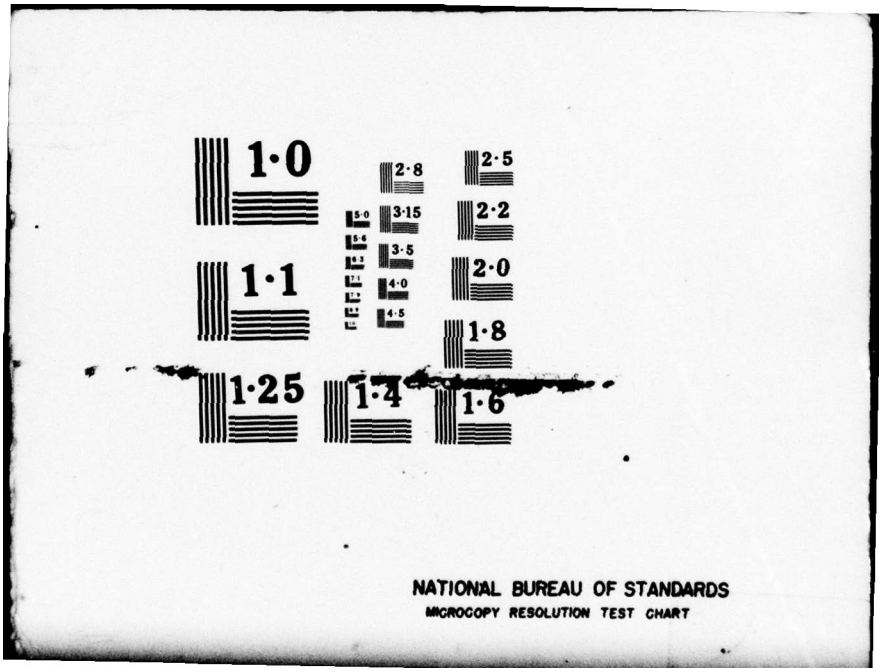
AD-A049 033 ARMY MEDICAL BIOENGINEERING RESEARCH AND DEVELOPMENT --ETC F/G 6/12
PROBLEM DEFINITION STUDY: PRODUCTION AND TESTING OF PYROGEN-FRE--ETC(U)
NOV 76 J J BARKLEY

UNCLASSIFIED USAMBRDL-TR-7612 NL

1 OF 1
AD
A049033

END
DATE
FILMED
2-78
DDC


1·0
1·1
1·25
1·4
1·6
2·8
3·15
3·5
4·0
4·5
5·0
2·5
2·2
2·0
1·8
NATIONAL BUREAU OF STANDARDS
MICROCOPY RESOLUTION TEST CHART

AD

TECHNICAL REPORT 7612

PROBLEM DEFINITION STUDY: PRODUCTION AND TESTING OF PYROGEN-FREE WATER UNDER FIELD CONDITIONS

JESSE J. BARKLEY, JR.

US ARMY MEDICAL BIOENGINEERING RESEARCH and DEVELOPMENT LABORATORY
Fort Detrick
Frederick, Md. 21701

NOVEMBER 1976

US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
FORRESTAL BUILDING
WASHINGTON, DC 20314

NOTICE

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Disposition

Destroy this report when it is no longer needed. Do not return it to the originator.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Date Entered)

(14) REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: USAMBRDL-TR-7612 | 2. GOVT ACCESSION NO. | 3. RECIPIENT'S CATALOG NUMBER (9)

4. TITLE (and Subtitle): (6) PROBLEM DEFINITION STUDY: PRODUCTION AND TESTING OF PYROGEN-FREE WATER UNDER FIELD CONDITIONS

5. TYPE OF REPORT & PERIOD COVERED: Technical Report. August [illegible] — November 1976

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s): (10) JESSE J. BARKLEY, JR.

8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS: US Army Medical Bioengineering Research & Development Laboratory, ATTN: SGRD-UBG, Fort Detrick, Frederick, MD 21701

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: (16) 6.27.78.A, 3S762778A838, 00/046, (17) 00

11. CONTROLLING OFFICE NAME AND ADDRESS: US Army Medical Research & Development Command, ATTN: SGRD-RO, Washington, DC 20314

12. REPORT DATE: (11) November 1976

13. NUMBER OF PAGES: 30

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office): (12) 28p.

15. SECURITY CLASS. (of this report): UNCLASSIFIED

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Activated carbon	In vitro testing	Production
Assay	Ion exchange resin	Pyrogen-free water
Bioassay	Limulus Amebocyte Lysate (LAL)	Pyrogens
Bio-filter	MUST Pharmacy Module	Rabbit pyrogen test
Endotoxins	Ozone contact chamber	Submicron filter

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The production and testing of pyrogen-free water under field conditions, such as in the Pharmacy Module of Army Mobile Unit Self-Transportable Hospitals (MUST), presents serious problems because of volume requirements, power constraints, and quality control requirements. This report defines these problems and makes recommendations concerning: the development of a treatment system to produce pyrogen-free water from potable water; the evaluation of an in vitro biological test to determine apyrogenicity in the field; and evaluation of a handling and storage system for field-produced pyrogen-free water.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Date Entered)

404838

19. KEY WORDS (Cont'd)

Testing
UV sterilization

EXECUTIVE SUMMARY

The production and testing of pyrogen-free water under field conditions, such as in the Pharmacy Module of MUST-equipped hospitals, presents serious problems because of resource constraints, and volume and quality control requirements. Historically, distillation has been used as the method for the preparation of pyrogen-free water. This method was excluded, however, since it is exceptionally taxing on the limited resources of the Pharmacy Module. The assay for pyrogenicity has involved the use of rabbits in a lengthy *in vivo* test. The use of rabbits in the field would be impractical due to added support requirements.

This report recommends the development of a treatment system consisting of ion-exchange, carbon absorption, UV irradiation and ozonation to produce pyrogen-free water under the resource constraints imposed upon the Pharmacy Module. The proposed assay method for pyrogenicity will utilize the *Limulus Amebocyte Lysate* test, a rapid, sensitive *in vitro* test for pyrogenicity. The report also recommends a handling and storage system and an outline for a feasibility study.

