SEVENTH ANNUAL REPORT

CHEMICAL CORPS BIOLOGICAL LABORATORIES

1 July 1953

YF-1850

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REPORT SERIES NO. 7
1 July 1953

SEVENTH ANNUAL REPORT

of the

CHEMICAL CORPS BIOLOGICAL LABORATORIES

(Fiscal Year 1953)

This document contains 126 printed pages.

CHEMICAL CORPS BIOLOGICAL LABORATORIES
Camp Detrick, Frederick, Maryland

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I. INTRODUCTION

The Seventh Annual Report of the Chemical Corps Biological Laboratories was written primarily for the purpose of presenting the status of various end-items under development at this installation. The reviews in this report are arranged according to major field: agents for man, animals, and crops; protection; and dissemination. In addition, a brief discussion is presented of some of the more important areas of supporting research. The report also contains a condensed review of the administrative activities during the fiscal year, such as organizational changes, fiscal matters, construction, and personnel.

Among the major accomplishments during FY 1953 has been the adoption as standard-type of two anticrop agents, butyl 2,4-dichlorophenoxyacetate (LNA) and 2,4,5-trichlorophenoxyacetate (LNB), and one munition for dissemination of wheat rust spores carried on feathers (the M115 bomb). Design criteria and guidance have been furnished the USAF in the development of an anticrop bomb bay spray tank. Assistance was also furnished in testing and evaluating its performance. Several other items under development are approaching the stage where consideration soon will be given to their adoption as standard-types. These include: (1) Bacillus anthracis. (2) Coxiella burnetii. (3) a toxoid for Clostridium botulinum, type A, (4) a new vaccine for rinderpest, (5) stem rust of rye, (6) an ampoule containing ethylene oxide, (7) a BW detection kit, (8) the E61 bomb, (9) the E86 bomb, (10) the E99 bomb, (11) the E4 marine mine, (12) the XB14B submarine mine, and (13) the E77 bomb.

LEROY D. FOTHERGILL, M.D.
Director

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SECTION II - TECHNICAL STATUS

A. ANTIPERSONNEL AGENTS

TABLE I. DEVELOPMENTAL STATUS OF BW ANTIPERSONNEL AGENTS

Screening	Laboratory Phase	Pilot Plant Phase	Standard Types
Malleomyces mallei	Pasteurella pestis	Bacillus anthracis	Brucella suis (AB1)
Malleomyces pseudomallei	Psittacosis virus	(Bacterium tularense)**	Coxiella burnetii *
Mycobacterium tuberculosis	Bacterium tularense	Venezuelan equine encephalomyelitis virus	
Corynebacterium diphtheriae		Brucella melitensis	
Enteric pathogens		Botulinum toxin, type A	
Rickettsia prowazeki			
Rabies virus			
Poliomyelitis virus			
Smallpox virus			
Coccidioides immitis			
Histoplasma capsulatum			
Nocardia asteroides			

* Adoption under review by the Chemical Corps Technical Committee

**In transition from laboratory to Pilot Plant

1. BACILLUS ANTHRACIS (N)

a. HISTORICAL

Extensive laboratory research and pilot plant development were carried on during World War II, which led to the design and construction of a large-scale production plant for this agent at Vigo, Indiana. This plant had been test-run with a simulant, Bacillus globigii (BG), and was preparing for production of Bacillus anthracis when the war terminated. During the postwar period, practically all development effort on the offensive aspects of this agent ceased, and the small effort that was expended was primarily for the development of an effective vaccine for use in man. This low degree of effort on N was in accord with program guidance, e.g., N was seventh in order in the priority list for development of antipersonnel agents prepared by the Joint Chiefs of Staff (September 1951), and major emphasis was directed to the first five items. There has been a recent renewal of interest in N for a number of reasons including the revision in service emphasis from debilitating to lethal antipersonnel agents and a reappraisal of the BW potentialities of N because of (1) successful trials at sea in OPERATION HARNESS, (2) demonstration of the marked influence of particle size on the number of spores required to infect, and (3) observation that antibiotic therapy following aerosol exposure to N was effective only as long as treatment continued, i.e., death was delayed but not prevented. Since April 1953 the development of a liquid suspension of N has been proceeding under an overriding priority by direction of the Chief Chemical Officer, in order that the development testing of a 750-lb. cluster of E61 bombs filled with this agent may be completed by 1 March 1954 and a service-tested agent-munition combination be available by 1 July 1954.

b. LABORATORY DEVELOPMENT

During World War II several media were developed. The first which received extensive study was based primarily on corn steep liquor. This was found to have several deficiencies, among them: (1) the commercially available corn steep liquor was quite variable in composition, (2) toxic materials were present which required removal, and (3) spores of reduced virulence were produced. Research led to the formulation of a medium of known composition for N which in turn led to the development of practical media for large-scale production based upon pepticase (a tryptic digest of casein) or other peptones combined with cerelose, yeast products, thiamin, and salts. As an outgrowth of postwar development of practical media for Brucella suis, Brucella melitensis, and Pasteurella pestis, an acid partial hydrolysate of casein is now recommended instead of the tryptic digest. Laboratory-grown cultures (100 ml volumes grown at 34 C on a shaker for 20 to 30 hours) produce approximately 1.25×10^9 spores per ml.

Three different practical methods for producing effective cell-free immunizing antigens have been developed and tested in rabbits, guinea pigs, monkeys, and sheep, with satisfactory evidence of immunity. A pharmaceutical manufacturer, under contract, is adapting these developments to large-scale production of vaccine.

d. DEVELOPMENT OF PROCESSES FOR DRYING

No extensively tested process for the drying of this agent is now available but effort in this area is underway on a priority basis. Fragmentary evidence exists indicating that spores of N can be dried without loss by the following methods: (1) selected solvents, (2) vacuum drying, and (3) freeze-drying. Critical sizing is required of the products obtained from each of these methods. Spray drying has been successfully applied to the spore simulant, BG, but not yet to N because of delays encountered in modifying the dryer to meet safety requirements and the higher priority of process development of the liquid suspension of N. The current effort in the development of a drying process for N will investigate (1) solvent drying, (2) freeze-drying, and (3) spray drying, in that order of priority.

e. PILOT PLANT STUDIES

During World War II the pilot plants produced about 8,000 gallons of concentrate assaying 40 x 10^9 spores per ml. Until very recently, very little effort was expended in the offensive aspects of the development of this agent in the postwar period. This minor effort was directed largely to studies of the concentration of spores by froth flotation. Concentration has been accomplished by this method on a laboratory scale, but the operation has not yet been successfully transferred to pilot plant equipment.

In 1953 a detailed new process recommendation was made for the production of this agent. Included are procedures for the preparation of inoculum control of quality of inoculum, preparation of a production medium, fermentation, concentration, antolysis and preservation of agent, filling into munitions, and storage. The principal characteristics expected of the agent produced by this process are described. This process is designed to be adaptable to the X-201 facility, with minimal alterations.

Piloting of the process began in June 1953. It may be well to emphasize that existing pilot plant facilities are not adequate for this purpose and in many respects the equipment, and therefore, the unit operations, differs from that at the X-201 plant. For example, the centrifuges at the pilot plant are a batch type as contrasted with the intermittent discharge

type at X-201. The size and proportions of the tanks differ, making doubtful the transferability of aeration data. These difficulties should be remedied when building PP-1, which contains a 1-cell counterpart of equipment at X-201, becomes available. This building, originally scheduled for occupancy in December 1951, will not become available until 31 July 1953 at the earliest, and will require several months of testing and shake-down thereafter before full use can be made of it with pathogens.

f. AEROBIOLOGICAL STUDIES

Certain characteristics of the N currently being produced by the pilot plant seem evident from the relatively small numbers of tests which it has been possible to perform thus far. The aerosol stability of N is high (decay rates are 0 to 6 per cent per minute) and not significantly different from that of the simulant, BG. Recoveries from aerosols 1 or 3.5 minutes old vary from 15 to 88 per cent. There are indications that (1) recoveries from aerosols prepared from different cultures may vary appreciably, (2) the presence of 1 per cent phenol may cause a marked reduction in recovery, and (3) recovery may decrease with increase in relative humidity to values greater than 70 per cent (partially an increased physical loss). Upon exposure of guinea pigs to aerosol clouds of N, LD_{50} values of about 30,000 inhaled spores have been obtained which agrees with British data. The available data with monkeys indicates an LD_{100} of about 5,000 spores which is about one-tenth the value reported from recent work in Porton. More extensive information is being obtained on the stability and infectivity of aerosols of N and correlation of the characteristics of aerosol clouds of N and BG.

A broad assessment program is underway in the large test sphere to determine the characteristics of N and BG when liquid suspensions of those organisms are disseminated from the E61 and the M114 bombs. This program is too current for any more detailed comment than to indicate that aerosol clouds of N infective for monkeys and guinea pigs can be produced by explosive dissemination from these munitions.

The scope of field trials which can safely be performed with N is seriously circumscribed because of the stability of the organism. Using the Mark I bomb (a predecessor of the M114) to disseminate a liquid suspension of N, the British were able to infect and kill sheep at Gruinard Island in 1943 and guinea pigs and monkeys at OPERATION HARNESS in 1949. At the present time, discussions of the feasibility, scope, objectives and site of future field trials with N are underway in various echelons of the Department of Defense.

2. BACTERIUM TULARENSE (UL)

a. STRAIN SELECTION AND GENETIC STUDIES

More than 50 strains varying in pathogenicity from avilulent to highly virulent have been collected from various locations throughout the world. A highly virulent culture, designated SCHU, originally isolated from a human ulcer, was used for most experimentation prior to 1950. Since that time, homogeneous colony types have been isolated from the highly virulent but heterogeneous SCHU strain. The variant presently being used, SCHU SD, possesses a low dissociation index and has a respiratory LD_{50} of less than 5 cells for the guinea pig and less than 30 cells for the monkey. The high virulence of this strain for man is substantiated by the high incidence of infection among vaccinated and nonvaccinated laboratory workers. Antibiotic resistant strains (e.g., resistant to 10 mg streptomycin) have been developed which, in preliminary study, retain the virulence of the parent strain.

b. DEVELOPMENT OF PRACTICAL GROWTH MEDIA AND STORAGE STABILITY

Two media have been developed which are suitable for large scale production of this organism: one is based upon hydrolyzed casein (MCPH medium), and the other based upon yeast. Viable cell populations of 25-35 x 10^9 per ml are attainable in MCPH medium after 12 hours incubation at 37 C with aeration. Continued incubation does not result in an increase in viable population, and studies are underway to determine the limiting chemical and metabolic factors involved. These cultures retain full virulence and 80-100 per cent viability after storage at 5 C for 4 weeks. Approximately 50 per cent viability is maintained after storage for 12 weeks; however, after 8 weeks storage, virulence (determined by interaperitoneal injection) decreases. This decrease in virulence can be eliminated if cultures are frozen and stored at -50 C.

c. DEVELOPMENT OF PROCESSES FOR DRYING

Only fragmentary studies on the drying of this agent have been performed. Reduction of virulence subsequent to freeze-drying has not been observed, in contrast to the experience with Pasteurella pestis and Brucella suis. Sufficient concentrated cells to support pre-pilot plant scale drying operations will not be available until the agent is produced in the pilot plant.

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d. PILOT PLANT STUDIES

Prior to 1953, 19 batches of an avirulent strain of this agent were grown primarily as a service to other divisions and agencies. Two batches of the virulent strain SCHU SD were grown in the fall of 1952. A program for the pilot plant development of this agent was initiated as a first priority item in 1953. A detailed experimental operating procedure and recommended process for piloting was prepared. The initial pilot plant runs were not successful. Although the cause for this failure has as yet not been determined, both the inoculum development and medium preparation procedures are suspect and being reviewed. Because of the overriding priority granted by the Chief Chemical Officer to the pilot plant development of Bacillus anthracis, the program on Bacterium tularense will be delayed.

e. AEROBIOLOGICAL STUDIES

The stability of aerosol clouds of this organism is dependent upon humidity, with best recoveries obtained at the higher humidities. Average death rates of suspensions prepared for recent field trials have been 19, 25, and 6 per cent per minute at relative humidities of 35, 60, and 90 per cent, respectively. No significant effect on aerosol stability was observed in cells stored for 11 to 47 days at 5 C.

Comparison of intraperitoneal and respiratory (Henderson apparatus) virulence titrations indicate an average LD_{50} of 1.2 cells for the former and 2.4 cells for the latter. The narrow 95 per cent confidence limits observed indicate that these titrations can be performed with a high degree of precision. The respiratory LD_{50} is seem to be in close agreement with the intraperitoneal LD_{50}.

The respiratory LD_{50} for strain SCHU SD is less than 5 organisms for the guinea pig and less than 30 organisms for the monkey. This latter figure has recently been confirmed in tests employing 36 monkeys.

Dissemination from munitions of liquid suspensions of this organism in the large test sphere has indicated that poor aerosol recoveries (0.1 to 0.22 per cent in the period 1 to 2 minutes after explosion) are obtained with the M114 bomb even at 95 per cent RH. In addition, guinea pig infections were low and fell off rapidly as the cloud aged, e.g., 46 per cent mortality on exposure between 1 to 2 minutes after explosion and 18 per cent mortality between 16 and 17 minutes after explosion. Preliminary tests indicate that superior results are obtained when dissemination is accomplished by a generator. Recoveries as high as 19 per cent are obtained 2 minutes after aerosolization, and 100 per cent of the exposed guinea pigs are killed at cloud ages of at least 34 minutes. Initial tests using the E61 bomb indicate that it approaches the generator in efficiency and is, therefore,

far superior to the M114 for dissemination of this organism. Definitive comparisons of the generator and E61 remain to be performed.

Preliminary information is available from a recent successful large-scale field trial in which 400 E61R4 bombs were placed on an 8,000 x 8,000 foot grid at a density of 3/8 of a cluster per square mile. (This is actually a more disperse pattern than is presently attainable by dropping from a plane.) A steady state was reached in the resultant aerosol cloud as judged by a constant level of deaths in the exposed guinea pigs (30-45 per cent). This steady state was attained 3200 to 4800 feet from the upwind edge of the munition layout and continued to between 8600 to 9000 feet. The cloud decayed from this point onward but still was capable of killing 13 per cent of the guinea pigs at a distance 4840 feet from the downwind edge of the minition layout. Of the 49 monkeys exposed in this test, 41 died of tularemia and 6 of the 8 survivors were infected as indicated by positive laboratory tests.

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3. PASTEURELLA PESTIS (Le)

a. STRAIN SELECTION AND GENETIC STUDIES

Over 100 strains are available at the Naval Biological Laboratory and 30 at the Chemical Corps Biological Laboratories. Strains of choice among the virulent strains (i.e., highest virulence together with greatest genetic stability) are those derived from H-16 (139-J.) which was originally isolated from a human case in India. The intraperitoneal LD_{50} for mice of this strai is 0.9 - 5.7 organisms. Several avirulent strains are also available for study.

A genetic study of morphological types recoverable from strains of Pasteurella pestis has revealed four types considered to be most important. Of these, two are smooth and two are rough types. One of the smooth types, the so-called "rugose cream" type, is more virulent than the others. Dissociation in liquid media has been found most frequently after 7 days incubation if cultures were unshaken, incubated at 26 C, and possessed a pH above 7.0. A strain (V-6 which is ultimately derived from H-16) has been tentatively selected, which evidences a satisfactory degree of uniformity and genetic stability as well as a high degree of virulence. The correlation study between virulence and colonial morphology is being continued employing strain V-6, with all detectable variants isolated and titrated in mice in an effort to establish the criteria of differentiation and to select a strain with improved stability. Strains resistant to dyes and antibiotics have been recovered.

b. DEVELOPMENT OF PRACTICAL GROWTH MEDIA

Two practical media have been developed, one whose basic component is a fish hydrolysate and the other a casein hydrolysate (DCPH medium). Of these, the latter is receiving current emphasis. One of the governing limitations in achieving high yields of viable cells has been found to be the relatively faster rate of glycolysis as compared with the oxidation of the resultant organic acids. Success has been achieved in overcoming this limitation by (1) the incremental addition of glucose and (2) by the substitution for glucose of other sugars (e.g., D-xylose) which have a slower rate of glycolysis. Maximum yields of viable organisms in the order of 60-70 x 10^9 per ml have been achieved.

c. STORAGE STABILITY

It has been shown that in the presence of suitable adjuvants, the storage stability of this organism can be markedly increased. Strain V

grown in DCPH medium and stored at 4 C with the addition of 3 per cent lactose as an adjuvant showed adequate maintenance of viability (65-75 per cent), genetic stability, and virulence for more than 3 months.

d. DEVELOPMENT OF PROCESSES FOR DRYING

Laboratory freeze-drying has indicated that when dry material is reconstituted, its virulence is considerably reduced. Processing conditions required for optimal recovery, storage, and retention of infectivity are under study. Survival of 40-75 per cent of viable cells has been obtained following freeze-drying by the snap-freezing technique when skim milk is added to the suspending fluid of the cells prior to drying.

e. PILOT PLANT STUDIES

This agent is not yet in the Pilot Plant. However, it has been successfully grown in relatively large volumes in the laboratory under conditions simulating Pilot Plant production. Also, work has been initiated on seed cycles, seed "build-up", aeration, temperature, intervals required for addition of carbohydrates, and bacteriostatic agents to control growth of contaminants.

f. AEROBIOLOGICAL STUDIES

This organism has a relatively low aerosol stability, especially at high relative humidities. Addition of 10 per cent skim milk solids to the suspending fluid prior to aerosolization partially neutralizes the adverse effects of high relative humidity and markedly improves initial recoveries but does not materially affect subsequent recovery. An LD_{50} of 20,000 inhaled organisms for rhesus monkeys has been calculated from exposures in the NBL chamber. Initial trials at sea in the British OPERATION CAULDRON have indicated that the efficacy of this agent dispersed from the B/E.1 bomb is much less than that of Brucella suis. However, these tests were performed at high relative humidities which may account for the erratic sampling and poor animal results.

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4. BRUCELLA SUIS (AB-1)

The development of a liquid suspension of the organism as an anti-personnel BW agent is essentially complete, and classification as "standard type" has been accomplished. The standard-type AB-1 munition fill has been authorized for use in the standard-type M114 bomb. A production plant for vegetative-type BW agents has been designed on the basis of experience gained in the design and operation of the Vigo plant during World War II, laboratory and pilot plant data and operating procedures developed at the Biological Laboratories, and engineering studies performed by several consultants from industry. This plant is approaching completion and should go into production early in FY 1954. Since large-scale production facilities for this agent are not yet in operation, it is being prepared by the pilot plant of the Biological Laboratories for field tests being performed not only in this country but also in the United Kingdom and Canada.

The present effort with this agent is devoted largely to studies in genetics and the development of processes for drying. The genetic studies with Brucella suis serve a dual purpose: (1) they provide a model system for the development of any bacterial BW agent, and (2) they offer the possibility of selection of strains of this organism with properties even more desirable than found in the presently recommended strain. Virulent strains resistant to available antibiotics have been selected, and the aerosol stability of these mutants as well as sodium chloride resistant mutants is being determined. Further genetic modification of this organism may result in selection of strains with increased resistance to drying, aerosolization and, in addition, may lead to more virulent strains which will fail to react to the commonly used criteria of identification.

Processes for drying this organism are under development. It has been noted that there is a significant loss of virulence (determined by intraperitoneal injection) following immediate use of reconstituted freeze-dried organisms without allowing a period of recovery of growth in broth. Pre-pilot plant studies have compared the state from which the material was dried (frozen pellets or frozen layers of varying depths in trays) and the method of heat application (conduction or infrared radiation). Viable cell recoveries of 23.3 - 58.8 per cent per gram of solids were obtained. Critical sizing of dried agent by means of ball milling or the Tanner grinder is being studied. The latter apparatus causes size reduction by rupture of agent particles from internal forces developed by centrifugal spin; it offers promise of successful application to the sizing of BW agents.

Information just received from the British OPERATION CAULDRON confirms and extends existing knowledge of the antipersonnel potentialities of this agent. These trials were performed with Brucella suis grown in the pilot plant of the Biological Laboratories in the manner being recommended for

large-scale production. The infectivity of the agent for guinea pigs (ID_{50} = 45 cells) as determined in the Henderson apparatus remained constant during a 6 month storage. An identical value was obtained when the agent was disseminated from a Collison spray in the open. When the agent was disseminated from the B/E.1 experimental unit bomb and the pre-impinger used in sampling, an ID_{50} of 27 cells for guinea pigs was observed (for the fraction less than 4 μ) and 81 cells for the total cloud (particles 30 μ and below). An ID_{50} of 100 cells was calculated for monkeys in these trials. The scatter (20 to 4800 cells) was quite wide, as might be expected on the basis of only 41 animals and the brief exposure of these animals to the infective cloud because of the high wind speed (17 miles/hour) at the time of the trials. The actual percentage of animals infected in the path of the cloud was not too different, being 85% for the guinea pigs and 59% for the monkeys.

5. BRUCELLA MELITENSIS

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a. STRAIN SELECTION AND GENETIC STUDIES

Strains of high virulence are available, which were originally isolated from human cases in Mexico. One of these cultures had an ID_{100} for guinea pigs by subcutaneous inoculation of less than 14 cells, although the culture proved to be heterogeneous when examined for colonial morphology. A streptomycin-resistant mutant has been isolated, which has a subcutaneous ID_{50} for guinea pigs of 3.3 cells and is resistant to 1 mg streptomycin per ml. An isolate from a human who had become infected by this strain now shows a subcutaneous ID_{50} of less than 2.2 cells for guinea pigs and causes severe gross pathological changes. This strain will be recommended for future pilot plant work.

b. DEVELOPMENT OF PRACTICAL GROWTH MEDIA

A medium based upon an acid hydrolyzed casein has been developed, in which anions are removed by passage through an ion-exchange resin. Under laboratory conditions (shake cultures) yields of 80-113 x 10^9 viable cells per ml are attainable after 48 hours growth. In the pilot plant (deep tank culture) the average yield has been 28.7 x 10^9 cells per ml. The seed cultures are grown in the presence of 0.1 mg per ml streptomycin, no streptomycin is added to the fermentors, and 1 mg per ml of streptomycin is added to the final product. This sequence of addition of streptomycin is based on laboratory studies and assures production of uniformly streptomycin-resistant seed and at the same time allows for maximum yield of cells.

A chemically defined medium has been developed which contains the amino acids, carbohydrates, vitamins, and salts required for growth of this organism. In the laboratory, yields of 100-120 x 10^9 viable cells per ml are obtained in 64-80 hours with this medium.

c. STORAGE STABILITY

No significant loss in viability and no significant population changes were observed during a storage period of 14 weeks at 5 C. No loss of respiratory virulence of pilot plant grown organisms was evident after 5 months storage.

d. DEVELOPMENT OF PROCESSES FOR DRYING

Practically all the effort in this area has been with Brucella abortus and Brucella suis.

e. PILOT PLANT STUDIES

In general, process development and pilot plant studies with Brucella melitensis have utilized the experience gained with Brucella suis with such modifications as are necessitated by the differences in the culture medium and addition of streptomycin. A relatively small number of batches of this agent have been produced in the pilot plant, primarily as a source of material for field trials. In 9 runs under uniform conditions, the mean yield of viable cells per ml was 39.8×10^9 at 57 hours mean harvest time. Non-smooth variants in the product ranged from 0 to 3 per cent. Upon storage at 4 C the mean survival in billions of viable cells per ml was 39.8, 35.9, and 23.4 after 0, 28, and 56 days.

f. AEROBIOLOGICAL STUDIES

Pilot plant grown organisms have demonstrated an ID_{50} of 48 cells with 95 per cent confidence limits of 23 to 101 cells when tested in the cloud chamber against guinea pigs. Death rates of laboratory cultures grown in and sprayed from deionized casein partial hydrolysate have shown similar relations to water vapor concentration as have those of Brucella suis. The death rates as the aerosol aged from 3.5 to 15 or 30 minutes at 25 C averaged 9.2 $\pm$ 3.9 per cent per minute between 20 and 50 per cent RH and 0.3 per cent per minute above 70 per cent RH. The estimate for cells dying immediately upon entering the air was 22 $\pm$ 4.8 per cent, which is similar to values previously obtained with Serratia marcescens.

Only fragmentary field tests have been performed with this agent. Successful dissemination has been attained with the E-114 bomb resulting in infection of 76 per cent of exposed guinea pigs on the 27 yard arc and 74 per cent on the 50 yard arc. In contrast, dissemination from the E-88 generator resulted in an extremely low infection rate.

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6. COXIELLA BURNETII (CB)

A liquid suspension (egg tissue emulsion) of this organism is being reviewed for type classification as a standard-type debilitating anti-personnel BW agent.

a. STRAIN SELECTION AND GENETIC STUDIES

Of the many strains of this organism made available for study, three have been selected as representative: the Henzerling, Nine Mile, and California AD strains. The bulk of the development work has been performed with Strain AD. This strain, which was originally isolated from infected milk of dairy cattle in California, was made available by the National Institutes of Health because of its higher mortality for guinea pigs as compared with other available strains.

Little success has been achieved, thus far, in attempts to develop strains resistant to aureomycin, terramycin and streptomycin. However, there is some indication that resistance to chloromycetin may be induced.

b. GROWTH ON A LABORATORY SCALE

Studies of the growth cycle of this organism in embryonated eggs and the influence of various experimental factors on this cycle have formed the basis for the selection of conditions resulting in maximal yields. Yolk-sac inoculation of 5-day-old embryonated eggs is the preferred method of cultivation. Allantoic inoculation is unsatisfactory. The optimal time of harvest is just prior to or shortly after the death of the embryo (approximately 9 days when 0.25 ml of a 10^{-3} dilution of infected yolk sac is used as inoculum). The concentration of the rickettsiae in the original inoculum influences only the time of death but not the ultimate titer in the embryos during the death cycle. The maximum concentration of this organism found in the yolk sacs of infected eggs is approximately $10^{5.5}$ to $10^{6.0}$ LD_{50} per 0.25 ml as measured in the embryonated egg and 10^{10} ID_{50} per ml as measured by inoculation into guinea pigs, using complement fixation as an index to infection. The presence of living rickettsiae in the inoculated egg is not necessarily manifested by death of the embryo, but frequently can be detected only by repeated subinoculation of pools of surviving eggs with observations of embryo mortality and microscopic examination of yolk sac smears. The evidence of infection obtained by the microscopic examination of smears is much more rapid and more sensitive than the specific death pattern of embryos in detecting the presence of small numbers of rickettsiae. There is at least a 5 log differential between the dosage of organisms required to infect (ID_{50}) as contrasted to the dosage required to result in death of the inoculated embryos (LD_{50}).

c. STORAGE STABILITY

A slurry of infected whole egg can be stored without significant loss in titer for at least 3 years at -40 C and for 3-6 months at 4 C. Stability is progressively lowered as storage temperatures are increased above 4 C. Freeze-dried infected yolk sac suspension in nutrient broth can be stored without significant loss in titer for at least 6 months at -40 C and for 6 months at 4 C.

d. DEVELOPMENT OF PROCESSES FOR DRYING

Exploratory experimentation both on a laboratory scale and on a pre-pilot plant scale have indicated that this organism can be successfully freeze-dried. Ball-milled freeze-dried agent has been disseminated from fixtures and from the E61 munition with formation of aerosol clouds infectious to guinea pigs and monkeys. A defatted dried powder, prepared by extracting a freeze-dried whole egg preparation with Freon 113, gave superior results when disseminated from the E61 bomb.

c. PILOT PLANT STUDIES

The pilot plant has produced approximately 13,700 lbs of a liquid suspension of the AD strain of this rickettsia. The liquid suspension is a slurry resulting from the homogenization of all components of infected embryonated eggs except the shells and shell membranes. Yields of 10^{10} guinea pig intraperitoneal ID_{50} per ml are attained. In addition, a contractor has performed a design study of a facility for the production on a large-scale of viral or rickettsial agents in the embryonated egg.

d. AEROBIOLOGICAL STUDIES

Cloud chamber experiments indicate that 1 guinea pig respiratory ID_{50} equals approximately 0.00072 egg LD_{50}, and 1 guinea pig respiratory LD_{50} equals approximately 6 egg LD_{50}. Aerosol recovery values for this organism are high, i.e., a mean recovery of 50.1 per cent at an average cloud age of 3.5 minutes. Aerosol clouds of this organism are quite stable over short periods of time and do not appear to be significantly altered by changes in relative humidity. The average over all half-life of clouds of this rickettsia is approximately 15 minutes, as compared with 21 minutes for similar aerosols of a nonliving substance (phenol red).

Both laboratory and pilot plant prepared agents were disseminated from M114 bombs at Dugway Proving Ground in the summer of 1952. A group of 37 bombs of each preparation was used and guinea pigs were exposed at regular

intervals up to 1 mile from the munitions. The resultant aerosol clouds were infective over the entire distance, with 93 per cent infection resulting from the laboratory preparation and 82 per cent from the pilot plant material. The cloud was still highly infectious at the end of the sampling array (1 mile distance). In another test in which this agent was disseminated from generators, 74 per cent of the guinea pigs and 73 per cent of the monkeys exposed became infected, with infections occurring at the end of the sampling array (2 miles distant from the munitions).

