified water or water for injection, it is automatically placed on a moving belt and conveyed into the tunnel which minimizes the chance for re-contamination. The tunnel typically consists of a pre-heat zone to raise the temperature of the glassware, a sterilization or holding zone, and a cooling zone to lower the glassware temperature back to a safe level. Finally, the moving belt is adjusted to deliver the treated and cooled glassware to a product filling line as it is required, thereby eliminating the need for storage.

Having previously introduced the topics of GMP and validation, let us trace the impact they have had on the evolution of continuous-belt sterilizers. In radiant heat tunnels, glass is exposed directly to IR emitted by electric resistance elements, and turbulent air flows counter current to the flow of glassware. This can cause a number of serious problems:

— the turbulent air flow in all zones contributes to non-uniform heat distribution;
— the differential air pressure across the tunnel from the aseptic processing area to the non-aseptic area has a dramatic affect on performance;
— the abrupt change in temperature from the holding zone to the cooling zone sometimes causes glassware breakage; and
— foreign particulate matter levels tend to be high.

Some of these problems are not apparent until an attempt is made to qualify and validate the equipment.

One of the most serious problems with conventional tunnel sterilizers is the level of particulate matter that is present in the vicinity of the glassware being processed. Particle concentrations of 1,000 to 20,000/L ≥ 0.5 µ have been reported. These particles are generated by the moving belt, by deterioration of the heating elements, and by the abrasive action of the glassware, etc. Particulates are not effectively removed by turbulent air flow in this type of tunnel.

Hortig (7) estimated for a continuous filling process that conventional dry heat sterilization presented the greatest potential contamination risk for glassware due to the number of particles in the environment and the long residence times. He then showed that particle counts could be reduced to < 1.0/L using LAF in a sterilizing tunnel. Since LAF was also more efficient in transferring heat to the containers, sterilization exposure times could be reduced by 40% which also contributed to a lower risk of exposure to particulate matter. The author concluded that with these improvements, “the possibility for contamination by airborne particles … has practically ceased to exist”.

Not all radiant heat tunnel sterilizers are necessarily as “dirty” as described by Hortig. Akers and colleagues (1) reported on an experimental design which used HEPA-filtered LAF at the tunnel entrance and in the cooling zone (Figure 1). The highest particle count recorded was 221 particles ≥ 0.5 µ/ft^3 in the tunnel entrance zone, and particle counts

overall were usually < 70 per ft^3. However, this tunnel apparently was not connected to an aseptic processing area, and did not have to cope with a ΔP across the sterilizer. The authors reported the velocity of air exiting from both end openings of the tunnel was approximately 100 ft/min which helped to purge out particulates.

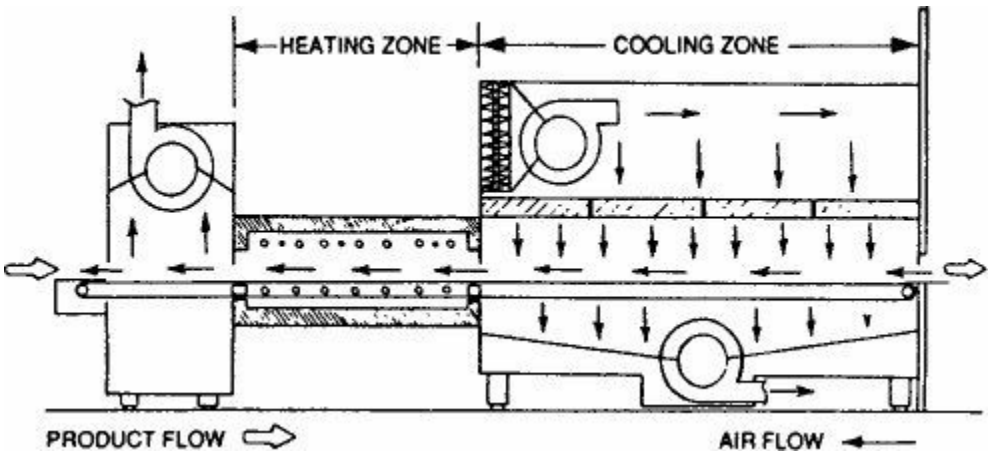

Figure 1. Diagram of a Continuous-Belt Radiant Heat Sterilizing Tunnel with LAF Cooling Zone

(○) electrical heating elements

(●) temperature sensors

The heating of various types of containers in a hot LAF steam using a laboratory test apparatus was investigated by Wegel (16). Heating curves were generated showing the relationship of air temperature and velocity to the geometric design of containers. He also demonstrated a border condition or temperature for glass containers having various relaxation times above which killing of bacterial spores occurred. Wegel's studies allowed him to predict the optimum design values for dry heat sterilization tunnels. Other authors also studied LAF tunnels and claimed that these sterilizers could be used effectively for both sterilization and depyrogenation of glassware (5, 6, 18) (see Figure 2). However, none of these authors described a comprehensive approach for validating the production of sterile, pyrogen- and particulate-free glassware.

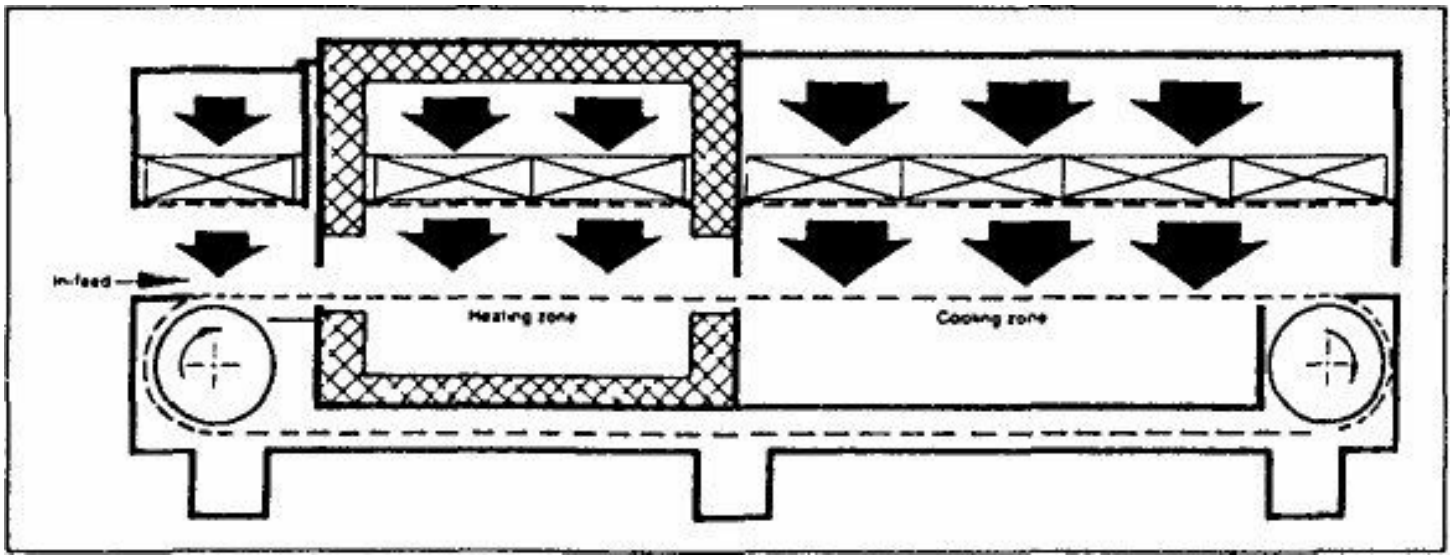

Figure 2. Longitudinal Section of a "Total" LAF Continuous-Belt Sterilizing Tunnel Adapted from Willing (18)

Process Validation

Elias (5) stated that, “high speed operations are difficult to control by procedure alone and require built-in integrity to obtain maximum quality and efficiency”. In a modern LAF sterilizing tunnel, the interplay of critical electronic monitoring and controlling devices, coupled with alarms, etc. is an important mechanism used to assure integrity of the process. However, the use of separate physical and biological measuring systems during validation provides final proof that the process is working properly. In this regard, problems that are not always apparent from process parameter measurements are sometimes discovered by the independent monitoring systems used during validation. Each critical sterilizer function is investigated during validation:

— equipment state-of-control conditions are evaluated;
— chamber air and container particulate levels are checked;
— temperature distribution profiles and container heat penetration are measured; and
— spore and/or endotoxin inactivation studies are conducted.

The first concern of validation is to verify state-of-control. State-of-control may be defined as “a condition in which all operating variables that can affect performance remain within such ranges that the system or process performs consistently and as intended” (4). Therefore, all operating ranges must be established, documented, and controlled in order for validation to proceed.

Air particulate levels in each zone of the sterilizer are determined with the belt running. An electronic particle counter is used, and samples from the hot zone must be cooled (by passage though a water condenser) to prevent damage to the particle counter. The HEPA filters used in the sterilizer are tested for integrity by the DOP test. Containers processed through the tunnel are rinsed with filtered water for injection (WFI) and evaluated for particulate content.

Temperatures within containers sent through the tunnel are measured using implanted thermocouples and are recorded on a data-logger. The data logging system is normally a portable, multi-channel temperature measuring and digital data output device used independently of the sterilizer recording and controlling system. The more sophisticated data loggers can be interfaced directly with microcomputers that are programmed to analyze time/temperature data automatically. The most useful information concerning sterilizer heating profiles is provided by multiple probed-containers processed continuously with full belt loads of glass.

There are several ways to evaluate the repeatability of these dynamic temperature distribution and heat penetration tests. For example, simply plotting the data and comparing the graphs side-by-side will show at a glance whether the sterilizing and cooling zones are heating and cooling the containers uniformly. Akers and colleagues (1) calculated the area under the curve of heat profiles measured during replicate runs with various bottle sizes and then compared the results statistically by the least significant difference test. It is also possible to compute the F value of each heat profile at 170°C and/or at 250°C. This will provide quantitative data which are not only useful for determining process repeatability, but which indicate the level of microbial inactivation or endotoxin destruction that can be

expected to occur during the process.

Heat penetration and temperature distribution studies are the final step before biological challenges are conducted. Bacterial spore and endotoxin preparations may serve as bioburden or pyroburden models during bio-validation. If a tunnel sterilizer is to be operated at temperatures below 260°C (or container temperatures are expected to be < 180°C), test loads should include some containers seeded with heat resistant spores to verify or estimate spore log reduction (SLR) levels. However, spore challenges are meaningless at operating temperatures above 260°C-270°C, or for processes intended to produce at least a 3 log cycle reduction of endotoxin. In this case it is appropriate to add aqueous solutions of endotoxin to test containers instead of spores, and to assay for the presence of residual undestroyed endotoxin after high-temperature treatment.

The validation studies that have been described provide significant benefits including:

— a comprehensive verification of process reliability;
— a measure of the margin of safety inherent in sterilization or depyrogenation cycles;
— information needed to adjust processing conditions to fit a wide range of container types and sizes; and
— information needed for optimum performance of the equipment which is a prerequisite for economical and trouble-free operation.

Summary

Dry heat sterilization and depyrogenation processes have been made more effective in recent years by the demand for process quality improvements in pharmaceutical manufacturing. Several unrelated developments have been responsible for these changes. The advent of the quantitative *in vitro* LAL test permitted an investigation for the first time of endotoxin destruction kinetics, and has enabled the routine verification of high temperature depyrogenation processes. The validation life cycle concept has caused investigators to have a more in depth look at problems inherent in dry heat processes, and this has led to a more comprehensive approach to process development and control. The use of LAF technology for hot air streams, made possible by the development of HEPA filters that can withstand temperatures > 400°C, led to the development of sophisticated continuous belt sterilizers to support highspeed aseptic filling operations. Rapid heat transfer sterilizers have been developed for dental and medical clinics which have made dry heat processing of surgical instruments a practical alternative to steam or chemical sterilization methods.

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Role of Glutaraldehyde and Other Liquid Chemical Sterilants in the Processing of New Medical Devices

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Introduction

The term "chemosterilizer" (chemical sterilizers) was described and defined by Borick in 1968. Chemosterilizers are chemicals (liquid or gaseous) which have the property of destroying (inactivating) all forms of microorganisms; it should be distinguished from the term "chemosterilants" which are chemical substances used to sterilize insects, i.e., render them incapable of reproduction. In recent years, the term "liquid chemical sterilants" has replaced chemosterilizers in defining those chemicals used in the hospital to sterilize used or reusable medical items (14).

During a search for an efficient substitute for formaldehyde as a liquid sterilant, Pepper and Lieberman (32) investigated a group of saturated dialdehydes, including glutaraldehyde, for disinfectant and sporicidal activity. The commercial formula later developed by this research group consisted of a 2% aqueous glutaraldehyde solution buffered by a suitable alkalinizing agent (0.3% sodium bicarbonate) to a pH of 7.5-8.0.

It was known before 1962 that short-chain saturated dialdehydes could be used as disinfectants and had moderate sporicidal properties. However, glutaraldehyde, if diluted from its acidic commercially concentrated form (25% or 50%), could not meet the Association of Official Analytical Chemists (AOAC) sporicidal test requirements which are mandatory if a product is to be registered as a sterilant under the U.S. Federal Insecticide, Fungicide, and Rodenticide Act. By 1963, a 2% alkaline glutaraldehyde solution was registered with the U.S. Government and marketed for use with utensils and instruments (reusable medical devices) in health care institutions (36). Table I lists the comparative sporicidal activities of formaldehyde and several short-chain dialdehydes.

Table I. Sporicidal Activity of Some Aldehydes*

ALDEHYDE	CHEMICAL STRUCTURE	SPORICIDAL ACTIVITY
Formaldehyde (methanal)	HCHO	Good
Glyoxal (ethanedial)	CHO-CHO	Good
Malonaldehyde (propanedial)	$CHO\text{-}CH_2\text{-}CHO$	Slightly active
Succinaldehyde (butanedial)	$CHO\text{-}(CH_2)_2\text{-}CHO$	Slightly active
Glutaraldehyde (pentanedial)	$CHO\text{-}(CH_2)_3\text{-}CHO$	Excellent
Adipaldehyde (hexanedial)	$CHO\text{-}(CH_2)_4\text{-}CHO$	Slightly active

* Based on data from several literature sources.

During the late 1950s, investigations by leather tanning chemists showed that glutaraldehyde would effectively crosslink collagen and other proteins in connective tissue (15). Glutaraldehyde crosslinks the various proteins contained in animal skins and thereby produces a mechanically strong leather which is resistant to both water and microbial attack (20). The commercial availability of glutaraldehyde led to further uses, particularly in electron microscopy, where it is a superior fixative for proteins in cells (21).

In 1968, Hancock and Angell made available the first commercially prepared tissue heart valve, a formalin-tanned porcine xenograft. The use of formalin as a sterilizing and tanning agent did not result in long term durability for this zenograft (10). The first use of

glutaraldehyde as a tissue fixative for a porcine valve was by Carpentier et al. (11). The implantation of glutaraldehyde-treated xenografts in humans has not resulted in any rejection (immunological) of the animal tissue. Glutaraldehyde reduces immunogenicity by crosslinking antigenic determinants in the animal tissue, thereby blocking recognition of these antigenic sites by the host's immune system (24). By 1980, Angell and Angell estimated that the total number of glutaraldehyde-treated xenograft valves implanted (both porcine and bovine pericardium) was 40,000 valves. By 1988, one U.S. manufacturer of tissue heart valves stated that in excess of 300,000 tissue valves had been implanted. Currently, tissue heart valves fill approximately 35% of the world's annual need for replacement heart valves.

This paper will review some of the past history of glutaraldehyde as a microbicidal agent and its extensive use as a protein crosslinking (tanning) chemical and sterilizing agent for tissue-derived medical devices. Its chemistry with biological tissue will be analyzed and then put into perspective as part of its ability to be a chemosterilizer. There is a definite relationship between its activity as a crosslinker in protein chemistry and how it functions as a liquid sterilant.

Chemistry

Glutaraldehyde is a five-carbon aliphatic dialdehyde with a molecular weight of 100.11, a boiling point of 187-189°C, a vapor pressure at 20°C of 15 mm Hg (50% solution), a freezing point of −21.0°C (50% solution), with complete solubility in water at 20°C. It is commercially available in the U.S. as 25% or 50% solutions at acidic pH. In water, glutaraldehyde exists in a very complex equilibrium mixture with the hydrated forms shown in Figure 1 (13) and the cyclic polymer in Figure 2 (7,34). The reactions depicted in Figures 1 and 2 occur in aqueous acid media and are considered reversible. At four to five glutaraldehyde residues in length, these oligomers readily revert back to the reactive, monomeric form of glutaraldehyde under relatively mild conditions, such as dilution coupled with slight warming or slight increase in pH.