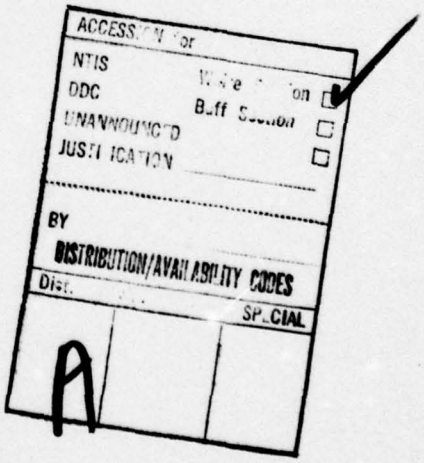


TABLE OF CONTENTS

EXECUTIVE SUMMARY 1

INTRODUCTION 5

OBJECTIVE 6

APPROACH 6

FINDINGS 6

Pyrogens 6
Assay of Pyrogens 9
Removal of Pyrogens 13
Storage 14

DISCUSSION AND CONCLUSIONS 15

RECOMMENDATIONS 17

LITERATURE CITED 19

APPENDIX A 27

APPENDIX B 29

DISTRIBUTION LIST 30

INTRODUCTION

In response to a request from US Army Medical Research and Development Command[1] (USAMRDC) a work unit entitled "Development of a Water Purification Unit and Field Test for Pyrogen Free Water" was established. The initial effort under this work unit has been to conduct a problem definition study concerning the production and testing for pyrogen-free water in the MUST Pharmacy Module. The capability to produce sterile, pyrogen-free water in a field situation would have a significant impact on increasing the tactical flexibility and reducing logistical support requirements of MUST-equipped medical units. The need for this type of unit is best exemplified by historical data indicating that some 154,000 1-liter bottles of 0.9 percent Sodium Chloride Injection solution were shipped to one theater of operation in Southeast Asia during the period of January through December 1969.[2] The approximate gross weight of these shipments was 681,000 pounds and they required approximately 25,700 cubic feet of cargo space. The weight of the medicinal agent involved was 3,000 pounds (0.4%); the weight of the water was 336,000 pounds (49.3%); and the weight of the glass containers and shipping material was 342,000 pounds (50.2%). If plastic instead of glass containers were used, the weight of the water would have been 88% of the total weight. It is apparent from these data that considerable resources have been expended in shipping processed water throughout the world.

The requirements for the water treatment unit were established in 1966.[3] The Pharmacy Module is required to provide "immediate and effective pharmaceutical support to combat casualties" and it has as its primary objectives the rendering of efficient service in the receipt, preparation, compounding, dispensing, and storage of drugs and the production, storage and dispensing of pyrogen-free sterile water for intravenous injections, drug compounding and wound irrigation. The Pharmacy Modules will be used in both the Evacuation Hospital and the Combat Support Hospital, with greater utilization in the Evacuation Hospital.

Various efforts have been made since 1968 to develop a water purification unit capable of producing pyrogen-free water. Among these were efforts by USAMRDC (at US Army Medical Bioengineering Research and Development Laboratory[4] [USAMBRDL] and commercial contractors (Beckman Instruments, Inc.).[5,6]

The results of these efforts have been unsatisfactory either because the units produced water that failed the USP test for pyrogenicity or they exceeded the power and water production constraints in the requirements document.[3]

OBJECTIVE

The objective of this study is to compile and evaluate technical information on the field production and testing of pyrogen-free water in order to identify currently-available methods for the production and assay of pyrogen-free water and, where knowledge gaps or inadequacies exist, to recommend research to develop methods for the field production and/or testing of pyrogen-free water.

APPROACH

To accomplish the objective, a literature search was conducted. The computer libraries Medline, Medlars and the Defense Documentation Center were searched using the key words: pyrogen-free water; pyrogens; production of pyrogen-free water; testing/assay of pyrogens; and endotoxins. Index Medicus and Chemical Abstracts were also reviewed. The pertinent articles found in the various searches were obtained and reviewed in detail. Personal communications with knowledgeable individuals were also utilized. Contacts were made with manufacturers of pyrogen-free water production units and trade literature was perused.

FINDINGS

Pyrogens

Nature and Sources: The word "pyrogen" means heat-producing. All pyrogens bring about an increase in body temperature, i.e., a febrile response, when introduced into mammals by any route other than ingestion.

For the most part pyrogens are derived from certain metabolic by-products of various microorganisms, such as bacteria, molds, some yeasts, viruses[7] and algae.[8] Some drugs such as epenephrine, methylene blue, dinitrophenol and some antibiotics have been reported to be pyrogenic.[7] Certain chemicals when injected intramuscularly (IM) result in a temperature elevation. Included in these are sulfur, certain steroids, native dextrans, tetrahydro-β-naphthylene, LSD, methyl cellulose, kaolin, 5-hydroxytryptophan, pentachlorophenol[9] and polysaccharides obtained from such diverse sources as mouse tissue, chick embryos, tangerines and Bryonia roots.[10]

Since all but the biological pyrogens can be removed from water by standard water treatment practices only the pyrogens of biological origin will be considered in this study. While the direct injection, either intraperitoneally (IP), IM, subcutaneously (SC), or intravenous (IV), of viable gram-positive or gram-negative bacteria, viruses or fungi will elicit a febrile response, the fever is due primarily to "infection"

rather than a "pyrogenic response."[11] These viable microorganisms are normally removed by standard sterile filtration procedures and are rarely a problem in parenteral drugs or irrigation waters. Because of this ease of removal of viable organisms, only those substances formed by the destruction of viable organisms will be considered.

Pyrogens will pass through most bacterial filters. They are of microbial origin, produced by a large number of organisms.

In 1950, Harkness found that filtrates from certain mold cultures would, upon IV injection, produce fevers in rabbits.[12] The most effective filtrates were those of lysed cells. In 1974, Rotta and Schleifer determined that material produced from lysed gram-positive bacteria when injected IV into rabbits resulted in pyrexia.[13] The causative agent was found to be a mucopeptide. Many authors have reported that a substance derived from the cell wall of gram-negative bacteria causes fever when injected into rabbits.[14-17] These substances derived from the three different classes of microorganisms are routinely referred to as endotoxins. The most potent of the three are those derived from gram-negative bacteria.[9] Because of the high toxicity of the gram-negative bacterial endotoxin the remainder of this report will almost exclusively deal with the endotoxins derived from this class of bacteria. It should be pointed out that the virulence of an organism and its endotoxin potency are not related.[18]

Chemical Structure - Endotoxins: The endotoxin, accounting for 30-40% of the cell wall of gram-negative bacteria, consists of a lipopolysaccharide.[10] This lipopolysaccharide has two components with distinct physicochemical character: a heteropolysaccharide and a covalently linked lipid, termed Lipid A. Lipid A is made up of acylated glucosamine disaccharides which are interlinked by pyrophosphate bridges.[19] It was found that the pyrophosphate bridges interlink β-1,6-linked glucosamine disaccharide units, which are esterified by lauric, palmetic and 3-D-myristoxymyristic acid and which are substituted at the amino groups by 3-D-hydroxymyristic acid.[20] The polysaccharide from most gram-negative bacteria consists, generally, of glucose, galactose, and mannose. Pentoses, hexosamines heptoses, octonic acid derivatives and deoxy sugars are frequently found. The most common aminoacids associated with endotoxin preparation, though possibly not constituents of the toxin, are aspartic acid, glutamic acid, cysteine, valine, lucines, alanine, serine, arginine and lysine.[21] The toxic center of the lypopolysaccharide resides in the Lipid A fraction.[19] The polysaccharide is not essential for toxicity, however, it is essential for solubility of the complete compound.