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7. VIRUS OF VENEZUELAN EQUINE ENCEPHALOMYELITIS (NU)

a. STRAIN SELECTION AND GENETIC STUDIES

The strain of this virus currently being recommended for use in pilot plant studies is VE-1, an egg-propogated strain of equine origin. Laboratory developmental work was performed with this strain which had been selected on the basis of higher virulence for laboratory animals, and the recommendation of an investigator in this field. No attempts have been made to select further among strains or substrains for desirable properties. However, current studies on mosquito transmission are expected to reveal any observable differences between the animal or egg-cultivated virus and mosquito-passed virus.

b. PRODUCTION OF AGENT ON A LABORATORY SCALE

After inoculation of 0.1 ml of virus seed containing 60-6,000 egg allantoic LD_{50} into 10-day-old embryonated eggs via the allantoic cavity, the growth of this virus follows a definite pattern: (1) an initial lag period characterized by titers of less than 10^2 per 0.1 ml, with a duration dependent upon the concentration of the inoculum, (2) a period of rapid multiplication during which a 5-log increase in titer may be achieved in 7 hours, and (3) a period of attaining a maximum virus level characterized by titers ranging generally between $10^{7.5}$ and $10^{8.1}$ per 0.1 ml. The peak period of death of the embryos is approximately 22 hours after inoculation; it will, of course, vary inversely with the dosage of the inoculum. Selective harvest of the chick embryo itself gives a product of higher titer than is encountered in other portions of the egg or in the whold egg. Comparison of the products of whole egg harvest and selected embryo harvest, with respect to pilot plant production and dissemination by explosive munitions, is being performed currently.

c. STORAGE STABILITY

Virus seed has been stored for at least 3 years at -40 C without significant loss of titer. At 4 C the titer is maintained for 3-6 weeks depending on whether the virus is present in whole egg slurry or selectively harvested embryo. Storage stability becomes progressively poorer as the temperature is raised. Storage stability at temperatures intermediate between 4 C and -40 C are under study.

d. PROCESS DEVELOPMENT AND PILOT PLANT STUDIES

Present methods and plans for the production of viral agents leave

no sharp line between laboratory development and process development. Such laboratory studies as the growth cycle of virus in embryonated eggs and the influence on yield of virus of such factors as age of the embryonated egg, route of inoculation, volume of inoculum, temperature of incubation, time and method of harvesting/the basis for the process recommendations for use in the pilot plant. Pilot plant studies of this virus have recently been initiated with the close cooperation of the laboratory development personnel.

e. DEVELOPMENT OF PROCESSES FOR DRYING

Only laboratory experimentation in the drying of this virus has thus far been carried out. Freeze-drying in ampoules has been accomplished without loss in titer. The dried material stored under a vacuum showed no loss in titer after storage at 4 C or 25 C for at least 45 days, and at 37 C for at least 30 days.

f. AEROBIOLOGICAL STUDIES

Animals have been exposed to aerosols of this virus in the modified Reyniers chambers and the following exposed respiratory LD_{50} values determined (all expressed in mouse intracerebral LD_{50} units): guinea pig, 9.68 and 21.1; rabbit, 14.6; and mouse, 1340. An average recovery of virus from the aerosols of 54 per cent was obtained. Low humidities and temperatures are the most favorable conditions for aerosol stability. The aerosol stability of this virus is adversely affected by high humidities (i.e., 90 per cent RH). In this characteristic it is similar to Pasteurella pestis and differs from Bacterium tularense and Brucella suis. In the aerosol chamber, environmental conditions of 8 to 11 per cent RH and 27 C have been found favorable for the stability of aerosols of this virus, while 90 per cent RH and 40 C are unfavorable.

Preliminary trials on the dissemination of whole egg suspensions of this virus in E61 munitions under optimum vapor pressure conditions in the test sphere have been successful. The agent has caused death of exposed guinea pigs, and analysis of impinger samples indicates recovery values of approximately 3 per cent. It is anticipated that this agent will be field tested during the 1953 British field trial program at sea (OPERATION HESPERUS). These field trials will be preceded by dissemination from munitions in the test sphere at the Biological Laboratories.

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8. PSITTACOSIS VIRUS (SI)

Investigation of this agent has revealed deficiencies in such characteristics as storage stability, aerosol stability, and aerosol infectivity, especially when comparisons are made with other egg-cultured agents such as Coxiella burnetii and, on the basis of limited data, the virus of Venezuelan equine encephalomyelitis. Therefore, the agent has been withdrawn from the pilot plant and returned to the laboratory for further investigation. Efforts to overcome these deficiencies have been concerned initially with concentration and purification studies which, although successful, have not materially improved the capabilities of this agent. Future efforts will look to selection of appropriate strains and study of the virus as excreted by infected birds.

a. STRAIN SELECTION AND GENETIC STUDIES

A number of strains have been screened of which the Cal 10 (derived from man or ferret), the 6BC (derived from a parakeet), and the Borg (derived from man) appeared most worthy of further study. On the basis of existing evidence, the Borg strain would be recommended for development as an antipersonnel agent because of its greater virulence for both man and animals.

The 6BC strain has been made resistant to sulfadiazine and retains this resistance after 38 serial passages in the absence of the drug. Some resistance to penicillin can be developed but it is only partially retained after passage in the absence of the antibiotic.

b. PRODUCTION OF AGENT ON A LABORATORY SCALE

Studies of the growth cycle of the psittacosis virus in the embryonated egg and the influence of various experimentally controllable factors on this cycle have led to the recommendation of standard procedures designed to give maximal yields of virus. For the Borg strain, 8-day-old embryonated eggs are inoculated into the yolk sac with 0.25 ml of diluted yolk sac suspension assaying 2000 LD_{50} per ml. The optimum time for obtaining maximum titer of virus (and therefore for harvesting) is just prior to or shortly after death of the embryos, i.e., 4 to 6 days after inoculation of the eggs. Based on studies of the mortality (LD) of the infected embryos, determination of infective dose (ID) by examination of surviving eggs for the presence of virus in yolk sac smears, and repeated subinoculation of pools of the surviving eggs into new groups of eggs with observations of embryo mortality and positive yolk sac smears, LD_{50} titers of $10^{8.4}$ have been obtainef for the Borg strain and $10^{7.6}$ for the 6BC strain with no evidence of a substantial difference between the infective

and lethal doses. This is in contrast to experience with Coxiella burnetii. Over 97 per cent of the titratable psittacosis virus is found in the yolk and yolk sac, 85 per cent being in the yolk itself.

c. STORAGE STABILITY

Liquid suspensions of psittacosis virus do not suffer any significant loss in titer for 1 month when stored in sealed glass ampoules at 4 C or 10 C. Stability decreases as environmental temperatures are raised. At -50 C there may be an initial loss in titer of up to 1 log because of the freezing; however, the frozen product is stable for 2 to 5 months.

d. DEVELOPMENT OF PROCESSES FOR DRYING

Freeze-drying of suspensions of homogenized whole egg (minus shell and shell membrane) containing psittacosis virus frequently results in losses of one to two logs in titer. Use of infrared radiation as a heat source in the freeze-drying process increases the rate of drying considerably but does not eliminate the loss in titer resulting from the process. Dried preparations stored under reduced pressure in sealed ampoules exhibited no decrease in titer after 5 months storage at -50 C, -20 C, or 4 c, but significant losses in titer occurred after storage for 1 month at 25 C or 1 week at 37 C. Extraction of lipoids from the dried product with Freon can be accomplished with a negligible loss in titer. A fine powder results which can be aerosolized more readily than unextracted powder.

e. PILOT PLANT STUDIES

Approximately 25,780 pounds of milled embryonated egg containing high concentrations of psittaccsis virus have been produced in the pilot plant. About 60 per cent of this production utilized the Borg strain. With a 95 per cent confidence, yields may be expected to fall within the limits of 0.35 to 13.8 x 10^8 mouse intracerebral LD_{50} per ml. A contractor has performed a design study of a facility for the production on a large scale of viral or rickettsial agents in the embryonated egg. This study has been reviewed and the design basis revised.

f. AEROBIOLOGICAL STUDIES

Various preparations of the Borg strain in liquid suspension have been aerosolized in a 4,800 liter tank. Average results indicate that at cloud age of 1 minute a recovery of 4 per cent may be expected.

Approximately 200 intracerebral LD_{50} were equivalent to 1 respiratory LD_{50} for the mouse. Aerosol recoveries are dependent upon the environmental temperature and humidity (vapor pressure) with best recoveries obtained at vapor pressures less than 13 mm Hg (about 55 per cent RH at 25 C). Recoveries at high vapor pressures were poor. This virus is quite fragile as is illustrated by losses of 85 per cent suffered upon atomization directly into an impinger fluid, i.e., omission of the aerosol cloud portion of the chamber studies. These results are in contrast to losses of about 2 per cent observed with phenol red and of 15 per cent with Coxiella burnetii.

A number of tests have been performed in which psittacosis virus has been disseminated by a nozzle or the M114 bomb in the test sphere. Both the stability and infectivity of the resultant aerosols have been poor.

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9. BOTULINUM TOXIN (X)

The bulk of the development work on botulinum toxin has been concerned with type A. In this report all referenced to toxin should be understood to be type A unless specifically identified otherwise.

a. STRAIN SELECTION AND GENETIC STUDIES

A large number of strains from various areas of the world have been screened. Much of the early laboratory and pilot plant work utilized the Hall strain. At least part of the variability in production of toxin has been demonstrated to be a result of nonuniform seed stock. A single colony stock, designated HE, has been isolated which is more stable and produces more than 1.5×10^6 mouse intraperitoneal LD_{50} per ml. This is the stock currently recommended for use in the pilot plant. Some strains that yield as high as 4×10^6 LD_{50} per ml have been selected, but they have not proved to be uniform when held as stock cultures. Stocks of Clostridium botulinum, type B have been selected which yield more than 2×10^6 LD_{50} per ml. Many strains of types C, D, and E have been screened for toxigenicity with the ultimate objective of conversion of toxin to toxoid.

b. LABORATORY DEVELOPMENT

Laboratory methods of culture have been developed which produce high yields of toxin. It has been possible to isolate and purify both the type A and type B toxin and to determine many of their physical and chemical characteristics, e.g., molecular weight, shape, frictional ration, amino acid content, electrophoretic mobility, etc. Both are extremely toxic, with mouse intraperitoneal LD_{50}'s per mg N of $220\text{–}240 \times 10^6$ for type A and $110\text{–}200 \times 10^6$ for type B. A process has been devised for preparing a clinically acceptable, aluminum phosphate precipitated, purified toxoid of type A. This process has been adapted for large scale production by a pharmaceutical manufacturer who has prepared several lots of toxoid which are currently undergoing clinical evaluation. Attention is being devoted to the development of procedures for large-scale production of a purified polyvalent botulinum toxoid.

c. DEVELOPMENT OF PROCESSES FOR DRYING

Partially purified toxin at pH 3.9 or 6.8 and free of salt can be freeze-dried and stored for at least 3 months at -18 C or 25 C without loss of toxicity. Purified toxin is quite stable in an acetate buffer solution at pH 4, but it cannot be frozen in the solution without a large reduction in toxicity. However, the purified toxin can be freeze-

dried when free of salts at pH 6.8 and maintained at -18 C. Complete retention of toxin activity over a period of 3 months storage was possible under these conditions.

The toxin can be spray-dried, yielding a product with a potency of 750 to 2,600 x 10^6 mouse intraperitoneal LD_{50} per gram. The loss in toxicity averages 10 per cent when compared with the concentrated liquid feed. In addition, a collection loss of about 20 per cent is sustained. The particle size of the dried product has a mass median diameter of 4 to 7 microns and a moisture content of 4 to 5 per cent. Stability studies with these spray-dried products indicate that a sample assaying 1 x 10^9 mouse intraperitoneal LD_{50}'s per gram can be stored at 20 C for 15 months without measurable loss of toxicity.

d. PILOT PLANT STUDIES

A recommended process is available for growth of Clostridium botulinum and for the precipitation, concentration, partial purification, and spray drying of the toxin. Much of the pilot plant production of toxin has been guided by the needs of other laboratories and agencies, although some experimental piloting has been accomplished. The major weakness of the purification process has been the high percentage loss of toxin encountered in the presently available pilot plant equipment which was not specifically designed for this operation. The partially purified toxin is of good quality, but recoveries are erratic and usually low. A design of a plant for large-scale production of toxin has been prepared by a contractor. The design has been reviewed and revised by personnel of the Biological Laboratories who have prepared a report summarizing the recommendations in this area as of 1952.

e. AEROBIOLOGICAL STUDIES

Respiratory toxicity studies, using aerosols of toxin created from liquid suspensions, have demonstrated that (1) innaled LD_{50} values for mice increased (i.e., toxicity decreased) from 180 to 1500 mouse IP LD_{50} units with increase in the mass median diameter of the inhaled toxin-containing particles from 0.46 to 2 microns, and (2) lung-retained LD_{50} values for mice remained fairly constant (coefficient of variation of ± 26.4 per cent) at 18.2 mouse IP LD_{50} units over the same range of particle sizes.

Aerosol stability studies at low RH have indicated that high recoveries are obtained at an aerosol age of 3.5 minutes (about 55 per cent) and the inactiviation rate is relatively low (4.6 to 6.4 per cent per minute).

Studies in a 4,800-liter tank on the toxicity for guinea pigs and mice of aerosolized spray-dried toxin show that (1) guinea pigs are much more susceptible than were mice (this may be a result of the particle size distribution of the toxin), (2) inhaled LD_{50} values for the guinea pig increased (toxicity decreased) with increase in relative himidity of the aerosols, particularly above 70 per cent, and (3) inhaled LD_{50} values for guinea pigs exposed in aerosols at RH less than 60 per cent are about 75 mouse IP LD_{50} units.

Wartime field disseminations of liquid suspensions of toxin from the Mark I (predecessor of the M114) bomb were not conclusive. Recently two field tests were conducted at Dugway Proving Ground in which a spray-dried toxin was disseminated from a generator. The results of the second test have not yet been officially analyzed. However, preliminary review of the available data indicates that in the first test the 490 grams of spray-dried toxin covered an area of 47,000 square yards with at least 1 guinea pig LD_{50}, and the total cloud area was 136,000 square yards as measured by the toxicity of impinger samples to mice. In the second test. 750 grams of spray-dried toxin were disseminated. The total cloud, as measured by impinger samplers, covered an area of 938,360 square yards. However, very few animals were killed in this second trial. A number of factors of possible pertinence to this low aerosol toxicity are being investigated. It may be noted that in this second trial the RH was 85 per cent, which chamber tests have shown leads to rapid deterioration of the toxicity of aerosols of this toxin.

Some further field trials with this toxin are planned with Suffield Experimental Station this summer.

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10. SHELLFISH POISONS (SS)

Sources of these toxic substances have been poisonous mussels, clams, and scallops. Most of the work has been done with the first two, since the supply of poisonous scallops has been too limited to allow more than exploratory experimentation. In the mussels, the origin of these toxins is the dinoflagellate protozoan Gonyaulax catanella. The origin of the toxin in the clams is unknown; however, all chemical and physical properties and pharmacological activity indicate that the clam poison is identical, or nearly so, to mussel poison. Some attempts have been made to develop methods for culturing Gonyaulax catanella to provide a ready source of toxic material; although considerable progress was made, the low yields experienced made it appear impractical to continue these attempts. Procurement of the poison from natural sources (toxic mussels from California and clams from Alaska) is considered more economical.

The interest of the Biological Laboratories in this poison is based on its possible synthesis for use as a new CW agent or as a BW agent for covert activities. The poison has a molecular weight of 327 and is believed to be within the realm of synthesis. It is more toxic than any other known poison of low molecular weight and its hydrochloride salt is unaffected by boiling in water and drying in air. It is stable in certain soft drinks, ordinary chlorinated drinking water, and in coffee.

Research on the poison has been directed toward establishing its structure and developing a method of synthesis. The first phase of this work involved the isolation of this material in a pure form from poisonous mussels found along the California coast and from poisonous clams obtained in southeastern Alaska. The difficult problem of purification was solved with the use of cation exchange resins Amberlite IRC-50 and XE-64 followed by chromatography on alumina. The purity of the product has been established and the MLD determined, by intravenous injection into experimental animals, to be 3 to 4 micrograms per kilogram of body weight.

The quantity of purified poison available for study has been extremely small and has necessitated carrying out much of the structural determinations on a micro scale. In spite of this shortage of purified material, considerable progress has already been made on the chemical studies of the poison. Its molecular formula has been shown to be $C_9H_{16}N_6O_3Cl_2$. The compound is optically active and has only end absorption in the ultraviolet region, which indicates the absence of aromatic or conjugated unsaturation. Infrared studies indicate the presence of amidic type groups and probably hydroxyl groups. Two titrable functions are present with pH_a equal to 8.0 and >11.4. Acidic functions and carbonyl groupings are not present. The presence of at least one guanidine grouping in the molecule has been established, as well as the presence of an arrangement of atoms capable of giving β-alanine and glycine on alkaline hydrolysis.

In view of the results obtained at this time, it appears that the poison must contain a heterocyclic structure involving the guanidine group. A large amount of additional evidence has been accumulated which will be more readily interpreted as larger structural fragments are identified.

Much of the tedious preliminary work on this problem has been completed at this time, and the solution of the problem of its structure is progressing very satisfactorily.

11. SCREENING OF AGENTS AND COMBINATIONS OF AGENTS

In this program, emphasis is placed on exploratory investigations designed to reveal the potentialities for BW purposes of various pathogenic organisms or combinations of organisms. Promising leads which are uncovered are then recommended for further laboratory investigation of a more detailed and definitive nature.

a. MALLEOMYCES MALLEI AND MALLEOMYCES PSEUDOMALLEI

A highly pathogenic mutant strain of M. mallei isolated in Canada is under investigation. Yields as high as 10^{11} cells per ml have been obtained. Hamsters are infected by 1-10 cells by intraperitoneal injection and by approximately the same number via the respiratory tract. Other animals have been incompletely tested. Recoveries of 5 per cent have been realized at 0/1 minute after aerosolization of liquid suspensions of this organism at 70 F and 50 per cent RH.

The strains of M. pseudomallei which were available from World War II studies have been found to possess reduced virulence, and attempts to increase their virulence by animal passage have been unsuccessful. Work utilizing other strains is underway at the Naval Biological Laboratory with special emphasis on strain selection, factors affecting dissociation, methods of growth, and determination of virulence.

b. MYCOBACTERIUM TUBERCULOSIS

An initial investigation of the susceptibility of monkeys to an aerosol of this organism has indicated that infectivity and virulence by the respiratory route are high with all animals exposed (700 particles was the minimal dose) dying. Deaths were more rapid in animals exposed to the aerosol than they were in animals given similar dosages intravenously.

c. ENTERIC ORGANISMS

Vibrio cholera is under study by contractors with the objectives of collection of strains and investigation of factors associated with their virulence.

Enteric pathogens, primarily the Shigella spp, were studied on a limited basis in 1951-52, but the effort at the Biological Laboratories was terminated at the end of 1952 and the personnel transferred to complete the development of other antipersonnel agents of higher priority.

A small effort in this area is continuing on contract. Strains involved in epidemic outbreaks of dysentery in man have been collected for use in laboratory screening studies. A major difficulty encountered in studies of the Shigella spp. is the lack of a good technique for measuring their virulence.

d. OTHER BACTERIA

A survey of the literature concerning diphtheria is underway and screening of Corynebacterium diphtheriae will be initiated shortly.

e. COCCIDIOIDES IMMITIS

A large number of strains of this fungus are available for study, most of which have been isolated from human cases. Growth on solid soy bean yeast extract agar yields 2.0 - 2.5 grams of dry organisms per liter of medium of which 55-90 per cent represents single spores. Mycelial content is generally less than 5 per cent. Counts have ranged from 4 to 8 x 10^9 spores per gram. Growth is also possible in liquid culture. Storage stability of this organism is good, with liquid suspensions showing no loss in viability for periods up to 30 weeks at 25 C or 4 C. Aerobiological studies of this organism are underway.

f. OTHER FUNGI

Histoplasma capsulatum is being studied under an interagency agreement with the Communicable Diseases Center of the U. S. Public Health Service. Epidemiological studies have stressed the importance of the airborne route of infection in histoplasmosis. This will be studied experimentally in a Henderson type exposure chamber.

The physiology and pathogenicity of strains of Nocardia asteroides will be studied by a contractor, with the aerosol infectivity aspects of the investigation to be accomplished at the Biological Laboratories.

g. VIRAL AND RICKETTSIAL AGENTS

A number of viruses and rickettsiae are under consideration for screening. A literature survey is in progress and laboratory effort will soon begin on Rickettsia prowazeki. A few exploratory aerosol exposures have been performed with the virus of rabies and of poliomyelitis. Respiratory exposure of guinea pigs to more than 2133 mouse IC LD_{50} of rabies virus did not cause infection. Fragmentary experiments with the

Lansing strain of poliomyelitis virus have demonstrated that this agent can infect when airborne. The aerosol stability of this virus appears highest at high relative humidities.

h. TOXINS OF MICROBIOLOGICAL ORIGIN

Very active dermatitic substances are produced in aerated liquid cultures of Myrothecium verrucaria. These compounds are of low molecular weight (250-500) and preliminary examination has indicated that they are more active as vesicants than is mustard (β,β'-dichlorodiethyl sulfide). Characterization of these dermatitic substances is underway.

Certain species of the blue-green algae are believed to produce a very toxic substance which has caused the death, within a few minutes to 24 hours, of animals drinking water from heavily contaminated lakes. A contractor is collecting various species of these toxic algae and investigating the potency, stability, and other properties of the toxins which these algae produce.

i. COMBINATION OF AGENTS

An active screening program is underway both at the Biological Laboratories and with two contractors. Examples of both antagonism and synergism have been found. As an illustration of the former, it has been noted that guinea pigs infected by Brucella suis are more resistant to infection by Coxiella burnetii than normal guinea pigs. As an illustration of the latter, it has been observed that exposure to aerosols of influenza virus appears to render the host more susceptible to agents such as Coxiella burnetii or psittacosis virus.

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B. ANTIANIMAL AGENTS

1. GENERAL

The discussion of antianimal agents has been divided into two categories, offensive and defensive. Within these categories the order of comments on agents is identical with the order of priority of the Sixth Program Guidance Report of the Committee on BW, RDB. Laboratory facilities for work on diseases of large animals are not yet available at the Chemical Corps Biological Laboratories; such facilities are now scheduled for completion in early 1955. The present BW program in the antianimal field is being carried on primarily by contract.

It should be recognized that existing regulations of the Bureau of Animal Industry prevent introduction into the United States of the causative agents of certain exotic diseases. Preliminary discussions with that Bureau have indicated that permission will be granted for the study of these diseases in the above-mentioned facilities which are now being constructed at the Biological Laboratories. Meanwhile, an active and productive project is being carried on in Africa with the cooperation of the Department of Agriculture and the military services for the study of rinderpest and African swine fever.

2. OFFENSIVE

a. RINDERPEST (including virus diarrhea of cattle)

Only one immunological type of this exotic disease (Rinderpest) is known. A strain has been found which was demonstrated to be so virulent and contagious that isolation facilities successfully used in rinderpest research for 20 years proved inadequate to contain this strain. Research has been resumed in new facilities made available in Africa in May 1953. The only source of the virus is tissue from infected bovine. This agent can be stored for a year in the frozen state. It loses about 1 log of titer in lyophilization. Cattle may be infected by respiratory or oral routes. Little quantitative data are available.

Virus diarrhea of cattle is believed to be without offensive significance.

b. FOOT-AND-MOUTH DISEASE (including vesicular stomatitis and vesicular exanthema of swine)

All foot-and-mouth disease research has been made the mission of the Fort Terry facility to be operated by the Bureau of Animal Industry under contract to the Chemical Corps.

Vesicular stomatitis virus is known to occur in two immunological types, and can be produced in bovines and in embryonated eggs. It stores well either frozen or lyophilized. No data are available concerning routes of infection, dosages, or quantity production.

Vesicular exanthema virus is known to occur in four immunological types. It can be produced only in the epithelial tissue of living swine. No production data are available. Virus preparations are known to withstand lyophilization or wet storage for over a year at 5 C, but no quantitative data are available. The virus is probably infective by the oral route.

c. RIFT VALLEY FEVER

No facilities have been available for research on this exotic disease. Spread among animals seems to be entirely dependent upon an insect vector. Little is known about the range and habits of this vector.

d. VENEZUELAN EQUINE ENCEPHALOMYELITIS

Data have been obtained concerning production and effect on animals, including horses. Please refer to the report on this virus under Antipersonnel Agents, Section IIA.

e. OTHER ANIMAL DISEASES

(1) African Swine Fever

No facilities have been available for work on this exotic disease; however, it is hoped to begin work on it in Kenya in FY 1954. To a large extent, the experience gained with the virus of hog cholera may be applicable in the development of this agent.

The literature suggests that the virus may mutate spontaneously in a single animal passage to any one of a number of immunological types. The source of virus is the blood of infected swine. In this form it is said to be extraordinarily stable at 5 or 20 C and can be lyophilized. This virus is infective by the oral route.

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(2) Hog Cholera

One immunological type is generally recognized, although various aberrant strains showing partial cross immunity with the classic have been studied.

The source of the virus is the blood of infected swine. It has been stored without appreciable loss in titer for 1 year at -25 C in the frozen state and at 4 C in the lyophilized state. It loses about 1 log during lyophilization.

The virus has been infective in small doses by respiratory or oral routes. Dry virus fed in pellets has infected in doses of 10^5 MID (subcutaneous), while wet preparations per os have infected in doses of about 10^3 MID. Little quantitative data on the respiratory dose are available, but it has been estimated as 1000 MID.

The dry agent has been disseminated successfully from feathers as carrier with the E73 bomb in a large-scale field trial, OPERATION GREEN. Successful small-scale trials with generators have also been made.

(3) New castle Disease Virus

Only one immunological type is know although different strains vary greatly in virulence and contagiousness. The virus may be produced in embryonated hen's eggs and stored without loss when frozen at -30 C or lyophilized and held at 4 C. Lyophilization has been accomplished without loss in titer. The virus has been successfully disseminated with feathers in a small-scale trial. Additional laboratory tests have shown the infectivity of aerosols and established that the disease may be transmitted in nature by this method. Estimations of dose cannot be made.

(4) Fowl Plague

No research has been conducted with this exotic disease. According to the literature only one immunological type is known. Some strains are extremely virulent and contagious. The virus can be grown in embryonated eggs, and wet preparations are said to have usually good resistance to storage at 5 C. It is readily lyophilized. Infection is said to occur by the oral route, but it can be assumed by the respiratory route as well.

(5) Glanders

No facilities have been available for large animal investigation. Data pertaining to production and effect on small animals have been summarized in the report on Screening of Agents.

f. In addition to the investigations of agents, contract work has been done on the use of pellets for dissemination of agents. These studies have led to the development of a food pellet which is acceptable to cattle, sheep, swine, and horses.

A good rate of pickup in 1 to 4 days was found with this pellet in all seasons of the year, on a variety of pastures, when the concentration was 1 pellet per 2.5 acres.

3. DEFENSIVE

(1)Foot-and-Mouth Disease (See comment under Offensive, above)

It is of great importance to distinguish rapidly the occasional outbreak of vesicular stomatitis from the rare and dangerous Foot-and-Mouth disease. Useful developments in diagnosis include neutralization tests in embryonated eggs, a complement fixation test, and a vaccine for immunization of laboratory animals.

Vesicular exanthema may also be confused with Foot-and-Mouth disease. No vaccine or useful diagnostic aids have been developed, although complement fixation tests may have some promise.

2) Rinderpest

An efficient killed vaccine incorporating an adjuvant has been developed. This vaccine can be stored for at least 7 months at 4 C or -30 C. Solid immunity for at least 9 months is conferred by a single 2 ml subcutaneous injection, a dose 1/30 the size of similar vaccines previously used. Promising results have been obtained on a complement fixation test for early diagnosis.

(3) Fowl Plague

No research was conducted during the past year on this disease. Neutralization and hemagglutination tests are available for diagnosis. Killed and attenuated vaccines have been developed but not perfected.

(4) African Swine Fever

No research was conducted during the past year. There is no serological diagnostic test, treatment, or vaccine.

(5) Rift Valley Fever

No research was conducted during the past year. Neutralization and complement fixation tests are available for diagnosis. An efficient vaccine has been produced commercially in South Africa. A Rockefeller Institute team is preparing to resume investigations.

(6) Hog Cholera

Methods of control of this disease are established. Commercially available vaccines and sera are generally efficient prophylactics.