Glutaraldehyde can also polymerize into alpha, beta, unsaturated dimers, trimers, or larger polymers by aldol condensation reactions (39,18). Progression to these polymeric forms (Figure 3) just described occurs at increasing alkaline pH and time and is not reversible with higher molecular weights. Decrease of reactive aldehyde groups by aldol condensation is responsible for the rapid loss of biocidal activity of alkaline solutions on storage.

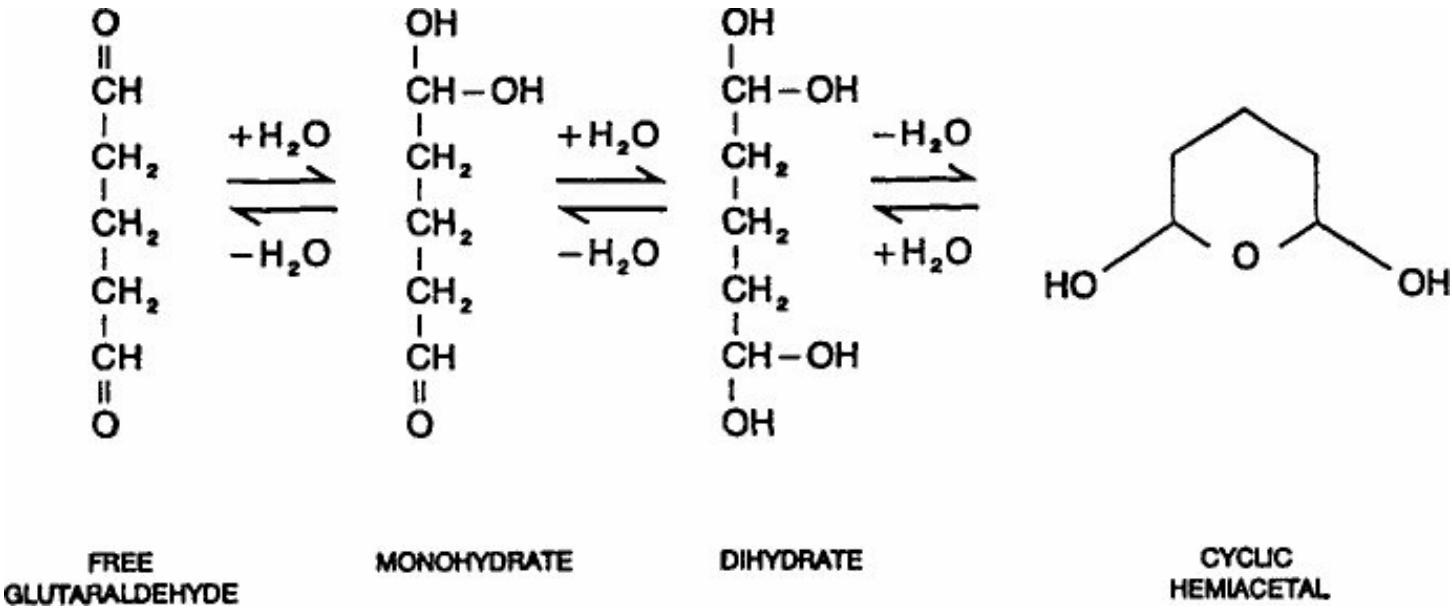

Figure 1. Monomeric Forms of Glutaraldehyde (Acidic pH)

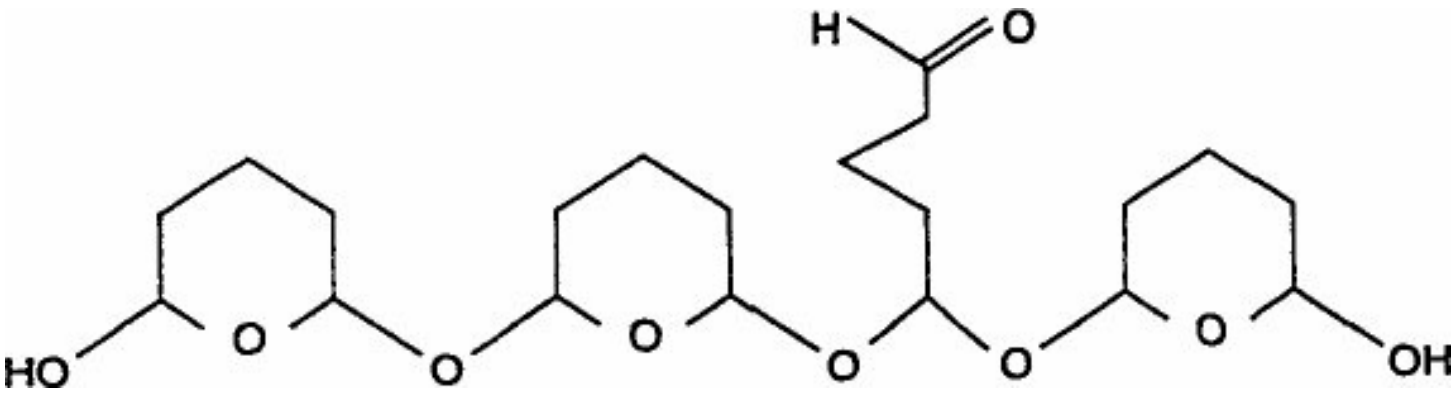

Figure 2. Hemiacetal Polymeric Glutaraldehyde (Acidic pH)

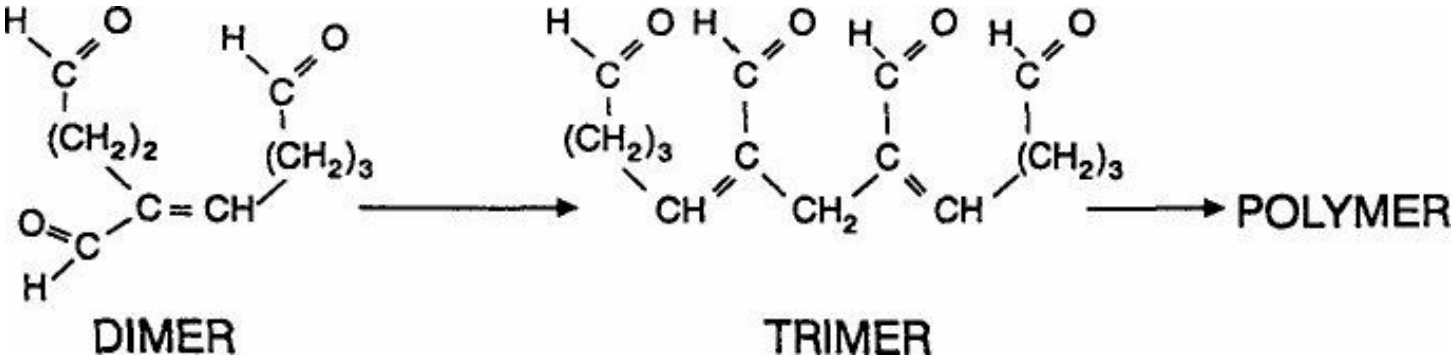

Figure 3. Aldol Condensation Products (Alkaline pH)

Manufacturers of tissue-derived medical devices can assess the polymeric content of their use solutions by comparing the UV spectra at 280 nm (free aldehyde) versus that obtained at 235nm. Some authors have claimed that cold storage temperature is the most important factor preventing polymerization. More recent work has shown that hydrogen ion concentration is very important, since there is a sharp rise in irreversible polymerization above pH 7. When the amount of polymers in solution increases, the microbicidal activity decreases as a direct relationship to the loss of free glutaraldehyde activity.

Reaction with Proteins

The mechanism of glutaraldehyde reactions with proteins is still being defined. The reactive moiety is primarily the free glutaraldehyde, which can react by several different mechanisms giving rise to a number of products. As an aldehyde, glutaraldehyde can undergo the typical chemistry associated with aldehydes including oxidation, reduction, and condensation reactions. The crosslinking chemistry (and microbicidal effectiveness) of glutaraldehyde is based on the ability of aldehydes to undergo alkylation reactions. Glutaraldehyde can alkylate sulfhydryl, carboxyl, and hydroxyl moieties. However, all of the protein crosslinking and microbicidal action of glutaraldehyde can be assessed by its reactivity with amino residues. The free aldehydes of monomeric, and possibly dimers and trimers of glutaraldehyde, could react with the primary amines of lysine, hydroxylysine or n-terminal amino acid residues to form a Schiff base. These bases are relatively unstable at neutral pHs and could revert to the free amine and aldehyde upon increases in temperature or decreases in pH.

Most glutaraldehyde chemists state that the reaction with protein gives rise to products that are stable to acid hydrolysis and a chromophore with an absorption maximum at 265nm. The stability of these crosslinkages to acid hydrolysis rules out a Schiff base, though a Schiff base may be reduced enzymatically or non-enzymatically to form a secondary amine (39). Hardy et al. (19) state that the reaction of free monomer with a primary amine of a protein is followed by condensation of additional free glutaraldehyde and leads to the formation of a 1,3,4,5-substituted pyridinium salt analogous to the amino acid desmosine. This explanation fits nicely with the observation of a new absorption peak at 265nm when proteins are crosslinked with glutaraldehyde. Hardy's group points out that the pyridinium linkage is not the only type of crosslink in glutaraldehyde-treated proteins. However, it probably represents a significant portion of the crosslinks.

Under typical use conditions, the reactivity of glutaraldehyde with amino groups is limited to primary amines. It can react with secondary amines; however, this reaction occurs only under relatively severe conditions (13). Reactions with tertiary or quaternary amines have not been observed.

All of the significant protein and microbicidal reactions of glutaraldehyde are based on its ability to function as a highly effective crosslinking agent with various types of proteins. Hopwood (22) has investigated the reaction of glutaraldehyde with nucleic acids. At temperatures up to 60°C, no reaction occurs between native DNA and glutaraldehyde. At temperatures above 75°C, the reaction follows pseudo-first order kinetics. The reactions between RNA and glutaraldehyde are similar except that they begin above 45°C. There was little evidence for the formation of intermolecular crosslinks, even at elevated temperatures. The interaction of glutaraldehyde with nucleic acids cannot account for its microbicidal effectiveness at room temperature.

In tissue valve fixation, the primary substrate for crosslinking is collagen. Glutaraldehyde crosslinks the proteinaceous strands of collagen and thereby produces a mechanically strong leather which is resistant to both water and microbial attack. Residues of lysine are quite abundant in collagen. Due to the presence of a relatively accessible amino group on its epsilon carbon, this amino acid serves as a prime target for glutaraldehyde-based

crosslinking. The crosslinks introduced by glutaraldehyde are stable to hydrolysis both in boiling water and dilute acids, and are quite complex in nature. These crosslinks typically contain two to three glutaraldehyde residues per crosslink (9). At least 10 distinctly different molecular pathways for crosslinking have been observed with glutaraldehyde.

It is well established that glutaraldehyde treatment of proteins in the tanning of tissues significantly decreases tissue immunogenicity. This reduction is probably brought about by the crosslinking of the haptens and other antigenic determinants. Mattila and Fogarty (24) found that processing of bovine and porcine vascular grafts with dilute glutaraldehyde gave greater reduction of immunogenicity in rabbits then did processing with dialdehyde starch. O'Brien et al. (31) showed that glutaraldehyde-tanned bovine pericardial valves had low immunological sensitivity in sheep and dogs. A similar response was noted by Villa et al. (37) for glutaraldehyde-tanned porcine valve tissue grafted into mice or for valvular homogenates injected intraperitoneally into mice. These animal data validate the lack of immunogenic response in humans to long-term implantation with glutaraldehyde-treated tissue valves.

Reactions with Microorganisms

Despite the fact that glutaraldehyde is known to be an important liquid chemical sterilant, comparatively few studies have been performed regarding its biochemical interactions with microorganisms. Its bactericidal action is thought to be similar to its mechanism of tanning collagen. The free aldehyde groups of glutaraldehyde react with the primary amino acid residues of the organism to form stable secondary amines or as crosslinks that serve to fix the organism and render it non-viable. The review by Borick (5) showed that vegetative bacteria, including the more resistant species, are destroyed within one minute, most viruses within 10 minutes, and bacterial spores within three hours at temperatures above 75°F at pH 7-8. In general, glutaraldehyde is most effective against vegetative bacteria and less effective against sporeformers or organisms with an unusually high lipid content in the cell wall such as *Mycobacterium*.

Some generalized remarks will now be offered about the biochemical mechanisms for the microbicidal activity of glutaraldehyde. If one considers a bacterium, the structural unit separating the inside of the organism from the outside environment is the cell wall and cell membrane. The cell walls of Gram-positive microorganisms are considered more resistant to aldehyde attack than those of Gram-negative cells. The Gram-positive organism cell wall is made up of a mucopeptide which consists of repeating polysaccharide units with attached peptide chains (Figure 4). In this particular example, the presence of lysine in the peptide side chain would be a prime target for glutaraldehyde crosslinking.

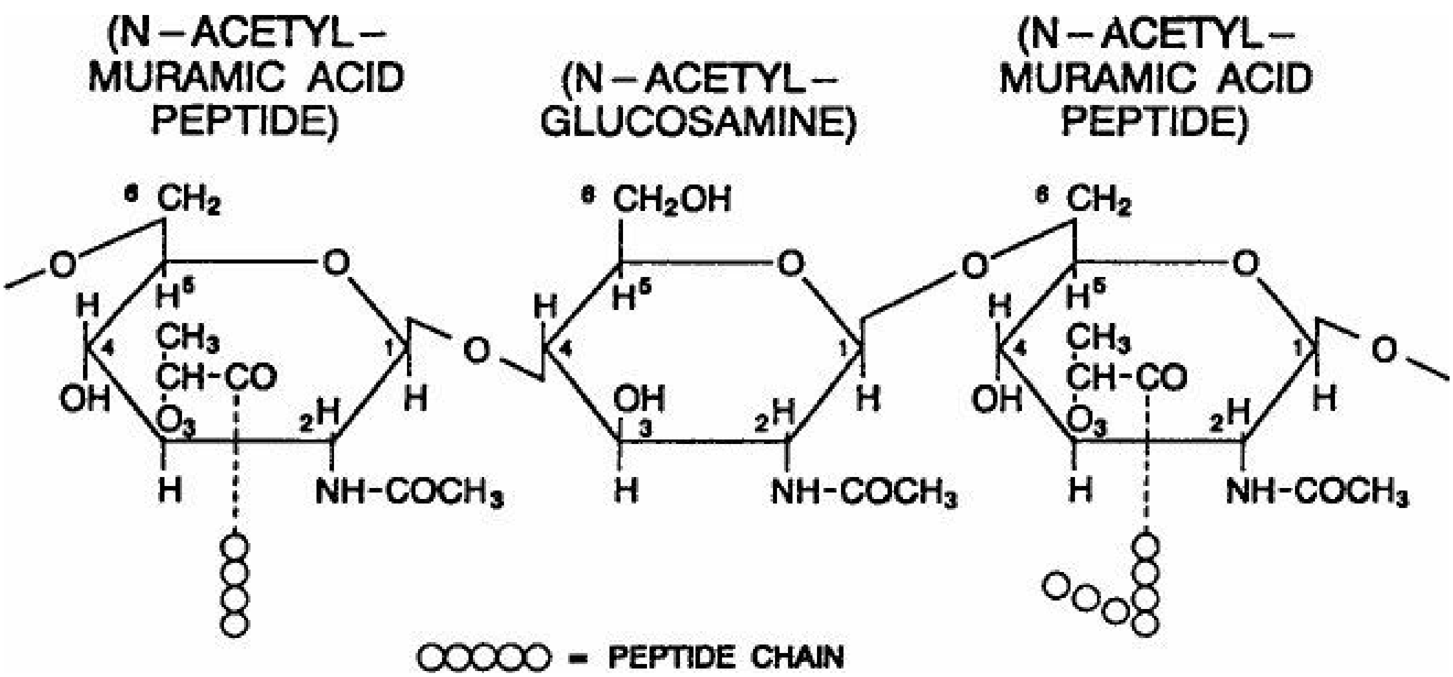

Figure 4. The Mucopeptide of A Typical Gram-Positive Bacterial Cell Wall

While this example is typical of a Gram-positive microorganism, the exact structure of the cell walls and membranes of microorganisms will vary from genus to genus. It is known that the outer surface of a Gram-negative bacterium is a layered structure of protein, lipoprotein and lipid. Regardless of the exact microorganism being considered, all will contain amino acids and thus sites for potential reaction with glutaraldehyde. As a reactive

microbicidal chemical, glutaraldehyde (as does ethylene oxide) irreversibly alkylates labile groups on the cell surface or within the cell. The reaction of glutaraldehyde with cytoplasmic constituents is still a fertile field for investigation. The inhibitory effect of the aldehyde on protein, RNA, and DNA synthesis in *Escherichia coli* is virtually complete within 10 minutes of its addition (25). Relatively few studies have been made of the effects of glutaraldehyde on cell enzyme activity. Dehydrogenase activity is inhibited by concentrations that have little effect on cell viability (29). Because the compound seals the outer cell surface, it disrupts cellular metabolism by preventing ready access of substrate to cell enzymes.