Physico-Chemical Properties - Endotoxins: Lipopolysaccharides are soluble in water and may be recovered from water as opalescent crystals.[10] Endotoxins possess a high molecular weight in the order of 4.8×10^6;[12] the molecule contains 45.5% carbon, 6.1% hydrogen and, 1.2% nitrogen

and 0.06% phosphorous; and it is moderately hydroscopic.[22] Most purified endotoxins are thermostable.[9] The particle size range for the lipopolysaccharides, derived from various gram-negative bacteria, is 1-50 nm.[23]

The lipopolysaccharide can be liberated from the intact cell by sonication; by mild extraction in a hypotonic solution; by EDTA; and by treatment of the cell with trichloroacetic acid, aqueous phenol, aqueous pyridine, diethylene glycol and phenol-chloroform-petroleum ether.[10] The lipid fraction can be removed from the polysaccharide by extraction with methanol and hydrolysis with weak HCl, and dissolved in chloroform.[24]

Endotoxins can be chemically detoxified by oxidizing and acetylating agents. Detoxification can also be accomplished by lithium aluminum hydride and transesterfication with boron trifluoride. The cleavage of the ester-bound carboxylic acids is also effective. Both alkaline and acidic hydrolysis destroys most endotoxins. Endotoxins may be detoxified by nonionic detergents.[21]

Health Implications - Endotoxins: Pyrogenic reactions to endotoxins can be regularly elicited in man, rabbits and certain other mammals. Fevers are lower and slower in onset after IM or SC inoculation[18] than those in response to IV injection of endotoxin and endotoxin contaminated material. Doses of as little as 0.1 to 0.3 µg/kg of endotoxin elicit a febrile response in rabbits, with man requiring 5 µg to 2 mg/kg to respond.[18]

In minimal dosages endotoxin will induce an elevation from the normal temperature of the recipient without significantly affecting body functions. In higher dosages the effects of endotoxin include fever, changes in leukocytes and platelets, production of hemorrhagic lesions in viscera and skin, hypotension, adrenal and renal necrosis, and metabolic changes involving electrolytes and glucose.[14]

Acute endotoxin poisoning leads to irreversible shock and death. The preceding symptoms are weakness, lethargia, hypo- and hyperthermia, cyanosis, profuse diarrhea and convulsions. Internal effects are gastrointestinal hemorrhage, decrease in venous blood return and cardiac filling attenuating increases and decreases in total peripheral vascular resistance and blood pooling in the vascular beds of the spleen.[25] The type and severity of the patient's injuries can enhance susceptibility to endotoxins.

The febrile response seems to be the result of endotoxin action on the blood leukocytes, lung macrophages, and phagocytic cells of liver, spleen and lymph node.[26] Under the influence of the toxin the cells release a protein, termed endogenous pyrogen.[27,28] The endogenous pyrogen (EP) then acts directly on the central nervous system (CNS).[29] In the CNS, the areas most susceptible are the anterior lobe of the hypothalmus and the preoptic area of the brain.[30]

EP has molecular weight of 1.3×10^4. It is primarily derived from the peripheral blood monocytes and from neutrophils,[31] both of which belong to the class of corpuscular elements known as leukocytes. Unlike endotoxins EP is non-dialyzable[32] and it is heat labile being deactivated at 70°C[33] and can be inactivated when incubated with pronase or trypsin.[34]

Repeated sublethal injections of endotoxin result in resistance, i.e., tolerance, not only to the fever-producing effect of endotoxins, but to their lethal effects.[14] This resistance is specific for endotoxin. Repeated injection of EP does not result in tolerance.[35] This tolerance will disappear 2 or 3 days after the final injection of endotoxin.[14]

There exists some protective effects of endotoxin. An enhancement to the resistance to infection has been found by various authors.[36,37] Zweifach found that rats made tolerant to endotoxin were able to resist traumatic shock.[38,39] The prior administration of a single dose of endotoxin protected mice from a lethal dose of irradiation.[40] The above three effects are related to the method utilized by the body to clear endotoxin.

After the invasion of endotoxin into the subject animal the reticuloendothelial system (RES) is mobilized to remove or inactivate the substance. The RES is a physiologic unit without anatomic localization. This cell group includes the endothelial and reticular cells of the spleen, lymph and hemolymph glands, the Kupffer cells of the liver, the reticulo-endothelium of bone marrow, and the clasmatocytes.[40] After the introduction of endotoxin, the material is rapidly taken up by the circulating leukocytes. The endotoxin then is accumulated in the RES and is completely cleared from the circulation within a short time.[41] Rosen found that calcium and other divalent cations would suppress the inactivation of endotoxin, indicating that certain anions normally present in the circulation were required for inactivation.[42] Lysozyme and other basic polypeptides combine with endotoxin, in vitro and possibly in vivo, to render it inactive.[40] By blocking the RES with colloids or other materials the effect of endotoxin is enhanced.[14,25]

Assay of Pyrogens

Bioassay: Ever since the introduction of inoculation there has occurred a common side effect, fever. It is now recognized that this was caused by gram-negative bacterial endotoxins. The medical profession, even before the discovery of the cause of the fever, has attempted, since the late 19th century, to test the inoculating agent to determine its ability to cause "injection fevers."

The earliest attempts to assay pyrogens were coupled with efforts to determine their chemistry and to find methods for their destruction or removal from solutions. Over the years numerous physical, chemical, and

microbiological tests were proposed. All of these tests tended to detect bacterial rather than pyrogen contamination. The need for an official pyrogen test was recognized in the early 1940's. Joint efforts were undertaken by the Food and Drug Administration, the National Institutes of Health, and pharmaceutical manufacturers to establish a standardized test. The culmination of these efforts resulted in the publication of the rabbit pyrogen test in the XIIth Revision of the US Pharmacopeia (USP) in 1940.

The present USP[43] test consists of the IV injection of 10 ml of the test material per kilogram of body weight into an ear vein of each of three mature rabbits weighing not less than 1.5 kg. The rabbits must be housed in individual cages, free of disturbances, and with a uniform constant ambient temperature. The temperatures of the three rabbits must be recorded 40 minutes prior to injection and for 3 hours after injection. If no rabbit shows an individual rise in temperature of 0.6°C or more above its respective control temperature, and if the sum of the three individual maximum temperature rises do not exceed 1.4°C, the product meets the requirements for the absence of pyrogens. If the two temperature criteria are not met, the test must be repeated using five different rabbits. The criteria for non-pyrogenicity in this case is a sum of less than 3.7°C or an individual rise of less than 0.6°C. The animals cannot be used more frequently than once every 48 hours nor prior to 2 weeks following a positive reaction.

Other bioassay methods exist, each of which is purported to be an improvement over the USP test. Among these are the chick embryo lethality test, the mouse lethality test of Pieroni[44] and others. None of these alternative bioassays are recognized by the USP, and no efforts are being made to include them in it.