(7) Newcastle Disease (See comments under Hog Cholera, above)

(8) Venezuelen Equine Encephalomyelitis

Studies are continuing on the epidemiology and particularly on the role of insect vectors. A killed vaccine suitable to production is available.

C. ANTICROP AGENTS

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TABLE II. DEVELOPMENTAL STATUS OF ANTICROP AGENTS

Screening	Laboratory Phase	Pilot Plant Phase	Standard Types
Various chemical agents	Fluorophenoxyacetic acids	Piricularia oryzae	Puccinia graminis tritici (TX)
Sclerotinium oryzae	Maleic hydrazide	Helminthosporium oryzae	Butyl 2,4-Dichlorophenoxyacetate (LNA)
Sclerotinium sclerotinium	N-4-chlorophenyl-N',N'-dimethyl urea	Phytophthora infestans	Butyl 2,4,5-trichlorophenoxy-acetate (LNB)
Helminthosporium spp.		Puccinia graminis secale	
Fusarium spp.		Isopropyl-N-phenyl carbamate	
Piricularia spp.			
Phytophthora spp.			
Soybean bud blight virus			
Tomato ring spot virus			
White tip nematode of rice			

Security Information

Security Information

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C. ANTICROP AGENTS

1. RUSTS OF CEREAL CROPS

The stem rust of wheat has been adopted as a standard-type anticrop agent. This agent is an obligate parasite and must, therefore, be produced on susceptible host plants. As of 1 Apr 1953, a quantity of agent was on hand containing approximately 400 pounds of viable spores. Within the past few months this stockpile has been tripled, resulting in a supply of agent sufficient to meet present USAF requirements. Production is continuing at two sites.

The stem rust of rye is under consideration for adoption as a standard type anticrop agent. As of 1 April 1953, a quantity of material was on hand containing approximately 2,200 pounds of viable spores. This amount will be increased to a limited extent as a result of summer operations. Production this winter (1953-1954) will be carried out at two sites. The present stock is equivalent to about 67 per cent of present USAF requirements. It is expected that sufficient additional agent will be produced this coming year to fulfill completely this requirement.

The production of these rusts is the responsibility of Edgewood Arsenal, with technical direction furnished by the Biological Laboratories. A manual of procedures to be used in field production of rust spores has been prepared by the Biological Laboratories and kept current. This manual is used as guidance to the commanders of the various production sites.

During the past year some 1980 grams of wheat stem rust and 1902 grams of rye stem rust spores have been produced in the greenhouse for use as inoculum by the field production group, for storage and surveillance tests, for experimental field tests, and for use by contracting agencies. Techniques have been improved to the extent that yields at present approximate 10 grams of spores per 100 pots as compared with 3.5 per 100 pots in 1951.

Contamination of field harvested rust spores with saprophytic organisms and leaf rust, and the serious reduction in yield of rust caused by bacterial infection of rust pustules has necessitated a study of factors involved in producing rust under dry-land farming where rust is not endemic. Low humidities encountered in arid or semi-arid regions make the initiation of infection difficult.

Studies leading to the field evaluation of inoculum in terms of the amount of infection resulting from a given density of inoculum applied over a selected area at present are receiving considerable attention. Data obtained in field experiments in 1949 indicated that adequate infection would develop following the application of inoculum at the rate of 0.1 gram per acre. The adequate prediction of the effects of an attack with this agent, however, necessitates a more thorough study of the problem to determine (1) the amount of infection that would result from a given dosage, (2) the amount of spread that could be expected from an initial infection locus, and (3) from this the reaction between the size of initial loci of infection and the spread that would result, and (4) the correlation of the above three points with the meteorological conditions and status of crop development. Quantitative data on such matters are extremely difficult to obtain. Surprisingly enough, despite the long period during which this disease has been an important factor in wheat production, little or no such data exist.

2. HELMINTHOSPORIUM ORYZAE

(BROWN SPOT DISEASE OF RICE)

Cultures of Helminthosporium spp. obtained from all possible sources are being screened for pathogenicity.

Maximum sporulation was obtained on sorghum grain alone in continual darkness at 23-29 C. An initial moisture content of the grain above 72 per cent inhibited sporulation. Sporulation increased with increasing rates of aeration up to the maximum rate used; with no aeration, no spores were produced. The shape of the culture vessel should be such as to provide maximum surface area. With sufficient aeration sporulation will continue indefinitely; but, for most efficient use of space, harvests should be made after 10 to 14 days.

Mycelium produced on a yeast extract (0.5 per cent) and dextrose (2 per cent) medium can be washed, dried and ground to produce fragments approximating spores in mass and having a viability of about 10 per cent. Vigorous aeration of the medium on a shaker was essential for maximal yields.

A modified version of the standard field harvester has been developed and used during the winter production program. Present work with harvester design is largely directed towards determining (1) the efficiency of present equipment, (2) methods of increasing the efficiency of the machines, and (3) whether a change in the design of the pickup heads will result in larger harvests.

The most urgent problem to be met at the present time is that of increasing the longevity of rust spores. The average monthly rate of decay of viability, calculated on data obtained approximately 8 months storage, has been 5.35 per cent per month. (Average half-life of 9.3 months). Decay rates vary from 1.4 to 10.8 (half-life 36 and 4.6 months, respectively). No consistent correlation has been found between any single factor and the viability decay rate. In general, the purer product has had the lowest decay rate. Material has not maintained well when obtained from plants growing poorly or from those on which rust pustules are badly attacked with the bacterial parasite of rust. A change in processing prodedure appears to offer some promise. Spores normally have been dried to approximately 10 per cent moisture content. When they are dried to a moisture content of approximately 2-3 per cent and not subjected to temperatures below freezing (i.e., by too rapid drying), they will germinate well after a period of rehydration in a saturated atmosphere. Results of assays made 60 days after such drying have shown a decay rate of less than 2.0 per cent per month.

The infection efficiency ratio (i.e., the number of pustules developing per 100 viable spores deposited), has been found to average approximately one. There is a considerable variation in the value of this ratio from experiment to experiment, indicating that a higher ratio is fully possible. The number of pustules developing on leaves (under controlled conditions) has been found to be proportional to the density of spore deposition. The viability of the inoculum is affected markedly by the treatment the spores received during processing and by the treatment plants receive prior to and after inoculation. The germination of low moisture (2-3%) spores on plants approximates that which occurs after rehydration, but appears to be slower than that of spores not dried below 9-10 per cent moisture. Assays made after periods of high sunlight indicate that much more infection is obtained under such conditions than when the light intensity is low during the preassay period. Thus, it appears that the preconditioning of the host plants is an important factor in the amount of infection which develops.

A modified rice-polisher was the most effective method of harvesting spores from dried grain-substrate. The product, when mixed with talc, was a readily dispersable powder containing 6.5 to 9.2 x 10^6 spores per gram (pure spores equal 43 x 10^6 per gram) with a viability of 95 per cent. To obtain mycelium, the liquid cultures were filtered, the mat washed thoroughly and dried overnight at 95 F. Before use the dried material was ground to pass a 40 mesh screen. Yields from Fernbach flasks averaged 9.1 gms. dried mycelium per liter of medium.

Storage of the organism poses no problem. Spores and mycelium have retained a high viability (95 per cent) for one year at 8 C, and the viability of spores, even without refrigeration, has not decreased appreciably.

Differences between isolates could be detected in the greenhouse to the extent that the type of initial infection is different. Sufficient incubation facilities have not been available to determine differences in lesion development. The mycelial preparations give only poor infection under greenhouse conditions.

The field tests of 1952 were the first ones after the reactivation of the project. The results were inconclusive but served to evaluate the various sites and to point up the specific problems involved. From the results obtained it was concluded that (1) work in the future will be concentrated at Texas and Florida, (2) a more pathogenic isolate is needed for effective use, and (3) more information is needed concerning the conditions required for infection.

Four isolates of Helminthosporium spp., more virulent than the strains of H. oryzae formerly used, have been obtained by isolation from rice seed from Manchuria, rice seed from Texas, and unidentified plant at Camp Detrick, and from a culture of H. oryzae No. 29 that had received ultra-violet radiation. Four species of Helminthosporium, hitherto not known to attack rice, have been demonstrated to be pathogenic.

Preliminary tests have been made with Sclerotinium oryzae and Fusarium moniliforme as agents or auxiliary agents against rice. The latter shows some promise as a further means of destroying the panicle.

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3. PIRICULARIA ORYZAE

(BLAST DISEASE OF RICE)

Piricularia oryzae can be cultured readily in the laboratory in a variety of media. There is considerable difference among isolates with respect to sporulation, colony type, rate of growth and stability.

The general principles of pilot plant production of this organism have been worked out. The mechanics of culture control and inhibition of contamination need considerable attention to make such operations reliable.

Sporulation of the organism is considerably increased in light with maximum sporulation at 200-500 foot-candles. Low humidity suppresses sporulation as does a saturated atmosphere. Approximately 80 per cent RH appears optimum for sporulation.

A natural epidemic of Piricularia spp. completely killed a plot of Zenith rice in Florida during the summer of 1952. This was the first time that this disease was seen in epidemic proportions in the course of this project. Other varieties were damaged severely but recovered sufficiently to yield some grain. It may be possible, by working in Florida, to overcome one of the most severe handicaps which has confronted the work with this agent, that of inadequate field testing sites.

A program has been started to obtain, by collection and by induction of mutants, strains of the organism with a wide host-variety range and good epidemiological characteristics. Two mutants have been obtained which show some improvement over the parent strains.

4. WHITE TIP NEMATODE OF RICE

A survey of literature and some preliminary work has indicated that the "white tip" nematode of rice has some promise of being useful as an agent against rice. Methods of culturing the organism have been worked out and it would appear that such methods could be adapted to large scale operations

for production purposes. The nematodes can be stored for relatively long periods of time if kept moist. The problems of large-scale storage would have to be studied. The nematode is generally distributed in many rice growing areas. The conditions under which they multiply rapidly and cause significant yield reductions need to be determined.

5. PHYTOPHTHORA INFESTANS

(LATE BLIGHT DISEASE OF POTATOES)

Sporangia of Phytophthora infestans are relatively fragile and susceptible to desiccation and concurrent loss of viability. The difficulty in handling sporangial suspensions led to the development of a pelletized substrate throughout which the organism would grow and form sporangia. It was envisioned that these pellets, when dropped into a target area, would absorb moisture, resume growth and produce sporangia on the surface of the pellet. These sporangia in turn would be carried on air currents to susceptible plants and initiate infection. No field trials were made prior to the cessation of work on the project in 1945.

A thorough evaluation of these pellets made after the projects was reactivated in 1951 showed that actually no new sporangia were formed on the surfaces of these pellets under field conditions. This was attributable largely to the rapid rate of drying which occurred when pellets were exposed to sunlight and to the fact that the pellets are quickly overgrown with soil fungi. Infection of potato plants could be obtained, however, when pellets were crumbled and the fragments distributed over the foliage. The amount of infection obtained was relatively low, but the principle appeared to have considerable promise. Consequently, the present research program is directed toward the study and development of the fragmented pellets as a means of disseminating the late blight pathogen.

Severe infection from sporangial suspensions occurred in less than 6 hours when adequate moisture was present on the plants. Pellet inoculum was slower acting and required several additional hours to initiate comparable infection. This corresponded roughly to the difference between the time required for zoospore formation (89 per cent at 4 hours) and direct germination (23 per cent at 12 hours). Maximum infection resulted when inoculum was stored at 20 C prior to use.

Deviations from this temperature resulted in an increase in the required incubation period and some reduction in amount of infection. A shift in type of germination from indirect (zoospores) to the direct germination appeared to account for this effect. The rate and amount of direct germination was markedly altered by brief exposure of sporangia to temperatures of 40 C. Certain adjuvants radically changed the type of sporangial germination. Two percent peanut hull extract prevented indirect germination. Such results suggest that the original inclusion of peanut hulls in the pellet composition may have been detrimental in that it prevented the rapid indirect germination.

Contrary to commonly accepted views, infection and spread of late blight occurred when the only moisture present was that supplied by nightly deposits of dew, demonstrating that while beneficial, it is not essential to have long rainy spells to insure spread of late blight.

Strains of P infestans differ with respect to the rate of germination, rate of infection, rate of lesion development, rapidity of sporulation, reaction to host variety resistance, heat tolerance, and resistance to desiccation. Isolates are being collected from all available sources to study the extent of the differences between strains and to build up a collection of the most effective isolates possible.

6. PLANT VIRUS DISEASES

The mechanics of initiating infection of plants by viruses constitutes the major problems in the development of plant viruses as BW agents and is receiving the major attention on this project.

Early attempts to establish infection by utilizing the various types of mechanical injuries to which plants are normally subject have resulted in only a low level of infection.

7. CHEMICAL ANTICROP AGENTS

Butyl 2,4-dichlorophenoxyacetate (LNA) and butyl 2,4,5-trichlorophenoxyacetate (LNB) have been classified as

standard-type BW anticrop agents for use against broadleaf crops. A large capacity spray system for B-29, B-50, and C-119 aircraft has been constructed and tested satisfactorily by the USAF. One hundred of these units have been procured.

4-Fluorophenoxyacetic acid and 4-fluoro-2-methylphenoxy-acetic acid appeared very promising against rice, substantial yield reductions resulting from applications of 0.1 lb/acre or less.

Maleic hydrazide, at 0.25 lb of active ingredient per acre, produced substantial yield reductions of wheat and rye, but improvement in formulation is desirable.

Isopropyl N-phenylcarbamate and isopropyl N-(3-chlorophenyl)-carbamate do not appear sufficiently effective to be employed as strategic anticrop agents but may have a place in tactical uses.

N-4-chlorophenyl-N', N'-dimethylurea produces substantial yield reduction of rice and kaoliang at 0.5 lb/acre or less.

Additional compounds of promise have been uncovered by primary screening and are being further investigated.

D. PROTECTION

1. DETECTION

The detection problem in BW has been a particularly difficult one. The low concentrations of BW aerosols and the fact that BW particulates lack any unusual physical or chemical properties make them extremely difficult to detect and identify. It was recognized early in the program that there are at least three distinct aspects to this problem. The first is a requirement for a rapid warning of the presence of BW aerosols so that protective measures can be taken promptly. The second is a need for careful sampling of the aerosol, maintaining viability of the organisms collected. The third problem is that of precise identification of the agent used. Sampling is, of course, necessary before identification can be made.

The first of these problems is the most difficult technically. BW aerosols are composed of particles averaging somewhat larger than one micron in size. They are organic in nature, consisting largely of simple proteins, and are viable. Normal air usually contains particulate matter, most of which is apt to be inorganic and nonviable; but, almost always, some proteinaceous matter and viable microorganisms are present. Moreover, if the purpose of a warning device is to tell one when to take protective measures and when to sample, it must work quite rapidly for BW aerosols can infect very quickly and are usually of short duration. Very precise particle counters, based upon photoelectric and sonic principles, have been developed and are under test to see if changes in the particle distribution in the atmosphere can serve as a warning. This approach is the least specific and most influenced by normal atmospheric interferences, but it is also the simplest approach and cannot be overlooked. Methods based on a rapid determination of the protein content of the air in particles under 15 microns in size are more specific, and chemical, colloidal, spectroscopic and polarographic methods of doing this are under intensive study. Correlative studies are also underway on the normal protein "background" of the atmosphere and on improved sampling techniques. The polarographic technique has progressed to a point that it is desirable to construct prototype automatic field devices based on this principle. More specific and more complicated techniques under study involve direct observation of microorganisms on membrane filters or under ultraviolet microscopic devices.

Work on the second problem in BW detection, that of sampling, is much further advanced. A field sampling kit, the E25R1, is well along in development and is illustrated in Figure D-1. The basic element of this kit is the Membrane Filter, first developed by the Germans in World War II, but greatly improved and made available under Biological Laboratories contracts since that time. (See page 52).

There are two approaches to the third problem in BW detection, which is identification of the organisms used. One approach has been to turn to physical techniques, by far the most promising of which has been the use of infrared adsorption spectroscopy which appears to give unique results for different species of bacteria. Instrumentation is available in this field, but the task of studying the adsorption spectra of as many microorganisms as possible under a variety of conditions is a laborious one which has been underway now for some time at the Biological Laboratories, in the laboratories of two of our contractors, and at the British Microbiological Research Department at Porton.

The second approach to BW identification lies in simplifying, improving, and, in particular, speeding up the standard biological methods long used by microbiologists. To that end programs are underway both in the Biological Laboratories and under contract, with most progress being reported in serological reactions and lysis by specific phages. The application of these two techniques to the identification of BW agents in mixed cultures would obviate the need for obtaining pure cultures.

2. VULNERABILITY

The vulverability program was set up two years ago to study the hazard to personnel under BW attack in various target areas and to determine what magnitude of physical protective measures must be taken for their safety. The work done under this program largely involves field studies using simulant BW agents.

Tests at Port Hueneme, California, showed that a simple wooden building could be made into an effective BW shelter utilizing forced air passing through an E35 collective protector unit, if as little as 0.01 inch positive pressure differential existed within the building. The aerosol contaminated the terrain outside the building to an extent of 5,000 organisms per square inch, but no dangerous secondary aerosol was raised when 21 men marched about over it for 30 minutes. Only when the terrain contamination was raised to around 30,000 organisms per square inch was a potentially dangerous aerosol created by the marching men. It was also found that personnel exposed as long as 15 minutes to a primary aerosol of 500 organisms per liter did not produce a dangerous secondary aerosol when they removed their clothing in the outer air lock of the building.

Tests at Camp LeJuene showed that the M-1950 Shower Bath Unit was efficient in reducing skin contamination to a minimum on human subjects.

Other studies done earlier in this field have concerned themselves with vulnerability of personnel in naval vessels, at air bases, and in

large cities, and with the protective effect of clothing and shelters.

3. DECONTAMINATION

The group working on BW decontamination has in the past concentrated its efforts on a screening program for new decontaminating agents, evaluating in the laboratory and in the field promising agents uncovered in this program, and in investigating the basic principles involved in decontamination or disinfectant processes. Prior to this fiscal year, emphasis has shifted from laboratory investigations to the development of definite end-items for use by the various services.

Ethylene oxide, the first promising material to arise from the screening program, is the decontaminating agent furthest along in the program. It was recommended for standardization in 1951 as the pure compound, sealed in glass ampoules, for use in the Quartermaster Corps delousing bag or the Chemical Corps vapor-proof sack. The Cml C Engineering Agency is conducting the final engineering tests and writing the specifications on this item. Other methods for the practical application of this compound are still under investigation. Its use under gas-proof tarpaulins to sterilize material in bulk has been demonstrated as feasible, tarpaulin construction has been studied, and a sufficient number of large tarpaulins of satisfactory design have been procured to permit extensive field testing of this procedure. The flammability of ethylene oxide has hampered its use and is the main reason why the E-7 Ethylene Oxide Ampoule is not yet standardized. The commercial mixture of ethylene oxide and CO_2, Carboxide, is not inflammable, but difficulties arise from the high vapor pressure of this mixture and its low concentration of ethylene oxide. Within the past year it has been shown that mixing it with Freon 12 results in a non-inflammable material, even with a 19 per cent ethylene oxide content, and the vapor pressure of the mixture is such that it can be packaged in a variety of light metal containers. This development will permit safe handling of ethylene oxide in much simpler equipment, and it is being actively followed.

Considerable progress has also been made this year in the use of formaldehyde as a decontaminating agent. An intensive survey has been conducted, under contract, of the best type of dispenser for liquid formalin, and considerable insight has been gained on methods of preventing, or overcoming the annoying residue of polymerized formaldehyde left behind on exposed surfaces following treatment. Small aerosol bomb-type dispensers for formalin have been developed and are under evaluation.

Ethylene imine and β-propiolactone, two highly effective compounds uncovered in the screening program are both under intensive chemical evaluation, with a view towards their possible inclusion in end-items.

The screening program is continuing, with special attention being given to fluorine and carbonyl-containing compounds.

As much of the activity of the decontamination group has been spent in developing specialized procedures as has been in uncovering new decontaminating agents or designing new equipment. During this year a series of Biological Laboratories Interim Reports on "Principles and Practice of BW Decontamination" was initialed, summarizing experience to date on particular applications, and reporting on new developments in the field.

4. PHYSICAL PROTECTIVE DEVICES

The Biological Laboratories are not charged with the development of masks, hoods and protective clothing, other than specialized items worn by our own personnel in certain hazardous tasks. The Chemical and Radiological Laboratories of the Chemical Corps design such items for their general troop issue, effective against all CBAR agents, as do certain groups in the other services. The Biological Laboratories evaluate these items for their efficiency against BW aerosols during the development program. These evaluations are done both in the laboratory and on human subjects in a large chamber in the presence of an aerosol of simulant BW agents.

In the past year, a comparative study was made on the relative aerosol permeability of poplin, sateen, and HBT fabrics, which did not differ significantly from one another. Many other items of regular issue clothing, Army, Air Force, Navy, and Marine, were also evaluated; in general, all offered good skin protection, particularly when two layers of fabric covered the skin in all areas.

The main problem in BW protection is respiratory, not body protection. A one part per million aerosol reduction has been accepted as a goal in this field by Tripartite agreement. The M11 canister has an efficiency of one part per 100,000,000. The standard M9A1 mask which uses this canister, however, is effective on the average only to about one part in 20,000, giving good but not ideal protection. Many experimental masks were evaluated during the past year including the E51R3 and the E51R15 noncombat masks, the E52R18 civilian mask, the E2 and E3 infant masks, the E10R24 and the E14R2 field protective masks, the E56 tank crew mask, and a new navy shipboard mask, none of which quite achieved the desired goal, but the majority of which still offered good protection. During the same period, Chinese, Czechoslovakian, East German, French, Swedish, and Russian masks were tested. All except the French mask fell below American prototypes in performance.

5. SAFETY

For the first time, it now appears possible that BW occupational infections among laboratory and pilot plant personnel may be reduced to the vanishing point. In the few buildings which have been fully equipped with the latest type safety features and devices designed within the Biological Laboratories, infections over the past year have been absent or negligible. Such equipment is expensive and can be installed in existing laboratories only after elaborate modification. The new facilities, discussed in Section III of this report, with latest safety design incorporated in them will offer a much safer environment for laboratory personnel. The influence of the introduction of ventilated safety cabinet systems which first began in 1951, is probably the deciding factor in a 33 per cent decline in occupational illnesses in the present fiscal year from the peak in FY 51.

In addition, continued progress in the development of fibre-glass air filters makes it seem possible that in some situations air filtration of laboratory exhaust air may replace incineration, with significant savings of money.

Employees are now receiving 10 vaccines and 6 skin tests. More than 250 employees received all of these. A new anthrax vaccine developed in the Biological Laboratories is included in this test and has now been administered to several hundred persons without untoward results. Research efforts towards combining vaccines is progressing. It is interesting to note that among the recent laboratory infections several occurred in immunized persons. There was one such case with anthrax, 10 with psittacosis and 4 with Q fever which required hospitalization, indicating that the immunity provided by vaccination may be overwhelmed by sufficient dosage. This demonstrates that adequate physical safety features, such as the ventilated cabinet systems previously referred to, will still be required even in work with agents for which good vaccines are available. The screening program for new agents recently instituted, and the shift of emphasis from debilitating to lethal agents in the laboratory program again emphasize the need for an adequate safety program and continued study on new safety devices.

[redacted] Security Information

FIGURE D-1. PROTOTYPE BW FIELD SAMPLING KIT.

[redacted] Security Information

E. DISSEMINATION

1. BOMB, BIOLOGICAL, 4-lb., M114

The M114 bomb, figure 1*, is a standard-type explosive munition for disseminating liquid, bacterial agent-fill. The bomb was initially conceived and tested for disseminating spores. However, the standard-type AB-1 fill, containing the vegetative organism Brucella suis, is an authorized agent-fill for the M114.**

The filled munition weighs 4.2 lb and is cylindrically shaped, about 21½ in. long and 1-5/8 in. maximum diameter. An M174 air-arming, impact, nose fuze initiates an explosive charge of 106 gm of tetryl contained in a central burster tube. The volume of agent fill is 320 ml. One hundred and eight M114 bombs are loaded into an M26 500-lb cluster adapter to make an M33 500-lb biological cluster, figure 2.

During the past fiscal year, assistance was furnished the production facility in solving difficulties encountered in obtaining a satisfactory mechanical and biological seal at the secondary closure of the bomb.

Many assessment tests with the M114 were performed to compare the performance of development-type munitions with a standard-type item and to assess candidate agent-fills.

2. BOMB, BIOLOGICAL, ½-lb., E61R4

The current experimental model of the ½-lb. biological bomb is the Bomb, Biological, ½-lb., E61R4, figure 3a. It is a tail-ejection type antipersonnel munition specifically designed to disseminate an aerosol of liquid fill of vegetative organisms. Assembled as a complete round, the bomb consists of a fuze assembly, an

* Illustrations of the munitions will be found on pp 66-72

** Refer to Section IIA, 4: p 12

E11 plastic container carrying 38 ml of agent-fill, and an E61R4 metal parts assembly. The approximate weight of the complete round is 1.2 lbs. Over-all height is 7.3 in.; maximum outside diameter, at nose and tail of bomb, 1.5 in.; and minimum outside diameter, at the waist of the bomb, 1in. The E108 type cluster, figure 4, consists of 474 E61 type bombs in an uninsulated E48 type adapter; 544 bombs in an insulated E53 type adapter for high performance aircraft comprise the E133 type cluster. The E108 cluster weighs about 750 lb, the E133 about 850 lb.

Statistically planned experiments were made with the E61R4 on (1) the thickness and design of the shear cup, (2) the type and loading density of propellant, (3) the method of propellant ignition, and (4) the closure design. These factors were evaluated on the basis of percentage aerosol recovery from a liquid fill of Serratia marcescens. Based on the evaluations, the most favorable design of metal components was selected with respect to the interior ballistics of the bomb.

Several static field tests with the E61R4 were conducted with S marcescens wherein four E61R4 bombs, laid out on the corners of a square with 30-foot diagonals, were compared with one M114 placed at the point of intersection of the two diagonals. The 4-to-1 ratio in the number of bombs were chosen to correspond to the ratio of the bombs in a single E108 type cluster. The following results were obtained:

a. Four E61R4 bombs produced an area coverage 1.42 to 1.90 times the area of equal aerosol dosage from a single M114 bomb.

b. No measurable difference in aerosol decay rates was found between the two munitions.

Preliminary sphere tests of the E61R4 with AB1 were not conclusive. However, among guinea pigs exposed for one minute at aerosol ages of 16, 30, and 60 minutes, approximately 90 percent were infected at the first and second ages, 70 percent at the third. The aerosol concentrations at those ages were 5.65×10^3, 3.06×10^3, and 6.25×10^2 cells per liter of aerosol, respectively.

A Sphere test was conducted to assess the ability of the E61R4 to disseminate a liquid fill of B tularense. Impinger recoveries were not considered reliable, but the following infectivity rates among guinea pigs were found for one-minute exposures; 97, 87, 45, 15, and 6 percent at respective aerosol ages of 2, 16, 30, 58 and 86 minutes.

Sphere tests with the E61R4 were performed with dry and wet

fills of Coxiella burnetii. The number of replicates for these experiments was exceedingly small because of the labor involved in performing the animal assessment. With dry, ground C burnetii, 100 percent of the guinea pigs were infected after exposure for one minute at periods up to and including two hours after functioning of the bomb when a relative humidity of 31-38 percent was used. At a humidity of approximately 85 percent, the infection rate was much lower. With wet fills of C burnetii, the guinea pig infection rate was generally lower, but the same trend was apparent with respect to relative humidity.

The E61R4 was used to disseminate ABl in three Dugway trials. In the first two of these trials, thirty-seven M114 bombs were detonated in one group and one hundred sixty-one E61R4's were simultaneously functioned in another, both groups being located in patterns of equal area. The numbers of bombs chosen represented proportionate fractions of the M33 and E108 cluster contents respectively. In the third test, the numbers of M114 and E61R4 were reduced to 7 and 31 respectively. From these tests it was generally concluded that the E61R4 on a cluster-to-cluster basis of comparison is roughly 10 times as effective as the M114 with S marcescens.

A trial utilizing four hundred E61R4 bombs, filled with B tularense, also was performed at DPG in connection with the testing of a new concept for assessing small munitions designed for large area coverage. The munitions were evenly spaced on a square with 1½ mile sides (2.25 sq mi), the concentration of bombs/an expenditure of 3/8 of an E108 cluster per square mile. The ½-mile horizontal grid was located on the downwind edge of this square pattern. Approximately 35 percent of the 2000 guinea pigs exposed on the ½-mile grid were infected. On three lines of animals placed within the bomb pattern between the upwind edge and the ½-mile horizontal grid, a distinct buildup of infection rate was shown as a function of downwind distance. Three similarly placed lines up to distances of nearly a mile downwind of the bomb pattern showed a sharp decrease in infection rates. Relative humidities and temperatures were favorable for the test. Monkeys also were exposed in this test; of the 49 used, 46 became infected.