Glutaraldehyde-Antimicrobial Activity

The factors which influence the antimicrobial activity of glutaraldehyde include pH, time, temperature, concentration, composition, and character (ratio of monomers/polymers) in the use system. It has already been pointed out that this dialdehyde is more bactericidal (rate of kill) at alkaline than at acidic pH, and that sodium bicarbonate is frequently added to "activate" the glutaraldehyde to the required pH (approximately 8). Although sodium bicarbonate itself possesses some bacteriostatic activity against vegetative Gram-negative organisms, this activity, in view of the highly sporicidal effectiveness of alkaline glutaraldehyde, is of little consequence. The enhanced biocidal activity of glutaraldehyde in alkaline solution could be due to an effect on either the glutaraldehyde molecule in relation to polymerization or to changes in the ionization of surface moieties on the outer layers of the microbial cell.

Amines, particularly those found on the cellular surface, are influenced by pH in the classical acid-base sense. The pK for amines is typically around 9, i.e., at a pH of 9, about 50% of the amino groups are protonated and the remaining 50% are not. These unprotonated or free amines serve as the reactive site for glutaraldehyde attack. Therefore, as pH increases from acidic to basic, more reactive sites for glutaraldehyde reaction are formed. If, at a slightly alkaline pH, glutaraldehyde formed complex crosslinks between the unprotonated amino residues on the cell surface, this reaction could be similar to applying a "seal" to the cell wall and membrane. The resulting crosslink surface could prevent the transport of nutrients into, or waste products out of, the cell.

If the pH of the medium surrounding the organism is acidic, few of the surface amino residues would be in the free (reactive) amine form. Monomeric glutaraldehyde is a relatively small molecule and would then penetrate into the cell or spore if few reactive sites are present on the microbial surface. Since microorganisms can maintain an internal pH near neutrality regardless of external pH, a reasonable percentage of internal amino groups will be in the free amine form. Thus, glutaraldehyde would alkylate these free amino groups and inactivate cell enzymes from the inside.

This analysis was anticipated by Boucher (8) when he postulated as to whether aldehydes mainly act externally by sealing the outer cell surface or within the cytoplasm by interfering with enzymes. He noted that it was of interest to study the shape of microbial inactivation curves as a function of time of contact between acidic vs. alkaline glutaraldehyde. His group found, in general, two types of curves as shown in Figure 5. The first curve, with alkaline glutaraldehyde, shows continuous microbial destruction, while the second curve, with acidic glutaraldehyde, indicates a lag time followed by a sharper sigmoid (destruction) curve. It could then be postulated that the acidic glutaraldehyde kills spores by penetration through the spore coat and reaction with spore cortex constituents, whereas the alkaline glutaraldehyde attacks the spore coat and destroys the ability of the cell to function through more of a surface phenomenon. If a rapid microbicidal action is wanted, then an alkaline-based glutaraldehyde solution should be employed. Contrariwise, if penetration into cells (or collagen) is wanted, then an acidic glutaraldehyde would give increased penetration and internal reaction of amine moieties. Furthermore, the stability of the glutaraldehyde solutions is longer at the acidic pH and allows more economic use of a

solution.

The rate of antimicrobial action of glutaraldehyde is also influenced by temperature. Chemical reactions are generally enhanced by increasing temperature. This is indeed true for the reaction of microbial cells with glutaraldehyde. Under alkaline conditions, microbial destruction rates increase slightly with increasing temperature. This is in contrast to a dramatic rate enhancement typically seen with acid glutaraldehyde and increased temperature. The heat provides additional kinetic energy which facilitates the penetration of glutaraldehyde into the cell, whereas with alkaline solutions, the heat increases significantly the rate of glutaraldehyde decay (polymerization).

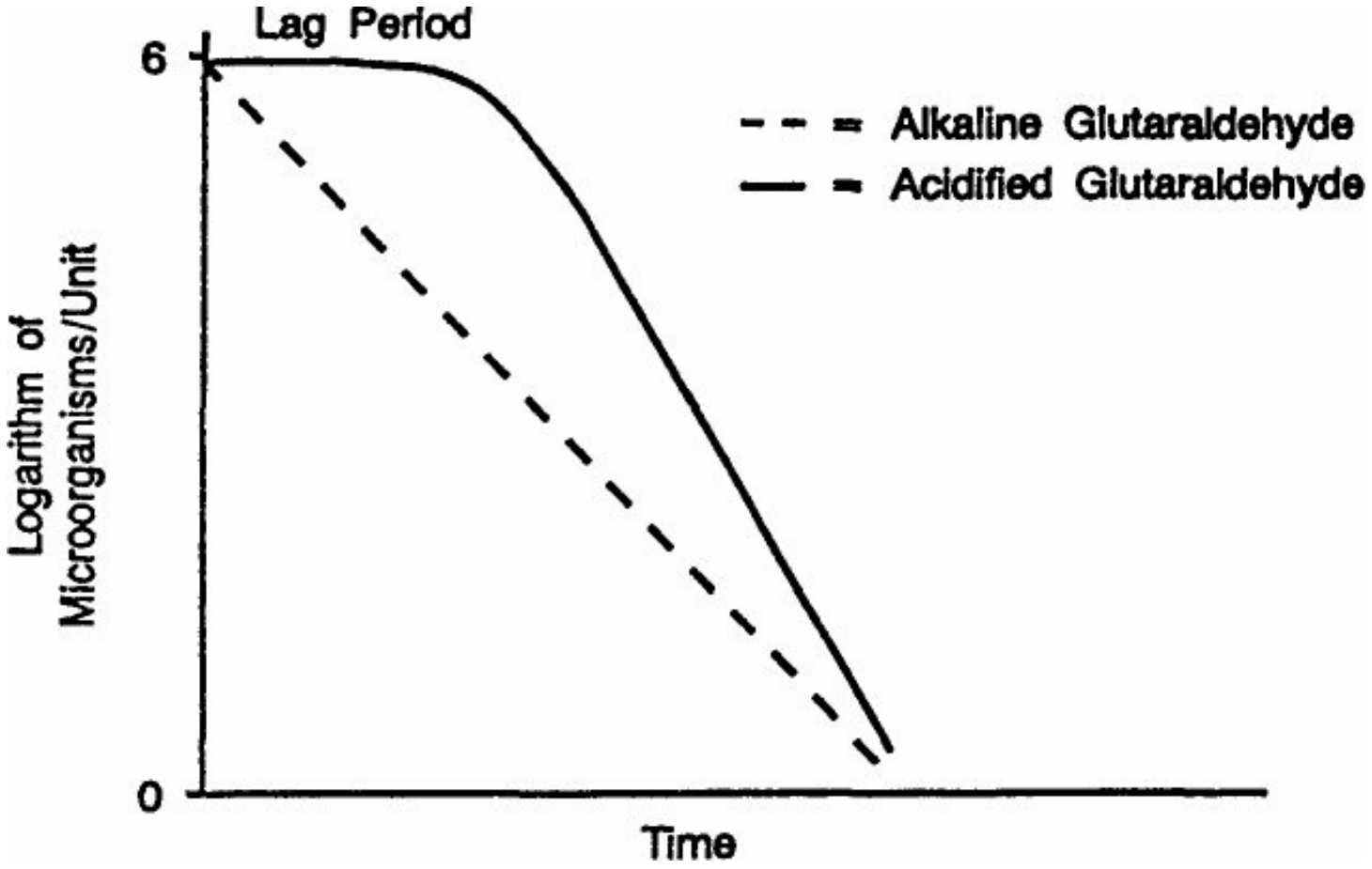

Figure 5. Generalized Inactivation Curves for Spores of *B. Subtilis* var. *Niger*

The factors of pH, time, concentration, and temperature become more closely intertwined when considering the matrix of components in the actual sterilizing system where glutaraldehyde is used. Organic matter is considered to have no effect on the activity of glutaraldehyde, although the interaction of this dialdehyde with amino groups in proteins suggests that this is a rather unusual finding. Organic matter can act either by protecting the microbial species from chemical attack (occlusion) or by competing with the microbial cell for active sites on the reactive chemical, thus reducing the effective concentration of the bactericide. When 2% alkalinized glutaraldehyde was tested in the presence of 20% blood serum, its activity remained the same as that in the absence of organic matter (4). Miner et al. (26) measured the concentration (%w/v) of sterile baker's yeast (called the soil neutralization number) that a chemosterilizer could tolerate and still be able to kill bacteria in 10 minutes at 25°C. These authors found that 2% alkaline glutaraldehyde had a maximum

neutralization number of 50% against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

These findings must be contrasted with the difficulty of determining the minimum inhibitory concentration (MIC) of glutaraldehyde in a microbial growth medium such as nutrient broth. Rubbo et al. (33) reported on the darkening of nutrient broth in the presence of glutaraldehyde, which could explain its poor bacteriostatic activity versus glutaraldehyde in water. Gorman and Scott (17) noted a 60% decrease in alkaline glutaraldehyde concentration after its addition to malt extract broth. This decrease occurred in a six hour period at 37°C and should be related to the rapid destruction (minutes) of vegetative cells under these same conditions. It is difficult to evaluate the microbicidal activities of glutaraldehyde in microbial culture media.

While the presence of amino groups in a matrix has the potential to decrease the bactericidal activity of glutaraldehyde, other system components may enhance its activity. Under alkaline conditions, a surfactant could aid glutaraldehyde penetration into cellular clumps or soiled surfaces. Under acidic conditions, it could help wet the cell surface and enhance the penetration of glutaraldehyde into the cell. Boucher (7) reported on 2% acid potentiated glutaraldehyde with a specific mixture of non-ionic ethoxylates of isomeric linear alcohols (0.25% w/w). These non-ionic ethoxylate molecules have a linear alkyl hydrophobic portion which is a mixture of C_{11} to C_{16} linear chains and a hydrophilic portion which is a polyoxyethylene chain containing from nine to 13 oxyethylene groups randomly attached to the linear aliphatic chain through an ether linkage. The combination of this surfactant with the acid glutaraldehyde was far more bactericidal than the acid glutaraldehyde alone. By and large, surfactants can enhance the activity of aqueous glutaraldehyde, particularly at acid pHs. Glutaraldehyde is fully compatible with most cationic, nonionic, and anionic surfactants.

Glutaraldehyde is unique among the short-chained aldehydes in its ability to serve as a leather tanning agent and also to have high sporicidal activity. Scott and Gorman (35) note that an activated 2% solution (alkaline) has a greater killing effect than 8% aqueous formaldehyde against a broad range of microbial spores. At the dilution of 2% used by most health care practitioners for sterilization of hospital instruments, alkaline glutaraldehyde is capable of killing spores of *Bacillus* and *Clostridium* species in three hours (4,36). Rubbo et al. (33) found that the spores of *Bacillus pumilus* were the most resistant among the *Bacillus* and *Clostridium* species they tested. Using the AOAC sporicidal test and vacuum-dried spores of *B. subtilis*, Boucher (7) reported that 10 hours at 20°C were necessary for complete kill with 2% alkaline glutaraldehyde, while 2% acid glutaraldehyde would inactivate the same challenge in one hour at 60°C and in 3.5 hours at 37°C. Babb et al (3), in an investigation including nine commercially available glutaraldehyde formations, reported that a challenge of 10^6 spores dried onto aluminum foil was inactivated in a three hour kill cycle by a majority of the preparations. Most manufacturers' recommendations are for a 10 hour minimum sterilization cycle to account for in-use dilution of the solution and slow inactivation by organic matter.

In 1984, Ascenzi et al. (2) showed that reuse of glutaraldehyde solutions has an adverse effect on efficacy, especially tuberculocidal activity. The existing AOAC tuberculocidal test

procedure was found to produce inconsistent results. They developed a new and more accurate quantitative test procedure which was allowed by the U.S. Environmental Protection Agency (EPA) to support their tuberculocidal label claims. With this new procedure they stated that not all commercially available glutaraldehyde solutions would kill *Mycobacterium bovis* BCG when tested according to manufacturers' label claims. Mycobacteria have been found to be very sensitive to temperature during testing with diluted glutaraldehyde solutions. This can cause misleading results unless accurate temperature control is maintained during the tuberculocidal testing.

Another factor that must be considered is the source of the organism. Carson et al. (12) reported that TM strains of *Mycobacterium chelonei* (isolated from peritoneal dialysis solutions) survived 60 minutes of exposure to 2% alkaline glutaraldehyde whereas no survivors of American Type Culture Collection (ATCC) strains of *M. chelonei* or *M. fortuitum* were detected after two minutes of contact time. With concentrations of 0.2% alkaline glutaraldehyde, both TM and ATCC strains showed survivors at 96 hours of exposure at room temperature. These data will be considered in the next section on the use of glutaraldehyde as a tanning and sterilizing agent for tissue-derived medical devices.

Glutaraldehyde Sterilization of Tissue Valves and Grafts

Surgical replacement of defective human heart valves and arteries with glutaraldehyde-tanned porcine aortic valve bioprostheses and bovine carotid arteries have been successful therapeutic procedures for the past 15-20 years. Such glutaraldehyde-treated animal tissues have been primarily studied for hemodynamic acceptability, lack of thrombogenicity, resistance to infection, and lack of rejection. Glutaraldehyde tanning proved to be the key aspect of the processing technique. Earlier, formaldehyde was employed both as the tanning agent and sterilant, but after several years of animal study it was determined that crosslinking with formaldehyde was reversible in body fluids (10). Other aldehydes were studied, and dialdehyde starch and glutaraldehyde were found to produce stable crosslinking of collagen macromolecules which was permanent. While dialdehyde starch is still used in the commercial tanning of some bovine heterografts, glutaraldehyde has become the crosslinking agent of choice in the preparation of tissue-derived heart valves. After the animal tissue has been tanned, it is still necessary to sterilize the xenografts, usually with a glutaraldehyde concentration higher than that used in crosslinking (tanning) the tissue. This section will review the efficacy of glutaraldehyde, either acidic or alkaline, as a sterilant for animal tissue xenografts.

In the manufacture of porcine xenografts, the industrial firm will purchase either whole or partial pig hearts from commercial slaughter houses. The fresh tissue is shipped in cold storage, usually under ice, to an initial processing facility. Within a few hours the aortic root is trimmed of excess tissue, inspected, and then fixed (tanned) under low pressure in concentrations of alkaline buffered glutaraldehyde from 0.2%-0.625%. The valve is then finally dissected, retaining a cuff of aortic root above the annulus and some fibrous tissue below the leaflet attachments. The roots are then mounted on a cloth-covered stent of suitable size to accommodate the valve. The valve is sutured to the stent being careful to assure competence of the leaflets.

During all of these manufacturing operations, careful attention is paid to reducing the bioburden by frequent changes of solutions, and by carrying the valves in glutaraldehyde solutions similar to the concentrations in which the valves were fixed. When the suturing steps are completed, the valve is then packed in a small container with the leaflets in closed position. A fresh solution of glutaraldehyde at a concentration equal to or slightly higher than the fixing concentration is then added to the container and the closed containers held for a fixed period of time. This is the sterilization step. Some manufacturers have chosen to use a sterilizing solution other than glutaraldehyde, usually 4% formaldehyde for eight to 24 hours at temperatures ranging from 25-37°C. A discussion will be presented later as to why some manufacturers will use liquid sterilants other than glutaraldehyde for sterilization of tissue xenografts. After sterilization, the sterility assurance is assessed by either assaying tissue coupons that have been carried along with the valve or by doing membrane filtration procedures of the glutaraldehyde solutions in which the valves were sterilized. The valves are shipped and stored in containers with 0.2%-0.625% buffered glutaraldehyde until use.

The processing of bovine carotid arteries follows in much the same fashion as has been described for the porcine aortic roots. The arteries are removed from the carcass very quickly after slaughter and shipped to the processing facility under ice. There is constant

concern that the range of environmental microorganisms contacting the arteries be limited, and that the bioburden counts be held to a minimum. At the start of manufacturing, the arteries are inspected, stripped of loose adventitia, the side vessels tied off, any holes sutured, and the lumens leak tested. The units then go into chemical processing where they may undergo an enzymatic digestion, usually with a proteolytic enzyme such as ficin, followed by several rinses with potable water. Additional chemical processing steps to modify the lumen surface may follow. The arteries are then placed on mandrels and tied; chemical fixation and sterilization occur simultaneously with processing in 1%-3% buffered glutaraldehyde (pH 5-7.5) for 12-24 hours. The sterilization solution is then decanted and sterile Dacron mesh applied to the grafts under aseptic manufacturing (sterile fill) conditions. The grafts then are sterile packaged in 40%-50% sterile ethanol with samples of product being taken for sterility assurance and quality testing for each batch.