The significant points favoring the rabbit test include the fact that the threshold response of the rabbit and man is correlated and established.[16] It is the only test accepted by law. The USP test is not particularly expensive after the initial cost of installing the cages and the temperature controlled room and purchasing the initial supply of rabbits. In 1972 the cost of an individual test was $4.60.[45]

There exist, however, numerous drawbacks in using the rabbit pyrogen test. Kobayashi has reported that rabbit temperature is notably affected by the quantity and time of feeding.[46] Noise, vibration and other emotional disturbances, including the type and position of restraints, will cause rabbit temperature fluctuations.[47] The degree of restraint can alter the rabbit's sensitivity to threshold pyrogenicity.[48] False negative results may be obtained when rabbits are used to test medicaments containing calcium gluconate, procaine, sodium citrate, strophanthin, chlorpomazine, and certain hypnotics and anesthetics, since these compounds lower body temperature.[15]

Because of toxic or other adverse effects, rabbits may not be used to test the pyrogenicity of products containing toxoids, viral and bacterial vaccines and other derivatives of biologically-active substances.[49] Finally, Martin and Marcus[50] reported that when testing a known non-pyrogenic material, predetermined by IV injection into human subjects, the rabbits yield 17% false positives.

In Vitro Assay: In order to avoid the problems of rabbits, various in vitro tests have been devised. In 1956 Bang reported a bacterial disease of the Limulus polyphemus (Horseshoe Crab). Gram-negative bacteria, native to the shore waters, infected the limulus and caused extensive intravascular clotting and death.[51] The blood of the limulus contains only one type of cell - the amebocyte. When withdrawn from the limulus the blood immediately clots, this clot shrinks spontaneously and a liquid phase appears. This liquid material has the capability of gelling when exposed to bacterial endotoxin. Levin and Bang used this information to begin studying the relationship of endotoxin and limulus amebocytes.[52] As a refinement in the technique, the liquid from the spontaneous clotting was collected from the mechanically disrupted amebocytes themselves. The amebocyte lysate was found to be more consistent in reacting to the presence of endotoxin. Further refinements have since been made, yielding a compound sensitive to endotoxin in the order of 10^{-6} mg/ml.[53]

The Limulus Amebocyte Lysate (LAL) assay method consists of mixing the test material with the lysate and incubating the mixture at 37°C in a water bath for 60 minutes. The formation, at this time, of a firm gel is taken, by most investigators, as a positive indication of endotoxin.[54] Some investigators continue the test for 24 hours at room temperature using as end points the appearance of a firm gel, increased viscosity or granules as a positive test.[55,56]

The LAL assay for endotoxins is, by far, the most sensitive (10^{-6} mg/ml), rapid (0.5 - 1 hour), specific[54] and simple of all of the pyrogen assays developed to date. Unlike other tests, the LAL method requires the least material per test; these being three test tubes (positive and negative control and the sample), 0.1-0.1 ml of sample and 0.3-0.6 ml of reagent, and a 37°C water bath. The analyst requires only rudimentary skills to perform the LAL assay.

This test is the only quantitative assay method available. The time required for formation of a firm gel is directly proportional to concentration.[53,57,58] For all vaccines and in other parenterals where the use of rabbits is precluded, the LAL is applicable.

The LAL assay has some shortcomings. According to Cooper the test is pH and temperature dependent.[59] Agitation of the test tube may irreversibly destroy a positive result.[55,59] The lysate is heat sensitive itself;

refrigeration, freezing or lyophilization is necessary for stability.[59] Various chemicals can inhibit the test; included in these are heprin,[60] nucleic acid substances, cysteine, sulfhydryl reagents and benzyl alcohol.[61] McAuley reported that certain surfactants and pyropylene glycol inhibit gel formation.[55]

Elin and Wolff report that thrombin and thromboplastin cause false positive results.[54] Levin found that the LAL is generally inhibited when undiluted plasma or serum is assayed; such as would be the case when LAL is used to diagnose endotoxemia.[62] This was confirmed by some other authors;[63,64] however, others disagree.[58,65,66] The consensus agreement is that the LAL is generally inapplicable for diagnostic purposes when testing whole blood or serum.

Other in vitro tests have been devised. Included among these are bacterial counts, electrical conductivity, ultraviolet absorption at 2650 nm and various wet chemical and color tests.[15] None of these have been universally applicable. In 1967 Heilman and Bast devised an in vitro test based on the inhibition of macrophage migration by endotoxin.[67] While this test requires considerable time, materials and expertise, it could be used as a replacement for the USP test. Another alternative assay was developed by Kritzingen. This test requires live rabbits and involves the count of total leukocytes. It avoids most of the problems of the USP test but it is dependent on live animal care and maintenance.[68] By use of membrane filtration Marcus was able to determine pyrogenicity indirectly through bacterial counts.[69] Good correlation was established between this test and the rabbit test; however, if the bacteria are lysed before testing, as could be the case in antibiotic preparations, his test would be the least acceptable of any of the in vitro assays.

Comparison - Rabbit Pyrogen Test vs LAL Assay: When comparing the USP rabbit pyrogen test to the LAL test it must be kept in mind that the LAL is 100 times more sensitive than the USP assay. Recognizing this, Eibert found that if the LAL was allowed to extend only over 30 minutes "perfect correlation between USP and Limulus was obtained."[70] Cooper et al., reported in their comparison of rabbit and LAL assays that the quantities required for the rabbit assay are at least 10 times greater than for LAL. They also found LAL gave positive results for 1.0 pg/ml of E. coli and 0.1 pg/ml of Klebsiella endotoxin, while the rabbit assay yielded negative results at these concentrations.[71] Rojas-Corona et al., compared the LAL assay with the immunoassay test of Skannes and the Epinephrine skin sensitivity assay method of Thomas and Kass.[72] They confirmed that the LAL test was "the most sensitive test available for the detection of endotoxin." In 1974 Ronneberger and Stark came to the same conclusions as the preceding citations.[73]

Removal of Pyrogens

From Water: Various researchers have devised many techniques to render water free of pyrogens. The common denominator in all these efforts has been the use of potable water as starting material. Among the methods used are ion-exchange resins, carbon columns, distillation, ultrafiltration, chemical treatment, reverse osmosis (RO), and ultraviolet (UV) and ozone treatment.

In 1968 Budyeszewski reported that a pyrogenic 10% d-manitol solution could be made apyrogenic when passed through columns containing both anionic and cationic-exchange resins.[74] Among the resins used were Amberlite resins IR-120 and IRA-400, and Zeolite-225. Later in 1968, Budziszewski found that cationic exchangers were more effective than anionic resins.[75] He found that at pH 6.2 a 240 mm column of Zeolite-225 would render the manitol solution apyrogenic. He also found that these columns could easily be regenerated. An earlier work by Whittet found just the opposite, that anion exchange resins were more effective than cation exchangers.[76] The water source for Whittet's work was ordinary tap water. In 1975, Holcombe reported the use of mixed bed ion exchangers employed by a large pharmaceutical manufacturer.[77] This method was an integral part of a larger treatment system.

In their review article in 1956, Berger et al., reported that carbon columns were effective in removing pyrogens from water and parenteral solutions.[15] They also reported that carbon has the disadvantage of removing some components from the parenteral along with the pyrogens. Recent experience with carbon columns indicates that they tend to allow bacterial growth when idle and may actually contribute to pyrogenicity.[78,79] This may also be true for ion exchange resin columns.