Intensive development work was done to solve serious problems of fuze malfunctioning and bomb instability which became apparent when airplane drop tests with E108 clusters were made. A stability and fuze-functioning test was run to test 2 designs of each of 6 fuzes, a total of 12 treatments. Each treatment was tested both

with standard E61R4 bomb bodies and with finned bodies. Satisfactory results were obtained with several treatments and the E34R1 fuze was selected as the most practicable choice of several models.

In view of recent directives, current assessment work has been focussed on the E61R4-Agent N combination. The E61R4 was specifically tailored for disseminating vegetative organisms and but few data are available on performance with spores.

3. BOMB, BIOLOGICAL, 2-oz., E93 TYPE

The E93 bomb, figure 5, is a miniature munition for disseminating dry agent-fill, approximately 3 in. long by 3/4 in. diameter. It has an air-arming, impact fuze carried in a 4-fin tail assembly. The bomb body is a modified, compressed CO_2 cartridge commercially produced for carbonating water or other beverages in the home. Six E93 bombs can be carried in a ribbed, plastic, 4-in hollow sphere. This assembly designated Subcluster, Biological Bomb, 2-lb., E119, figure 6, is opened above ground by a barometric device being developed by the Minneapolis Honeywell Regulator Co.

The preliminary investigation on the E93 was started by the Chemical Corps Biological Laboratories and later was assigned to the Ralph M Parsons Co as a development task under contract No. DA-18-064-CML-2283. Design studies, wind tunnel runs, and dissemination tests with the E93 led to interior changes influencing the mechanism of dispersion and to improved designs of the arming and firing device and the stabilizing tail element. These studies resulted in the E93R1, R2 and R3 models of improved design.

4. BOMB, BIOLOGICAL, 1½-lb., E99

The E99 bomb, figure 7, is a cylindrical munition utilizing pneumatic atomization to disseminate liquid, antipersonnel agent-fill through a 2-fluid nozzle. The munition is approximately 8-7/8 in. long and 1½-in. diameter. It weighs about 1½ lb when loaded with 15-25 ml of fill. Pressurized gas for dissemination of the fill is obtained by controlled vaporization of liquid propene. Propane also has been used as a gas source. Agent-fill is carried in a separate-loading, plastic container. The bomb is initiated by a nonexplosive, inertia type mechanism. Four

wrap-around, tabbed fins are attached to the bomb to impart rotational forces to arm the fuze and to induce ballistic stability. Two test fixtures, the E88R1 and the E88R3 incorporating the aerosolizing principles of the E99, have been used in preliminary aerosol assessment trials.

Mechanical design of the E99 bomb is being carried forward under contract with Arthur D. Little Inc. Wind tunnel and laboratory tests were made to confirm engineering studies and calculations; air drops and mortar firings were made to test component functioning under simulated field conditions; and, other studies relating to design, assessment, and operation of the unit were made both by the contractor and by Biological Laboratories personnel.

Seven clusters each composed of 395 dummy E99 bombs in an E48R2 adapter were dropped under oporational conditions at Holloman Air Force Base to determine the aerodynamic stability of the bomb and the dispersion pattern of the cluster. Discouraging results from these drops indicated a serious mechanical weakness in the tabbed fin assembly which was damaged or torn from the bomb body. Of 1138 bombs recovered from 3 clusters released at 26,000 ft and opened at 24,000, 20,000 and 10,000 ft, the percentage of undamaged units decreased progressively with increased distance of fall from 43 to 18 to 3. Modifications of the fin assembly are being designed by the contractor and new models are being fabricated for further testing.

In addition to the above work, the design of an improved 2-fluid nozzle was studied at the Biological Laboratories. A commercial, swirl-type nozzle was modified and tested. Aerosol characteristics equal to or better than those obtained with the contractor's experimental nozzles were obtained in chamber tests with Serratia marcescens. From an engineering viewpoint, the modified commercial nozzle is more desirable; it is smaller, lighter, and easier to fabricate.

Other Biological Laboratories investigations included studies on the orifice plate, which controls the flow of fill to the nozzle, and on the effect of various propellent gases on the agent fill.

5. BOMB, BIOLOGICAL, 1½-lb., E103

The E103 bomb is a cylindrical munition for disseminating 10-25 ml of liquid fill by pneumatic atomization through a 2-fluid

nozzle. The bomb is about 8 in. long and 2 inches in diameter. A preliminary investigation of this bomb was started by the Biological Laboratories; later, work on the E103 was transferred to the R. M. Parsons Co. as a task under contract No. DA-18-064-CML-2283 with the Chemical Corps.

Studies were started on several phases of the bomb design, such as slenderness ratio, location of center of gravity, nozzle design, agent container, liquid flow rate, clustering, propellant, flight stablization, and method of arming.

Wind tunnel tests were started with solid-vaned dummy bombs to obtain aerodynamic stability data. Dummies with wire vanes were scheduled for wind tunnel tests, and additional solid-vaned models were designed and fabricated for dynamic tests to obtain data on flight stability, terminal velocity, and impact effect.

6. BOMB, BIOLOGICAL, SPHERICAL, 1¼-lb., E96R1

The E96 bomb, figure 8, is a self-dispersing munition for disseminating a liquid antipersonnel agent-fill. The bomb weighs about 1¼ lbs and is composed of three basic components: (1) a 3-in., metal, spherical generator containing a two-fluid nozzle, a flexible agent-container holding 12-18 ml of fill, a propellent chamber charged with liquid propene and wet, shredded balsa wood, and an E31 all-ways, air-arming fuze; (2) a shock-absorbing material, 3/8 in. thick, surrounding the generator; and (3) a 4-in., 9-ribbed, plastic outer casing which incloses the cushioned generator.

The E96R1 bomb is a modification resulting from laboratory testing of the E96. The E96R1 uses the E35 all-ways, air-arming fuze, an improved version of the E31 used in the E96 bomb. Tests were made with the pre-armed, water-filled E96R1's to determine functioning on impact when launched from an air gun. Impact surfaces ranged in hardness from soggy turf to concrete. The test results showed that it is possible to absorb the impact against concrete surfaces and to protect the generator so that it disseminates the fill satisfactorily. Against soggy turf, the bombs penetrated to a depth of about 3 inches, and the plastic outer casing did not shatter. Against soft turf and rubber-covered wood, the bomb functioned as well as against concrete.

7. BOMB, BIOLOGICAL, SPHERICAL, 1¼-lb., E94 TYPE

The E94 type bomb, figure 9, is a self-dispersing munition composed of 3 basic parts: (1) an aerosol generator, 3 in. in diameter, surrounded by (2) a layer of shock-absorbing material, all contained in (3) a ribbed, plastic, 4-in. hollow sphere. The generator sphere will disseminate 75 ml of fill through a single-fluid nozzle in about 15 seconds. In test fixtures, pressurized gas for operating the munition was provided by a slow-burning propellant. An all-ways, air-arming, impact fuze, the E36, will be used to initiate the bomb.

The E94 type bomb is similar to the E96 type with the major exception of the generator sphere. As stated above, a single-fluid nozzle system is used in the E94, whereas a 2-fluid nozzle system is used in the E96. Common problems of study included fuzing, methods of carry and dispersion, aerodynamic stability, area coverage, shock-absorbing material, and outersphere configurations. Specific work on the E94 bomb dealt with testing candidate nozzles and experimental propellent charges developed by the Naval Research Laboratory under an interagency agreement with the Chemical Corps.

8. BOMB, BIOLOGICAL, 2-lb., E97

The E97 is a spherical munition for disseminating liquid agent-fills by explosive means. Two candidate designs, the E97 and the E97R1, now are being studied. The body of the E97 is a ribbed, plastic, 4-in. sphere, whereas that of the E97R1 is a composite sphere formed by sections of 4-13/16 in. and 4-in. spheres. Both models are being designed to receive a VT fuze functioning from 2 to 8 feet above terrain. The feasibility of such a fuze is being investigated by the Ordnance Department. Two shaped explosive charges and two agent containers are used in each bomb. Figure 10 shows an E97 with a dummy fuze.

The preliminary investigation of this bomb covered the following: (1) a literature search on the aerodynamics of spheres, the physics of aerosol dissemination by explosive means, the physics of shaped charges, and related subjects; and (2) a study of the engineering problems involved in design of the agent containers, the casing, the explosive components, and necessary test equipment.

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9. CLUSTER, BIOLOGICAL BOMB, 750-lb., E123

The E123, figure 11, is a glide cluster which will attain lateral deviation from the line of flight of the aircraft before opening and scattering the component munitions. Lateral deviations of the cluster up to two miles right or left are anticipated during a fall of 15,000 ft when the cluster is released at an altitude of 30,000 feet. The E123 is part of an integrated area-bombing system which also includes the Cluster, Biological Bomb, 750-lb., E124 described on page 60 of this report. The basic item of the E123 is an E48 type cluster adapter with folding delta wings for imparting the necessary lift. A roll stabilizing mechanism and controllable tabs are required to impart aerodynamic stability and to direct the flight of the cluster. The E123 is being developed by Aircraft Armaments, Inc. under contract with the Chemical Corps.

Quarter-scale models of the delta wing E123 were manufactured and flight tested to determine the effect of various modifications on the over-all flight characteristics of the model. Eight full-scale test missiles were fabricated for test at Holloman Air Force Base. Four of the missiles were dropped. Data from the tests now are being used as a basis for modifying the remaining test items.

10. CLUSTER, BIOLOGICAL BOMB, 750-lb., E124

The E124 is a cluster which when released from aircraft at 30,000 feet will attain a lateral deviation of 1,250 yards to the right or left of the line of flight during a fall of 15,000 feet, before opening and scattering the component munitions. The code name "DEVRON" has been assigned to this unit. The E124 is part of an integrated area-bombing system which also includes the Cluster, Biological Bomb, 750-lb., E123 described in section 9 of this Part. The basic component of the E124 is an E48 type cluster adapter with controllable tail surfaces. A directional and rate gyroscope is provided for control of stability and trajectory. DEVRON is being developed by WADC cooperatively with the Biological Laboratories. Figure 12 shows a model of the E124 cluster.

Wind tunnel trials were made on test models of the DEVRON and the data used to establish design parameters. The Sommers Gyroscope Company was awarded a contract by WADC to design and fabricate models for full-scale flight testing. As a result of a

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recent policy decision, the E53 cluster adapter was adopted as a major component of DEVRON in place of the E48 adapter originally used.

11. BOMB, BIOLOGICAL, 750-lb., E86R1

The E86 type bomb, figure 13, is an anticrop munition for disseminating TXI from high performance aircraft. It is a successor to the E73R1 500-lb. Biological Bomb, figure 14.* The E86R1 consists of an E53 750-lb cluster adapter into which are placed 7 cylindrical agent containers each loaded with about 2¼ lbs of feathers and anticrop pathogen. Insulation and other means for controlling the temperature of the fill are incorporated in the bomb to preserve agent viability. After release from the aircraft, the bomb falls for a predetermined time and is opened. The agent containers then fall free and decelerate to terminal velocity, after which the containers are opened and the fill released.

The E86 bomb is an earlier model which resembles the E86R1 but uses an E48R2 cluster adapter, rather than the E53 type adapter. Both the E86 and the E86R1 bombs overcome the major deficiencies of the original E73R1 munition, i.e., unsatisfactory release from high-performance aircraft and lack of temperature control to preserve viability of the agent.

The Ralph M. Parsons Co. is developing the E86R1 as one of its tasks under contract DA-18-064-CML-2283. Various designs of agent containers and opening mechanisms were studied, methods for controlling the temperature of the fill between the limits of 43 F and 75 F were devised, and ballistic problems were investigated.

12. BOMB, BIOLOGICAL, 80-lb., E77

The E77 bomb, figure 15, is a balloon-delivered munition for disseminating dry anticrop pathogens over large target areas. It is being developed by General Mills, Inc, under contract DA-18-064-CML-2104. The basic configuration of the E77 bomb is a right circular cylinder 32 in. in diameter and 24 in. high. This cylinder, termed the gondola, is made of wood-reinforced fibreboard and is lined with 2 in. of styrofoam insulation. Five agent containers, each holding approximately 3½ lbs of feathers and anticrop pathogens, are grouped centrally around a chemical type heater which maintains the proper temperature control for

*See CCTC Item 2709: The E73R1 is now the standard-type Bomb, Biological, TX1, 500-lb., M115

preserving viability of the fill. A combination barometric and mechanical-time mechanism mounted as an assembly on the gondola provides the control for (1) neutralizing the fill in the event of bomb malfunctioning over friendly territory, (2) termination of flight, and (3) release of the bomb from the balloon.

When the balloon has traveled a pre-selected time long enough for it to reach the intended target, the bomb is released from the balloon and falls to a pre-selected altitude of from 1000 to 5000 ft above terrain. A drag chute is used to reduce the velocity of fall. The gondola then opens in a clamshell fashion and ejects the containers. Opening of the gondola is controlled by the master barometric switch that also controls a portion of the neutralizer circuit. As the containers leave the gondola, a gas pressure mechanism is activated and forces the fill from the container. To minimize radar detection of the bomb, it is constructed of nonmetallic materials where possible.

Five models of the bomb have been made and tested jointly by the contractor, the Chemical Corps and USAF. Four were dropped from an airplane to obtain functioning data and one from a balloon. These models were dropped with simulant fills. In the balloon test, the release of the bomb was accomplished by radio control rather than by a time mechanism in order to assure the release at a pre-determined location. All of the bombs functioned satisfactorily from a mechanical point of view. Bombs now are being made for final engineering tests.

13. BOMB, BIOLOGICAL, SPHERICAL, 10-oz., E95

The E95 bomb is a self-dispersing spherical munition for disseminating dry anticrop pathogens. The complete munition consists of a 3-inch, plastic, hollow sphere, externally ribbed to induce rotation during flight; a barometric opening device to separate the sphere at a selected altitude; and agent-fill. The approximate internal volume of the hollow sphere is 11 cu in., about 3 cu in. of which is taken up by the opening mechanism, the remainder being loaded with approximately 2½ oz of agent-fill. The total weight of the loaded bomb is estimated at 12 oz. Until the barometric opening device is developed, two interim bombs, the E95R1, figure 16, and E95R2 models with mechanical time-delay opening mechanisms are available for test use only. The opening device is the major difference between the E95 and the E95R1 models. However, the E95R2 model also included a change from a ribbed, plastic body to a smooth aluminum body.

The E95 is being developed for carrying in and delivery from either a bomb bay hopper in bomb aircraft or an externally mounted dispenser on fighter or reconnaissance aircraft.

Dugway Proving Ground tests with single and multiple E95R1 bombs loaded with nonviable TX established that spores released at heights up to 1000 ft above terrain can be deposited onto large target areas, under meteorological conditions of neutral or inversion temperature gradient. For example, spores from releases of five E95R1 munitions, opened at 1000 ft above terrain under inversion conditions with low wind speed, were deposited on a target grid 15 miles wide by 30 miles long. Preparations for tests to correlate the density of spore deposition, as determined from sampling plates, to plant infection were initiated to add further information concerning the number of bombs to be released at low altitudes in order to obtain effective crop infection.

The design of the barometric opening device, including a remotely controlled setting mechanism for selecting the opening height just prior to release of the bomb from the aircraft, is a major part of the over-all munition development work being done under contract with the Minneapolis-Honeywell Regulator Company. First designs of the fuze and setting mechanism were made, and a limited number of models now are being fabricated for tests of accuracy of the barometric sensing element, reliability of functioning, and general safety and soundness of design. Methods of filling and sealing the munition also are being investigated.

Design of the E21 biological bomb dispenser carrying ten E95 bombs was initiated. First models were made for flight functioning, heat transfer studies, and other engineering work.

14. MINE, BIOLOGICAL, XB-14-B

The XB-14B is a submarine-launched torpedo mine for offshore dissemination of liquid agent-fills by a 2-fluid nozzle system. After launching, the mine will sink to the bottom, remain there for a predetermined period up to 2 hours, then surface and disseminate the fill; and, after completion of dissemination, the mine will scuttle itself. The over-all weight of the mine will be about 1400 lbs, its diameter and length about 21 in. and 120 in. respectively. A standard torpedo tube will hold 2 mines, each of which has a maximum capacity of about 42 liters of agent fill.

The mine will float with the longitudinal axis in the vertical position and will disseminate the fill about 4 ft above the

water from an extensible mast incorporating a nozzle manifold which resembles a wagon wheel. Twelve nozzles placed to eliminate spray-cone interference will be manifolded on the rim.

The mine will consist of three basic sections that can be shipped, stored, and/or assembled as required: (1) bottom section including the anchor, timing and actuating mechanisms, and the propellent container; (2) middle section containing the agent-fill; and (3) a top section holding the buoyance chamber and mast assembly.

The development and testing of the agent-fill and the generating system is the responsibility of the Chemical Corps; of the complete munition, the BuOrd, Department of the Navy. Close coordination between these agencies is maintained in the cooperative effort.

Because of the hazards associated with the use of propane, the Navy specified that any material with explosive or toxic properties could not be used as a source for pressurized gas. A study for an acceptable gas was undertaken, consideration being given to such properties as vapor pressure, latent heat of vaporization, specific volume, explosiveness, toxicity, and availability of supply. On the basis of these qualities, CO_2, N_2, Freon 12 and Freon 22 were selected for testing at several operating pressures in various candidate 2-fluid nozzles under consideration. As a result of these tests, CO_2 was chosen as the preferred source of pressurized gas for a modified, commercial, swirl-type nozzle at operating pressures from 300 to 400 psi.

Two field trials with XB-14B test fixtures were run, coded as OPERATIONS SELTZER and WHITEHORSE. SELTZER, a land trial, was carried out locally to supply reference data and other information useful in performing WHITEHORSE, a sea-to-land trial, conducted in the vicinity of Panama City, Florida. A combined fill of S marcescens and B globigii was disseminated in both trials. Cloud widths between 300 and 400 yds were obtained in SELTZER; S marcescens was recovered by impingers 2700 yds downwind, and B globigii, 5600 yds downwind. Final reports of WHITEHORSE are not yet available, but indications are that results will be better than predicted by extrapolation of SELTZER data.

15. MINE, MARINE, BIOLOGICAL, 300-lb., E4

The E4 mine is a continuous aerosol generator that can be used as a weapon in amphibious warfare against harbor and shore

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installations. The munition is primarily intended for use by UDT personnel who will tow it to a planting site in a towable container known as a "water boy." When the site is reached the mine will be released from the water boy and planted by the swimmer. Upon release it will sink to the bottom in depths not over 75 feet, remain there for a predetermined time, then surface and disseminate its fill, and will finally sink again. The mine also may be planted from surface craft, in which event the water boy is not used.

The E4 is a cooperative effort between the Bureau of Ordnance, Department of the Navy, and the Chemical Corps. Areas of developmental responsibility have been defined; the Chemical Corps will be responsible for development of the aerosol generating system, and BuOrd for the over-all design of the mine. Close cooperation is being maintained. Work on the E4, originally started by the Biological Laboratories, now is assigned to Ralph M. Parsons Co. under Chemical Corps contract No. DA-18-064-CML-2283.

Because the E4 must fit the already existent water boy, maximum limitations of 300 pounds total weight, 17 in. external diameter, and 47 in. over-all length are imposed on the mine.

The dissemination system used in the E4 mine is based on the formation of aerosol particles from bursting foam globules suddenly expanded by release from a region of high pressure to one of atmospheric. This principle was used in the "C" generators previously developed and tested by the Biological Laboratories. Pressurized gas formed by the evaporation of a liquid propellant is used to force the fill through a metering orifice into a mixing chamber wherein the fill is mixed with a metered amount of gas under high pressure. A foam is formed in the chamber and is forced through an exit orifice to the atmosphere.

Studies are being made on the factors which control the design of the generating system. These include the agent and agent-fill container, the propellant and its container and the nozzle and mast assembly, all interrelated with the basic dimensional and weight limitations imposed by the water boy. Test fixtures were fabricated to investigate these factors.

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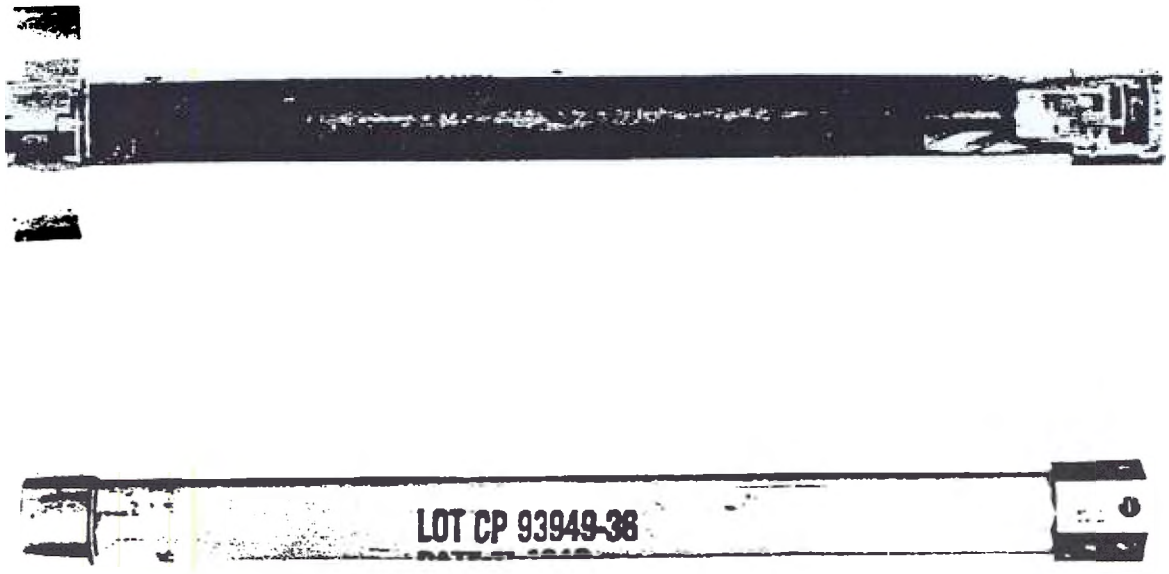

FIGURE 1. BOMB, BIOLOGICAL, 4-lb., M114 (SIDE & SECTIONAL)

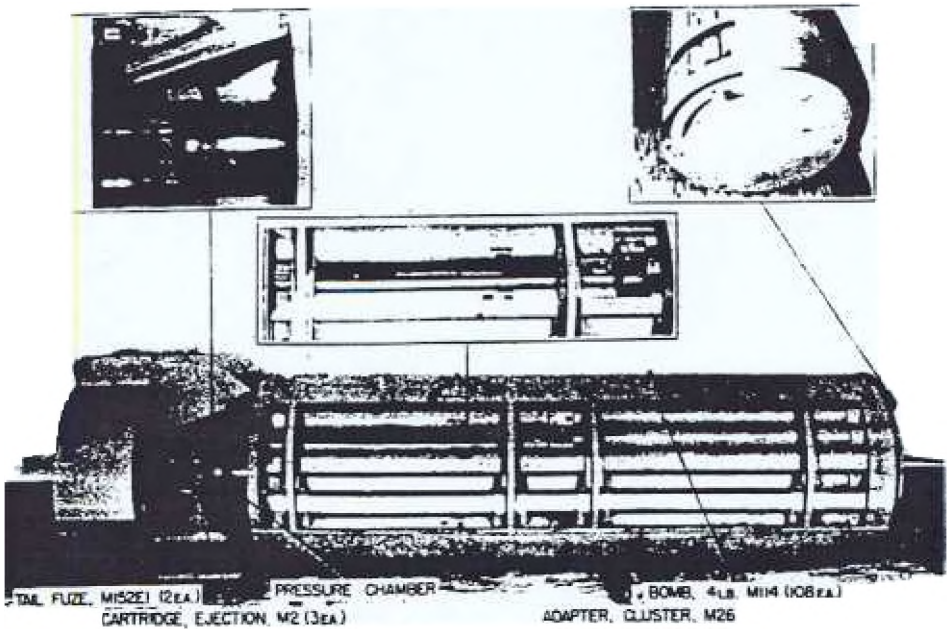

FIGURE 2. CLUSTER, BIOLOGICAL BOMB, 500-lb., M33 (SECTIONAL)

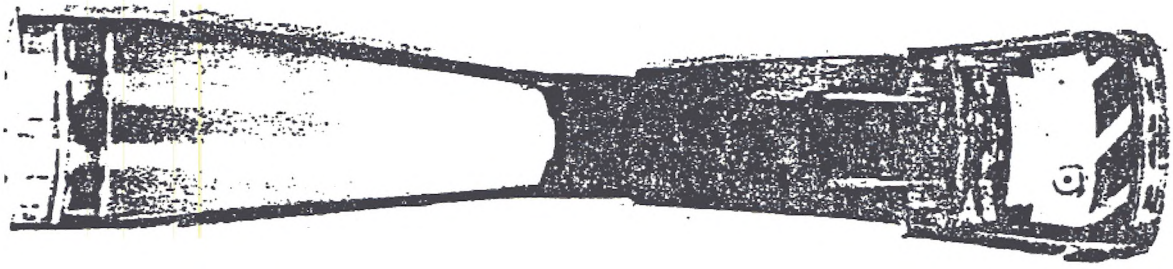

FIGURE 3. BOMB, BIOLOGICAL, ¼-lb., E61 (SECTIONAL)

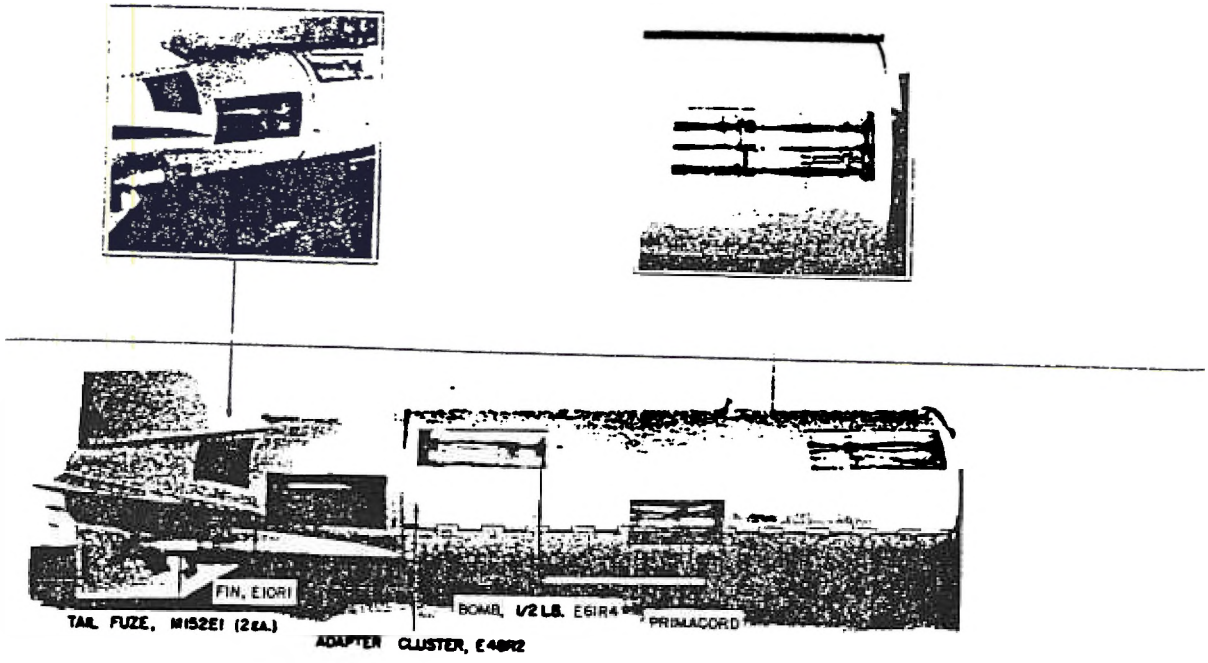

FIGURE 4. CLUSTER, BIOLOGICAL BOMB, 750-lb., E108R3 (SECTIONAL)