The tanning of animal tissue occurs rapidly when exposed to glutaraldehyde concentrations of 0.1%-5%, pHs of 5-8, and temperatures from 25-37°C. Woodroof (39) stated that at these conditions (pH 7.4) the tissue tanning reaction in one hour was 84% complete at a glutaraldehyde concentration of 5%, and 74% complete with 0.1% glutaraldehyde. The absorbance at 265nm of proteins being tanned (crosslinked) with glutaraldehyde will increase for at least another seven days.

While the crosslinking of the collagen and other proteins occurs rapidly, the sterilization of the xenografts, particularly the inactivation of microbial spores, is a slower phenomenon. This section will now review the organisms of concern in the sterilization of bioprostheses and offer additional data on rates of microbial inactivation in the presence of animal tissue.

In 1977, the ability of certain species of microorganisms, particularly *Mycobacterium*, to resist glutaraldehyde sterilization of porcine valves was detected. The U.S. Centers for Disease Control (CDC) reported in the Morbidity and Mortality Weekly Report (MMWR) of February 11, 1977 that reports had been received from six medical centers regarding 14 isolations of mycobacterial species from pre-implantation cultures of tissue coupons from porcine heart valves manufactured by a U.S. firm. These mycobacterial cultures became positive after approximately two weeks of incubation at 37°C in thioglycollate medium. All 14 valves in which the tissue coupons tested positive were used for cardiac valve replacement before the hospital/clinic test results were available. The CDC report indicated that there had been no documented mycobacterial disease associated with the valves.

The particular crosslinking/sterilization process being used by the U.S. company at that time consisted of valve fixation with 0.2% buffered glutaraldehyde solution. The valves were carried throughout processing and shipping, except for a brief sterilization exposure to 1% glutaraldehyde in a 0.2% buffered glutaraldehyde solution. Before implantation, the valves and test coupons were aseptically removed from the shipping container, rinsed, and held several times in sterile physiological saline. The test coupons were to monitor the adequacy of sterile technique being used in preparing the xenografts for surgical use.

In a response to the CDC in early 1978, the U.S. firm reported that there was no evidence of clinical mycobacterial endocarditis in patients implanted with their porcine bioprosthesis. The manufacturer indicated that the glutaraldehyde sterilization cycle had been modified to include the presence of an alcoholic component added to the 1% buffered

glutaraldehyde sterilization solution, an increase in the time at which the valves were held in this solution, and the addition of the alcoholic component to the carrying and shipping solutions of 0.2% buffered glutaraldehyde. Furthermore, the manufacturer provided sterilization kinetic data that included the measurement of the D-values from the death rate curve for pure cultures of *M. chelonei* and for this organism inoculated into porcine tissue. (D-value is the time to reduce microbial population by lone-logarithm [90%] and assumes approximately first-order reaction kinetics). It had been determined by the CDC as well as by other hospital laboratories that the mycobacterial species resistant to the original glutaraldehyde sterilization process was a variant of *M. chelonei*, probably *M. chelonei* var. *abcessus*.

The above cited report from the CDC forced other manufacturers using buffered glutaraldehyde as a tissue prosthesis sterilant to review the adequacy of their specific procedures. In 1979, another U.S. manufacturer of porcine heart valves reported that their valves were final sterilized with 4% formaldehyde (as formalin) and then shipped in dilute glutaraldehyde. It was also stated in the firm's product literature that the fungus, *Chaetomium globosum*, was resistant to glutaraldehyde up to concentrations of 10% at one stage of its life cycle. This porcine heart valve manufacturer had previously established a sterilization procedure that involved 0.625% buffered glutaraldehyde as both a sterilant and shipping concentration. The manufacturer determined that this fungus, in its most resistant form, was sensitive to kill by 4% formaldehyde (as formalin) in a 24-hour holding cycle. Following this exposure, the valve was rinsed several times with 0.625% buffered glutaraldehyde and then shipped in that concentration.

During the late 1970s another U.S. manufacturer of tissue valves, whose process involved both sterilization and shipping of valves in glutaraldehyde concentrations of 0.5%, sent to the CDC a sterility test isolate labeled as a *Corynebacterium sp*. for species confirmation. This organism was later identified by Dr. Robert Weaver (38) of the CDC as *Vibrio extorquens*. It has since been reclassified as *Methylobacterium extorquens*. There have been no reports in the open literature on the ability of this Gram-negative organism to resist glutaraldehyde concentrations of less than 1% for extended periods of time. The fact that the organism was supposedly isolated from a sterility test of a tissue valve held continuously in 0.5% buffered glutaraldehyde would indicate that under certain defined ecological niches this organism had glutaraldehyde resistance.

As a result of these incidences of microbial survival due to the tanning/sterilizing concentrations of glutaraldehyde then being used by tissue valve manufacturers, the U.S. Food and Drug Administration requested manufacturers to provide additional data on the sterilization kinetics of their sterilization regimens. The types of studies provided by the various tissue prosthesis manufacturers were to indicate that the most resistant organism being encountered in their manufacturing process could be killed by the sterilization cycle to the extent that the probability of a non-sterile unit (valve in shipping container) was 10^{-6}. Manufacturers were encouraged to obtain resistant isolates from their bioburden through the use of incremental doses of their sterilization cycles. During this effort a U.S. manufacturer of a tissue valve demonstrated that the mature sporeform of *Microascus cinereus* (a fungus) exhibited resistance to varying concentrations of glutaraldehyde and

formaldehyde. This fungus is a common environmental isolate and had been reported in the literature as being associated with minor dermal lesions (nail-bed infections). In response to the resistance of *M. cinereus* spores to both glutaraldehyde and formaldehyde, the manufacturer undertook an extensive program that resulted in the development of a sterilant solution using formaldehyde in conjunction with an alcohol and a surfactant, which rendered the mature fungal spores susceptible to the process.

During the mid 1980s, the author had the opportunity, while working at a contract testing laboratory, to evaluate the resistance of the bioburden on bovine carotid arteries to a proposed glutaraldehyde tanning/sterilization process. This testing laboratory marketed a biological indicator comprised of spores of *Bacillus subtilis* var. *niger* for use in ethylene oxide and dry heat sterilization cycles. It was decided to use this sporeformer as baseline marker of the glutaraldehyde resistance of the bioburden of the raw arteries.

In conformance with U.S. FDA requirements for sterilization of implantable medical devices, validation studies were conducted of the in-use glutaraldehyde solutions. Glutaraldehyde concentrations of 0.5% and 1.5% were prepared from the commercial 50% concentrate. The sterilizing/tanning solutions had an initial pH of between 5-5.5 from the carryover of sodium bicarbonate from previous steps in graft manufacturing. The spores of *B. subtilis* var. *niger* at room temperature in 1.5% glutaraldehyde had D-values of 1.8 hours in an aqueous suspension, and of 1.6 hours in the presence of artery tissue (Figure 6). As shown in Figure 7, the D-values for this organism in 0.5% glutaraldehyde (pH 5-5.5) were 11.8 hours for the aqueous solution and 11.0 hours for the same solution with tissue present. These results also support the point made earlier that the presence of organic matter does not interfere with the microbicidal activity of fresh solutions of glutaraldehyde.

The resistance of the artery bioburden to these same solutions is shown in Table II. The bioburden which consists of a broad group of Gram-negative and Gram-positive organisms had an estimated D-value of 1.5 hours for the 1.5% solution and a D-value of 10.3 hours for the 0.5% solution. Sterility tests (per U.S. Pharmacopeia) performed on process samples and solutions after six hours of glutaraldehyde exposure at 1.5% glutaraldehyde revealed no survivors. These data illustrate, for this particular product sterilization system, that a concentration of glutaraldehyde of 1.5% for at least 12 hours would be necessary to render a product sterile with a probability of a survivor of 10^{-6} per final packaged xenograft.

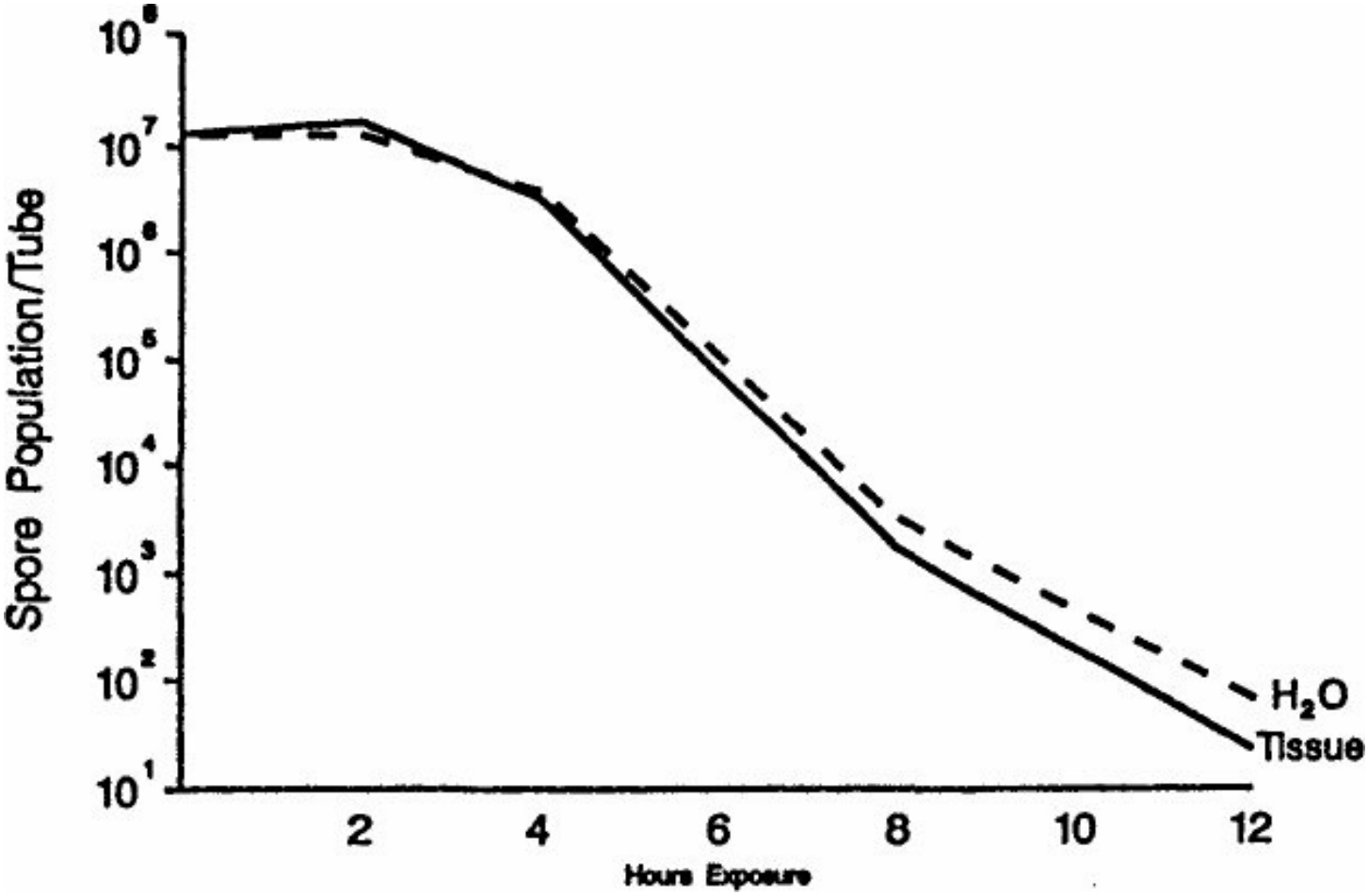

Figure 6. Inactivation Curves for Spores of *B. Subtilis* var. *Niger* in H_2O or H_2O + Tissue: Room Temperature, pH 5-5.5, 1.5% Glutaraldehyde
D-Value(H_2O) = 1.8 Hours; D-Value(H_2O + Tissue) = 1.6 Hours (from Skyland Scientific Services, 1985)

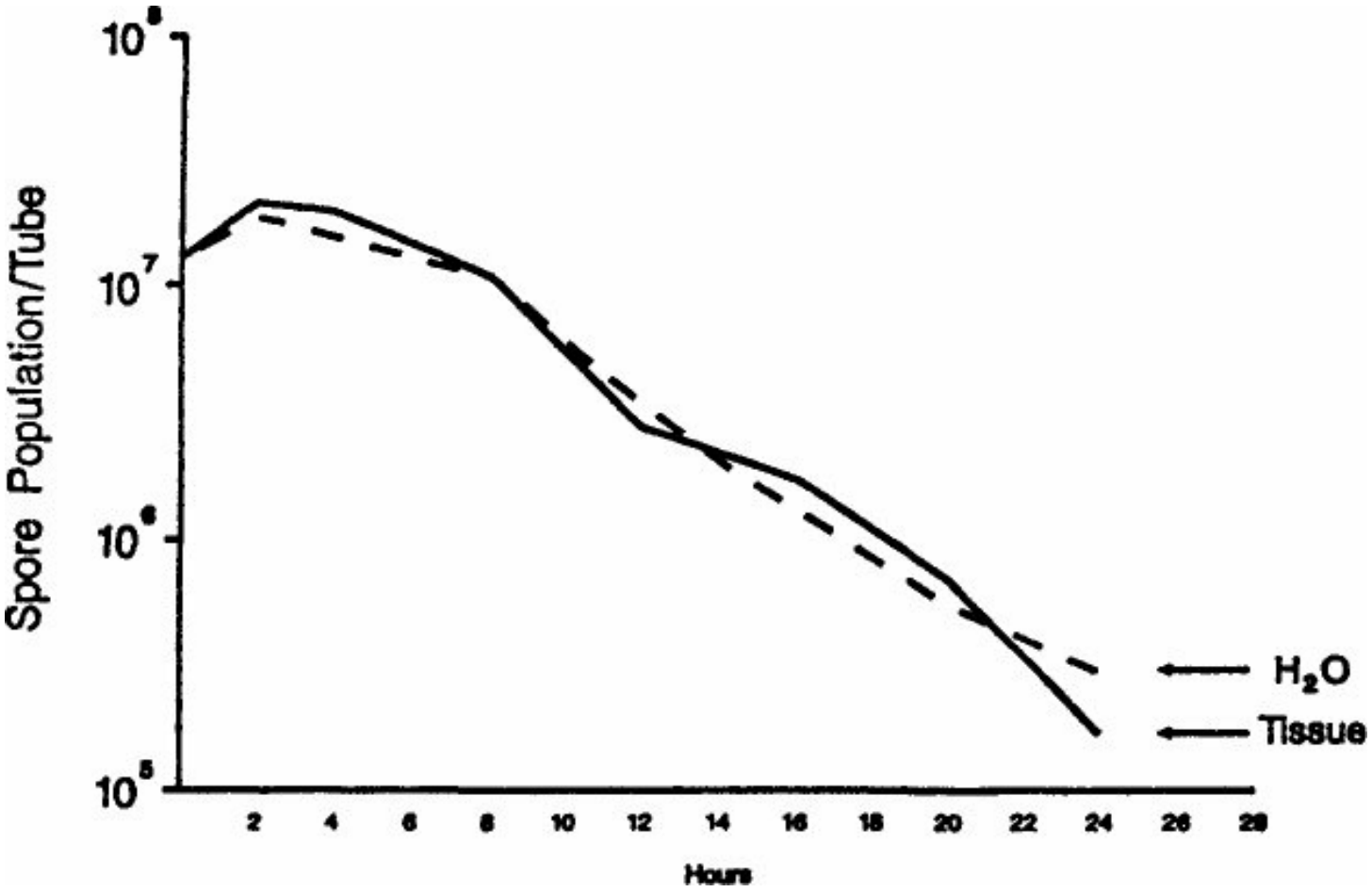

Figure 7. Inactivation Curves for Spores of *B. Subtilis* var. *Niger* in H_2O or H_2O + Tissue: Room Temperature, pH 5-5.5, 0.5% Glutaraldehyde
D-Value (H_2O) = 11.8 Hours; D-Value (H_2O + Tissue) = 11.0 Hours (from Skyland Scientific Services, 1985)

Figures 8 and 9 represent another approach to the control and validation of sterilization cycles by a non-U.S. manufacturer of porcine heart valves. Figure 8 shows the kill of spores of *B. subtilis* var. *niger* and Figure 9 the kill of bioburden of porcine aortic roots exposed at room temperature to 0.5% glutaraldehyde buffered to a pH of 7.3-7.5. At six month intervals, the in-use solutions from the manufacturing operation are challenged with spores of *B. subtilis* var. *niger* from a commercial manufacturer of biological indicators. The D-values obtained over a two year period show good stability of the test organism and the glutaraldehyde solutions. In addition, the bioburden from the porcine valves is also exposed to aliquots of the same use-dilutions (0.5% glutaraldehyde). These repeated audits of the product bioburden have shown that it is routinely killed within 60-90 minutes. This audit program is a constant check on the possibility of a glutaraldehyde-resistant organism occurring (and developing) in the bioburden from the hog slaughter house.