The most widely used method for pyrogen removal is distillation. Many authors have claimed that distillation is the only practical method for the removal of pyrogens from water. Among these are Castor,[7] Dawids,[79] DeRoos,[23] and Karamian.[80] In 1976 Karamian reported the development of an all glass still that would effectively produce pyrogen-free water. Included in the design of the still were baffles to prevent the entrainment of contaminated water droplets in the stream and air filters to prevent the introduction of airborne contaminants.

Ultrafiltration has been reported to be applicable for the production of pyrogen-free water. In 1975, Rusmin and Deluca challenged various in-line membrane filters with gram-negative bacteria contaminated pyrogenic solutions.[81] They found that the filtrate was apyrogenic until the bacteria were killed by antibiotics. As a direct remover of pyrogens, membrane filtration has no applicability, this being confirmed in various review articles.[15,79]

Various chemical treatments have been proposed to remove pyrogens from water and parenteral preparations. Menczel[82] and Cherkin[83] found that hydrogen peroxide (H_2O_2) was effective. Berger et al., report that acid and alkalies, quinones, and oxidizing agents, including H_2O_2 could effectively remove pyrogenicity from contaminated solutions.[15] A difficulty in the chemical treatment lies in the removal of the chemical and its reaction products after the completion of the treatment process.

Recently, reverse osmosis (RO) has obtained some prominence as a treatment system for the processing of pyrogenic fluids. Dawids,[79] Hindman et al.,[8] and Madsen[84] report the effectiveness of RO. However, as Dawids points out, the RO system must be used continually to maintain sterility. When RO is properly used, it will fulfill the requirements for the production of high quality water for pharmaceutical uses.

Recently, a system utilizing UV and ozone has been developed that is claimed to be capable of delivering pyrogen free water.[85] Neither was found to be effective alone, but when used in combination pyrogenic water is rendered apyrogenic within 2 hours after initial contact.

Other techniques have been tried to generate pyrogen-free water. These include simple heating, enzyme treatment additives and filtration through asbestos, various clays,[15] vapor compression distillation,[84] and aluminum hydroxide absorption.[86] Heating and enzymes are ineffective. Certain additives such as polyvinylpyrolidine and Chlor-Trimeton have a potential degree of success, but may have other, adverse, health effects. The filtration techniques were also ineffective. Vapor compression distillation is effective but the treatment unit is large. Richter, et al., found that the addition of aluminum hydroxide to protein solution would remove pyrogens through absorption and precipitation, but the effectiveness in large columns of water is doubtful.[86]

From Hardware: The removal of pyrogens from glassware, needles, syringes, and other medical hardware can easily be accomplished by using either dry heat, extended autoclaving or chemical treatment followed by rinsing with pyrogen-free water.[7] USP recommends the heating of these items to 250°C for 30 minutes. Other sources vary the time or temperature but essentially concur with the USP method. Strong alkali on strong trisodium phosphate solution will remove pyrogens from glass and hardware.

Storage

After the production of pyrogen-free water it must be stored until used. USP recommends that pyrogen-free water and end items containing pyrogen-free water be packaged in sealed glass containers. There are no recommendations for the storage of pyrogen-free water prior to final packaging. The Pharmacy specification document[3] anticipates the storage of end items in plastic bags; it also makes no recommendations for storage prior to packaging.

In their review article, Berger et al., noted that, on standing, some pyrogenic solutions become apyrogenic.[15] However, the reverse is most likely to occur. Distilled, sterile water has been found to be pyrogenic after standing for a period of time.[17] Holcombe reported that pyrogen-free water stored in sealed stainless steel containers, heated to 160-190°F, would remain apyrogenic.[76] The apparatus developed by Karaminan uses a closed glass carboy with a filtered air inlet.[80] The key to maintaining apyrogenicity in pyrogen-free water is the denial of access of airborne materials. The added safeguard of heat sterilization is also desirable.

DISCUSSION AND CONCLUSIONS

A pyrogen is a chemical or biological substance that causes a rise in temperature, i.e., febrile response, when intravenously introduced into a subject mammal. Endotoxins derived from the cell walls of lysed gram-negative bacteria are the primary sources of pyrogenicity in parenteral drugs. Most endotoxins, being high molecular weight lipo-polysaccharides, are thermally stable, water soluble and have particle sizes of 1 to 50 nm. Other contributors to febrile responses include viable gram-negative and gram-positive bacteria, viruses, fungi (both air and waterborne), material from the cell walls of gram-positive bacteria and numerous other organic, inorganic and bio-chemicals.

In order to determine the pyrogen-free condition of water produced in a field purification unit, a suitable field test is required. Many tests were found in the literature. The USP rabbit test and the Limulus Amebocyte Lysate (LAL) test were found to be the two most likely candidate tests.

The rabbit test is at this time the only USP recognized test and rabbit responses to pyrogens are threshold correlated to human responses. The disadvantages of the rabbit test are: maintenance of test animals; a minimum of 3 hours for performance; the requirement for at least three rabbits per test; and the requirement for skilled personnel to administer the sample and record temperature changes. The advantages of the LAL test include commercial availability, speed, simplicity, sensitivity and specificity. It can be used under MUST field conditions. Disadvantages of the LAL test are; it is specific for endotoxins alone and will not detect the presence of other substances that can induce a hyperthermic response in man; it is subject to false positive reactions when used in the presence of certain non-pyrogenic serum proteins; and it will yield false negative results under the influence of changes in temperature and pH and excess motion. In conclusion, the only available test for pyrogens found in the literature that is practical in the MUST Pharmacy is the Limulus Amebocyte Lysate test. The USP rabbit test is impractical due to its requirement for live animals. If the USP rabbit test were used, another

module in the MUST system would be required to house the rabbits. This module would need an environmental control system to maintain temperature and humidity, and it would have to be sound-proof to avoid upsetting the rabbits by the noises of battle and transports. Trained animal handlers and a veterinarian would have to be added to the staff.

The LAL test requires little space, no special equipment and no unusual skills or personnel. In short, the LAL test is the only test recommended for use in the MUST.

While being the most effective historical method for producing pyrogen-free water, distillation is, in terms of resources, the most costly system available. For each gallon of water produced, 8 gallons of cold water are consumed for cooling. To produce 100 gallons of pyrogen-free water in 16 hours the electrical requirement would be 18.2 kilowatts and 0.9 gallons of cooling water per minute would be needed. The USP does not specify distillation for the production of pyrogen-free water.

The best system for generating pyrogen-free water in the MUST Pharmacy module would consume less than 0.5 gallons per minute (gpm) of cold potable water (3 gpm available) and need no more than 3 kw (10 kw available). The system would treat potable water, however, a mixture of potable and non-potable water should not be precluded. The system would be wall mounted or free standing, have a minimum of moving and glass parts and consumables.