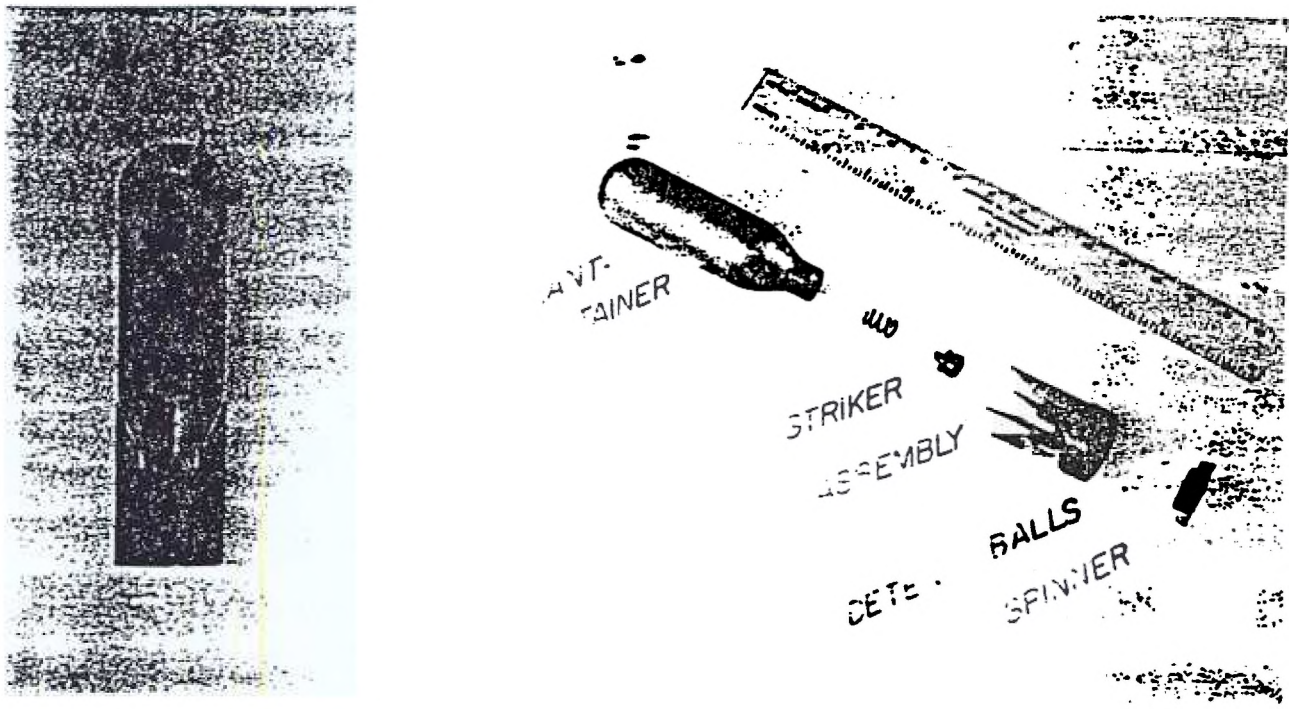

FIGURE 5. BOMB, BIOLOGICAL, 2-OZ., E93 (SIDE & EXPLODED)

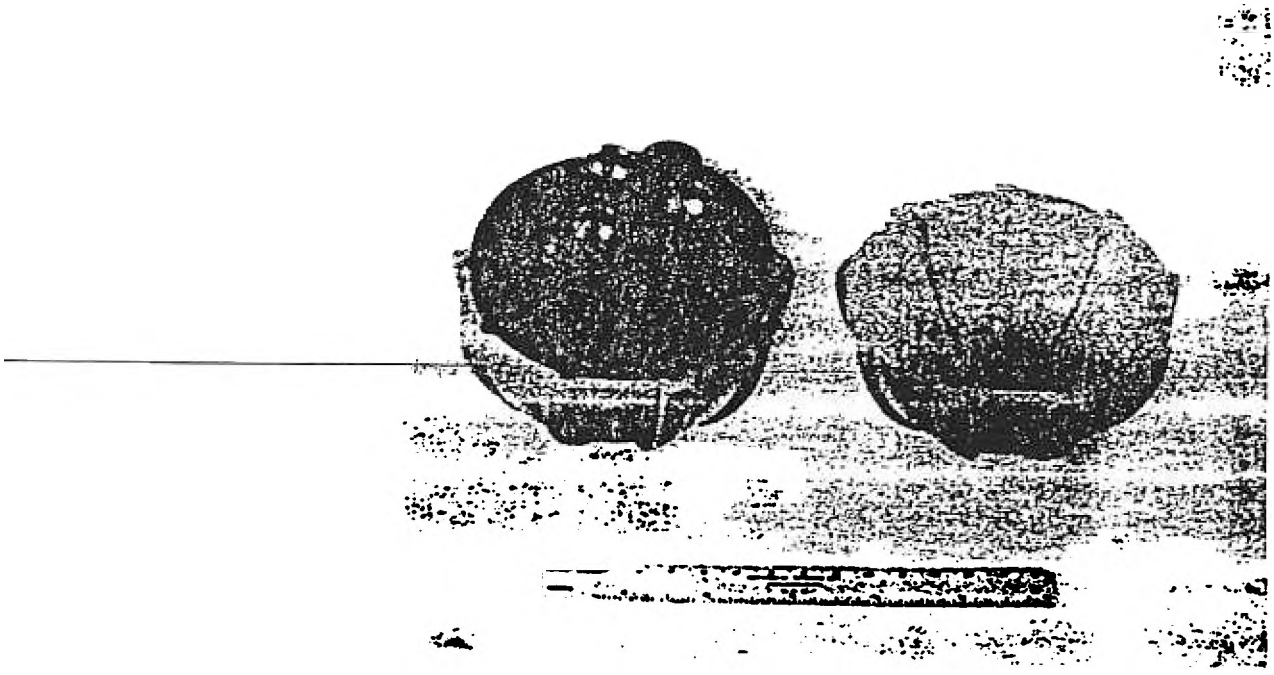

FIGURE 6. SUBCLUSTER BIOLOGICAL BOMB, 2-lb., E119

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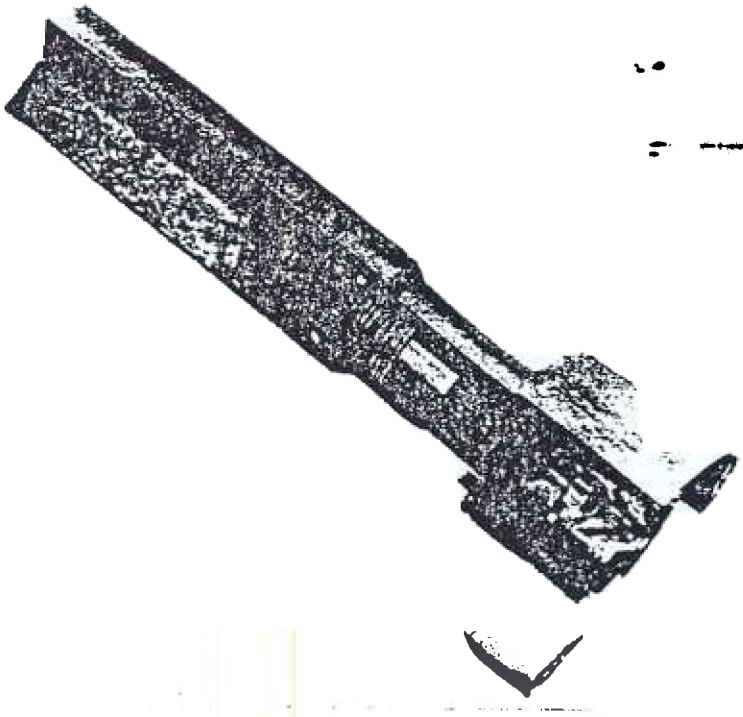


FIGURE 7. BOMB, BIOLOGICAL, 1½-lb., E99 (SECTIONAL)

FIGURE 8. BOMB, BIOLOGICAL, SPHERICAL, 1¼-lb. E96 (SECTIONAL)

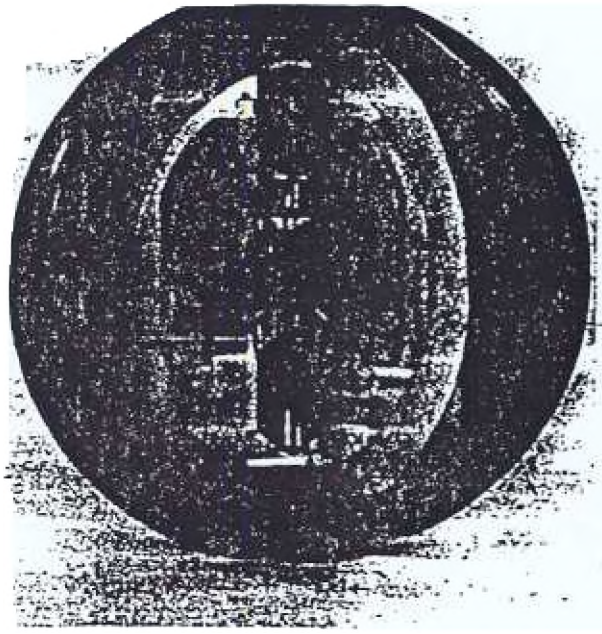


FIGURE 9. BOMB, BIOLOGICAL, SPHERICAL, 1¼-lb. E94 (SECTIONAL)

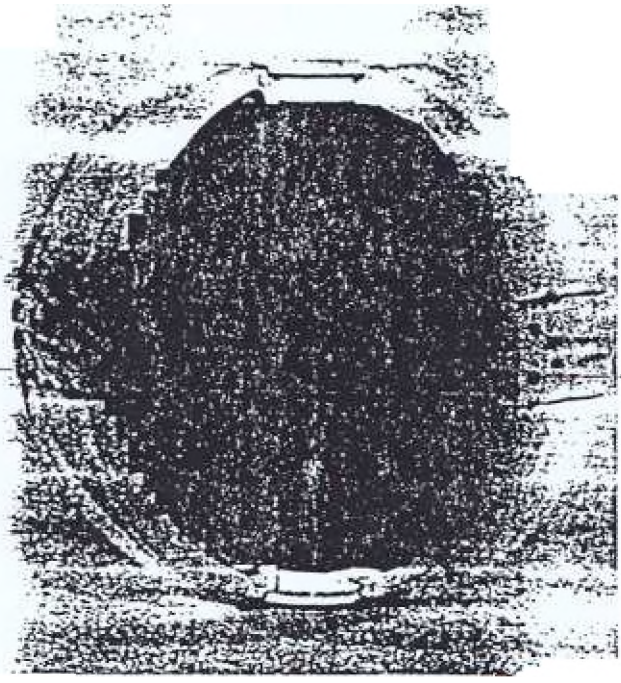


FIGURE 10. BOMB, BIOLOGICAL, 2-lb., E97 (SECTIONAL)

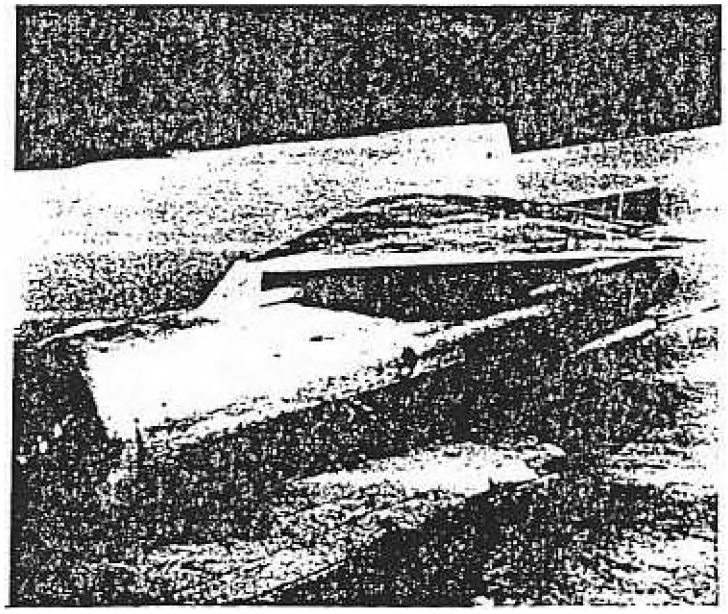


FIGURE 11. CLUSTER, BIOLOGICAL BOMB, 750-lb, E123

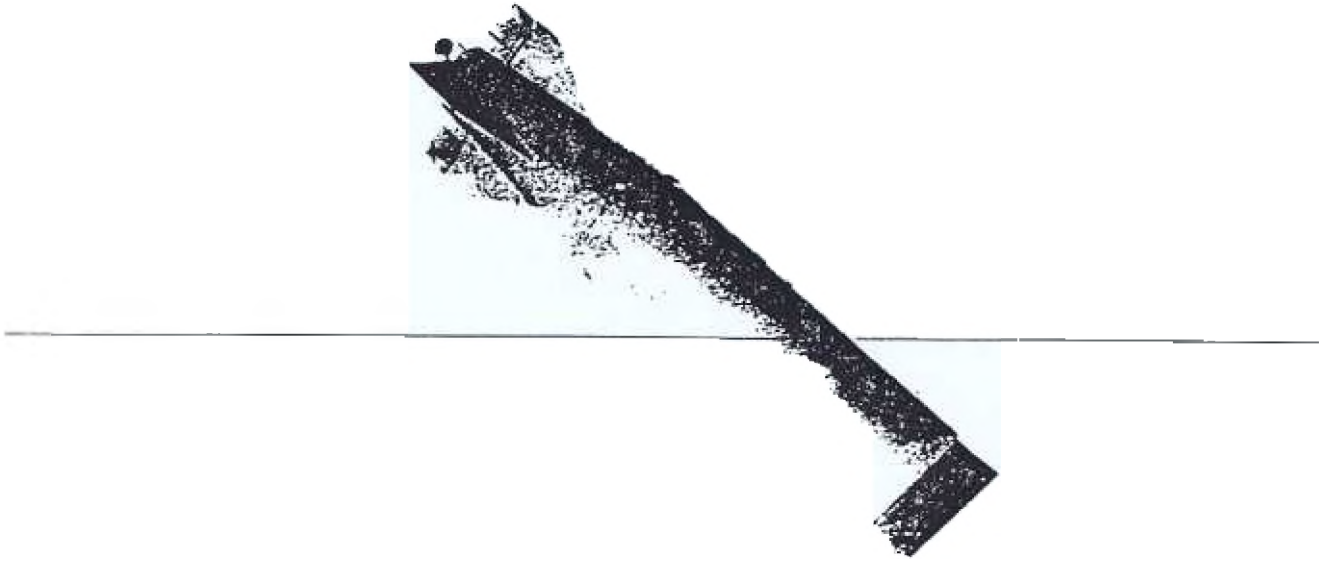


FIGURE 12. MODEL OF CLUSTER, BIOLOGICAL BOMB, 750-lb, E124

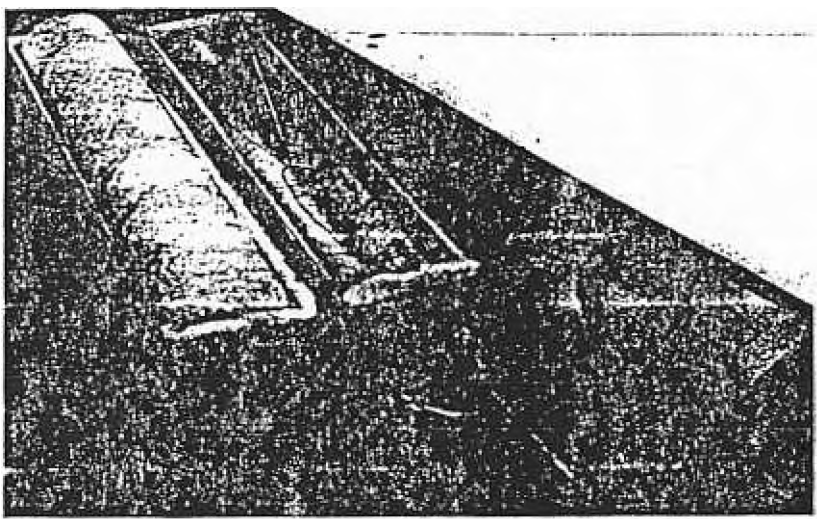


FIGURE 13. BOMB, BIOLOGICAL, 750-lb., E86

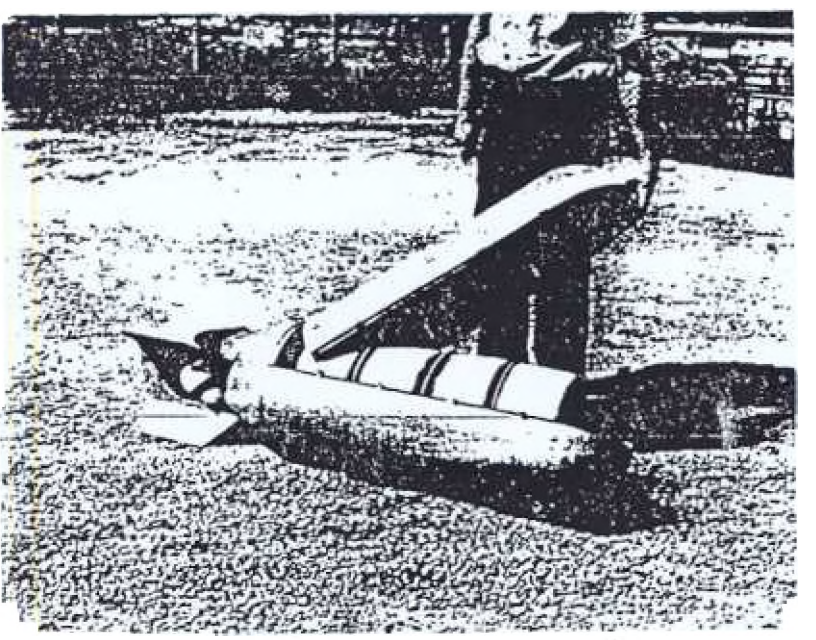


FIGURE 14. BOMB, BIOLOGICAL, 500-lb., E73R1 (OPEN, SHOWING E2R1 2¼-lb. BIOLOGICAL CONTAINERS)

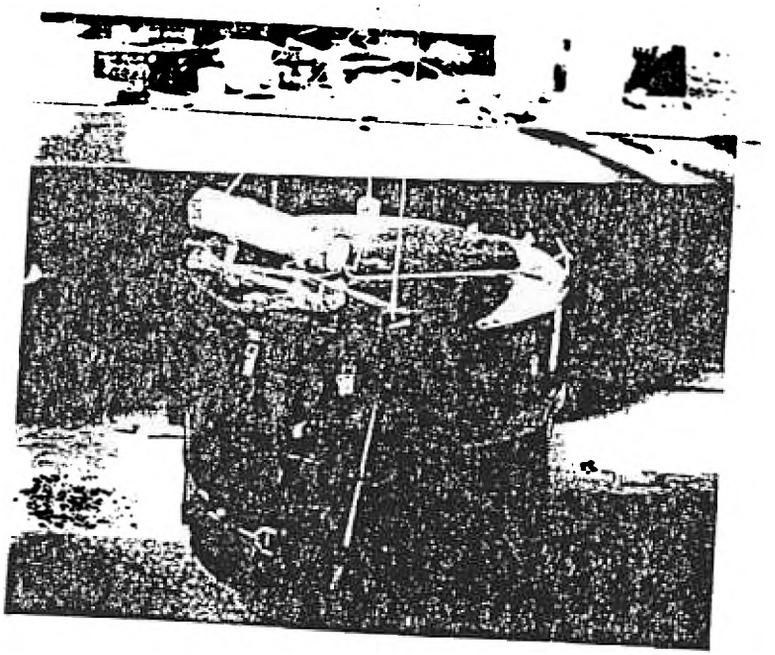


FIGURE 15. BOMB, BIOLOGICAL, 80-lb., E77

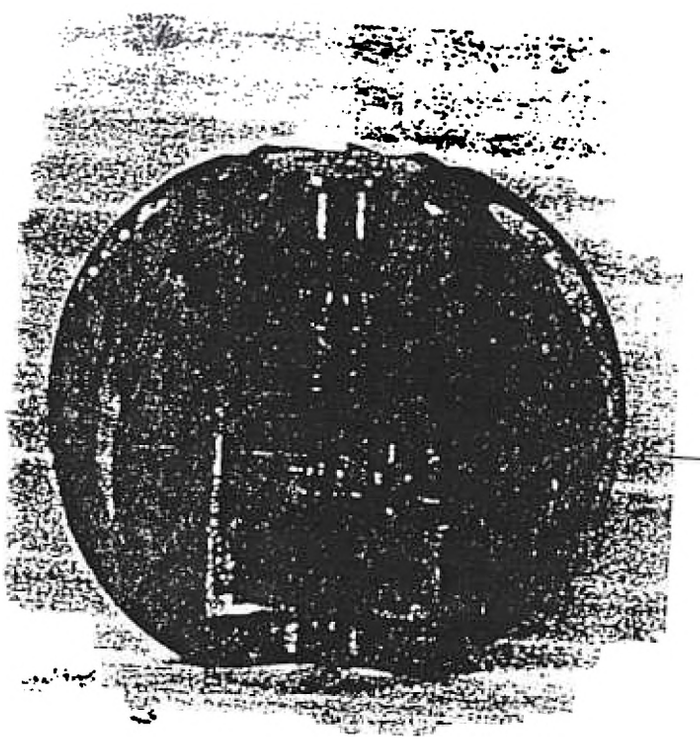


FIGURE 16. BOMB, BIOLOGICAL, SPHERICAL, 10-oz., E95R1

F. SUPPORTING RESEARCH

1. DEVELOPMENT AND TESTING OF PILOT PLANT EQUIPMENT

a. The design and development of processing equipment to produce BW agents is governed by the following criteria (listed in the order of their influence):

Assurance of producing the best possible product

Safety

Cost

The process equipment necessary for the production of bacterial BW agents is markedly different from that required for viral and rickettsial BW agents. The bacterial agents are grown in fermentation vessels in liquid media; whereas, on the basis of present knowledge, the viral and rickettsial BW agents must be produced in living tissues, e.g., embryonated eggs. Because of the basic differences between the processes for producing these BW agents, the development of process equipment for each of these two types of organisms is discussed separately.

b. BACTERIAL BW AGENTS

Most bacterial BW agents are fastidious and all are highly pathogenic. This gives rise to two major problems in the design of processing equipment: (1) prevention of ingress of contaminant organisms so that a pure culture of the desired pathogen may be produced and (2) prevention of the egress of the pathogenic organisms for the safety of operating personnel and the surrounding community. The problems involved in producing pure cultures arise from the following: (1) the conditions required for growth of pathogenic organisms are generally optimum also for airborne nonpathogens, (2) the generation time of most pathogens is longer than that for the common nonpathogenic contaminants, and (3) pathogens do not produce antibiotics or other substances which would retard or prevent the growth of most contaminants.

Past experience in the design of process equipment to successfully produce pure cultures of bacterial BW agents has forcefully demonstrated the need for making marked departures from

the techniques commonly employed by the fermentation industry. In 1943-44, the best design information available from industry was collected and used in the construction of pilot plants to study production processes for bacterial pathogens at Camp Detrick. Over 50 per cent of the cultures produced from this plant were not acceptable because of contamination. As a consequence, a major effort was directed to obtain the design information necessary for the construction of plants which would successfully produce pure cultures. The developments resulting from this work have made possible the production under safe conditions of pure cultures 95 per cent of the time. These developments include the following items:

(1) A processing system designed to permit gravity flow between the major elements of the processing equipment in order to eliminate the possibility of contamination from pumps.

(2) Satisfactory specifications on welds, process lines, valves, and vessels to greatly reduce leaks, eliminate pockets, minimize crevices and stagnant areas, in order that all surfaces may be maintained in a sterile condition.

(3) Use of materials of construction (in most cases, stabilized stainless steel) which prevents poisoning of the organism and greatly minimizes metal failures resulting from granular corrosion.

(4) An effective air purification system and the use of orifices operated at sonic velocity for the aeration and agitation of cultures in fermentors.

(5) Fermentor design with a minimum number of openings, no flanges or packing glands, and hot tanks between vessels, in order to further minimize the possibility of contamination and leakage. This is probably the most significant contribution in the field of process equipment design.

(6) Equipment for concentration by centrifugation or by specially designed settling tanks.

(7) Equipment for drying final cultures.

(8) Equipment for filling munitions.

(9) Effective waste sterilization.

All of the applicable above-mentioned developments have been incorporated in the design and construction of the production facility for vegetative BW agents (X-201 plant), the process

design of the plant for sporulating BW agents (X-301 plant) and the design and construction of new pilot plants at the Biological Laboratories.

c. VIRAL AND RICKETTSIAL BW AGENTS

Many of the principles discussed above in connection with the development of process equipment for bacterial BW agents are also applicable to the production of viruses and rickettsia. The production of these agents is currently being studied in the pilot plant. The principal direction taken in this work is the minimization of the manual handling required and the insurance of safe conditions for operating personnel. The developments in the production of these agents include the following items:

(1) The use of a small portable candling device to reduce egg handling to a minimum.

(2) An incubator room designed to increase the safety and efficiency of storing infected embryonated eggs.

(3) An inoculating hood installed to provide greater safety for operating personnel in the inoculation process.

(4) The design of a harvest table to permit selective harvesting, in order that the portion of the embryonated egg having the highest titer may be selected manually for further processing.

(5) Equipment for milling to produce a product with low viscosity and small particle size.

(6) Equipment for filling munitions.

(7) Effective waste sterilization.

(8) Effective procedures for cleaning equipment and process lines used in processing egg slurry.

An automatic inoculating apparatus has been investigated but has not yet been successfully applied to production. All of the applicable above-mentioned developments have been incorporated in the process design of a production facility for egg-cultured BW agents (X-401 plant).

In addition to the design of process equipment, an intensive effort has been devoted to the development and

application of quality control methods for BW agents produced in pilot plants.

Development work is continuing in many of these fields. As the demand increases for greater production of BW agents, further development work is necessary to obtain scale-up data for the design and construction of larger process equipment.

2. DRYING

In order to centralize the responsibility and coordination in the field of drying BW agents, a drying task force (DTF Division) was instituted during the fiscal year. This task force has a small integral laboratory staff for conducting investigations in this field, and, in addition, cooperates with other divisions of the Biololical Laboratories and monitors work carried out by extramural contractors.

The internal program of the DTF is principally concerned with (1) attainment of standard material for processing, (2) development of drying processes for BW agents, and (3)evaluation of dry agent-fills. In addition, facilities and services are provided for cooperating divisions. The freeze drying method is receiving primary emphasis for application to vegetative bacteria, viruses, and rickettsiae. Among the factors being investigated are the type of operation (batch versus continuous), the method of application of heat (conduction from platens versus radiation at selected wavelengths in the infrared region), the physical form of the material being dried (pellets versus thin layers in trays), the suspending fluid from which the organisms are dried, the influence of concentration of organisms upon recovery of viable cells, the importance of residual moisture after drying, the length of the drying cycle, and the sizing of the dried product. A process for spray drying of botulinum toxin has been developed, and this method of drying will receive consideration for application to the drying of Bacillus anthracis. The drying of this organism by solvents is now under investigation. Comments on the experience in drying specific agents are included with the reports of the individual agents (See Parts A, B, and C, this Section).

Other divisions of the Biological Laboratories are cooperating with the DTF in such studies as (1) procedures for restoration and enumeration of dried agents, (2) virulence (Contd next page)

of dried agents, (3) morphological alterations resulting from drying organisms, (4) evaluation of analytical techniques, and (5) determination of the influence of extraction of lipoid materials upon the drying characteristics of egg-cultured agents. The pilot plants serve as a source of material to be dried and will pilot recommended drying processes.

A large part of the effort in drying is represented by extramural contracts, some examples of which will be cited. A summary literature survey is being prepared by the University of Minnesota which will cover the technical literature, including patents, related to drying processes and equipment. The Bureau of Agriculture and Industrial Chemistry of the USDA is investigating the drying of vegetative bacteria and determining the characteristics of dried products. Avirulent organisms are being utilized in their experimentation with the understanding that the contractor's key personnel will assist in the translation of their findings to the drying of selected pathogenic agents at the Biological Laboratories. Arrangements have been completed with Lederle Laboratories to cooperate in developing a drying process for Bacterium tularense by furnishing advisory supervision, training in techniques, recommendations with regard to process equipment, and evaluation of experimental results. Spray drying and the characteristics of spray dried organisms are being investigated by a combined team of chemical engineers, bacteriologists, and biochemists at the University of Wisconsin. In addition, a small effort is being supported at Purdue University to develop a high velocity spray dryer and determine its applicability to drying BW agents.

Since conclusive evidence is available of the critical importance of particle size in determining the infectivity of airborne BW agents, the development of methods for reduction of dried agents to the 1-5 micron size range, and the collection and classification of these sized particles is an essential portion of the drying program. All these operations must be performed with minimal effects upon the infectivity of aerosols of the agent. These problems of sizing, collection, and classification are being investigated by General Mills.

3. ARTHROPOD DISSEMINATION OF BW AGENTS

Insects and other arthropods act as vectors and reservoirs of some of the most promising and highest priority BW agents affecting man and animals Arthropods provide a tactical concept of BW agent dissemination, as they can efficiently carry agents to specific targets. Military use of agents in an enemy target area requires detailed knowledge of the role of native arthropods in incrementing or maintaining an epidemic in the area, or spreading the agent outside of the area.

The primary objectives in this field are (1) to provide methods of dissemination of various agents by means of arthropods in both overt and covert operations, (2) to provide required information on the arthropods present in potential BW target areas and their effect on the planned military use of agents, and (3) to attempt to increase or maintain the virulence of various BW agents by passage through arthropods.