The data from Figures 7, 8, and 9 and Table II allow a comparison of the effect of pH with a glutaraldehyde concentration of 0.5% on a defined organism (spores of *B. subtilis* var. *niger*) and two different sources of tissue bioburden. The kill curve in Figure 7 shows an initial lag before first-order inactivation kinetics occur. This finding agrees with the discussion presented earlier on the difference in inactivation rates between alkaline (faster kill) and acidic (slower kill) glutaraldehyde solutions. The D-value for spores of *B. subtilis*

var. *niger* of 1.1 hours at pH 7.3-7.5 (Figure 8) is about one-tenth of that found at pH 5-5.5 (11.8 hours, Figure 7). The different product bioburdens show that the D-value (Figure 9) from alkaline solution exposure (0.22-0.30 hours) is about one-fortieth (1/40) of that estimated for the acidic solution (Table 2).

Table II. Survival of Bovine Tissue Bioburden to Glutaraldehyde*

TIME	ORGANISMS/mL	
(Minutes)	0.5% GLUTARALDEHYDE	1.5% GLUTARALDEHYDE
0	4.9×10^4	4.9×10^4
15	62	55
45	50	30
90	42	17
240	22	0

* Room Temperature, pH 5-5.5
(From Skyland Scientific Services, 1985)

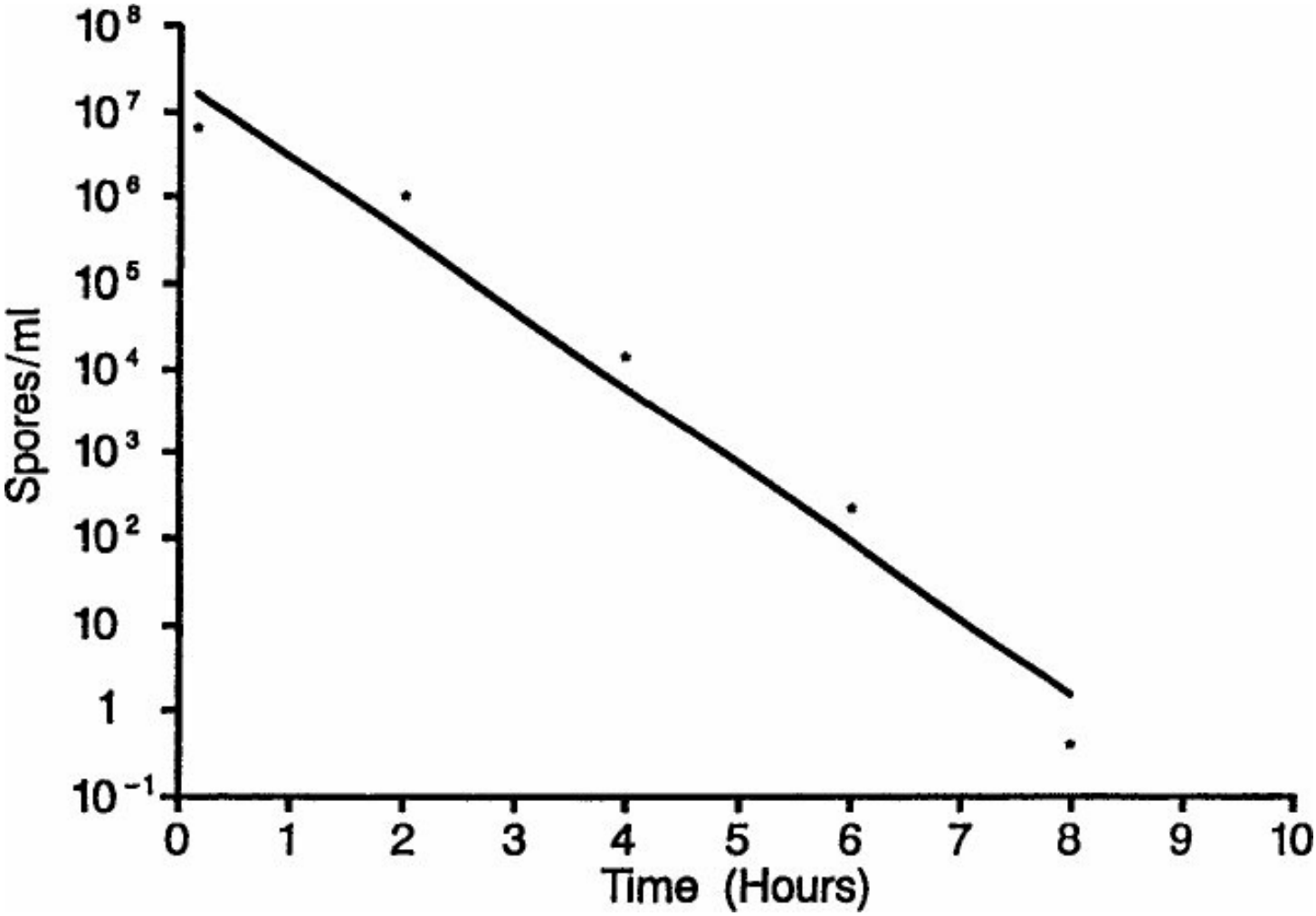

Figure 8. Inactivation Curve for Spores of *B. Subtilis* var. *Niger* in H_2O: Room Temperature, pH 7.3-7.5, 0.5% Glutaraldehyde
D-Value = 1.1 Hours

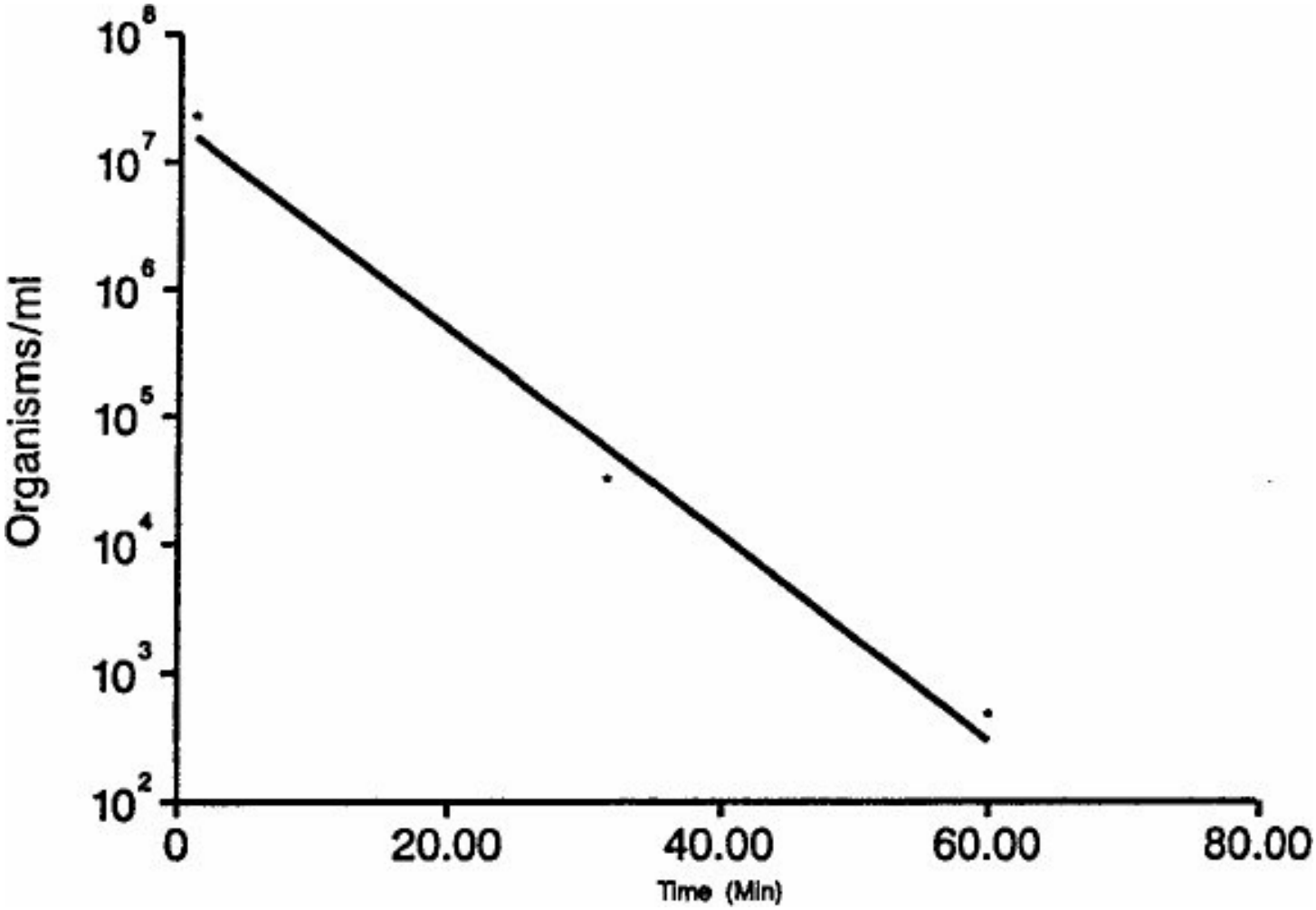

Figure 9. Inactivation Curve for Bioburden of Porcine Heart Tissue in H_2O: Room Temperature, pH 7.3-7.5, 0.5% Glutaraldehyde
D-Value = 13 Minutes (0.22 Hours)

The values reported in the previous paragraph for 0.5% glutaraldehyde at pH 7.4 can be compared with the data reported in Table III by Woodroof (39). These data include the *M. chelonei* discussed previously as a resistant organism to 0.2% glutaraldehyde solutions. Woodroof's data (0.5% glutaraldehyde, pH 7.4, 22°C) indicate that his strain of *B. subtilis* had an average D-value of 9.3 hours, which would indicate that it was approximately nine times more resistant than the spores of *B. subtilis* var. *niger* reported in Figure 8. It is interesting that Woodroof's data show that all vegetative organisms and a fungal sporeformer (*Aspergillus niger*) had very small D-values and were easily destroyed by either 0.5% glutaraldehyde or 4% formaldehyde (pH 5.5). These data showed that vegetative microbial forms are 100-1000 times easier to kill than bacterial spores.

Table III. D-Values (in hours) for Inactivation of Several Microorganisms

ORGANISM	0.5% GLUTARALDEHYDE*	4% FORMALDEHYDE**
Mycobacterium chelonei	1.06×10^{-1}	1.05×10^{-1}
Bacillus subtilis (spores)	9.30	2.48
Clostridium sporogenes (spores)	1.95	2.48
Aspergillus niger	8.61×10^{-2}	3.09×10^{-2}

Candida albicans	3.46×10^{-2}	6.01×10^{-3}
Escherichia coli	1.29×10^{-3}	2.98×10^{-3}
Pseudomonas aeruginosa	1.32×10^{-3}	1.32×10^{-3}
Staphylococcus aureus	1.37×10^{-3}	2.38×10^{-3}

* 0.5% glutaraldehyde at pH 7.4 and 22 C
** 4% formaldehyde at pH 5.5 and 22 C

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Since the FDA published its Good Manufacturing Practices (GMP) regulations in December 1978, validation of the sterilization procedures used in the manufacture of implantable medical devices must be demonstrated. The previous paragraphs have given indications of how some manufacturers have demonstrated the microbicidal effectiveness of their sterilization processes. The use of high challenge levels or resistant organisms to incremental doses (either less time of exposure or reduced glutaraldehyde concentrations) of the sterilizing system are now routine. There is still the expectation of lot-by-lot release sterility assurance.

In the early 1970s (before the advent of GMP regulations), tissue valve manufacturers used tissue coupons carried along with each valve through all stages of manufacturing and sterilization processing to be sterility tested per U.S. Pharmacopeia requirements at the time of batch release. In addition, because tissue prostheses shipped either in dilute glutaraldehyde or sterile 40%-50% ethanol must be rinsed in the operating room before insertion into the patient, additional tissue coupons were supplied in the final packaged product to be carried through all the package opening and product rinsing procedures within the operating room. Unfortunately, some hospitals misunderstood the use of these tissue coupons and immediately placed them into sterility test media without carrying them through all of the sterile rinsing steps normally encountered in the operating room.

Most manufacturers found over time that the positive findings with tissue coupons in the hospital testing laboratories were more indicative of operating room or laboratory mishandling then actual product contamination. Because hospital personnel felt that any positive finding with coupons that they tested meant that a non-sterile valve had been implanted, the patient then was subjected to unnecessary antibiotic/drug therapy. In addition, the hospital assumed that the manufacturer was at fault for the contamination and would force the manufacturer to test reserve specimens of coupons or actual product from the same shipping lots to assess that the product indeed was sterile. This led to significant unnecessary retesting of mistakes basically caused by hospital personnel. In the late 1970s, the FDA allowed U.S. manufacturers of tissue bioprostheses to discontinue the inclusion of tissue coupons in the shipping containers of products. All available evidence from GMP inspections and from epidemiological sources over the past 10 years indicates that manufacturers have increased the degree of sterility assurance for their products and the use of tissue coupons as test vehicles outside of the manufacturing site is not necessary.

While some manufacturers still test tissue coupons or tissue samples from each device

or each batch of devices processed with glutaraldehyde, increasing reliance has been placed on the detection of resistant organisms in the glutaraldehyde solutions used to carry or to sterilize the tissues. What is involved here is the decanting or recovery of fluid from the holding or packaging container (this fluid is replaced with fresh glutaraldehyde solution) and processing this recovered fluid through a sterile membrane filter. These filters can be placed either into various types of sterility test broths or onto agar media surfaces and assessed for the recovery of any resistant organisms. For those hospitals or institutions which still have doubts about the effectiveness of glutaraldehyde (or formaldehyde) sterilization procedures by manufacturers, the option remains to take the solutions in which the valves are shipped and test them using neutralization or membrane filtration procedures. Again, sterility testing is an art which requires an excellent testing environment combined with trained technicians to carry out this task without picking up extraneous contamination.

Other Biological Properties of Glutaraldehyde

Concern has been expressed in the past about the contact of either manufacturing workers or recipients of bioprosthesis to the toxic effects of residual glutaraldehyde. Miner et al. (27) showed that a 2% solution of non-activated glutaraldehyde had an oral LD_{50} of 17.5ml/kg in the rat. This is a low order of toxicity. Animal studies indicate that atmospheres containing glutaraldehyde vapor are not acutely toxic. Humans will find exposure to very low vapor concentrations intolerable, thereby giving sufficient warning of any potential overexposure. The current American Conference of Governmental and Industrial Hygienists (ACGIH) threshold limit value (TLV) is 0.2 ppm based on this known irritancy.

Dilute solutions of glutaraldehyde (0.25%-2.5%) will produce acute inflammatory effects in animal eyes. The primary irritant response on human skin is determined by a number of factors, including body site of contact, concentration of glutaraldehyde, duration, and conditions of contact. In general, prolonged contact on sensitive skin areas by concentrations exceeding 0.2% will produce primary dermal irritancy.

There have been many case reports of allergic contact dermatitis from contact with glutaraldehyde preparations being used for cold sterilization procedures of medical instruments in hospital or clinical practices. Allergic contact dermatitis is an immunologically-mediated hypersensitivity reaction expressed as an inflammatory skin lesion, which is produced at the contact site in response to amounts of the chemical significantly less than those producing a threshold primary irritant effect. Available data indicates that the concentrations causing such allergic dermatitis to be at 0.5% or greater. Cross sensitization to formaldehyde does not occur. Animal studies have shown that glutaraldehyde is not teratogenic (23), and there is extensive evidence that it is not a mutagenic chemical when measured by either the Chinese hamster ovary test, the sister chromotid exchange test or by an assay for induction of unscheduled DNA synthesis in rat liver cells.