The proposed system would begin by pumping source water from a stainless steel reservoir through a coarse filter to remove gross particulate contamination. This would be followed by an activated carbon column to remove both organics and any chlorine present. The water would then pass through two mixed bed deionizers for endotoxin and ion removal. The effluent from this would pass through a second carbon column to safeguard against resin throw from the deionizers. Following the second carbon column would be a 0.22 μm filter to trap any viable bacteria. From the filter effluent the water would then pass through an ozone contact chamber equipped with ultraviolet lights. This would be followed by a membrane filter and an enclosed 50 gallon, heated, stainless steel storage tank equipped with a biofilter outlet valve. Two pumps are required for this system: one to pump the water from the untreated reservoir to the coarse filter; and one to recycle the treated water. The purpose of the recycle pump is to keep the system continually wet to preclude the growth of bacteria on any of the filters or in any of the columns. The recycle pump would also be used to backflush the mixed bed deionizers and the carbon columns.

Quality control of the system would start at the first carbon filters with the effluent tested every 8 hours for the absence of free chlorine. The second carbon column and the UV-ozone unit will be supplied with

resistance meters, set to sound an alarm if the resistance falls below 400,000 ohms. The final effluent will be checked once each 4 hours for pyrogenicity.

Experience of Armour Pharmaceutical Company,[74] for example, indicates that when treating chlorinated tap water the carbon column should last at least 3 months (treating approximately 2 million gallons of water). The deionizers were found to be able to treat 29,000 gallons of water before regeneration (each had a flow rate of 1500 gallons per hour). The membrane filters were changed once a month.

The above system would require replacement filters, activated carbon, resin, bio-filters, UV lamps, and a continuous supply of dry compressed air. This system could be constructed from commercially available components.

The requirement of the specification document[3] to produce pyrogen-free water from non-potable waters compounds the complexity of the system, significantly reduces the probability of a successful development, will increase both end item and R&D costs greatly, and appears to be a requirement that was not well thought out. Water of potable quality will be required for numerous other hospital functions and will be routinely available. Therefore, the pyrogen-free water system should be developed to upgrade potable quality water to pyrogen-free water quality only.

RECOMMENDATIONS

The Limulus Amebocyte Lysate (LAL) test should be evaluated for use as a field test for pyrogen-free water under the conditions expected to exist in the MUST Pharmacy Module.

A treatment system consisting of the various filters, columns, and contact chambers detailed in this report should be evaluated to determine the system's chemical and engineering feasibility for the production of pyrogen-free water from chlorinated potable water under the constraints of the MUST Pharmacy Module.

The system proposed for the storage and handling of field-produced pyrogen-free water (plastic bags) should be evaluated to determine the ability of that system to maintain the pyrogen-free condition of the water under the conditions expected to exist in the MUST Pharmacy Module.

The resource requirements and research program recommended for the implementation of these recommendations are shown in Appendixes A and B.

LITERATURE CITED

1. Letter, HQDA, SGRD-SDM, 21 June 1976, Subject: Purified Water Production Unit, MUST Pharmacy Module, 816.00.009.

2. Letter, HQDA, CDCMR-O, 21 February 1971, Subject: Department of the Army Approved Qualitative Material Development Objective for a Pyrogen Identifier, Rapid Response.

3. HQDA, Medical Unit Self-Contained, Transportable - Statement of Requirements for Pharmacy Equipage, 18 April 1966; Amended 6 June 1966.

4. Personal Communication, A.Ismach, Chief, Engineering Division, USAMBRDL, Ft Detrick, MD (October 1976).

5. Beckman Instruments, Inc. System for Field Preparation of Electrolytic Solutions, Vol. II. System Evaluation. Contract DA-49-193-MD-2834 (July 1968).

6. Donner, W., "A Small Rotary Vapor Compression Still for the Production of Pyrogen Free Water," Report Contract DA-49-193-MD-2261 (January 1963).

7. Castor, G.B., "Pyrogens: Their Nature and Measurement in Parenteral Preparations," Bull. Parenter. Drug Assoc., 23(1):23-28 (Jan-Feb 1969).

8. Hindman, S.H., M.S. Favero, L.A. Carson, N.J. Pelersen, L.B. Schonbergen and J.T. Solano, "Pyrogen Reactions During Haemodialysis Caused by Extramural Endotoxin," Lancet, 2:732-734 (18 October 1975).

9. Palmer, C.H.R., "Pharmaceutical Aspects of Pyrogens in Hospital and Industry," In: Pyrogens and Fever, Wolstenholme, G.E.W. and J. Birch (eds), (Ciba Foundation Symposium) Churchill Livingston, Edinburgh (1971).

10. Work, E., "Production, Chemistry and Properties of Bacterial Pyrogens and Endotoxins," In: Pyrogens and Fever, Wolstenholme, G.E.W. and J. Birch (eds), (Ciba Foundation Symposium) Churchill Livingstone, Edinburgh (1971).

11. Matsen, J.M., "Pathogens, Their Sources and Control," Part I, Hosp., J.A.H.A., 48:71-76 (Dec 1974), Part II, Hosp., J.A.H.A., 49:63-65 (Jan 1975).

12. Harkness, W.D., W.L. Loving and F.A. Hodges, "Pyrexia in Rabbits Following the Injection of Filtrates of Typical Mold Cultures," J. Am. Pharm. Assoc., 39:502-504 (1950).

13. Rotta, J. and K.H. Schleifer, "Pyrogenic Activity of Bacterial Mucopeptides," J. Hyg. Epidemiol. Microbiol. Immunol., 18(1):50-59 (1974).

14. Bennett, I.L. and L.E. Cluff, "Bacterial Pyrogens," Pharm. Rev., 9:427-475 (1957).

15. Berger, A., G.D. Elenbogen and L.G. Ginger, "Pyrogens," Adv. Chem. Ser., 16:168-197 (1956).

16. Cotui, F.W. and M.H. Schrift, "Production of Pyrogen by Some Bacteria," J. Lab. Clin. Med., 27(5):569-575 (1942).

17. Favero, M.S., N.J. Petersen, L.A. Carson, W.W. Bond and S.M. Hindman, "Gram Negative Water Bacteria in Hemodialysis Systems," Health Lab. Sci., 12:321-334 (1975).

18. Atkins, E.A., "Pathogenesis of Fever," Physiol. Rev., 40:580-646 (1960).

19. Rietschel, E.Th., "Chemical Structure and Biological Activity of Endotoxins (Lipopolysaccharides) and Lipid A," Arch. Pharmacol., 287:73-84 (1975).

20. Luderitz, O., C. Galanos, V. Lehman, M. Nurminen, E.Th. Rietschel, G. Rosenfelder, M. Simon and O. Westphal, "Lipid A, Chemical Structure and Biological Activity," J. Infect. Dis., 12(Suppl):517-519 (1973).

21. Nowotny, A., "Molecular Aspects of Endotoxic Reactions," Bact. Rev., 33(3):72-98 (1969).

22. Castor, G.B., N. Kantor, E. Knoll, J. Blakely, J.K. Nielsen, J.O. Randolph and A. Kirshbaum, "Characteristics of a Highly Purifield Pyrogenic Lipopolysaccharide," J. Pharm. Sci., 60:1578-1580 (1971).