In studies on dissemination of agents, a number of species of mosquitoes have been mass reared in large numbers and their dispersal and flight range have been measured by means of radioisotope marking. Mosquito transmission experiments on Venezuelan equine encephalitis have been carried out: of 8 species of mosquitoes of 4 genera tested, 7 species became infected and transmitted the virus to laboratory animals. The incubation and infective periods, the infection thresholds, and transmission rates of several species of mosquitoes have been determined. Transmission of this virus from horse to horse by Aedes triseriatus, and from guinea pig to guinea pig by Mansonia indubitans has been demonstrated in work contracted at the USPHS Virus Laboratory at Montgomery, Alabama. This laboratory has also carried out extensive mosquito transmission experiments with Eastern equine encephalitis. The infection thresholds, incubation periods, and infection and transmission rates have been determined for A aegypti and A triseriatus, as well as infection thresholds and incubation periods for 10 other species of mosquitoes.

The transmission of simulant agents by mass reared houseflies has been intensively investigated in both the laboratory and field. Streptomycin resistant E coli, S marcescens, and Proteus mirabilis were fed to houseflies and the persistence and dissemination of these bacteria were determined. Individual flies were suspended over saline and the persistence and multiplication of the bacteria were determined. Effects of various foods and handling methods were determined on the longevity of flies and bacteria. Bacteria labeled with P32 were fed to houseflies in an attempt to use the radioactivity to measure bacterial dissemination, but only a limited degree of correlation was obtained. Field tests on persistence and dissemination of E coli and S marcescens were carried out with houseflies in a large outdoor field cage ($20' \times 40' \times 8'$). Several thousand flies were released in each test, and the vector ability of the flies was determined by exposing for 30 minutes four petri dishes containing nutrient agar and proteose peptone. In 13 field tests the flies were infected by allowing them to feed on bacterial suspensions and were then released into the field cage. The flies contained over 10^5 organisms per fly after 24 hours, and about 10^3 organisms after 48 hours. They disseminated about the same numbers of bacteria at the same time intervals on 4 petri dishes exposed for 30 minutes each day.

Peat bait containing a fly attractant and over 10^9 E coli ml were exposed to uninfected flies. In 9 field tests with about 5,000 flies

in each test, the flies ingested over 10^6 E coli fly in 24 hours, and disseminated 10^4 - 10^5 test organisms daily on 4 petri dishes exposed for 30 minutes each day for 6 successive days. These experiments indicate that this type of peat bait could be infected with various enteric agents or dried anthrax spores for dispersal by plane into populated enemy areas containing high fly populations. The research on housefly dissemination of simulants has been completed. Housefly studies will not be continued unless enteric pathogens or orally induced anthrax are considered to be promising agents.

Epidemiological studies on diseases and arthropods in potential target areas have been carried out. A detailed study has been completed on the distribution, abundance, biting habits, and importance as disease vectors of the 90 species of mosquitoes occuring in Russia and adjacent areas. About 800 references were compiled and the most important translated and abstracted. A detailed study on the housefly and its importance in transmission of various diseases has been completed. Similar studies on ticks, fleas, body lice and other arthropods of medical importance are in progress. These studies will provide required information on the arthropods present in potential BW target areas and their effect on the military use of agents. The presence of arthropods can affect an aerosol or munition produced epidemic by spreading an epidemic, converting an epizootic into an epidemic (e.g., VEE), or maintaining an agent. In scheduling the use of most BW agents, the presence of arthropods in test areas and in enemy target areas should be taken into account.

Passage of BW agents through various arthropods offers an opportunity to increase the virulence of the agents or to alter certain of their characteristics. This has been studied under contract at the USPHS Rocky Mountain Laboratory in Montana. Tularemia, Q fever, and plague have been passed through appropriate vectors and the resulting strains are being compared with the original strains supplied by the Biological Laboratories. Q fever rickettsiae multiplied so rapidly in the tick Dermacentor andersoni that the majority of the test ticks died. The Rocky Mountain Laboratory also offers a site for studying transmission of yellow fever in mosquitoes.

Studies are in process to demonstrate the capability of arthropods to act as efficient means of overt dissemination of BW agents. The agents selected are lethal for humans and include mosquito transmitted yellow fever, Japanese B encephalitis, Eastern and Venezuelan equine encephalitis, louse transmitted epidemic typhus, flea transmitted bubonic plague, and tick transmitted Rocky Mountain spotted fever. All of the vectors for these diseases have been reared in large numbers and millions can be produced and infected. Virulence of the agents and ability to infect lasts for several months to several years in all of these vectors (except the body louse). Insecticide resistant strains of most of these vectors have been developed or discovered in the field. In all cases, there is abundant

evidence documenting the ability of the above arthropods to transmit the diseases and to cause severe epidemics under naturally occurring conditions.

4. MICROMETEOROLOGICAL RESEARCH

The Biological Laboratories are charged with conducting a program of meteorological research for the Chemical Corps as a whole, applicable alike to CW, RW, and BW dissemination. The program has been conducted along four lines: meteorological sampling in the ground layer; aerosol cloud travel; a study of the intermediate atmosphere layers; and correlation between the micrometeorological structure and general meteorological pattern.

The first of these problems has required the adaptation or development of instrumentation necessary for micrometeorological observations, the construction of a low speed wind tunnel in which they could be tested calibrated, the establishment of a micrometeorological sampling network on the grid area at Camp Detrick, aid in equipping Dugway Proving Ground for similar measurements, and the use of such set-ups to study verticle wind profiles and temperature structure in the lower levels of the atmosphere.

Studies on aerosol cloud travel have extended over several years. They have included both theoretical treatments of the problem and field studies on an increasingly large scale. Early studies on cloud travel and diffusion were done with ammonia, but the tempo of the field work was greatly facilitated by the development of the fluorescent particle technique by the Stanford University group, under contract. These inert particles of zinc cadmium sulfide, 1 to 5 microns in size, behave physically in the atmosphere in the same fashion as toxic aerosols. These particles are easily detected with simple sampling devices because of their characteristic fluorescence under ultraviolet light. They have been used in large-scale trials over the city of San Francisco, in two operations covering the southeastern states from Georgia to Virginia, in sea to land trials off the Florida coast, and are presently in use in extensive trials over mountainous terrain and over the cities of St. Louis, Minneapolis, and Winnipeg. This latter study is being performed by the Ralph M. Parsons Company and Stanford University under contract and is designed to show the behavior of aerosols traveling over large built-up areas.

Data on the intermediate atmospheric layers have been accumulated through several long-distance cloud travel experiments and also through special meteorological soundings taken to intermediate heights. These have led to development of concepts relating to long-range aerosol travel which have been successfully put to test in the trials mentioned in the previous paragraphs.

The prediction of suitable target conditions for toxic aerosol attack can be made only after a knowledge is gained of the correlation between micrometeorological structure and the general synoptic pattern. Partial knowledge accumulated over past years has permitted the forecasting of suitable conditions for our own field trials. Special studies have also been made on the climatology of the Ukraine, Crimea, Roumania, Bulgaria, and various typical Russian cities. The selection of American cities for the aerosol travel studies now under way was based on the similarity of their climate with that of possible urban Russian targets.

5. SUMMARY OF FIELD TRIALS

Following the close of the war, Dugway Proving Ground and other test sites of the Chemical Corps were closed and, for a period, almost no field testing in BW was done, other than very small trials conducted on the grid area at Camp Detrick. About 1949, however, field testing was resumed. Operating divisions of the Biological Laboratories went to the field with developmental problems, utilizing various military installations when non-pathogenic or simulant agents could be used. Dugway was re-opened also in 1949, becoming the only site where pathogenic antipersonnel agents could be tested in the open, although it did not become operable with its own staff until 1951. By December 1950 the volume of field testing had become such that a Field Test and Meteorology Division was established within the Biological Laboratories, to aid mainly in the nonpathogenic trials carried on outside of the Dugway Proving Ground program. A brief resumé of the more important of the BW trials conducted since the war follows.

Joint trials with the British using Brucella suis, Pasteurella tularensis, and Bacillus anthracis were conducted at sea in the Carribean area in the winter of 1948-49. In 1949 and again in 1950 operating personnel from the Biological Laboratories tested the prototype M114 4-lb bomb at Dugway using only simulants the first year and B suis, as well, the second year. The agents causing Q fever and psittacosis were tested at Dugway in 1951, again by Biological Laboratories personnel. From 1951 on, Dugway Proving Ground personnel have run BW trials on a continuing basis, testing agents and munitions developed in the Biological Laboratories. B suis, Brucella melitensis, B tularense, botulinum toxin and the virus of Q fever as well as simulants have been tested, utilizing the M114 bomb, the E61 bomb, the ADL and C generators, the E73 or feather bomb, and other devices.

Simulant trials demonstrating possible tactical application of BW agents were performed at Ft. McClellan, Alabama, in 1952. A naval amphibious generator was tested at Little Creek, Virginia, in 1950. The prototype of a navy BW mine was tested at Key West and Panama City, Florida.

In addition to these trials with antipersonnel agents and munitions, airplane spray trials utilizing chemical anticrop agents were performed at Avon Park, Florida, in 1951 and 1952 and at Eglin Air Force Base, Florida, in 1952 and 1953. Trials with cereal pathogens in the E73 or feather bomb were conducted at Pine Camp, New York, in 1950.

Antianimal trials using hog cholera virus as the agent in the E73 feather bomb were carried out at Eglin Air Force Base in Florida in 1951. Tests with the Newcastle disease of poultry were done in 1951 at Madison, Wisconsin.

Many of the field trials performed have been in the area of physical defense. These have included vulnerability or decontamination tests on naval vessels at Norfolk in 1949, 1950, and 1952 and at Boston in 1952; similar trials involving aircraft at Eglin Air Force Base in 1950 and 1953, at Wright Patterson AFB in 1952; protective clothing trials at Quantico Marine Base in 1949; tests involving protective shelters at Fort Story, Virginia in 1947; at Port Hueneme, California in 1950 and 1952, at Camp Detrick and at Mound Laboratory in Ohio in 1951. Vulnerability studies were conducted at the Pentagon in 1949; over the city of San Francisco in 1950; at Carswell Air Base, Ft. Worth, Texas in 1951; and at the Naval Supply Base at Mechanicsburg, Pennsylvania in 1951. Decontamination trials were performed in 1953 in a naval warehouse at Scotia, New York, as well as personnel decontamination trials the same year at Camp LeJuene, North Carolina. Trials utilizing prototype detection and sampling devices were included in the 1952 Ft. McClellan test.

Two trials seeking basic meteorological data on long range travel over several southeastern costal states were performed in 1952. Trials involving aerosol travel over build-up areas are now in progress at St. Louis, Minneapolis, and Winnipeg, Canada.

6. PHYSICAL AND BIOPHYSICAL STUDIES

The group in biophysics have not only functioned as a service group, offering their specialized techniques in cooperative projects with other divisions, but they have carried out an independent research program within their group, working particularly on basic problems of interest to the whole program.

The cooperative program has utilized many pieces of physical instrumentation which are not available elsewhere in the laboratories. The electron microscope and the low-angle X-ray diffraction apparatus have been used particularly in studies of morphology, fine structure and particle assay. Electrophoretic studies have been particularly fruitful

in studying blood plasma and sera constituents, and even utilized to differentiate variant strains of bacterial species. Thin film techniques have provided a two-dimensional model for investigations of the properties of aerosol particles and various biological materials. Spectroscopic analysis, tracer techniques, and irradiation studies have found application in many fields.

Special studies of fundamental interest conducted by this group have included those on aerosol formation and the physics of dissemination. From these studies arose a new method of BW dissemination, that of ultrasonic generation of aerosols utilizing the Hartman whistle-principle. The practical application of this technique is now being carried out by the Munitions Division. Basic studies have been made in the field of rapid detection of BW agents by physical means. Again practical exploitations of promising principles are transferred to another group charged with developing detection devices. Another field of basic studies has been that of drying of biological suspensions and physical characterization of dry powders.

Biophysical study of botulinum toxin has revealed that one-tenth size "fragments" of the toxin molecule may be more basic than the parent molecule, but lack the hemagglutinating property formerly assumed to be an essential part of the toxic principle. The toxic principle itself has been studied electrically on isolated nerve-muscle preparations. The toxin appears to be a simple protein containing usual amino acids. Similar biophysical studies have been made on the capsular glutamyl polypeptide of Bacillus anthracis. Studies of the physical characterization of viral agents and their reaction with living cells have been carried out utilizing several methods of approach, particularly electron microscipy.

7. STUDIES BASIC TO MUNITIONS DEVELOPMENT

Many studies conducted by the munitions group have contributed basic information pertinent to munitions development and assessment in general, and hence cannot be reported under the sections of this report dealing with the development of specific munitions. Investigations in three overlapping fields of study fall in this category: dissemination of aerosols, physical properties of aerosols, and aerosol sampling techniques.

In the first of these fields, that of aerosol dissemination, fundamental studies have been conducted both on instantaneous or explosive dissemination and on continuous generator principles. A special laboratory is being equipped to study problems such as the effect of shaped charges and other kinetic mechanisms on explosive aerosol formation, utilizing high speed photography and other techniques.

Many approaches are being pursued to gain fundamental knowledge concerning continuous aerosol production. They include studies of solid propellants, break-up of jets, and various types of nozzles. Of prime importance in this field is the application of shock waves of ultrasonic frequency to break-up liquid films into aerosol droplets, based upon the Hartmann whistle mentioned in the Biophysical section of this report. Out of this latter work, BW munitions operating on an entirely new principle of dissemination are expected to develop.

The most promising developments in the area of physical properties of aerosols have arisen from fundamental studies on evaporation of bacterial suspensions. Controlling the rate of evaporation and the degree of dehydration of aerosol droplets may answer many problems concerning the maintenance of viability of BW aerosols particularly under conditions of adverse relative humidity. Sedimentation rates of BW aerosols have also been examined theoretically and experimentally. From this work a better understanding of the reasons for biological and physical decay of BW aerosols should arise.

The main problem in sampling has been that of developing better techniques for routine particle size determinations. Under contract, an improved cascade impactor is being developed as a tool in this field. Samplers which are selective as to particle size are being studied to develop samplers which closely parallel the human respiratory tract in their action. A device employing a microwave refractometer principle to measure the instantaneous mass concentration of a BW aerosol appears most promising. A device called the aerosoloscope to determine optically the size distribution and concentration of an aerosol is under development. A "flying-spot" microscope, of the type recently developed in England, is being constructed here and should offer an entirely new tool for the study of aerosol particulates. Under contract, studies are progressing on the flow of fine particulates of interest in dry-fill munitions, and on various mathematical models of aerosol behavior.

SECTION III - ADMINISTRATION

1. ORGANIZATION

The reorganization of the Chemical Corps Biological Laboratories was effected during FY 1953 in accordance with recommendations of the Killian report (6 May 1952).

The concept of bolstering the organization by strengthening the management was implemented by reorganizing the laboratories along functional lines and providing the necessary staff support to the Director.

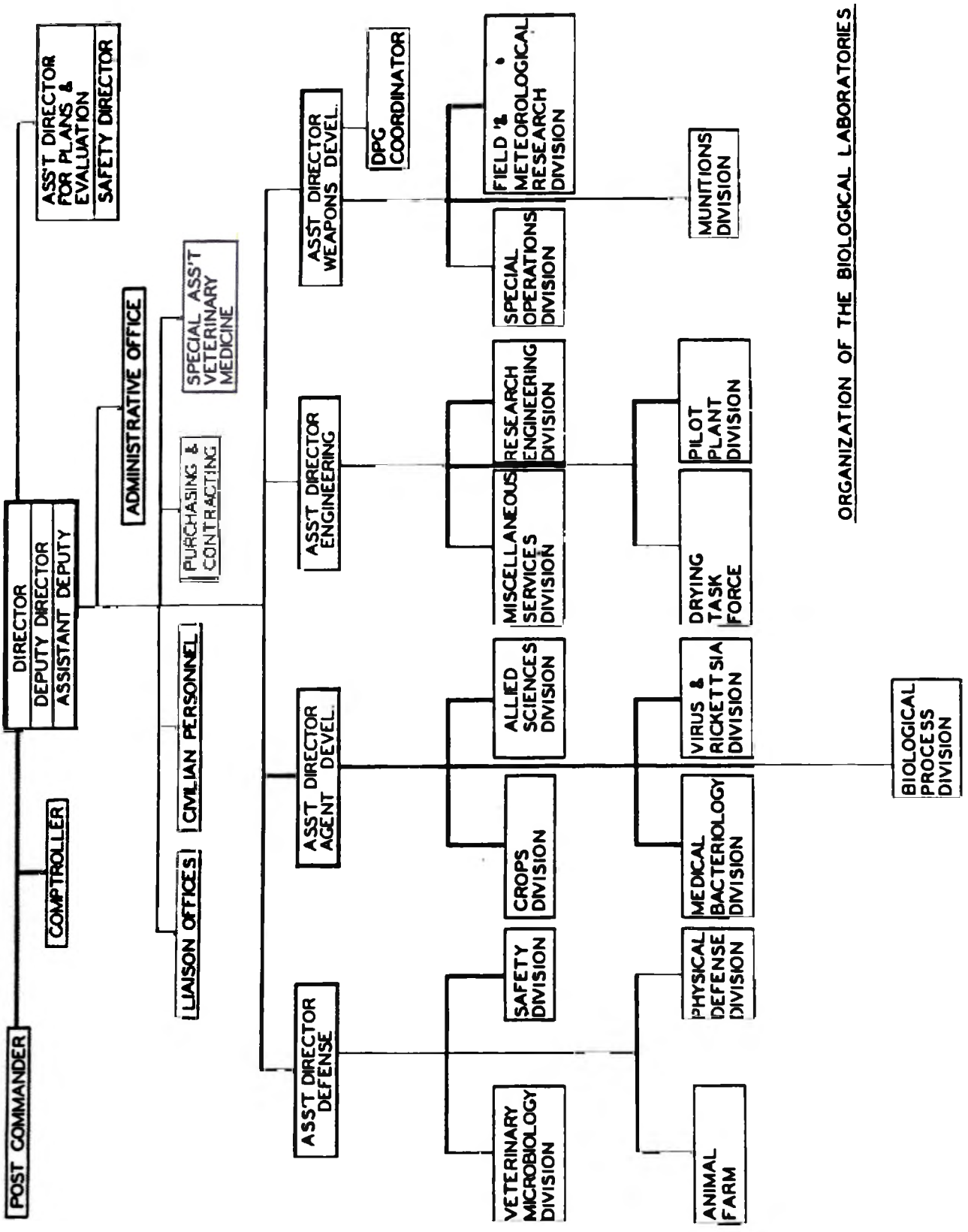
Managerial and executive support was established by organizing the positions of Deputy Director, Assistant Deputy Director, and Assistant Directors for Defense, Agents Development, Weapons Development, Engineering, and Plans and Evaluation. A listing of personnel occupying these and other key positions will be found on page 118.

A table of organization of the Chemical Corps Biological Laboratories is presented on page 85.

2. STATUS OF CONSTRUCTION AT CAMP DETRICK

During the past year, substantial progress was made in providing new technical facilities for the Chemical Corps Biological Laboratories at Camp Detrick. Other than the Horton Test Sphere, a Munitions Loading Building, and two greenhouses, no technical facilities had been erected between the end of World War II and the end of FY 1953.

Most facilities for which the H. K. Ferguson Company undertook construction early in 1951 under the "Expedited Program" were turned over to the Biological Laboratories by the District Engineer during FY 1953. These facilities include: PP-2, -3, and -4, providing laboratory and supporting office facilities for Pilot Plants Division; PP-6, a Simulant Pilot



ORGANIZATION OF THE BIOLOGICAL LABORATORIES

Plant; C-3, a Pilot Plant for obligate parasites of crops; BS-1, for the Aerobiology Branch; M-1 and M-1A providing office and laboratory space for munitions development (the three test tanks in M1-A have not yet been completed by the contractor); and the decontamination building which provides an up-to-date plant for the sterilization of contaminated effluent from the Laboratories. It is anticipated that Building PP-1 and the addition to Building 431, both of which are Pilot Plants, will be completed by 31 Aug 1953 (shown in Figure F-1 as completed). Total cost of the facilities included in the Ferguson contract is currently estimated at $15,975,980. This estimate is exclusive of the costs of $2, 024, 424 for providing a supporting Central Boiler Plant and Steam Loop and conducting a utilities survey. The total value of facilities which have been or are about to be turned over for use during this period is $18,000,404.

The following were placed under contract during FY 1953 but are not yet completed: new facilities for the Animal Farm including a Service Building and 18 small breeding barns; a laboratory and supporting corral for research on large animals; BS-PD-1 which will house the Biophysics Branch, the Chemistry Branch and the Statistics Branch; S-1 for use by Agent Control Branch of Safety Division; E-1, a Shop Facility for Research Engineering Division; C-2A, Crops Development Storage Shed; the addition to the Water Sewer and Electric Distribution Systems, and the addition to the Boiler Plant and Fuel Oil Storage. The total cost of these will be $9,729,225. The estimated completion dates of these facilities vary from October 1953 to July 1954. (Figure F-1)

The following items, for which funds were appropriated in the FY 1951 and 1952 MCA programs, were deferred early in FY 1952 in order to provide adequate funds for the completion of the Ferguson contract: Civilian Dormitory, $200,000; Special Laboratories F-1, -2, -3, $1,404,000; QM Warehouse, Motor Pool and Repair Shop, $680,000; and Emergency Power Building, $290,000. Deferment of other items in these same programs has been recommended recently by this Installation so that more urgently needed facilities, for which enabling legislation is being requested in the FY 1954 MCA program, can be funded. The deferred facilities are: Laboratory BS-2, $892,800; Clinical Investigation Building, S-3, $226,500; Engineering Office and Laboratory, E-6, $521,000; Pilot Plant, C-4, $1,344,560: Equipment and Storage, E-4 and E-5, $58,000; Laboratory Building, BS-3, $521,000; Meteorological Building, MT-1, $169,000; and Warehouses W-7 and -10, $122,000.

The Department of Defense has not released for construction certain other items for which Congress had appropriated money in FY 1951, 1952, and 1953. Certificates of Essentiality have been provided the Secretary of Defense for each of these since January 1953; however, authority to construct has not yet been granted (See Table F-1).

Title to the additional 529 acres of land (Area C) for Camp Detrick has finally been vested in the United States Government, but only after condemnation action was resorted to for several tracts. The drawing below shows the configuration of this new land and previously acquired tracts, as well as a summary Master Plan for utilization of the land. Area C is presently used for a Crops Outdoor Laboratory. No construction has been started in that area, since releases for construction have not been issued.

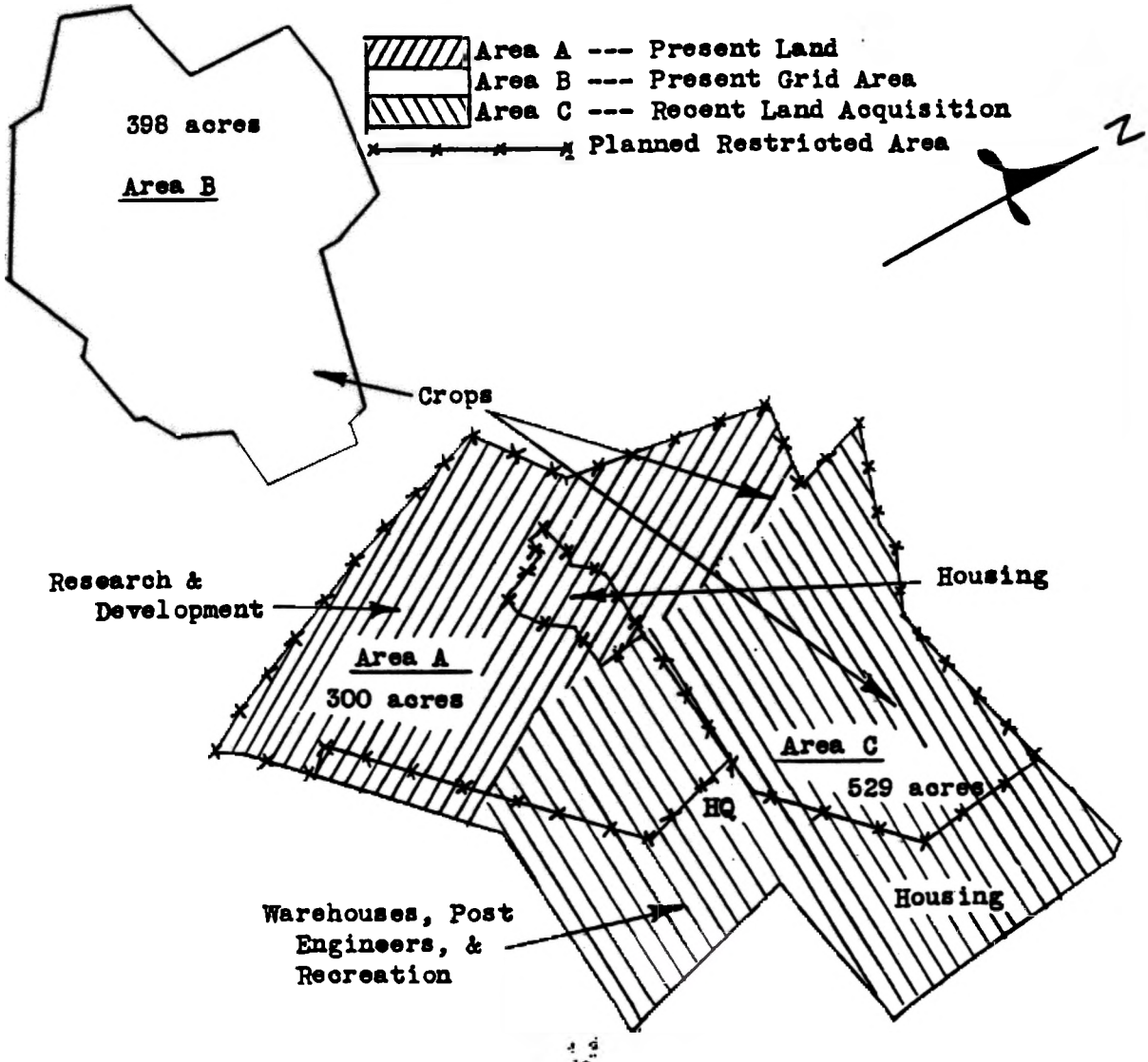


TABLE F-1. ITEMS IN FY 1951, 1952, 1953, and 1954
MCA PROGRAMS NOT YET STARTED

Project No.	Fiscal Year	Project	Current Working Est.
A206-28	1951	Detection Lab PD-1	$ 597,000.
-44,45	1952	Lab,MV-2,-3,-4,-5(Facility for bacterial and viral agent devel)	6,825,100.
-25	1953	SO Div Biological Lab	4,746,620.
-85	1953	Utilities (for newly acq. land)	1,367,000.
-2.2	1951	Plant Science Bldg C-1	1,112,000.
-2.6	1951	Soils Preparation Bldg.	274,700.
-34	1952	Two Classified Bldgs.(Greenhouses)	120,450.
-93	1954	Decontamination Bldg. (Not to be confused with Lab.Bldg. PD-3)	900,000.
-94	1954	Addn. to Bldg. 527 (Diln Cham.&Lab)	1,430,000.
-99	1954	Decon. Incinerator	150,000.
-98	1954	Communications (Areas A and B)	125,000.
-48	1954	Extension to Existing Roads	143,000.
-29	1951	Bacterial Nutrition Lab. (BS-4)	880,300.
-41	1952	Research Lab. S-2	1,041,300.
-52	1952	Addn. to M-1 Bldg.	1,231,900.
-81	1952	Classified Bldg. (Munitions Load.)	115,000.
-78	1952	Two Igloos	40,000.
-27	1952	Laboratory, PD-2	575,900.
-33	1952	Laboratory, PD-3	663,300.
-54	1952	Addn. to Meteorological Bldg. (Field Test Activities)	557,200.
-37	1952	Two classified Bldgs.(Greenhouses)	120,450.
-38	1952	Whses W-1,-2,-3,-4,-5,-6,-8,&-9	642,600.
-53	1952	Whse for Animal Feed	119,900.
		TOTAL	$23,798,720.