Angell and Angell (1) reported on a possible mode of action for perivalvular leak with tissue bioprostheses. Usually, perivalvular leak is considered to be a technical surgical complication associated with the suturing of the valve into the tissue annulus. They indicated that in one situation there was observed tissue destruction with severe necrosis of the fibrous annulus which suggested some form of chemical necrosis. In this situation, it was suspected that the valve had been incompletely rinsed of residual glutaraldehyde. Most tissue valves are shipped in glutaraldehyde concentrations ranging from 0.2%-0.625% and are rinsed before implantation. It was suggested that the glutaraldehyde may have polymerized and the short chained polymers remained absorbed onto the fabric surface of the valve cuff due to limited rinsing techniques. Their experimental studies with trace amounts of glutaraldehyde (1-5 ppm) present on the fabric of the implanted valve resulted in both acute reactive thrombosis and fibrosis, and destructive tissue processes resulting in perivalvular leak. As part of their experiments, they showed that poorly-rinsed fabric that had been soaked in dilute glutaraldehyde concentrations and implanted subcutaneously, produces sterile abscess formation and a marked inflammatory response in the surrounding tissue.

The most practical deactivation of microbicidal or toxic concentrations of glutaraldehyde

is to dilute it with water to levels below 10 ppm. The reaction with amino groups provides another convenient method for deactivating dilute concentrations of glutaraldehyde. Usually this is accomplished by adding ammonium hydroxide until the solution is alkaline. In sterility testing where the bactericidal properties of glutaraldehyde solutions or treated tissues should be neutralized before addition to test media, the use of 1% glycine has been found to be effective and non-toxic (16). The use of sodium bisulfate (a general inactivating agent for aldehydes) to neutralize glutaraldehyde solutions is not recommended because of the microbial inhibitory action of the bisulfite itself (28).

The storage stability of glutaraldehyde solutions is dependent on pH and temperature. Dilute solutions (0.5%-2%) at neutral to acidic pHs are stable over 12 months at 4°C, but will degrade at 25 and 37°C, losing 25%-50% of their active concentration. It is known that the glutaraldehyde in which valves are shipped will lose up to 60%-70% of its activity over the three-year shelf life claimed for most tissue prostheses.

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Practical Application of Liquid Sterilants In Health Care Facilities

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Introduction

The purpose of this presentation will be to, first, discuss the proper use of chemical germicides in health care facilities and second, to discuss sterilization or disinfection of medical devices and the risk of infection, and the activity level of chemical germicides.

Some of the more common techniques used for sterilization of medical devices include heat, gases, radiation, and liquid chemical germicides. This article will focus exclusively on liquid chemical germicides.

In the United States, chemical germicides that are used in the health care field as sterilant/disinfectants are regulated by the U.S. Environmental Protection Agency (EPA) and in some instances by the U.S. Food and Drug Administration (FDA). The Centers for Disease Control (CDC) has the responsibility for recommending strategies and guidelines for the disinfection and sterilization of medical devices, but has no regulatory authority over these formulations. In other countries such as Canada, the methods by which chemical germicides used as disinfectants are approved are similar to the United States. However, in parts of Europe and other parts of the world, the determination of efficacy with resultant approval or recommendation of specific disinfectant formulas is made in the universities. I am not sure how some other countries test germicides for efficacy and approve specific products for sale in those countries.

Liquid Chemical Germicides

Two points concerning the use of liquid chemical germicides in the United States should be made. First, all procedures in health care facilities that deal with disinfection or sterilization incorporate a cleaning step prior to the use of the liquid disinfectant. If this procedure is done correctly and the chemical germicide is used according to the manufacturer's directions, the occurrence of adverse events or transmission of infections (associated with disinfected devices) rarely occur. Second, formulations that have been approved by the U.S. EPA as liquid chemical sterilant/disinfectants are almost never used to accomplish sterilization. They are primarily used to accomplish high level disinfection.

A number of previous papers have stressed various factors that influence the inactivation of microorganisms by physical or chemical agents. Liquid chemical germicides can be affected by a number of these factors, including the amount of organic matter on the device to be disinfected. It is primarily for this reason why the first step in any disinfectant procedure involves cleaning of the medical device to remove organic material. The number of microorganisms on the device to be disinfected is also an important factor regarding the effectiveness of a germicide. The higher the number of microorganisms present, the longer it takes for the chemical germicide to inactivate the microbial population, or the larger the amount of chemical germicide needed to accomplish total inactivation.

Another important factor is the type of microorganisms present, along with their innate resistance. For example, bacterial spores, mycobacteria, and some types of viruses are innately more resistant to some types of chemical germicides than are other microorganisms, such as vegetative gram-negative bacteria and some viruses. In addition, it has been shown that, in their naturally occurring state, microorganisms are significantly more resistant to chemical germicides than microorganisms after they have been subcultured and stocked as laboratory strains. Two good examples of this phenomenon are gram-negative water bacteria such as *Pseudomonas aeruginosa* or non-tuberculous mycobacteria which, when grown in water, exhibit much more resistance to chemical germicides than the same organisms that have been subcultured once on culture media. Gram-negative water bacteria have an added feature. When grown in water, they are able to colonize surfaces and form a biofilm (referred to as a glycocalyx) which prevents penetration of chemical germicides to the interior of the biofilm mass containing bacterial cells. The result is an extraordinary resistance to chemical germicides such as povidone-iodine (1, 4). Another example is naturally occurring bacterial spores in soil. These microorganisms exhibit much greater resistance to heat and chemical germicides compared to the same organisms that have been subcultured and subsequently sporulated on laboratory culture media (2).

The type of germicidal agent selected as a sterilant/disinfectant is important because some are more effective at inactivating organisms than others. The concentration of the germicidal agent is also important; in general, the higher the concentration, the more rapid the rate of inactivation. Additionally, the length of exposure to a chemical germicide influences its germicidal effectiveness; the longer the time of exposure, the more extensive the level of inactivation. The temperature of the exposure is another factor because the higher the temperature of the liquid chemical germicide, the more rapid the inactivation of

microorganisms. With some systems, the pH of the liquid chemical germicide is important and in many cases, must be controlled. This can be accomplished by building in buffering systems within the chemical germicide or by being aware of the effect of pH on the germicidal efficacy. For example, with halogens such as chlorine, pH levels above 8.0 result in most of the free chlorine being absent. Although moisture influences the effectiveness of both ethylene oxide gas sterilization and dry heat sterilization, it is not a problem usually associated with liquid chemical germicides.

Sterilization

Sterilization classically is defined as a procedure that kills all microorganisms, including high numbers of bacterial spores. Sterilization can be accomplished by moist and dry heat, ethylene oxide gas, radiation, or by relatively long periods of contact with chemical germicides that are sporicidal. Bacterial spores, because of their resistance to germicides, are used to test the efficacy of sterilizing procedures. In the United States, the particular spore strains usually used are *Bacillus subtilis* var. *niger* as well as several other species of *Bacillus*.

Sterilization from an operational standpoint is defined as a carefully designed and monitored process that will assure that the probability of an item being contaminated after sterilization is equal to or less than one in a million, or 10^{-6}. In a hypothetical inactivation curve using bacterial spores, one can start with one million bacterial spores (10^6), and measure the inactivation rate over time to about 10^0 or 10^{-1}. At that point the line is extrapolated. The probability of one spore surviving is one in a million at a particular point on the graph. It is this type of procedure that is used by the medical device industry in the United States to produce medical devices that are sterile.

The following is a list of some of the components of a typical sterilization plan by U.S. industry which would involve Good Manufacturing Procedures (GMPs):

— a validated sterilization process;
— the use of biological indicators;
— sterility testing;
— process controls;
— quality control on materials; and
— post-sterilization reliability testing of the particular medical device.

For sterilization processes in hospitals using steam autoclaves or ethylene oxide gas sterilizers, many of the elements listed above are not performed. Some of the elements are automatically incorporated into the cycles of sterilizers.

It is clear that there is a much higher degree of quality assurance performed by industry than by health care facilities. In addition, when chemical germicides are used either as sterilants or as disinfectants, the level of assurance and testing is significantly different than that practiced by industry. It is virtually impossible, for example, to verify by use of a biological indicator, the efficacy of a disinfection or sterilization procedure using liquid chemical sterilants. It is for this reason that many of the protocols which have been developed rely on tightly controlled procedures. Furthermore, it is for this reason that certain types of disinfection procedures need to have higher levels of the active chemical germicide than might be thought necessary. The use of higher levels of germicides are to compensate for human error.

When recommending strategies for disinfection and sterilization, my colleagues and I at the CDC have used the classification system developed 25 years ago by Dr. Earl Spaulding of Philadelphia. Dr. Spaulding recommended two classifications, and divided medical devices into three categories: critical, i.e., those that penetrate skin and see blood; semi-critical, those that touch mucous membranes; and lastly, non-critical, those that do not touch

broken skin. The second part of Spaulding's classification described the germicidal power of liquid disinfectants used with each of these categories. For example, with those medical devices considered critical, sterilization is the method of choice. Semi-critical devices can either be sterilized or, at a minimum, be subjected to high-level disinfection. Non-critical devices, depending on the device or the surfaces, might be subjected to an intermediate or low-level disinfection process.

High- and Low-Level Disinfection

High-level disinfection kills all microorganisms, except high numbers of bacterial spores. In practice, it involves a relatively short contact time of 10 to 30 minutes and the chemical germicide is sporicidal. Moist heat at 75° to 100°C can also accomplish high-level disinfection and examples of chemical germicides used in the United States are glutaraldehydes, hydrogen peroxide, chlorine dioxide, and peracetic acid. It should be emphasized that in the United States, a chemical germicide that has been approved by the U.S. EPA as a sterilant/disinfectant can be used as a high-level disinfectant. In other words, the same formulation can accomplish sterilization if the exposure time is long enough.

An intermediate level disinfectant kills *Mycobacterium tuberculosis* and is considered tuberculocidal; it also kills all other vegetative bacteria, fungi, and almost all viruses. Examples of tuberculocidal chemical germicides include phenolics, iodophors, some chlorine compounds, glutaraldehydes, and alcohols. The chemical germicides recommended for decontamination of massive blood spills or spills of pathogenic agents are those that at least have the capability of being a tuberculocide. This is recommended not because of a fear of transmission of mycobacterial infections, but rather because mycobacteria are resistant and consequently germicides that are effective against them have a higher degree of germicidal power.

Low-level disinfection is a process that kills most vegetative bacteria, some viruses, and some fungi, but not *M. tuberculosis*, and thus these germicides are not tuberculocidal. They are used primarily for routine housekeeping procedures; a good example would be quaternary ammonium compounds. Table I contains the activity levels of selected liquid germicides. The EPA Product Classification is compared to the CDC Process Classification in Table II.

Disinfection of Microorganisms Not Previously Tested

When the etiologic agent in question might not have been tested in the laboratory, i.e., Hepatitis B virus, a different rationale for procedures of disinfection or sterilization using chemical germicides is used. These viruses cannot be grown in tissue culture and consequently, there are no data using tissue cultures to determine efficacy of various types of chemical agents. Furthermore, when a pathogenic agent produces a serious illness as in the case of the Hepatitis B virus (HBV), or the human immunodeficiency virus (HIV), it is often assumed that these agents have extraordinarily high resistance to physical and chemical agents. Table III shows a rationale that was used some years ago to determine the procedures for decontamination or disinfection when HBV was thought to be present. It is acknowledged that bacterial spores are the most resistant microorganisms present, which is followed in order by mycobacteria, fungal spores, small or non-lipid viruses, fungi, vegetative bacteria, and medium-sized or lipid viruses. Originally it was assumed that HBV had the same degree of resistance as *M. tuberculosis*. All disinfection and decontamination strategies made use of that assumption when formulating germicidal concentration and time of exposure. In fact, the resistance of HBV lies towards the bottom of Table III between vegetative bacteria and medium-size and lipid viruses. HIV is even more sensitive to the common chemical germicides. Two studies have been performed on HBV; in one, Walter Bond and I used 10^6 HBV in dry plasma and showed that exposure for 10 minutes at 20°C to a variety of germicides inactivated the viruses, i.e., they did not produce infection in susceptible chimpanzees (3). The chemical germicides were 70% isopropanol, 50 mg/L sodium hypochlorite; 80 mg/L iodophor disinfectant; 2% glutaraldehyde; and a 1 to 16 dilution of a glutaraldehyde-phenol formulation. Kobayashi and his co-workers, using 10^5 to 10^6 HBV, showed that even lower concentrations of glutaraldehyde and alcohol inactivated large numbers of the virus (5). As I mentioned, there have been several studies on HIV and the results have shown that common germicides used as disinfectants throughout the world are very effective in inactivating it.

Table I. Methods of Sterilization or Disinfection Activity Levels of Selected Liquid Germicides[a]

Method	Concentration or Level[b]	Disinfectant Activity
STERILIZATION		
Heat		
Moist (Steam Under Pressure)	121°C; 132°C for various time intervals	
Dry	171°C for 1 hr; 160°C for 2 hr; 121°C for 16hr.	
Gas (Ethylene Oxide)	450-500/L at 55-60°	
Liquid		
Glutaraldehyde	Variable[c]	
Hydrogen Peroxide	6-30%	
Formaldehyde	6-8%[d]	
Chlorine Dioxide	Variable[e]	
Peracetic Acid	Variable[f]	
DISINFECTION		

Heat		
Moist (Includes hot water pasteurization)	75-100°C	High
Liquid[g]		
Glutaraldehyde	Variable	High to intermediate
Hydrogen Peroxide	3-6%	High to intermediate
Formaldehyde	1-8%	High to low
Chlorine Dioxide	Variable	High
Peracetic Acid	Variable	High
Chlorine Compounds[h]	Free/Available Chlorine	
Alcohols (Ethyl, Isopropyl)[i]	70%	Intermediate
Phenolic Compounds	0.5 to 3%	Intermediate to low
Iodophor Compounds	30-50 mg/L free iodine; up 10,000 mg/L available iodine	Intermediate to low
Quaternary Ammonium Compounds	0.1 to 0.2%	Low
ANTISEPSIS[k]		
Alcohols (ethyl; isopropyl)	70%	
Iodophors	1-2 mg free iodine/L; 1-2% available iodine	
Chlorhexidine	0.75-4.0%	
Hexachlorophene	1-3%	
Parachlorometaxylenol	0.5-4.0%	

[a] This list of chemical germicides centers on generic formulations.

[b] For sterilization or disinfection, refer to the manufacturers' instructions for exposure times and conditions as well as recommendations for rinsing and subsequent handling of process items.

[c] Several glutaraldehyde-based proprietary formulations are marketed in the U.S., e.g., low-, neutral or high-pH products recommended for use at normal or elevated temperature with or without ultrasonic energy. It is imperative that the manufacturer's instructions regarding use as a sterilant or disinfectant be followed.

[d] Because of the ongoing controversy of the role of formaldehyde as a potential occupation carcinogen, it's use is limited to specific circumstances under carefully controlled conditions, e.g., for the disinfection of certain hemodialysis equipment. Currently there are no EPA-registered products designed for liquid chemical sterilizing or disinfecting that contain formaldehyde.

[e] Chlorine dioxide is claimed to be the active chemical resulting from mixing water, sodium chlorite and lactic acid in varying proportions depending upon the intended use as a sterilant or disinfectant.

[f] In recent years, a limited number of trade name products combining low concentrations (< 0.1%) of peracetic (peroxyacetic) acid with low concentrations (< 1.0%) of hydrogen peroxide and the in-use dilutions have been registered with the EPA as sterilants or sterilant/disinfectants. This combination minimizes negative effects, such as corrosiveness, seen at higher concentrations of peracetic acid.

[g] As of December 1988, approximately 85 proprietary formulations were registered with EPA as "hospital disinfectants" that are also tuberculocidal, virucidal and fungicidal. Among this list are formulations that contain an array of "active" chemical agents which may make it difficult to define a generic classification for the product. The user is urged to pay particular attention to the information on the product label and accompanying package literature.

[h] Concentrations between 500 and 1000 mg/L chlorine are appropriate for the vast majority of uses requiring an intermediate level of germicidal activity; higher concentrations are extremely corrosive as well as irritating to personnel, and their use should be limited to situations where organic material is difficult to clean (e.g., porous surfaces) or contains unusually high concentrations of microorganisms (e.g., spills of cultured material in the laboratory).

[i] The effectiveness of alcohols as intermediate level germicides is limited, since they evaporate rapidly and lack the ability to penetrate residual organic material. Items to be disinfected with alcohols should be carefully pre-cleaned and then totally

submerged for an appropriate exposure time (e.g., 10 minutes).

[j] Only those iodophors registered with EPA as hard-surface disinfectants should be used. Antiseptic iodophors are not suitable for disinfecting medical instruments or devices or environmental surfaces.

[k] This is not a complete listing of formulations categorized by FDA, but it includes selected germicides which are commonly used as antiseptics in hospitals.