23. DeRoos, R.L., "Purifying Water for Special Hospital Uses," Hospitals, 46:100-104 (1972).

24. Leive, L., V.K. Shovlin and S.E. Mengenhagen, "Physical, Chemical, and Immunological Properties of Lipopolysaccharide Releases from Escherichia coli by Ethylenediaminetetraacetate," J. Biol. Chem., 243(24):6384-6391 (1968).

25. Rosen, F.S., "The Endotoxins of Gram-Negative Bacteria and Host Resistance," N. Engl. M. Med., 264(18):919-923 (1961).

26. Bodel, P., "Studies on the Mechanism of Endogenous Pyrogen Production III. Human Blood Monocytes," J. Exp. Med., 140:954-965 (1974).

27. Allison, E.S., W.I. Cranston, G.W. Duff, R.H. Luff and M.D. Rawlins, "The Bioassay of Human Endogenous Pyrogen," Clin. Sci. Mol. Med., 45:449-458 (1973).

28. Atkins, E. and W.B. Wood, "Studies on the Pathogenesis of Fever. II. Identification of an Endogenous Pyrogen in the Blood Stream Following the Injection of Typhoid Fever," J. Exp. Med., 102:499-516 (1955).

29. Feldburg, W. and P.N. Saxena, "Mechanism of Action of Pyrogens," J. Physiol., 211:245-261 (1970).

30. Cooper, K.E., W.I. Cranston and A.J. Hanour, "Observations on the Site and Mode of Action of Pyrogen in the Rabbit Brain," J. Physiol., 191:325-337 (1967).

31. Dinanello, C.A., N.P. Goldin and S.M. Wolff, "Demonstration and Characterization of Two Distinct Human Leukocytic Pyrogens," J. Exp. Med. 139:1369-1381 (1974).

32. Lorber, D., M. Tenenbaum, S. Thurston, G.W. Gander and F. Goodale, "The Fate of Circulating Leukocytic Pyrogen in the Rabbit," Proc. Soc. Exp. Biol. Med., 137:896-901 (1971).

33. Snell, E.S., F. Goodale, F. Wendt and W.I. Cranston, "Properties of Human Endogenous Pyrogen," Clin. Sci., 16:615-626 (1957).

34. Bodel, P. and H. Miller, "Pyrogen from Mouse Macrophages Causes Fever in Mice," Proc. Soc. Exp. Biol. Med., 151:93-96 (1976).

35. Atkins, E. and E.S. Snell, "Pyrogenic Properties of Various Tissue Extracts in the Rabbit," J. Physiol. 169:47p-48p (1963).

36. Cohn, Z.A. and S.J. Morse, "Functional and Metabolic Properties of Polymorphonuclear Leukocytes. II. Influence of Lipopolysaccharide Endotoxin," J. Exp. Med., 111:689-704 (1960).

37. Raskova, H. and J. Vanecek, "Pharmacology of Bacterial Toxins," Pharm. Rev., 16:1-45 (1964).

38. Zweifach, B.W. and L. Thomas, "Relationship Between Vascular Manifestations of Shock Produced by Endotoxin, Trauma and Hemorrhage. I. Certain Similarities Between Reactions in Normal and Endotoxin Tolerant Rats," J. Exp. Med., 106:385-401 (1957).

39. Zweifach, B.W., B. Benacennaf and L. Thomas, "Relationship Between Vascular Manifestations of Shock Produced by Endotoxin, Trauma and Hemorrhage. II. Possible Role of the Reticulo-endothelial System in Resistance to Each Type of Shock," J. Exp. Med., 106:403-414 (1957).

40. Rosen, F.S., "The Endotoxins of Gram-Negative Bacteria and Host Resistance," N. Engl. J. Med., 264(19):980-985 (1961).

41. Cremer, N. and D.W. Watson, "Influence of Stress on Distribution of Endotoxin in RES Determined by Fluorescein Antibody Technic," Proc. Soc. Exp. Biol. Med., 95:510-513 (1957).

42. Rosen, F.S., R.L. Skarnes, M. Landy and M.J. Shear, "Inactivation of Endotoxin by Humoral Component. III. Role of Divalent Cation and Dialyzable Component," J. Exp. Med., 108:701-711 (1958).

43. U.S.Pharmacopeia, XIX, Monograph - Pyrogen Test, p. 613 (1976).

44. Pieroni, R.E., E.J. Broderick, A. Bundeally and L. Levine, "A Simple Method for the Quantitation of Submicrogram Amounts of Bacterial Endotoxin," Proc. Soc. Exp. Biol. Med., 133:790-794 (1970).

45. May, C.N. and R. Wiseman, "Establishment and Operation of a Hospital Pyrogen Assay Program," Am. J. Hosp. Pharm., *29:684-686* (1972).

46. Kobayashi, M., Folia. Pharmacol., Japan, 47:75 (1975).

47. Braun, H.A. and V. Klein, "Some Problems of Pyrogen Testing," Bull. Parenter. Drug Assoc., 14(5):9-15 (1960).

48. Dane, J.G. and G.A. Mogey, "Rabbit Responses to Human Threshold Doses of a Bacterial Pyrogen," J. Pharm. Pharmacol., 6:325-332 (1954).

49. Personeus, G.R., "Pyrogen Testing of Biologicals and Small Volume Parenterals," Bull. Parenter. Drug Assoc., 23(5):201-207 (1969).

50. Martin, W.J. and J. Marcus, "Studies on Bacterial Pyrogenicity. III. Specificity of the 'USP' Rabbit Pyrogen Test," Appl. Microbiol., 12(6):483-486 (Nov 1964).

51. Bang, F.B., "A Bacterial Disease of Limulus Polyphemus," Bull. Johns Hopkins Hosp., 98:325-351 (1956).

52. Levin, J. and F.B. Bang, "The Role of Endotoxin in the Extracellular Coagulation of Limulus Blood," Bull. Johns Hopkins Hosp., 115:265-274 (1964).

53. Yin, E.T., C. Galanos, S. Kinsky, R.A. Bradshaw, S. Wessler, O. Luderitz and M.E. Sanmiento, "Picogram-Sensitive Assay for Endotoxin: Gelation of Limulus polyphemus Blood Cell Lysate Induced by Purified Lyposaccharides and Lipid A from Gram-Negative Bacteria," Biochim. Biophys. Acta., 261:284-289 (1972).

54. Elin, R.J. and S.M. Wolff, "Nonspecificity of the Limulus Amebocyte Lysate Test: Positive Reactions with Polynucleotides and Proteins," J. Infect. Dis., 128(3):349-352 (Sep 1973).

55. McAuley, R.J., R.D. Ice and E.G. Curtis, "Quality Control and Drug Analysis - The Limulus Test for In Vitro Pyrogen Detection," Am. J. Hosp. Pharm., 31:688-691 (Jul 1974).

56. Levin, J. and F.B. Bang, "Clottable Protein in Limulus: Its Localization and Kinetics of Its Coagulation by Endotoxin," Thromb. Diath. Haemorrh., 19:186-197 (1968).