Security Information

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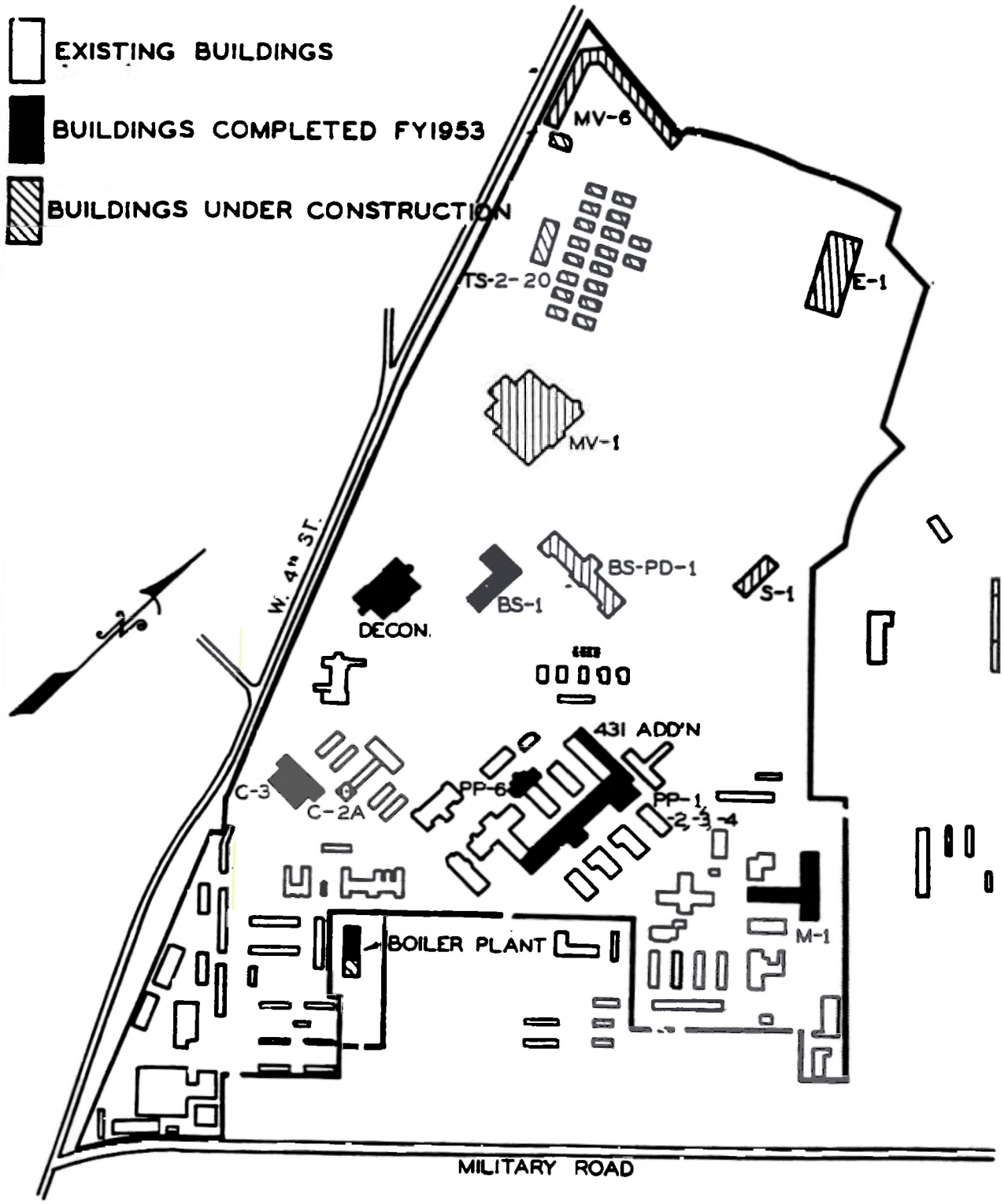

FIGURE F-1 BUILDING STATUS BIOLOGICAL LABORATORIES AS OF 30 JUNE 1953. (AREA "A")

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3. BUDGETARY CONSIDERATIONS

Funds for operation of the Biological Laboratories are from four sources. Figure B-1 shows these sources and the amounts for each. It will be noted that Army R&D, the USAF, and Navy funds have been reduced for FY 1954. These reductions will mean that not only are entire areas eliminated, but other planned developments will be extended over a longer period of time.

The total obligations for FY 1953, $22,140,000, represent a 45% increase over FY 1952. The major portion of this increase over FY 1952 was for research and development contracts. This included $3,500,000 obligated for the extension of the Ralph M. Parsons Company operations contract on the "Design and Development of BW Munitions," which is presently funded to 14 August 1954. A total of $5,750,000 representing 26% was obligated for the pay of personnel of the Biological Laboratories and supporting activities. Figure B-2 compares the amount for contracts and local operations at the Biological Laboratories. Actual obligations for research and development contracts, including inter-agency agreements, were 70% higher in FY 1953 than in FY 1952, and will be reduced in FY 1954 by 55% from actual contract obligations FY 1953. As of 30 June 1953, there were 154 contracts in force with a per-annum value in excess of $9,600,000, as shown in Table B-1.

As shown in Table B-2, the total funds being provided the Biological Laboratories for FY 1954 are approximately the same as actual obligations for FY 1952. As of 15 July 1953, no funds are included for the antianimal nor the defensive anticrop programs; the offensive crop program has been reduced to approximately 35% of FY 1953 (see Figure B-3).

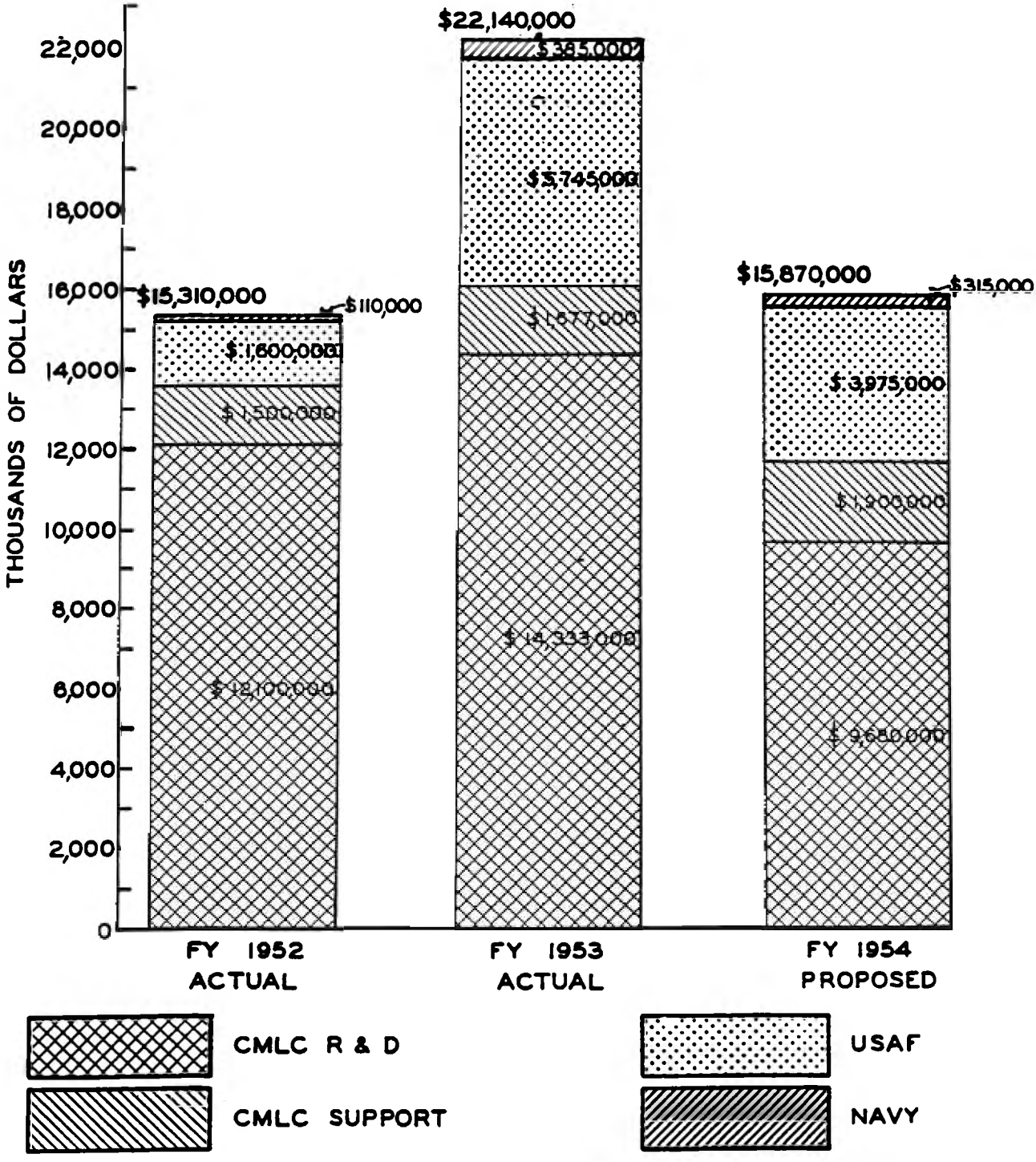

FIGURE B-1. SOURCES OF FUNDS OBLIGATED BY THE BIOLOGICAL LABORATORIES.

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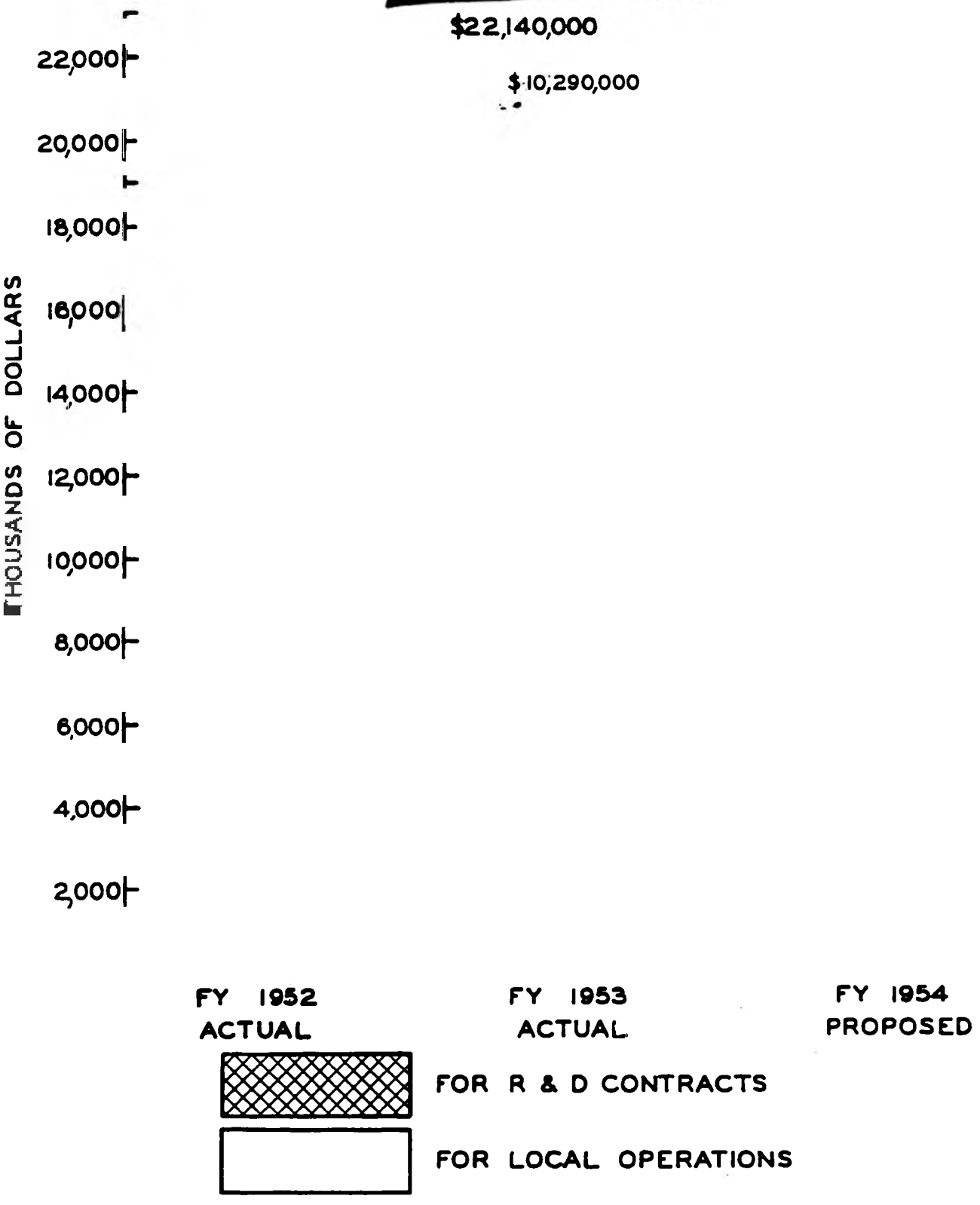

FIGURE B-2. FUNDS OBLIGATED FOR R & D CONTRACTS COMPARED WITH TOTAL OBLIGATIONS OF THE BIOLOGICAL LABORATORIES.

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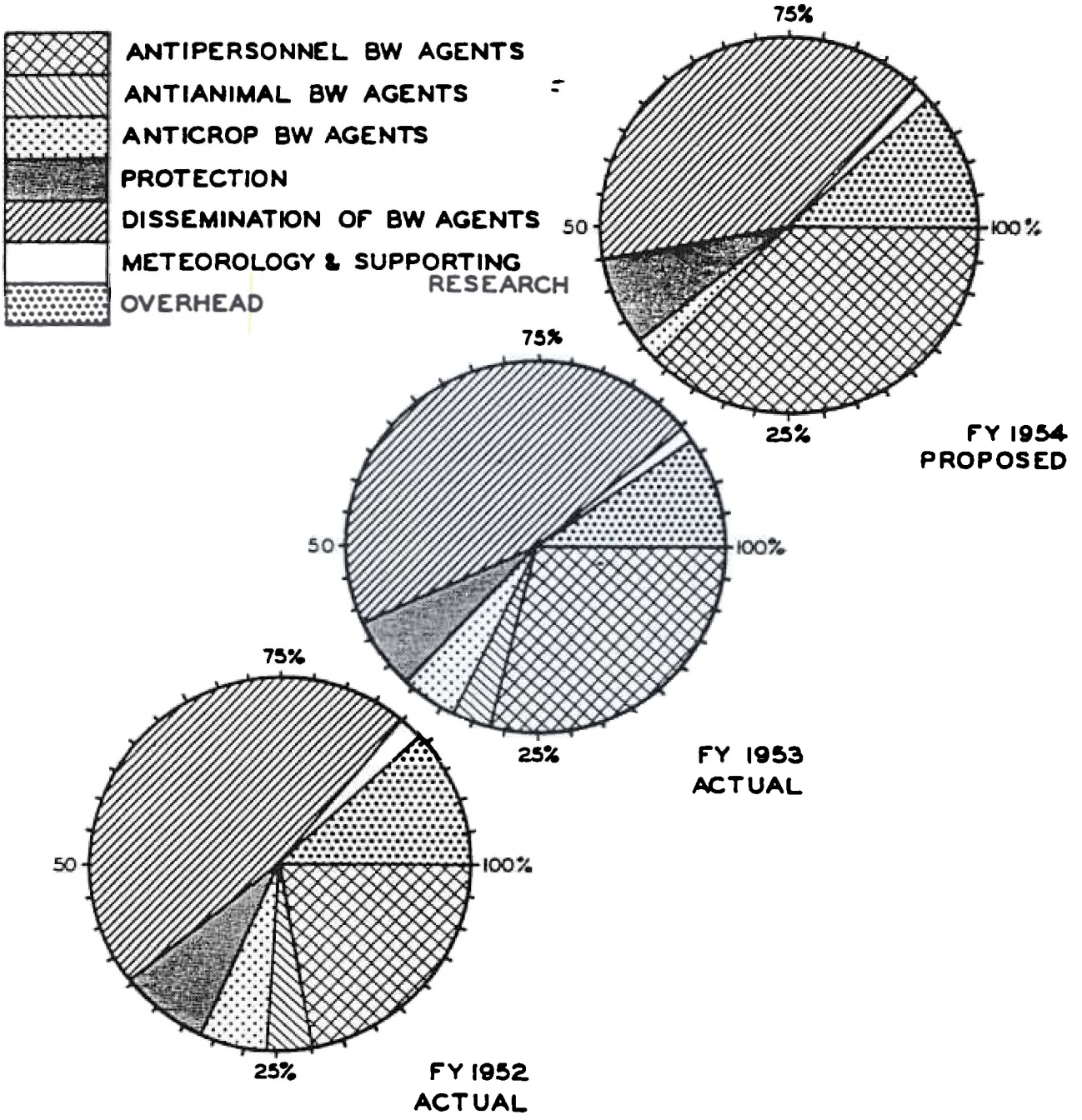

FIGURE B-3. SUMMARY OF ACTUAL OBLIGATIONS FY 1952 & FY 1953 AND PROPOSED OBLIGATIONS FY 1954 BY TECHNICAL OBJECTIVES.

Table B-2. SUMMARY OF ACTUAL OBLIGATIONS FY 1952 AND FY 1953 AND PROPOSED OBLIGATIONS FY 1954 BY TECHNICAL OBJECTIVES

Tech. Obj.	Short Title	FY 52		FY 53		FY 54	
				In Thousands			
		%	$	%	$	%	$
BW-1a	Offensive Antipersonnel BW agents	16.3	2,500	21.7	4,800	34.0	5,394
BW-1b	Defensive Antipersonnel BW agents	5.6	850	7.2	1,590	3.5	553
BW-2a	Offensive Antianimal BW agents	1.0	150	.8	180		0
BW-2b	Defensive Antianimal BW agents	3.0	460	2.5	550		0
BW-3a	Offensive Anticrop BW agents	4.9	750	4.2	930	2.1	333
BW-3b	Defensive Anticrop BW agents	.7	100	.5	100		0
BW-4a	Detection of BW agents	1.6	250	2.5	550	3.6	563
BW-4b	Protection Against BW agents	4.2	650	2.2	490	2.7	431
BW-4c	Decontamination of BW agents	1.7	260	1.7	375	1.4	225
BW-5	Dissemination of BW agents	46.9	7,180	45.9	10,175	39.3	6,241
IO-14	Meteorology	.3	50	.2	55	.3	50
SR-4	Supporting Research	1.8	270	1.1	245	1.1	180
Overhead		12.0	1,840	9.5	2,100	12.0	1,900
	Totals	100.0%	$15,310	100.0%	$22,140	100.0%	$15,870

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Table B-1. CONTRACTS AND INTER-GOVERNMENT AGENCY AGREEMENTS IN EFFECT AS OF 30 JUNE 1953

Contracts in the Field of Antipersonnel Biological Warfare Agents

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
Univ. of California DA-18-064-CML-471	Develop a Method for Culturing Gonyaulax catanella	4-61-14-001	$ 16,580.00
Univ. of Pittsburgh DA-18-064-CML-474	Studies on the Biophysical Characterizations of Botulinus Toxin and Other Protein	4-64-06-002	13,110.00
Univ. of Illinois DA-18-064-CML-494	Research on Shellfish Poison	4-61-14-001	14,667.00
Univ. of Mississippi DA-18-064-CML-1858	Physiology and Pharmacology of Botulinus Toxin	4-64-06-002	6,000.00
Univ. of Minnesota DA-18-064-CML-2302	An Investigation of the Toxic Principles which Occur in Certain Blue-Green Algae	4-64-01-001	14,800.00
Northwestern University DA-18-064-CML-2331	Investigations and Tests on Shellfish Poisoning	4-61-14-001	21,818.00
Long Island Bio Labs DA-18-064-CML-2360	Research Studies of Factors Inducing Resistance to Transmissible Mouse Leukemia	4-64-09-001	26,625.00
Methieson Chem. Corp. DA-18-064-CML-2385	The Purification & Characterization of a Shellfish Poison	4-61-14-001	33,500.00
Univ. of Wisconsin DA-18-064-CML-2110	Investigations on the Drying and Stabilization of Bacteria	4-11-02-045	59,580.00

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Contracts in the Field of Antipersonnel Biological Warfare Agents - continued

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
Purdue University DA-18-064-CML-2119	Development of High Velocity Spray Dryer for Use with Highly Thermaviable BW Materials	4-92-02-025	$ 14,230.00
General Mills, Inc. DA-18-064-CML-2336	Processing of Dry Materials to Produce and Collect Particles less than 5 Microns in Diameter	4-92-02-025	298,900.00
USDA--BAIC CD3-2460	Drying Vegetative Bacteria	4-11-02-045	568,380.00
Univ. of Notre Dame DA-18-064-CML-468	Preparation & Evaluation of CO-i Particles	4-11-02-045	23,450.00
American Type Culture Collection DA-18-064-CML-2108	Factors Influencing Recovery of Viable Organisms after Freeze Drying	4-92-02-025	10,846.00
Univ. of Minnesota DA-18-064-CML-2408	A Survey of Scientific Literature on Drying	4-11-02-045	16,000.00
Univ. of West Virginia DA-18-064-CML-2393	The Properties of Exudates Associated with Plant Pathogenic Bacteria	4-11-02-045	9,950.00
Lederle Labs., Inc. DA-18-064-CML-2413	A Study of Additives and Drying Techniques	4-92-02-025	26,250.00
Wahl-Henius DA-18-064-CML-2410	Means to Maintain Viable Organisms Subjected to Freeze Drying	4-92-02-025	97,700.00

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Contracts in the Field of Antipersonnel Biological Warfare Agents - continued

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
Univ. of Chicago DA-18-064-CML-495	Investigation of Methods of Active Immunization	4-11-02-025	$ 24,000.00
Univ. of Cincinnati DA-18-064-CML-1656	Virulence Relations in Shigella	4-11-02-020	7,650.00
Univ. of Cincinnati DA-18-064-CML-1661	Metabolic Differences Associated with Virulence - Avirulence in Bacterium tularense	4-11-02-019	6,750.00
Univ. of Texas DA-18-064-CML-1852	Infectivity & Virulence of Vibrio cholerae	4-11-02-020	10,700.00
Univ. of Wisconsin DA-18-064-CML-1911	Genetic Recombination of Bacteria	4-11-02-024	10,050.00
Trudeau Foundation DA-18-064-CML-2112	Tuberculin Reaction in Guinea Pigs	4-11-02-025	15,040.00
Univ. of Kansas DA-18-064-CML-2115	Immunology Virulence & Metabolism of Bacterium tularense	4-11-02-019	14,000.00
Univ. of Chicago DA-18-064-CML-2281	An Investigation of the Virulence of Cholera Vibrio	4-11-02-020	35,700.00
Univ. of California DA-18-064-CML-2291	Research Studies of Factors Governing the Virulence & Immunizing Potency of Members of the Genus Brucella & Mechanisms by which the Infection & Immunity May be enhanced	4-11-02-027	19,285.00

Contracts in the Field of Antipersonnel Biological Warfare Agents - continued

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
Univ. of Wisconsin DA-18-064-CML-2298	Research Services Concerning the Physiology of Brucella Species	4-11-02-027	$ 9,000.00
Long Island Biol. Assn. DA-18-064-CML-2299	Research Studies of the Effects of Environment on the Origin & Establishment of Bacterial Variants	4-11-02-024	24,300.00
Univ. of Texas DA-18-064-CML-2301	A Study of the Factors Involved in Immunity in Brucellosis	4-11-02-027	8,800.00
Wesleyan University DA-18-064-CML-2362	Research Studies on the Basic Mechanisms of Mutation in Bacteria	4-11-02-024	13,900.00
Univ. of Utah DA-18-064-CML-2363	Immunochemical Studies of Bacterium tularense	4-11-02-019	9,000.00
USDA--BAIC CD3-553	Effect of Photo-oxidation on Biological Activity of	4-11-02-025	20,000.00
USPHS--CDC CD3-555	Airborne Transmission of the Causative Agent of Histoplasmosis Histoplasma capsulatum	4-11-02-035	39,500.00
Duke University DA-18-064-CML-485	Testing Fungistatic Activity of Organic Compounds Against Human Pathogenic Fungi	4-61-09-005	9,933.00
Johns Hopkins Univ. DA-18-064-CML-470	Preparation of a Toxoid for Immunization Against Type "C" Botulinum Toxin Food Poisoning	4-61-09-004	16,135.00

Contracts in the Field of Antipersonnel Biological Warfare Agents - continued

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
Parke-Davis Co. DA-18-064-CML-1445	Investigation of Combined Botulinum Toxoid	4-61-09-004	$ 25,285.00
Parke-Davis Co. DA-18-064-CML-2339	Development of Methods of Large Scale Production of Anthrax Antigens	4-61-09-004	44,375.00
Parke-Davis Co. DA-18-064-CML-2386	Development of a Multiple Viral-Rickettsial Vaccine	4-61-09-004	111,500.00
Univ. of Chicago DA-18-064-CML-2032	Methods of Concentration & Purification of Staphylococcus Enterotoxin	4-04-14-004	33,400.00
USDA--BAIC CD3-553	Development of a Practical Fermentation Method for Producing Enterotoxin	4-04-14-004	41,500.00
Univ. of Florida DA-18-064-CML-2358	Study of Toxins of Higher Fungi	4-11-02-029	16,500.00
Univ. of North Carolina DA-18-064-CML-1664	Studies of Psittacosis Virus Toxin	4-11-05-010	9,550.00
Univ. of Chicago DA-18-064-CML-1909	Biochemical & Immunological Investigations on the Psittacosis Lymphogranuloma Group of Viruses	4-11-02-021	19,750.00
Johns Hopkins Univ. DA-18-064-CML-2308	Research Studies of the Synergism Between Viruses and Bacteria	4-11-02-023	22,000.00
Johns Hopkins Univ. DA-18-064-CML-2365	Research Studies on Combinations of Rickettsiae & Selected Agents	4-11-02-023	24,200.00

Contracts in the Field of Antipersonnel Biological Warfare Agents - continued

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
Indiana University DA-18-064-CML-2372	Tissue Culture Studies of Viruses & Rickettsiae	4-11-02-021 4-11-02-047	$ 11,950.00
USPHA--NIH, Rocky Mt. Lab. CD3-1547	Laboratory Studies on Selected Micro-organisms in Relation to Their Anthropod Vectors (Project No. 1)	4-11-04-004	73,616.00
	The Study of Multiple Infections Produced by Microorganisms of Their Products (Project No. 2)	4-11-02-023	
USPHS--CDC CD3-1548	Studies & Experimental Investigations with VEE Virus	4-11-02-041	32,543.00
Calif. State Dept. of Health DA-18-064-CML-2337	Airborne Transmission & Dissemination of "Q" Fever	4-11-02-047	36,300.00
Southern Research Institute DA-18-064-CML-2395	A Search for Antiviral Agents	4-61-09-005	59,950.00
Univ. of Calif. N7-ONR-29536	Research on Malleomyces pseudomeallei	4-11-02-034	142,000.00
	Research on Coccidioides immitis	4-11-02-035	98,000.00
	Research on Pasteurella pestis	4-11-02-036	71,000.00
	Experimental Epidemiology	4-61-05-001	71,000.00
	Total		$2,422,618.00

Contracts in the Field of Antianimal Biological Warfare Agents

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
Univ. of Minnesota DA-18-064-CML-2029	Investigations of the Effects of Swine Influenza	4-11-02-044	$ 12,000.00
Univ. of Wisconsin DA-18-064-CML-2034	A Study of Vesicular Stomatitis in Domestic Animals	4-11-02-043	28,668.00
Iowa State College DA-18-064-CML-2035	Investigation & Study of Hog Cholera Virus	4-11-02-044	24,350.00
Johns Hopkins Univ. DA-18-064-CML-2300	An Analysis of the Destruction Effects (Virulence) of Certain Pneumotropic & Neurotropic Viruses	4-11-02-030	17,500.00
Michigan State College DA-18-064-CML-2304	Modification of Animal Viruses	4-11-02-042	30,330.00
Cornell University DA-18-064-CML-2359	Investigations of Viral & Rickettsial Agents Causing Epizootic Diseases of Ruminants	4-11-02-042	35,000.00
Univ. of Minnesota DA-18-064-CML-2370	Research Studies on the Development of a Genetically Small-Sized Pig for Laboratory Use in Veterinary and Human Medical Research	4-11-02-044	24,900.00
Univ. of Wisconsin DA-18-064-CML-2377	Research Studies of Virulence & Immunogenecity Among Strains of Newcastle Disease Virus	4-11-02-030	17,800.00
USDA--BAI CD2-6686	Studies of Feed Pellel. Collection of Exotic Viruses in Foreign Countries	4-11-02-042	120,300.00

Contracts in the Field of Antianimal Biological Warfare Agents - continued

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
USPHS--CDC CD3-136	Q Fever Vaccination	4-11-02-047	$ 40,000.00
USDA--BAI CD3-730	Studies of Viruses of Foot & Mouth Disease in Suckling Laboratory Animals	4-11-02-043	52,000.00
Univ. of Calif. N7-ONR-29536	Research on Vesicular Infections	4-11-02-043	92,000.00
Texas A&M College DA-18-064-CML-2397	Field Assessment of Anthrax Vaccine	4-11-02-042	23,000.00
	Total		$517,848.00

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Contracts in the Field of Anticrop Biological Warfare Agents