Table II. Comparison of Environmental Protection Agency (EPA) Product Table Terminology and Centers for Disease Terminology Control (CDC) Germicidal Process

EPA Product Classification	CDC Process Classification
"Sterilant/Disinfectant" (e.g., glutaraldehye-, chlorine dioxide-, hydrogen peroxide-, or peracetic acid-based products)	"Sterilization"[a] (Sporicidal chemical, prolonged contact time) "High-Level Disinfection"[b] (sporicidal chemical, short contact time)
"Hospital Disinfectant" with label claim for tuberculocidal activity (e.g., phenolics, iodophors, or chlorine compounds)[c]	"Intermediate-Level Disinfection"[d]
"Hospital Disinfectant" with NO label claim for tuberculocidal activity; includes "Sanitizers" (e.g., quaternary ammonium compounds, some iodophors, and some phenolics)	"Low-Level Disinfection"[e]

[a] This type of sterilization procedure should be used ONLY with those instruments (critical or semi-critical) that are not heat stable. As indicated in the text, sterilization with liquid chemical germicides is not a biologically monitorable procedure and requires many cumbersome post-exposure manipulations such as rinsing with sterile water and drying with sterile towels prior to use on a patient.

[b] High-level disinfection, as defined above and in the text is appropriate for those semi-critical instruments that are not heat stable and therefore cannot be sterilized by steam autoclaving between uses. As with liquid chemical sterilization, thorough rinsing with sterile water after the required exposure time is appropriate.

[c] This class of germicide includes a number of generic hypochlorite formulations that are EPA-registered but do not have specific label designations of "Hospital Disinfectant" or "tuberculocidal".

[d] Intermediate-level disinfection is appropriate for between-patient processing of certain non-critical instruments or devices for environmental surfaces, particularly after significant spills of blood in any area or spills of microbial cultures in the laboratory.

[e] Low-level disinfection is appropriate for between-patient processing of certain non-critical instruments or devices or for routine cleaning and housekeeping. Manufacturer's label information, particularly for EPA-approved patterns of use, should be closely examined and followed.

Table III. Descending Order of Resistance to Germicidal Chemicals

BACTERIAL SPORES
Bacillus subtilis
Clostridium sporogenes
MYCOBACTERIA
Mycobacterium tuberculosis var. *bovis*
NONLIPID OR SMALL VIRUSES
poliovirus
coxackievirus
rhinovirus

FUNGI

Trichophyton, sp.
Cryptococcus, sp.
Candida, sp.

VEGETATIVE BACTERIA

Pseudomonas aeruginosa
Staphylococcus aureus
Salmonella choleraesuis

LIPID OR MEDIUM-SIZE VIRUSES

herpes simplex virus
Acytomegalovirus
respiratory syncytial virus
hepatitis B virus
human immunodeficiency virus

Infection Transmission

Finally, I would like to discuss, from a theoretical standpoint, the likelihood of infection transmission resulting from the use of a device after a particular treatment. For example, if a medical device is subjected to heat sterilization, we know by definition that the probability of a microorganism surviving this procedure is one in a million. If this procedure has been done correctly, the probability of infection occurring because of microorganisms contaminating this device in my opinion would be zero. On the other hand, if a device was subjected to chemical sterilization, e.g., an exposure over a long period of time to a chemical germicide that was a liquid sterilant, the probability of contamination is about a thousand times higher than that of heat sterilization, i.e., one in one thousand or 10^{-3}. There is less assurance and less means to validate this process. The probability of infection occurring under these circumstances would vary from zero to an unknown level.

Using this same chemical sterilant for a shorter period of time in a high-level disinfection procedure, the probability of contamination would range from one (it would be contaminated with microorganisms) to a one in 100 probability of being contaminated. The associated probability of infection from this device would range then from a probability of one to an unknown level. Lastly, if a low-level disinfectant is used on a medical device, the probability of microbial contamination on the device after the procedure almost certainly is one. The probability of infection, although presumably higher than some of the other categories, would range from one to an unknown amount. There are many examples in the literature where the incorrect use of low-level disinfectants on medical devices have been associated with hospital acquired infections.

Conclusion

In conclusion, I would like to point out that chemical germicides are used to disinfect, rather than to sterilize, devices in hospitals in the United States. The higher the germicidal activity, the less chance there is for a failure of the disinfection procedure. Lastly, the correct use of chemical germicides help to prevent infection transmission in health care facilities.

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General Discussion
Chairman: R.F. Morrissey, Ph.D.
Johnson & Johnson, U.S.A.

GENERAL DISCUSSION

Comments by Dr. Morrissey, Johnson & Johnson, U.S.A.

We are going to answer questions from this morning's session on Radiation Sterilization and then answer questions from this afternoon's session on Sterilization by Heat and Chemicals. We will then go back to the questions from the opening session, from Monday afternoon's session, and Dr. Prokopenko will read several questions from our Russian colleagues.

Question for Ms. Rakitskaya, All-Union Scientific Research Institute of Medical Polymers, U.S.S.R.

Are doses lower than 25 kGy used for sterilization of hospital products in the U.S.S.R.? If so, could you please give some examples of these products?

Answer by Ms. Rakitskaya, All-Union Scientific Research Institute of Medical Polymers, U.S.S.R.

Doses lower than 25 kGy are not used alone in the U.S.S.R.; they are used only in combination with the thermoradiation process when temperature alone is not bacteriocidal.

Question for Ms. Rakitskaya, All-Union Scientific Research Institute of Medical Polymers, U.S.S.R.

In your presentation you discussed significant enhancement of the bacteriocidal efficacy of radiation with magnetic field application. Could you tell us the strains used in this work? Did you suggest that the synergistic effect took 24 hours to develop?

Answer by Ms. Rakitskaya, All-Union Scientific Research Institute of Medical Polymers, U.S.S.R.

The radioresistance of microorganisms can be changed and it is possible to influence radioresistance. A bacteriocidal effect was seen with doses which were one and a half times less than normal when there was exposure to an alternating magnetic field. I don't have information with me regarding the field intensity.

Question for Mr. Stepanov, All-Union Scientific Research Institute of Radiation Engineering, U.S.S.R.

How many industrial irradiators are operating in the U.S.S.R., and how many have been operating since 1976? Are they wet-storage irradiators and what is the loading quantity of curies?

Answer by Mr. Stepanov, All-Union Scientific Research Institute of Radiation Engineering, U.S.S.R.

There are presently eight industrial gamma-irradiators in the Soviet Union. The activity of each irradiator varies from 200 kilocuries to 15 million curies. The total power of these irradiators accounts for approximately 5 megacurium.

Question for Dr. Whitby, University Hospital, University of Western Ontario, Canada

Have you conducted any studies on the sample item proportion used in methods B1 and B2 in North America? If so, what was the most frequently used sample item proportion that you found?

Answer by Dr. Whitby, University Hospital, University of Western Ontario, Canada

We did get some data regarding this issue recently in our survey. Surprisingly, relatively few of the manufacturers who responded were using anything other than the whole item for their work. Of course, really large devices, such as something nearing 2 square meters, are far too big to be handled uniformly.

Question for Dr. Whitby, University Hospital, University of Western Ontario, Canada

Regarding exposure to D-star dose, what is the rationale behind allowing two positives out of 100 samples tested for sterility? And the follow-up question, do the B1 and B2 methods of dose setting have equal rigor, and do they yield equivalent sterilizing doses if applied to the same product?

Answer by Dr. Whitby, University Hospital, University of Western Ontario, Canada

Regarding the question of equal rigor, if anything, method B2 would yield a lower dose than B1 because there is more detailed information on the bioburdens' behavior in the radiation sterilization process using B2. With the B1 method we have literally taken the population of microbes, done the calculations as to when it should yield one positive response out of 100 tested, and indicated that the product be tested at that dose. With the B2 method, one actually tests with a series of doses to see what you get. If you have a more sensitive bioburden, you would get a lower dose. In practice, however, there wasn't much difference. In answer to the first question, we felt it would be a high price to pay for a false positive sterility test if we allowed only one positive response. After considerable debate, it was considered more practical to permit two positive responses out of every 100 tested.

Question for Mr. Saylor, Falls Church, Virginia, U.S.A.

Does the use of a scanning mechanism on the beam mean that the doses are delivered to the product in a pulsed mode? Could this be important in determining the killing action of radiation?

Answer by Mr. Saylor, Falls Church, Virginia, U.S.A.

The answer to both questions is no. The scanning process should be considered continuous, and therefore, there are no pulses involved. If one were working at 10 MeV with a linac, there would be a pulsing mode involved in the process. However, you would be scanning with the beam of pulses across the surface of the target much faster than the product would be moving. In essence, then, it would appear as if cobalt-60 were shifting around the space very rapidly at a very high intensity.

Question for Dr. Pflug, University of Minnesota, U.S.A.

Since there are adequate physical methods of measuring cycle parameters, why should we use biological indicators in the steam sterilizing process?

Answer by Dr. Pflug, University of Minnesota, U.S.A.

It comes back to the objective of the sterilization process, which is to kill microorganisms. Somewhere you need to show that you can kill microorganisms. To clarify variability, each species of organism or each spore crop has its own characteristics, but for a given spore crop, the characteristics are reproducible. For any single spore crop, when you treat it in an identical way, you will get an identical result.

Comment by Dr. Morrissey, Johnson & Johnson, U.S.A.

Does that answer the question?

Answer by Dr. Tallentire, University of Manchester, U.K.

Yes. I realized that the cycle parameters have to meet the objectives of the sterilization process and it must be shown that microorganisms are being killed, but must this be shown repeatedly? I asked the question to determine whether you have to use biological indicators all the time. If you know the lethal effect of a given cycle, is it necessary to repeatedly show that effect, or can you monitor it by physical means?

Answer by Dr. Pflug, University of Minnesota, U.S.A.

I think you can monitor it by physical means, and I think one thing we always have to keep in mind is that we have to apply judgment to the situation. If hardware is put inside an autoclave, the physical parameters should certainly give a good indication and would not be expected to show much deviation. However, to sterilize a solution in a plastic pouch that had ports on it would certainly require careful biological monitoring. Potentially, the moisture content in the port area might change during sterilization, essentially going from dry to wet heat. Thermocouple monitoring alone would not give sufficient information.

Question for Dr. Hoxey, Department of Health, U.K.

What is the lowest temperature at which the LTSF process would be usable? During your presentation you gave a temperature range; how low can we use the process?

Answer by Dr. Hoxey, Department of Health, U.K.

I suggested during my presentation that there was a considerable decrease in efficacy below 70°C or certainly a big drop in efficacy between 70 and 65°C. There was some work conducted in Scotland in the 1970s in which reasonably good results were reported when the temperature went down to 65°C. I think that the bottom limit is probably 65°C but the process is more effective if the temperature is above 70°C.

Question for Dr. Hoxey, Department of Health, U.K.

Could the process be scaled up to the order of say 250-300 cubic feet?

Answer by Dr. Hoxey, Department of Health, U.K.

That's a difficult question to answer. The pulsing cycle was developed because of problems with formaldehyde stratification within the chamber. If the size of the chamber is increased, then the magnitude of that problem would increase. That is something you would have to look into very carefully, and I would not say categorically that you could do it.

Comment from the Floor

I have a comment that you may be able to elaborate on. In discussions and during these presentations, we tend to compare low temperature steam formaldehyde with ethylene oxide. I think that this is a somewhat unfair comparison, if one considers that ethylene oxide is effective below the formaldehyde temperature range. On an industrial scale, low temperature steam formaldehyde really is not used, probably because ethylene oxide is effective at the low range of temperatures.

Answer by Dr. Hoxey, Department of Health, U.K.

I agree that in some ways a comparison between LTSF and ethylene oxide isn't appropriate. The critical temperature of many polymer materials is around 80°C, so there are a lot of polymers that can actually be processed through LTSF, although it certainly is not widely used as an industrial process. The point I was making, however, is that LTSF has considerable advantages as a low temperature process in the hospital setting. You do need to know the sterilization process that you are going to have available in the hospital, and choose your equipment and materials accordingly.

Question for Dr. Hoxey, Department of Health, U.K.

How do you protect those people who are associated with the operation of the sterilizer?

Answer by Dr. Hoxey, Department of Health, U.K.

In some respects, the situation is very similar to an ethylene oxide machine. There would be a ventilation system over the doors of the sterilizer which automatically would come on when the sterilizer is opened. I disagree in some ways with Dr. Bruch's comment about ethylene oxide and formaldehyde. I think that you can smell two parts per million (or lower) formaldehyde, but that's not true with ethylene oxide.

Question for Dr. Wood, Pfizer, Inc., U.S.A.

Is it possible to use the method of dry heat sterilization in hospitals and in smaller institutions?

Answer by Dr. Wood, Pfizer, Inc., U.S.A.

Yes, I would think it would be possible to use small batch oven sterilizers for items in hospitals, although it may not be practical because of the long times involved with the sterilization. In hospitals, you are looking for methods that utilize low temperatures and less exposure time. But for items like surgical instruments and knives, for example, small batch oven sterilizers are ideal because this method does not corrode the sharp edge.

Comment from Dr. Hoxey, Department of Health, U.K.

Just a comment about the preservation of edges on instruments in steam sterilizers. There has been a lot of debate in the U.K. as to whether sharp instruments do lose their edge after steam sterilization. There still is some debate about it, but I am aware that a number of hospitals very successfully steam sterilize sharp instruments with no problems of corrosion, provided that you buy instruments with quality metal and the right material. This gives a lot of advantages in terms of turnaround times.

Question for Dr. Bruch, St. Jude Medical Inc., U.S.A.

Have you done any studies on the resistance of *Mycobacterium tuberculosis* to 2% glutaraldehyde used on rubber surfaces? If so, what time was required to kill a population of one million organisms in the presence of organic matter?

Answer by Dr. Bruch, St. Jude Medical Inc., U.S.A.

I have not personally tested that particular organism, but my experience is that glutaraldehyde is not affected by the presence of organic matter.

Comment by Dr. Morrissey, Johnson & Johnson, U.S.A.

I would now like to turn the questions over to Dr. Prokopenko.

Comment by Dr. Prokopenko, All-Union Scientific Research Institute for Preventive Toxicology and Disinfection, Ministry of Health, U.S.S.R.

The first question is for Dr. Russell. Have you done any studies on the use of quaternary ammonium compounds in sterilization and disinfection?

Answer by Dr. Russell, Welsh School of Pharmacy, Wales

Yes, we have done many studies with quats with both gram-negative bacteria and gram-positive bacteria. With gram-positive bacteria we have been looking at wild-type strains and envelope mutants. There is no doubt that the mutants are considerably more sensitive than the wild-type strains. With *Staphylococcus aureus* there is often a slow degree of kill, which has been noticed by other investigators as well, but not really explained. MRSA strains apparently can show some resistance to quats, which is often on a plasmid. It has been suggested that with these organisms, the quats are actually selecting for antibiotic resistant strains.

There is no doubt that quats are not sporicidal at normal temperatures. However, they will effect the outgrowth of bacterial spores at concentrations equivalent to those inhibiting the growth of gram-positive bacteria, but they are certainly not sporicidal.

Question for Mr. Danielson, HCA Wesley Medical Center, U.S.A.

In the U.S.A., what guidelines do you follow in a hospital environment for protection against ethylene oxide?

Answer by Mr. Danielson, HCA Wesley Medical Center, U.S.A.

U.S. hospitals adhere to OSHA requirements for guidelines on personnel exposure. One ppm is a PEL, permissible exposure limit, for an 8 hour period. The action level is 0.5 and the one excursion limit is 5 ppm over a 15 minute period. We monitor on a frequency basis in order to make those determinations.

Comment by Dr. Prokopenko, All-Union Scientific Research Institute for Preventive Toxicology and Disinfection, Ministry of Health, U.S.S.R.

I have three questions for Dr. Addy. Firstly, what is the power consumption for low temperature plasma sterilization?

Answer by Dr. Addy, Johnson & Johnson Medical Inc., U.S.A.

It depends on whether the technique uses microwave or radio frequency or other electrical discharges. It also depends on the size of the chamber and the material that has been sterilized. People have reported power levels in the literature anywhere from 0.1 watts up to over 20 watts per cm^3.

Second Question for Dr. Addy, Johnson & Johnson Medical Inc., U.S.A.

What are the maximum and minimal exposures for sterilization by this method?

Answer by Dr. Addy, Johnson & Johnson Medical Inc., U.S.A.

It's been reported that to inactivate exposed spores which are not inhibited by any materials, you could sterilize within one or two minutes. I think that when we get to more practical loads or material, the time will be much longer.

Third Question for Dr. Addy, Johnson & Johnson Medical Inc., U.S.A.

What medical items are restricted from microwave sterilization?

Answer by Dr. Addy, Johnson & Johnson Medical Inc., U.S.A.

I don't know yet. In our lab we have sterilized a wide range of instruments, and I think other people have done the same thing. I would broaden the question beyond just microwave, because plasma can be generated by any number of means, and some applications that cannot be done with microwaves can be done with radio frequency discharges.