57. Cooper, J.F., H.D. Hockstein and E.B. Seligman, "The Limulus Test for Endotoxin (Pyrogen) in Radiopharmaceuticals and Biologicals," Bull. Parenter. Drug Assoc., 26:153 (1972).

58. Longensen, J.H., H.F. Caravajal, B.E. Chips and R.F. Smith, "Rapid Detection of Gram-Negative Bacteriemia by Use of the Limulus Endotoxin Assay," Appl. Microbiol., 26:38-42 (1973).

59. Cooper, J.F., "Principles and Applications of the Limulus Test for Pyrogen in Parenteral Drugs," Bull. Parenter. Drug Assoc., 29(3): 122-130 (May-Jun 1975).

60. Sullivan, J.D. and S.W. Watson, "Inhibitory Effect of Heparin on the Limulus Test for Endotoxin," J. Clin. Microbiol., 2(2):151 (1975).

61. Kobayashi, M., T. Morimoto and M. Yamamoto, "Studies on the Gelation Reaction of Limulus Lysate (Pre-Gel). III. Influences of Various Substances on the Gelation Reaction of Limulus Lysate," Yakugaku. Zasshi., 95(6):720-727 (1975).

62. Levin, J., P.A. Tomasulo and R.S. Osen, "Detection of Endotoxin in Human Blood and Demonstration of an Inhibitor," J. Lab. Clin. Med., 75:903-911 (1970).

63. Martinez-G, L.A., R. Quintiliani and R.C. Tilton, "Clinical Experience on the Detection of Endotoxemia with the Limulus Test," J. Infect. Dis., 127:(1):102-105 (1973).

64. Stumacher, R.J., M.J. Kovnat and W.R. McCabe, "Limitations of the Usefulness of the Limulus Assay for Endotoxins," N. Engl. J. Med., 288(24):1261-1264 (1973).

65. Levin, J., T.E. Poore, N.P. Zauber and R.S. Osen, "Detection of Endotoxin in the Blood of Patients with Sepsis Due to Gram-Negative Bacteria," N. Engl. J. Med., 283(24):1313-1326 (Dec 1970).

66. Reinhold, R. and J. Fine, "A Technique for Quantitative Measurement of Endotoxin in Human Plasma," Proc. Soc. Exp. Biol. Med., 137:334-340 (1971).

67. Heilman, D.H. and R.C. Bast, "In Vitro Assay of Endotoxin by the Inhibition of Macrophage Migration," J. Bacteriol., 93(3):15-20 (1967).

68. Kritzinger, E.E. and B.J. Meyer, "Temperature and Leukocyte Response as Tests for Pyrogens in a Calcium Gluconate Solution for Injection," S.A. Med. J., 42:1145-1149 (Nov 1968).

69. Marcus, S., "Determination of Pyrogens Using Membrane Filtrations," Bull. Parenter. Drug Assoc., 18:18-24 (1964).

70. Eibert, J., "Pyrogen Testing: Horseshoe Crabs vs. Rabbits," Bull. Parenter. Drug Assoc. 26(6):253-260 (Nov-Dec 1972).

71. Cooper, J.F., F. Levin and H.N. Wagner, "Quantitative Comparison of In Vitro and In Vivo Methods for the Detection of Endotoxin," J. Lab. Clin. Med., 78(1):138-148 (1971).

72. Rojas-Corona, R.R., R. Skarnes, S. Tamakuma and J. Fine, "The Limulus Coagulation Test for Endotoxin, A Comparison with Other Assay Methods," Proc. Soc. Exp. Biol. Med., 132:599-601 (1969).

73. Ronneberger, V.H. and J. Stark, "Comparison of the Limulus and Pyrogen Tests in Rabbits," Arzn.-Forsh., 24(6):933-934 (1974).

74. Budziszewski, A., "Sorption of Pyrogenic Substances in Sodium and Chloride Cycles," Arch. Immunol. et Ther. Exper., 16:688 (1968).

75. Budziszewski, A., "On the Usefulness of Some Cation Exchangers for Sorption of Pyrogenic Substances in the Sodium Cycle," Arch. Immunol. et Ther. Exp., 16:850-854 (1968).

76. Whittet, T.D., "The Action of Ion Exchange Resins on Pyrogens," J. Pharm. Pharmac., 8:1034-1041 (1956).

77. Holcombe, J.C., "Pyrogen Free Water - Our Most Important Ingredient," Bull. Parenter. Drug Assoc., 29(2):153-159 (May-Jun 1975).

78. Personal Communication with Dr. Stephen A. Schaub, USAMBRDL, Ft Detrick, MD (Sep 1976).

79. Dawids, S.G., "Large Scale Production of Sterile, Distilled Water for Hospital Dialysis," Acta. Med. Scand., 193:387-392 (1973).

80. Karamian, N.A., "High-Purity, Bacteria-Free, Endotoxin-Free Water," Am. Lab., pp. 24-28 (Mar 1976).

81. Rusmin, S. and P.P. DeLuca, "Effect of Antibiotics and Osmotic Change on the Release of Endotoxin by Bacteria Retained on Intervenous Inline Filters," Am. J. Hosp. Pharm., 32:378-380 (Apr 1975).

82. Menczel, E., "A Note on the Depyrogenization of Infusion Solutions by Hydrogen Peroxide," J. Am. Pharm. Assoc., 40(3):175-176 (1951).

83. Cherkin, A., "Destruction of Bacterial Endotoxin Pyrogenicity by Hydrogen Peroxide," Immunochemistry, 12(6-7):625-627 (Jul 1975).

84. Madsen, R.F., B. Nielsen, O.J. Olsen and F. Raaschou, "Reverse Osmosis as a Method of Preparing Dialysis Water," Nephron, 7:545-558 (1970).

85. Personal Communication with Jack D. Zeff, Westgate Research Corp. (17 Sep 1976).

86. Richter, R., J. Lajos and I. Thury, "The Production of Pyrogen-Free Immunoglobulins," Annales Immunologiae. Hung., 160(0):377-381 (1972).

APPENDIX A

RESOURCE REQUIREMENTS

Man-Months of Effort (by discipline)		
Chemistry		4.5
Physical Science Technician		7.5
Engineering Technician		1.0
Statistician		0.4
Administration		0.1
	Total	13.5
Funding Requirements ($000)		
Supplies: Laboratory consumables		2.2
Equipment: Cartridge holder system; UV-ozone system		10.3
Travel: Visits to other laboratories; meetings		1.0
Salaries, benefits, base support		58.5
	Total	72.0

APPENDIX B

FY 78 PROGRAM SUMMARY

DISTRIBUTION LIST

Project No. 3S762778A838/00/046

No. of Copies	
5	US Army Medical Research and Development Command ATTN: SGRD-PL Washington, DC 20314
12	Defense Documentation Center ATTN: DDC-PCA Alexandria, Virginia 22314
1	Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, Texas 78234
2	USAMBRDL Technical Library