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
Regents of the Univ. of Calif. DA-18-064-CML-303	The Inactivation of Certain Plant Growth Regulators	4-11-02-009	$ 7,350.00
Univ. of Wisconsin DA-18-064-CML-476	Investigation on Stem Rust of Cereals	4-11-05-003 4-11-02-026	30,050.00
Iowa State College DA-18-064-CML-1602	Preparation of Compounds for Studies Relating Chemical Structure to Growth Regulatory Activity	4-11-02-008	12,000.00
Oklahoma A&M College DA-18-064-CML-1658	Investigation of the Effects of Herbicides and Growth Regulators on Wheat, Sorghum, and Other Crops	4-11-02-010 4-11-02-011	7,200.00
Univ. of Nebraska DA-18-064-CML-1659	Virus Infection of Plants	4-11-02-039	19,000.00
Rutgers University DA-18-064-CML-1662	Effects of Plant Growth Regulators on the Nitrogen Metabolism of Plants	4-11-02-009	5,200.00
Iowa State College DA-18-064-CML-1922	Varietal Responses to Growth Regulators	4-11-05-001	6,960.00
Univ. of Minnesota DA-18-064-CML-2405	The Ecology of Cereal Pathogens	4-11-02-026	16,000.00
Univ. of Illinois DA-18-064-CML-2295	The Synthesis of Organic Fluorine Compounds	4-11-02-008	11,360.00

Contracts in the Field of Anticrop Biological Warfare Agents - continued

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
Southern Calif. Univ. DA-18-064-CML-2306	Research Studies of the Chemical & Physical Properties of Phenoxy-acetic Acids	4-11-02-009	$ 12,450.00
West Virginia Univ. DA-18-064-CML-2334	Investigation of Races & Strains of Phytophthora Infestans	4-11-02-040	10,950.00
Tracerlab, Inc. DA-18-064-CML-2356	Carbon-14 Analyses	4-11-02-009	20,400.00
Univ. of Rhode Island DA-18-064-CML-2374	Studies on Varietal Differences in Response of Potatoes to Selected Plant Growth Regulators	4-11-02-010	4,200.00
Mississippi State College DA-18-064-CML-2384	Research Studies Relative to Responses of Cotton to Selected Plant Growth Regulators	4-11-02-010	10,000.00
Auburn Research Foundation DA-18-064-CML-2387	Synthesis of Indole Derivatives and Related Compounds	4-11-02-008	8,900.00
Smithsonian Institute DA-18-064-CML-2388	Research Studies on the Influence of Plant Growth Regulators on Oxidative Phosphorylaxation and Related Biochemical & Physiological Processes	4-11-02-009	7,500.00
USDA--BAIC CD3-500	Production, Purification, & Identification of Gibberellin Produced by the Organism Fusarium Moniliforme	4-11-02-012	15,500.00

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Contracts in the Field of Anticrop Biological Warfare Agents - continued

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
USDA--BAIC CD3-553	Synthesis of Some Compounds for Tests as Plant Growth Regulators	4-11-02-008	$ 5,000.00
USDA--BPI CD3-1000	Screening of Chemicals as Plant Growth Regulators	4-11-02-008	12,000.00
	Effects on Crops of Chemicals Applied in Irrigation	4-11-02-011	3,000.00
	Rice Diseases and Herbicide Responses	4-11-02-012	5,000.00
	Cereal Rust Epidemiology	4-11-02-026	20,000.00
	Soil Survey	4-11-03-001	3,000.00
	Rust Resistant Germ Plasm in Small Grains	4-11-05-003	45,000.00
USDA--BPI CD3-3450	Furnishing, Preparation, & Maintenance of 6,000 sq.ft. of Greenhouse Space	4-11-03-001	17,000.00
USDA--BPI CD3-3545	High Temperature Tolerant Strains of Phytophthora Infestans	4-11-02-040	8,975.00
USDA--OFAR CD3-3666	Phenological Maps of Specified Crops in Countries of Eastern Asia	4-11-02-010 4-11-02-026	24,000.00
USDA--BPI CD3-4295	Bibliography of Foreign Plant Diseases	4-11-02-012 4-11-02-039	20,000.00

Contracts in the Field of Anticrop Biological Warfare Agents - continued

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
USDA--BAIC CD3-4390	Antigenic Properties of Rust Spores	4-11-05-003	$ 8,800.00
	Total		$376,795.00

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Contracts in the Fields of Detection, Protection, and Decontamination Against BW Agents

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
Armour Research Corp. DA-18-064-CML-2106	Methods for Field Detection of Proteins & Bacteria	4-11-05-007	$ 53,370.00
Univ. of Mississippi DA-18-064-CML-2296	Research on & Development of an Acoustic Type Particle Counter	4-11-05-007	13,600.00
Aeroprojects, Inc. DA-18-064-CML-2309	Study & Development of Specialized Aerosol Samplers	4-11-05-007	29,550.00
Southern Research Institute DA-18-064-CML-2361	Research Studies on Detection of Biological Warfare Agents by Means of Particle-Size Distribution	4-11-05-007	22,665.00
Polaroid, Inc. DA-18-064-CML-2425	Development of a Bacterial Aerosol Field Detection Device	4-11-05-007	54,400.00
USPHS--EHC CD2-6220	Development of Rapid Identification Procedures by Infrared Spectrophotometry	4-11-05-007	43,182.00
USDA--BAIC CD3-119	Differentiation of Microorganisms by Infrared Spectra	4-11-05-007	30,500.00
Nat'l. Bur. of Standards CD3-1596	Detection & Determination of Suspended Protein Material in Air	4-11-05-007	35,000.00
USPHS--CDC CD3-1633	Bacterial Air Sampling in a Large Industrial Area	4-11-05-007	18,181.00
USP[redacted]-EHC CD3-[redacted]207	Determination of Protein Content in Air	4-11-05-007	19,732.00

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Contracts in the Fields of Detection, Protection, and Decontamination Against BW Agents - continued

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
Lovell Chemical Co. DA-18-064-CML-2407	Develop Membrane Filters of Graded Porosity	4-11-05-007	$ 20,000.00
USPHS--CDC CD3-550	Studies on the Microbiology of Air - Laboratory Processes	4-11-05-010	63,725.00
Florida State Univ. DA-18-064-CML-2383	Investigation of Tarpaulin Materials	4-11-05-005	32,000.00
University of Florida DA-18-064-CML-2030	Preparation of Substances Gaseous	4-11-05-004	25,000.00
Southern Research Institute DA-18-064-CML-2102	Methods for Dispensing Formaldehyde	4-11-05-004	22,600.00
Henry Ford Hospital DA-18-064-CML-2294	Investigation of Chemical Viricidal & Sporicidal Agents	4-11-05-004	33,918.00
Univ. of Michigan DA-18-064-CML-2376	Research Studies of the Mechanism of Action of Ethylene Oxide on Microorganisms	4-11-05-004	9,400.00
Bucknell University DA-18-064-CML-2381	Investigations of Kinetics of Ethyl-enimini Reactions	4-11-05-004	17,915.00
USDA--BEPQ CD2-5762	Development of a Dispenser for Formaldehyde Vapor	4-11-05-004	18,250.00
B. F. Goodrich Co. DA-18-064-CML-2396	Method for Using Beta-propiolactone as a Sterilizing Agent	4-11-05-004	30,000.00

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Contracts in the Fields of Detection, Protection, and Decontamination Against BW Agents - continued

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
Warner Institute for Therapeutic Research DA-18-064-CML-2400	Study the Mechanism of Action of Viricidal, Sporicidal, & Bacterial Action of Reactive Compounds	4-11-05-004	$ 56,487.00
Battelle Memorial Institute DA-18-064-CML-2378	Research Studies on the Evaluation & Design of Procedures & Equipment to be used in Bacteriology	4-61-04-001	49,775.00
USPHS--CDC CD3-520	Developing of Methods for Recovering & Recognizing Viral & Rickettsial Agents in Their Natural Environments Including Air, Water, Soil, etc.	4-11-02-041	19,119.00
	Total		$718,369.00

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Contracts in the Field of Dissemination of Biological Warfare Agents

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
Harvard College DA-18-064-CML-1654	Effect of Atmospheric Conditions on the Viability of Organisms in Aerosols	4-11-04-003	$ 20,750.00
Mellon Institute of Industrial Research DA-18-064-CML-2293	Retention and Distribution of Particulates in the Respiratory Tract of Experimental Animals	4-11-04-003	16,300.00
Lehigh University DA-18-064-CML-2332	Effect of Biologically Active Substances on Persistence of Airborne Microorganisms	4-11-04-003	21,500.00
Georgia Inst. of Technology DA-18-064-CML-2379	An Investigation of the Factors Determining Aggregation of Fine Particle Matter Suspended in Air	4-11-04-003	21,486.00
Univ. of Maryland DA-18-064-CML-2380	Studies of Aerosols with a Simple Cloud Chamber Technique	4-11-02-029	22,850.00
Nat'l. Bur. of Standards CD3-6940	Research in Application of Mathematical Statistics to Problems of the Chemical Corps	4-64-05-001	30,000.00
Leland Stanford, Jr. University DA-18-064-CML-1856	Research Concerning the Propagation of Airborne Agents	4-11-04-005	343,800.00
The Ralph M. Parsons Co. DA-18-064-CML-2282	Research Studies of Aerosol Cloud Travel over Cities	4-11-04-005	378,100.00
Prime, Inc. DA-18-064-CML-2399	Design & Fabrication of Special Aerosol Sampler	4-98-05-020	32,295.00

Contracts in the Field of Dissemination of Biological Warfare Agents - continued

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
Penna. State College DA-18-064-CML-1402	A Basic Study of the Physics of Aerosol Formation	4-04-14-001	$ 60,850.00
The Ralph M. Parsons Co. DA-18-064-CML-1533	Design and Development of Equipment for Test Sphere	4-11-04-001	69,120.00
Aircraft Armaments, Inc. DA-18-064-CML-1857	Development & Fabrication of Prototype Glide Clusters	4-04-14-008	118,480.00
New York University DA-18-064-CML-1860	Mathematical Theories Applicable to Formation & Behavior of Aerosols	4-64-06-002	10,200.00
Univ. of Notre Dame DA-18-064-CML-1907	Development of Protective Garments	4-11-04-001	13,567.92
Battelle Memorial Institute DA-18-064-CML-1914	An Investigation Leading to the Development of a Method for Determining the Particle Size Distribution & Concentration of Aerosols	4-11-04-001	83,565.00
Aeroprojects, Inc. DA-18-064-CML-2026	Investigation & Development of Ultrasonic Aerosol Devices	4-04-14-001	174,000.00
Rheem Mfg. Co. DA-18-064-CML-2028	Development of Experimental Dispenser System for Small Pellets	4-04-14-001	49,527.00
General Mills, Inc. DA-18-064-CML-2104	Development of a Special Munition	4-04-14-011	100,070.00

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Contracts in the Field of Dissemination of Biological Warfare Agents - continued

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
The Ralph M. Parsons Co. DA-18-064-CML-2120	Design & Modifications to Test Sphere	4-11-04-001	$170,950.00
The Ralph M. Parsons Co. DA-18-064-CML-2283	Design & Development of BW Munitions	4-04-14-001 4-04-14-002 4-04-14-008 4-04-14-013 4-11-04-001 4-04-14-014 4-04-14-016	2,633,892.00
Univ. of Minnesota DA-18-064-CML-2389	Wind Tunnel Time & Related Services at Rosemont	4-04-14-006	17,520.00
Knapp-Monarch Co. DA-18-064-CML-2292	Development of Equipment for Filling, Sealing, & Leak Testing Small Cylinders	4-04-14-002	39,857.00
Ohio Research Found. DA-18-064-CML-2305	Quantitative Determination of Protein at High Dilution	4-11-04-001	15,000.00
George Washington Univ. DA-18-064-CML-2307	Research Studies on the Physics of Explosive Dissemination	4-04-14-001 4-04-14-002	197,975.00
A. D. Little, Inc. DA-18-064-CML-2329	Research & Development Services to Bring E-99 Bomb to Engineering Acceptability as a Candidate Munition	4-04-14-013	405,000.00
Minneapolis-Honeywell DA-18-064-CML-2335	Research Studies & Tests of a Cushioned Sphere	4-04-14-013	49,000.00

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Contracts in the Field of Dissemination of Biological Warfare Agents - Continued

Contractor & Contract No.	Short Title	R & D Project No.	P. A. Value
Minneapolis-Honeywell DA-18-064-CML-2338	The Design & Development of a High Level Opening Device for Spherical Munitions	4-04-14-001	$ 58,800.00
Minneapolis-Honeywell DA-18-064-CML-2340	The Design & Development of Specific Munition with Low Level Opening Device	4-04-14-002	118,935.00
Univ. of Maryland DA-18-064-CML-2364	Analyses & Development of Prototype Munitions by Wind Tunnel Tests	4-04-14-001 4-04-14-002	45,040.00
Battelle Memorial Institute DA-18-064-CML-2375	The Application of Ultrasonics to Biological Systems	4-11-04-001	94,300.00
USDI--Bureau of Mines CD3-679	Study of Flow Properties of Fine Particles	4-04-14-001	21,000.00
Naval Research Labs. PO-CMLRE-13-53	Development of Solid Powder Grains	4-04-14-001	60,000.00
Danielson Mfg. Co. DA-18-064-CML-2406	Development of Molded Nylon Sampling Devices	4-11-04-001	28,200.00
Lovell Chemical Co. DA-18-064-CML-2407	Develop Membrane Filters of Graded Porosity	4-11-04-001	51,500.00
Dumont Laboratories DA-18-064-CML-2438	Development of an Automatic Colony Counter	4-11-04-001	93,000.00
	Total		$5,666,429.00

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4. PERSONNEL REVIEW

a. GENERAL REVIEW OF PERSONNEL ACTIVITIES

As of 30 June 1953, the staff of the Chemical Corps Biological Laboratories totaled 1562 and consisted of 1267 civilian and military personnel. The figures indicate a growth of 364 during the fiscal year which represents an increase of 386 civilians and a decrease of 22 military. Most of the civilian growth results from the organizational transfer of functions such as the Comptroller's Office, Purchasing and Contracting Office, Civilian Personnel Office, and the Miscellaneous Services Office to the Biological Laboratories from Post Headquarters during the first quarter of FY 1953. There was an increase of 118 wage board (ungraded) personnel. Compared with June 1952, professionals increased 68, while the sub-professionals decreased 29. In addition to the full-time staff, the services of 16 consultants and experts were used for a total of 104 days during the fiscal year. /2 (See page 119).

Not included in any of the figures given in the preceding paragraph are the contract personnel working for the Biological Laboratories on research and development projects in Frederick and Braddock Heights, Maryland, as follows: Ralph M. Parsons Co., 403 (contract period 28 June 1952 to 31 August 1954); George Washington University, 15 (contract period 15 November 1952 to 14 November 1954).

The graph P-1 shows civilian strength from FY 1950 through FY 1953. In addition, there are 140 Army enlisted scientific and professional personnel and 47 Navy enlisted men assigned to the Laboratories; most of the Navy personnel have scientific training.

The officer strength assigned to the Laboratories at the end of FY 1953 was 42 Army, 11 Navy, and 53 Air Force, or a total of 106. Further, there were 21 officers and 79 enlisted men assigned to the supporting elements. Also, 6 additional enlisted men from Dugway Proving Ground served a total of 90 days on a detached basis for special assignments in the Biological Laboratories.

The graph P-2 shows the strength in Biological Laboratories and Supporting elements for FY 1947 through FY 1953. The total strength (for planning purposes) is shown for FY 1954 and FY 1955 on the same graph. It will be noted that the planning strength for the supporting elements has decreased for FY 1954 and 1955 when compared with previous years, whereas the planning strength for Biological Laboratories for the same two years shows a substantial increase.

These requirements are in line with the change in guidance (outlined in General Directive No. 4 and its supplements 1 and 2 dated 24 February, 20 March, and 10 April 1953). The guidance placed emphasis on end-item development instead of research, which necessitates increases in personnel particularly in such areas as the Pilot Plants and others in development activities. There was a shift of emphasis from the development of debilitating to lethal agents which require safer facilities for operating personnel, cause increased difficulty in developing such agents, and necessitate the institution of screening programs on candidate agents. This too requires additional personnel.

The net requirement for additional personnel caused by the change of guidance has been assessed as 417 additional people for FY 1954 and an additional 347 for FY 1955. The new facilities will permit many of these activities to be handled much more safely than has been possible in the past, and they will permit more efficient utilization of existing personnel as well as provide adequate space for the planned increase.

Considerable difficulty has been experienced this year as a result of fluctuating personnel policies. For several years the Department of Defense has had an over-all personnel ceiling of 500,000 graded employees placed upon it by Congress. This has resulted in each Installation in turn receiving its own allotments for the number of personnel it is authorized to employ. In February 1953, a directive was received which prohibited the hiring of additional personnel or the filling of vacancies until new authorizations were set. The new authorization, when received, set a total authorized figure less than the number actually employed, thus enforcing a reduction in force. RIF notices were issued to 52 persons employed in the Biological Laboratories and in supporting elements under the jurisdiction of the Commanding Officer of Camp Detrick. Two weeks prior to the effective date of the RIF notices (31 May 1953), authorization was received to reach the new personnel ceiling by attrition. This resulted in the withdrawal of all but 4 RIF notices, and these were in supporting elements controlled by Second Army. In June, the number of authorized allotments was increased by 126 (3 Graded and 123 Ungraded) along with the additional authorization to recruit 10 percent (136) over the 30 June allotment. Fiscal year 1953 ended with the Biological Laboratories under authorized strength. Long term planning, employee morale, and recruitment efforts have suffered as a result of the issuance and withdrawal of RIF notices and the subsequent attempt to recruit additional personnel.

A listing of key personnel of the Chemical Corps Biological Laboratories is given on the following page (See also the Table of Organization, page [redacted]).

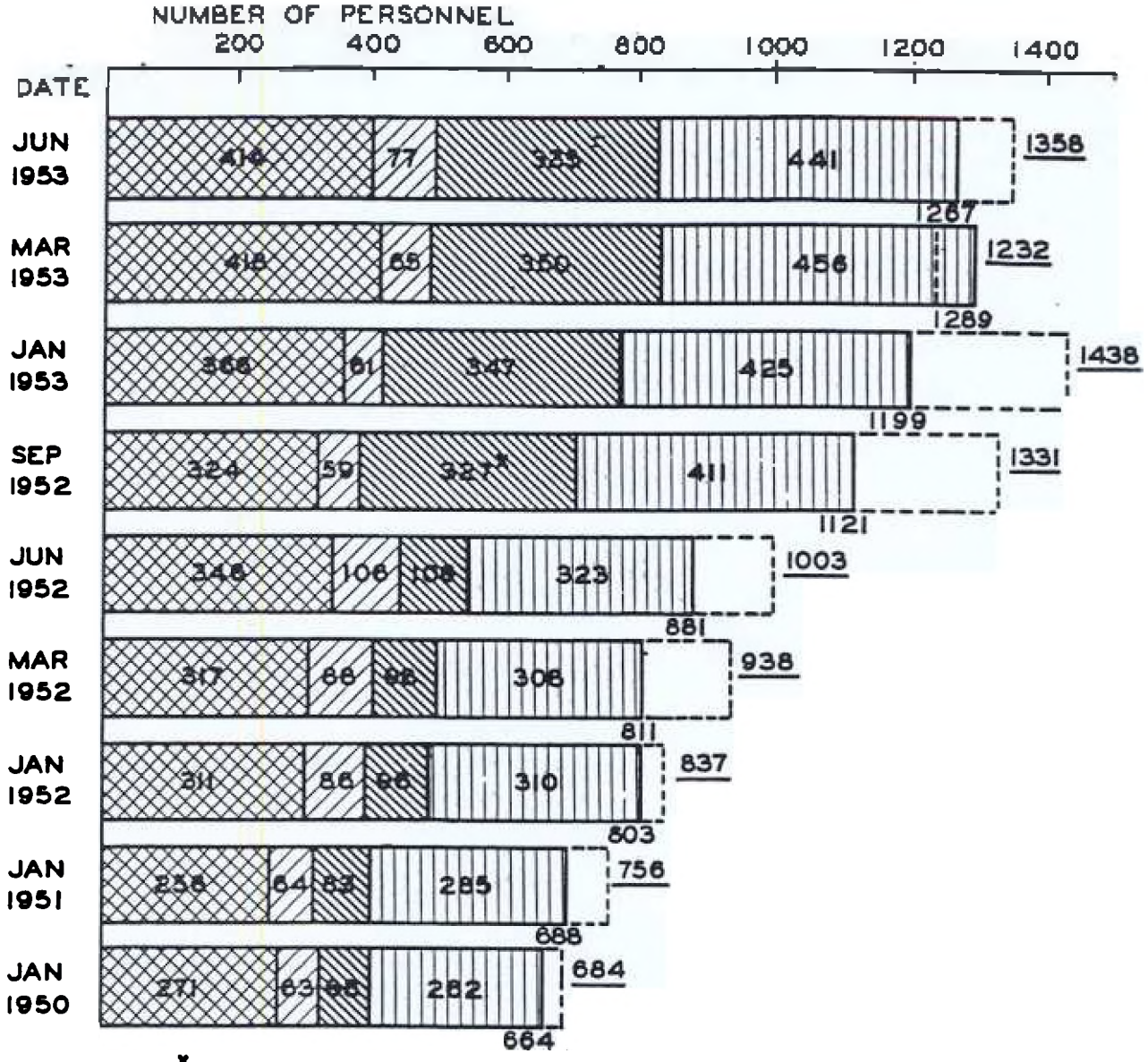
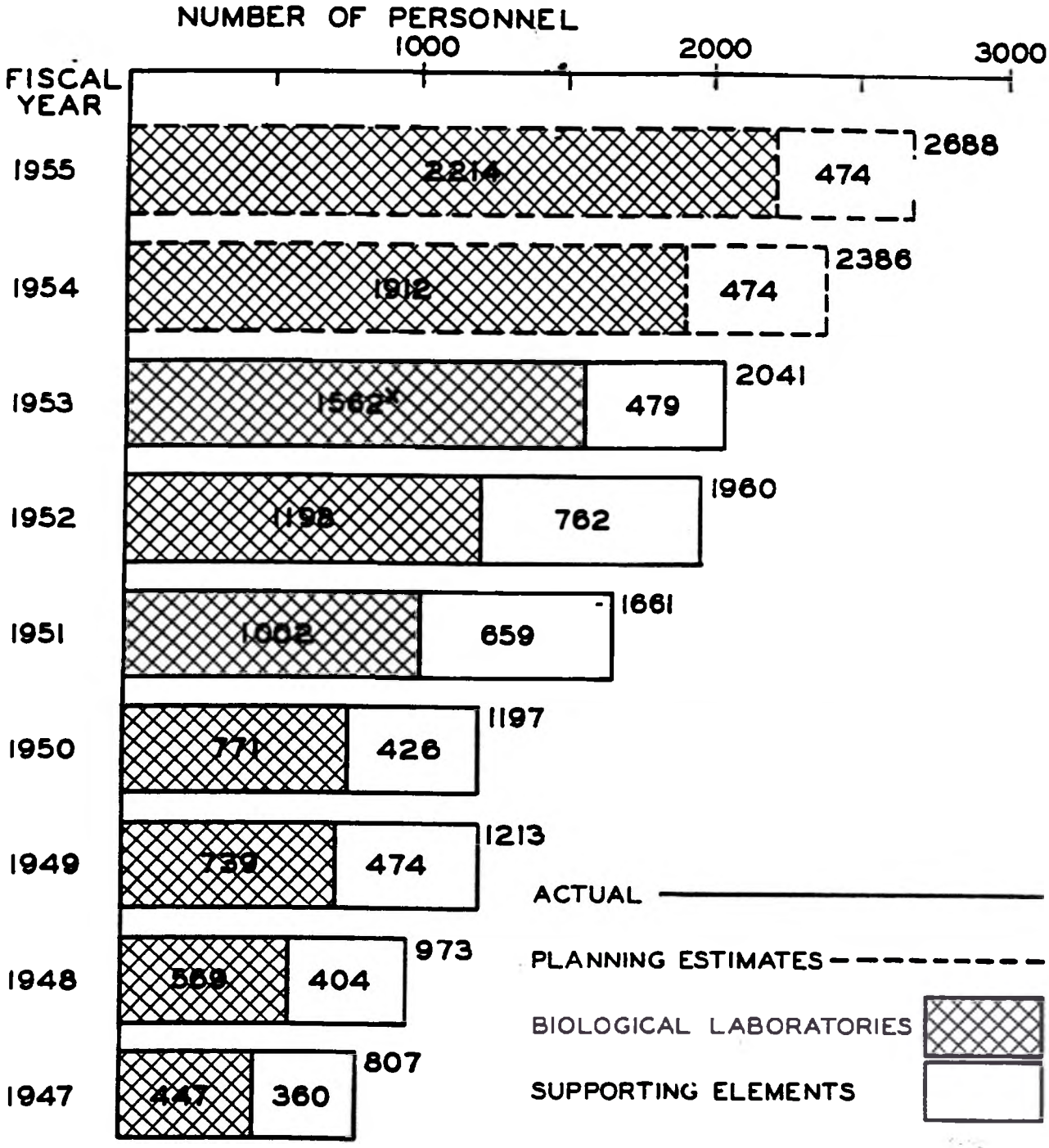


FIGURE P-1. CIVILIAN PERSONNEL OF THE BIOLOGICAL LABORATORIES BY GROUPS — JAN 1950-JUN 1953.



*REPRESENTS TRANSFER OF CERTAIN SUPPORTING ELEMENTS TO BIOLOGICAL LABORATORIES.

FIGURE P-2. CIVILIAN AND MILITARY PERSONNEL FOR THE BIOLOGICAL LABORATORIES AND SUPPORTING ELEMENTS — FY 1947 - FY 1955.

b. KEY PERSONNEL IN THE CHEMICAL CORPS BIOLOGICAL LABORATORIES

Director . . . . . .Leroy D. Fothergill, M.D.
Deputy Director . . . . . John L. Schwab, Ph. D.
Assistant Deputy Director . . . . . V. L. Ruwet, Lt Col, Cml C
Safety Director . . . . . A. G. Wedum, M. D.
Assistant Director, Plans and Evaluation . . . . . C. R. Phillips, Ph. D.
Assistant to the Director for Administration . . . . . R. D. Chapman, Lt Col
Liaison Offices . . . . . (See page 123)
Civilian Personnel Office . . . . . J. R. Thompson
Purchasing & Contracting Office . . . C. M. Willoughby, Major
Special Assistant for Veterinary Medicine . . . . . R. Randall, Colonel

Assistant Director, Defense . . . . . G. L. Orth, Colonel, MC

Veterinary Microbiology (VM Div) ... W. R. Hinshaw, D.V.M.
Safety (S Division) ..... A. G. Wedum, M.D.
Animal Farm (AF) ..... M.M.Rabstein, D.V.M.
Physical Defense (PD Division) ..... R. Porter

Assistant Director, Agents Development..E. V. Hill, M.D.

Crops (C Division) ..... C. E. Minarik, Ph.D.
Allied Sciences (AS Division)..... C. R. Brewer, Ph. D.
Medical Bacteriology (MB Div)..... R. D. Housewright, Ph.D.
Virus & Rickettsia (V&R Div) ..... F. B. Gordon, M.D.
Biological Process (BP Div)..... J. L. Roberts PA D

Assistant Director, Engineering . . . E. E. Champlin, Lt Colonel

Miscellaneous Services (MS Div) .... C.S.V. Sanner
Research Engineering (RE Div) ..... A. J. Rawson
Drying Task Force (DTF Division).... R. S. Hutton, Ph.D.
Pilot Plant (PP Div)..... G. L. Achorn

Assistant Director, Weapons Development..W. H. Kayser

Special Operations (SO Division) ....M. R. DeCarlo, Lt col
Field & Meteorological Research .....K. L. Calder
Munitions (M Division) .....G. A. DeShazer
DPG Coordinator .....G. E. Hoeffler, Lt Col

Post Commander, Camp Detrick . . . . . .John W. Fitzpatrick, Lt Col
Comptroller . . . . . .J.J.M. Beecher

c. CONSULTANTS, FY 1953

Name	Specialty	Institution
Addicott, Dr. Frederick T.	Plant Physiology	Univ of Calif., Los Angeles
Dayton, Mr. Arthur R.	Industrial Engineering	Clayton Mfg. Co. El Monte, Calif.
Fluck, Dr. Paul H.	Infectious Diseases	Private Practice M.D., Lambertville, N. J.
Herriott, Dr. Roger M.	Biochemistry	School of Hygiene & Public Health Johns Hopkins Univ. Baltimore, Maryland
Hertz, Dr. David B.	Research Administration	Columbia Univ. New York, N. Y.
Hildebrandt, Dr. Frank M.	Engineering	U. S. Industrial Chemical Co. Baltimore, Md.
Hopkins, Mr. Edward Scott	Water Treatment-Sewage & Sanitation	Bureau of Water Supply Baltimore, Md.
Hutchings, Dr. Leslie M.	Diseases of Large Animals	Purdue Univ West Lafayette Indiana
Lovell, Dr. Stanley P.	Chemistry	Lovell Chemical Company Watertown, Mass.
May, Dr. Jacques M.	Medical Geographical Research	American Geographical Soc., New York, N. Y.
Menotti, Dr. Amel R.	Antibiotics	Bristol Laboratories Syracuse, N.Y.