Comment by Dr. Prokopenko, All-Union Scientific Research Institute for Preventive Toxicology and Disinfection, Ministry of Health, U.S.S.R.

We have several questions for Dr. Ramkova. You said that different sterility assurance levels were used depending on the end use of the sterilized product. Is there a Soviet standard which tells you how to classify medical products? For example, what is the sterility assurance level required for a surgical wound dressing. Is there a variable scheme in the Soviet Union that would permit a sterility assurance level from anywhere in the area of 10^{-3} to 10^{-6}?

Answer by Dr. Ramkova, All-Union Scientific Research Institute for Preventive Toxicology and Disinfection, Ministry of Health, U.S.S.R.

There is no sterility assurance standard for wound dressing sterilization. However, the conditions of sterilization, in particular for the radioactive method, were calculated using the safety indicator or assurance indicator. The dressing materials are sterilized with an assurance indicator of 10^4 and obstetrics packages with 10^3. They do not calculate the assurance indicator for steam sterilization, particularly in hospitals.

Second Question for Dr. Ramkova, All-Union Scientific Research Institute for Preventive Toxicology and Disinfection, Ministry of Health, U.S.S.R.

Can you tell us more about sterilization with the combination of ethylene oxide and methyl bromide? Is this technique described in any published papers? How does the technique compare to other ways of using ethylene oxide?

Answer by Dr. Ramkova, All-Union Scientific Research Institute for Preventive Toxicology and Disinfection, Ministry of Health, U.S.S.R.

A mixture of ethylene oxide and methyl bromide in the ratio of 1:2.5 is used. The sterilization is conducted with the mixture, not sequentially by ethylene oxide and then by methyl bromide. There are official recommendations concerning this matter, as well as a lot of published papers which preceded the recommendations. I do not remember the exact titles of these papers now.

Question for Dr. Russell, Welsh School of Pharmacy, Wales

Could you comment on the efficacy of ethylene oxide against HIV?

Answer by Dr. Russell, Welsh School of Pharmacy, Wales

I don't have any information about this and I don't know if anybody else can comment. At the very end of my lecture, I started dealing with viruses but I was not suggesting that HBV and HIV were resistant viruses. I would assume HIV is sensitive to ethylene oxide but I have no personal information.

Comment by Dr. Prokopenko, All-Union Scientific Research Institute for Preventive Toxicology and Disinfection, Ministry of Health, U.S.S.R.

We have some additional questions for Dr. Russell. In your discussion you stated that bovine spongiform encephalopathy (BSE) prions are resistant and can withstand being processed at 132°C for up to six cycles. What is the nature of the biological assay for these agents, and what are the implications for pharmaceutical sterility tests?

Answer by Dr. Russell, Welsh School of Pharmacy, Wales

I regard myself as a bacteriologist although many people ask me about viruses. There have been reports from the United States that it requires up to six autoclaving cycles at 132-134°C to actually kill these particular organisms because they are so highly resistant. I know neither the mechanism of action nor whether there are implications for sterility testing.

Comment by Dr. Prokopenko, All-Union Scientific Research Institute for Preventive Toxicology and Disinfection, Ministry of Health, U.S.S.R.

How do you measure the biological assay?

Answer by Dr. Russell, Welsh School of Pharmacy, Wales.

They have to be put into an animal system of some sort; I believe into the appropriate brain tissue. It takes a long time, about a year, to develop.

Question for Dr. Russell, Welsh School of Pharmacy, Wales

Were reactivated glutaraldehyde-treated bacterial spores treated with alkaline-buffered or an acid-buffered glutaraldehyde and what were the conditions necessary for reactivation?

Answer by Dr. Russell, Welsh School of Pharmacy, Wales

Most of our work involved spores treated with alkaline glutaraldehyde, but we have done some work with acid-treated spores. We studied about half a dozen different types of *Bacillus* species. After exposure to a certain optimum level of sodium hydroxide, we found that the viable count had increased by a factor of about 10^2 even after a prolonged exposure to 10%. We postulated that the increase is due to the effect of sodium hydroxide on the outer layers of the spore, possibly rendering them more labile after glutaraldehyde treatment.

Comment by Dr. Prokopenko, All-Union Scientific Research Institute for Preventive Toxicology and Disinfection, Ministry of Health, U.S.S.R.

We have several questions for Dr. Graham concerning biological indicators. Were you able to identify the growth promoting factor in the standard recovery media or the inhibitory factor in the media that would explain the differences in efficiency?

Answer by Dr. Graham, American Sterilizer Co., Inc., U.S.A.

On the particular media that we examined and reported, we have not identified what the inhibitory or promotional factors were.

Question for Dr. Graham, American Sterilizer Co., Inc., U.S.A.

With regard to biological indicators used in hospitals, what is the minimum inoculation period for achieving the necessary results with these biological indicators?

Answer by Dr. Graham, American Sterilizer Co., Inc., U.S.A.

Different manufacturers have labeled different incubation times. The manufacturers are required to label incubation times. Guidelines were developed by the Food and Drug Administration for manufacturers to reduce the incubation time from the standard 7-day period. If manufacturers have not conducted that testing, the time should be listed as 7 days. Based on testing according to those guidelines, the shortest incubation times that I have seen on the market were 24 hours for steam sterilization and 48 hours for ethylene oxide.

Question for Dr. Graham, American Sterilizer Co., Inc., U.S.A.

You mentioned that a device called a BIER vessel is needed to certify biological indicators. I am sure that there are many people in the Soviet Union and in other countries who do not have this type of equipment. Can one certify or calibrate a biological indicator without the use of one of these BIER vessels?

Answer by Dr. Graham, American Sterilizer Co., Inc., U.S.A.

I think that it is possible to achieve that with steam sterilization. If one knows the Z-value of the organism, one can integrate the lethality times during the come-up and cool-down phases and compensate for the fact that it would not have a squarewave vessel. Because the Z-value is dependent on other factors, such as recovery media and heating medium, this is difficult but it could be done to some extent. For ethylene oxide or other chemical sterilants where you have multiple factors involved, I think it becomes too difficult because you can't integrate the physical equations as easily as you can with steam sterilization.

Question for Dr. Graham, American Sterilizer Co., Inc., U.S.A.

So, the best way is to obtain the resistance information from the manufacturer?

Answer by Dr. Graham, American Sterilizer Co., Inc., U.S.A.

I would say, obviously, to look for the certificate that comes with the biological indicator since this states all the performance parameters.

Question for Dr. Favero, Centers for Disease Control, U.S.A.

It would be very interesting to learn how the problem of the usage of syringes and other disposable medical products is solved in the West. Doesn't it complicate environmental problems? Is it possible in any way for infection to be carried out of the hospital? And are there reliable means for stopping infections? Are the means of preventing agents from leaving the hospital reliable?

Answer by Dr. Favero, Centers for Disease Control, U.S.A.

In the United States, the question of disposable syringes, as well as other medical waste, is driven primarily by emotional concepts and not necessarily by public health considerations. In the specific instance of the disposal of needles and syringes, the guidelines that existed for many years are still adequate.

According to the guidelines, the entire syringe is placed in a container that is not penetrable by needles. From this point, that container can then be transported directly to a landfill, or in some states, it is required that the process of decontamination be performed prior to disposal. Although hypothetically it would be possible to transmit infection from nondecontaminated needles and syringes, I am not aware of a single instance in the United States where an infection such as hepatitis or HIV has been transmitted from this type of medical waste to those individuals who have handled the waste.

Question for Dr. Young, Penn State University at Erie, U.S.A.

From your point of view, what is the present status of sterilization by formaldehyde in the United Sates and what about the future of alternative chemicals?

Answer by Dr. Young, Penn State University at Erie, U.S.A.

In the United States, formaldehyde is not used in hospitals as a gaseous sterilant, and to my knowledge, it is not used to any significant extent in industry. I doubt personally that we will see a switch from ethylene oxide to formaldehyde in the United States. I think, given the concerns in terms of toxicity, the chances of switching are extremely small. I do think, however, that in the next few years, some of the specialty sterilants that we have talked about will come into special segments of the ethylene oxide market.

Question for Dr. Jorkasky, Health Industry Manufacturers Association, U.S.A.

So much has been said, and with little scientific data, on the toxicity, mutagenicity or carcinogenicity of ethylene oxide in humans; does anyone have any information on the benefits of EO sterilization to humans?

Answer by Dr. Jorkasky, Health Industry Manufacturers Association, U.S.A.

HIMA has assembled information on the benefits of ethylene oxide. We've completed a list of devices that are life-saving and life-enhancing which rely on EO for sterilization. It is difficult to validate the statement that EO has played an important role in the decrease of nosocomial infections. I am not aware of any objective data that would quantify the role which EO has played. However, it is my opinion that EO has played an important role since it is used on devices such as flexible fiber optics that cannot be steam sterilized. I can't quantitate the degree of importance, but since EO has been used to produce sterile materials in both hospitals and in industry, it enjoys an important role. Industry is able to control the use of EO more so than in a hospital environment.

The one thing we found is, that if you tell people about the risks of EO and then you tell them about the benefits, by specifically saying it saves lives by making life-saving and life-enhancing products available, people are more favorably predisposed to the continued use of EO.

Question for Mr. Danielson, HCA Wesley Medical Center, U.S.A.

What is the rationale behind the composition and construction of the EO test pack used in your hospital? How many test packs do you use per day or cycle, and what is the frequency of failure of these packs?

Answer by Mr. Danielson, HCA Wesley Medical Center, U.S.A.

Rationale for the development of the pack came basically from AAMI. When we conducted a survey, we found that a number of hospitals were using various types of material in order to test sterilizers. The AAMI committee felt that it was extremely important to develop a standard method for hospitals. In our hospitals we recommend that they use either a quality assurance or routine test pack in every cycle. The quality assurance test pack consists of four towels, syringes, latex tubing, and a wrapper; a routine test pack consists of a BI within a syringe, wrapped in a towel, and then put in a peel pouch or nonwoven pouch.

Question for Mr. Danielson, HCA Wesley Medical Center, U.S.A.

In the context of using these packs, what would be a reasonable failure rate to expect?

Answer by Mr. Danielson, HCA Wesley Medical Center, U.S.A.

Over the last four years of testing our sterilizers on an every cycle basis, we've only had two positive BIs. We maintained our supplies in quarantine for 48 hours, and, therefore, we recalled and reprocessed all of those devices that had been processed in those loads. I would venture to say though, that in a lot of the hospitals, there are possibly one or two a year that grow and they probably would not be able to recall every item because not every hospital quarantines like we do.

Question for Dr. Addy, Johnson & Johnson Medical Inc., U.S.A.

When will your unit be commercially available? What is the concentration of hydrogen peroxide? Can free radical species with hydrogen peroxide be identified and quantified?

Answer for Dr. Addy, Johnson & Johnson Medical Inc., U.S.A.

I would like to keep within the spirit of this non-commercial conference and just note that it is probably premature to talk about when the unit will be commercially available. We are testing our system, looking at a number of alternatives, and when we are finished with the testing, we will decide about commercial introduction. The concentration of hydrogen peroxide is related to a specific part of design so I would rather not answer that. Lastly, free radical species can be identified by doing mass spectrometry measurements. One of the difficulties, however, is that the free radicals change very quickly, generally within one millisecond. As time goes on, one set of radicals is changing to more predominant types. It is more difficult, however, to quantitate them.

Comment by Dr. Morrissey, Johnson & Johnson, U.S.A.

Thank you very much. The last question is directed to me from Deputy Minister Kondrusev concerning my thoughts on sterilization in the 21st century. There are several things to comment on. The first is the area that Mr. Saylor spoke about, that is, the use of machine sources for radiation sterilization. I think that the need is heightened in more recent times due to concern about the environment. There are concerns, in some circles, about storage and handling of radioactive isotopes. Also, isotopes used in sterilization decay must be replenished and spent isotopes must be removed. Machine-generated x-rays, on the other hand, have the capability of processing the same type of bulky medical products and thus, is an attractive alternative. So that's one area under development within this timeframe. I personally expect to see major advances with x-rays over the next five years.

Secondly, some comment should be made on ethylene oxide, the chemical agent that received many questions during this conference. Many of us feel that ethylene oxide will be around for many years to come. It is a hazardous agent. If it was not a hazardous agent, it would not be a good sterilant. But it certainly can be used under proper conditions with proper safeguards for both the environment and people operating the sterilizer. It is a very unique chemical agent. Having said that, however, I believe that we are going to see specialty chemicals and special processes. These processes may not be as broad in their application as ethylene oxide, but will be designed for specific types of medical devices. Certainly we talked about plasma. There are other chemicals and mention was also made of hydrogen peroxide and chlorine dioxide. There are many agents that may have been looked at in the past but now need to be looked at again closely for specific niche applications.

From the manufacturing point of view, I think we are going to see more automated manufacturing and a lower extent of contamination (bioburden). As Dr. Whitby pointed out in his presentation, the lower the number of microorganisms, the less rigorous the sterilization process, the less damage to the product, and the less use of chemical agents in the environment. So I think that is very important. Probably the most fundamental thing of all, since we are all involved in this as part of our responsibilities, is the area of education and training. It has been 100 years or more since Lister and Pasteur elucidated the germ theory of disease. Yet we know that there are many people around the world today that are infected simply because of lack of hygiene and a lack of proper aseptic technique. We all need to be aware that it is not enough to have an effective sterilization process. We must ensure that the product is delivered to the patients in a sterile manner. This means improvements in sterile packaging and in education from the broadest perspective.

Concluding Remarks
General Chairman: Mr. A. Kondrusev
Deputy Minister of Health, U.S.S.R.

Concluding Remarks

Mr. A. Kondrusev

Deputy Minister of Health, U.S.S.R.

Dear colleagues, ladies and gentlemen. The Seventh International Conference on the Sterilization of Medical Products has finished its work. More than 250 people from 22 countries have participated. Scientists from ten countries have made presentations. I would like to emphasize the great importance of this conference for the development of healthcare throughout the world.

The conference included discussion of two major problems related to health care—nosocomial infection, its features, and ways to prevent it; and the sterilization of medical products as one of the most important means by which nosocomial, as well as other, infections can be fought and prevented. The characteristics and the causes of nosocomial infections in different types of hospitals were discussed. Data presented on the influence of various physical conditions and chemical substrates on microbial cells are of great interest. At the molecular level, this work can influence the direction of further research to develop new means and methods of sterilization and disinfection. Modern principles of monitoring and preventing nosocomial infections were discussed in a number of presentations. The importance of medical personnel training as a means of preventing and fighting nosocomial infections has been emphasized; as noted here, this training should start in the colleges and be continued into the hospital practice. The presentations concerning the epidemiological features and measures necessary for the reduction of morbidity and prevention of hepatitis B, hepatitis non A-non B, and Legionnaires' disease were especially important. Furthermore, presentations devoted to the methodological aspects of various sterilization processes as well as to new sterilization techniques were met with considerable interest. Of particular relevance were discussions on the use of low-temperature plasma, combination methods of sterilization using ionizing radiation and/or other physical and chemical means, and the status and ways of optimizing the traditional sterilization methods involving gas, steam, and air.

To facilitate the analysis of information and to improve the possibilities for the international exchange of medical products and sterilization facilities, it is very important to standardize the requirements for the sterilization of medical products. The discussion on standards received the interest it deserved. I think that it would be very useful to unify a number of the requirements which can be standardized. The majority of presentations in this area stressed the necessity of monitoring the outcome of sterilization processes and highlighted the development of biological and chemical indicators. A number of interesting presentations were devoted to the status of traditional steam and air methods of sterilization, and in particular, to showing the possibilities in optimizing the air method, in the efficient and safe usage of ionizing radiation, and in making the work of personnel in the centralized departments easier. Attention was given in presentations to the requirements for

safety when applying physical and chemical means of sterilization and to the need for protecting human health as well as the environment. The methodology and approaches to the study of the toxicologic effects of sterilizing agents were further discussed, and the specific recommendations for acceptable residual quantities were provided.

The presentations during these last few days were made by leading scientists and company specialists, individuals with the highest scientific and professional credentials. Their work will undoubtedly make an important contribution to the development of means for the nonspecific prevention of nosocomial infections. I regret that this conference is over. I wish all the participants further successes, good health, and well-being. I would like to thank, on your behalf, all those who served us here, including our interpreters. I would also like to thank Johnson & Johnson for their efforts in sponsoring this conference and for their commitment to future conferences in this area. All the best until the next meeting.

[1] These examples reflect federal laws in the United States

[1] This article appeared in Transplantation Proceedings, 1988, 20(6), Suppl. **8**; 7-11.

[2] A special paper included in the Proceedings because of its intrinsic interest and because of its importance in the prevention of infection.