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SPECIAL REPORT

NO. 185

PREPARED BY

SAFETY DIVISION

CHEMICAL CORPS
BIOLOGICAL LABORATORIES

[redacted]

SPECIAL REPORT NO. 185

SAFETY PROGRAM AT CAMP DETRICK

1944 to 1953

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Cml C Biological Laboratories

CHEMICAL CORPS BIOLOGICAL LABORATORIES
Camp Detrick, Frederick, Maryland

July 1953

[redacted]

SPECIAL REPORT NO. 185

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SUMMARY

The principal objective of all safety efforts is to prevent injury, illness, and death in employees and in the surrounding community. All other interests are subsidiary. The following statements will be better evaluated if this is kept in mind:

1. 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.

2. The record of illnesses, for 1952-53 compared to preceding years, in Buildings T-429, T-430, T-432, T-433, T-459, and T-527 shows that (these) buildings, adequately equipped with safety devices and adequately supervised, may handle the most infective human agents without infection of employees (See Table 4.1, Section II,4).

3. The buildings in which infections are still occurring are (a) those which are not equipped with known safety features and devices, and (b) the obsolete pilot plant T-431. (A replacement for this plant will be operative before the end of 1953). In some of these buildings, supervisory cooperation in safety is incomplete.

4. The single most important reason why BW illnesses are still occurring in Buildings T-434, T-522, T-524, and T-525 is that, largely because of a shortage of personnel, Research Engineering Division is unable to construct, fabricate, procure and/or install known safety features and devices where they are needed in a reasonable time. Safety considerations make it very difficult to contract out such work; immunizations and risk of illness bar most contractors.

This situation puts a grave burden on the Safety Director, Biological Laboratories, who is given the hard choice of permitting additional illnesses among employees, with an accompanying risk to health and life, when means to reduce or eliminate such illnesses and risks are known, or of ordering the stopping of these operations.

5. Although the Safety Director may acquiesce in the continuation of some, but not all existing operations involving calculated risks, provided some definite hope exists of remedial action, he cannot acquiesce in the initiation of new hazardous operations, unless adequate safety is initially provided.

6. Failure to provide adequate known safety facilities now prevents a complete program in three important areas involving new

hazardous operations: (a) bacterial screening, (b) viral-rickettsial screening, (c) combination of agents. Certain important aspects of these programs are not permissible with present facilities. All new hazardous programs will face a similar obstacle.

7. 1952-53 is outstanding in the progress made in developing safety devices. RE Division has borne the major burden of design, but it is particularly gratifying that the operating divisions studying infectious diseases have voluntarily taken an aggressive interest in making specific contributions.

There still exists to varying degrees, among supervisors in different places, some lack of initiative in safety, some failures to enforce or suggest workable modifications in safety regulations, some passive resistance to safety, and some failure to realize the extent and nature of supervisory responsibility for the health of subordinates. But, on the whole, the past year has been distinct improvement in these matters.

8. Introduction of B anthracis into the Pilot Plant T-431 and into M Division T-527 and P-567 has served to prove that no highly persistent agent can be prevented from contaminating the employees and the environment, unless a completely closed safety system is used (the Class III safety cabinet typical of SO Division in Building T-459).

The weak point in the closed system is the germicidal dunk bath through which objects are passed to the outside of the cabinet or aerosol chamber. No known disinfectant has killed the spore in a reasonable operating time. An improved technique utilizing a plastic bag is under study by contract; cost of the bag at present is too high.

Only when an organism dies quickly is it possible to use a safety cabinet without attached gloves, or remove unsterilized objects from a safety cabinet, without detectable organisms being spread to the employee or to the environment outside the safety cabinet.

The implication of this situation is that there must be an increase in the amount of Class III safety equipment, and more Class I equipment with an attached autoclave or other easily attached items such as incubators. The cabinet module system now under design by S. Blickman, Inc., on contract with RE Division, offers a probable solution.

The next weakest point in the closed cabinet system is the gloves. There is difficulty in standardizing a suitable glove and in maintaining adequate supplies.

9. There is a serious deficiency in safe animal holding facilities, particularly in ultraviolet cage racks and ventilated cages for aerosol-challenged animals.

10. Continued progress in the commercial development of fiber glass air filters makes it seem probable that in some situations air filtration may replace air incineration. This will save a significant amount of money.

11. The number of BW occupational illnesses in employees during 1952-53 has significantly decreased from the peak reached in 1951. It is believed this is due to the effect of the ventilated safety cabinet systems first introduced in any number in 1951-52. 1952 was the first year in the history of Camp Detrick when all the personnel of a building (T-430) engaged in the study of brucella for 12 months (now 16) without a single case of brucellosis (See Table 3.1 of Section II,3).

12. Employees are now receiving 10 vaccines (smallpox and diphtheria are being added to thoselisted in Section II,1,a) and 6 skin tests. More than 250 employees receive all of these. The tolerance of employees to such a schedule of injections is an eloquent testimonial of the confidence of employees in the high quality of medical care provided by the nurses and physicians in the Clinical Investigation Branch.

The various screening and agent combination programs present a threat to this confidence, by requiring even more vaccines. Combined vaccines offer some hope in this connection. A combined typhus, Q fever, and Rocky Mountain spotted fever vaccine will be in use in May or June 1953.

13. One recent case of cutaneous anthrax in an immunized person (2 injections of vaccine 5 months previously; lesion at a site of injury, rapid healing), 10 hospitalized cases of psittacosis in immunized persons, and 4 hospitalized, 3 non-hospitalized cases of Q fever in immunized persons (not all in 1952-53), again indicate that the immunity provided by vaccination may be overwhelmed by sufficient dosage.

14. The new anthrax vaccine developed by Dr. George Wright has now been administered to several hundred persons without untoward results.

15. The accomplishments of the Immunology Branch is perfecting a new anthrax vaccine, and the progress on the botulinum toxoids, justifies utilization of the research talents of this branch to maximum capacity of the building, by adding personnel to that point.

16. There are four areas of deficient activity:

a. Immunological evaluation of vaccines, single and combined, now being administered to employees.

b. Duplication of accidents, with a view to estimating possible human infective doses.

c. Safety engineering checks on new buildings, before, during, and after erection.

d. Routine safety control in all infectious units.

PLANS

1. Various specialized safety cabinets are being developed in S and other divisions, for the refrigerated centrifuge, the Warburg apparatus, shaking machines, the Henderson aerosol apparatus, autopsy of animals, changing of cages, and for other purposes.

2. Unless some increase in personnel is forthcoming, it is planned to drop the in-service training program as far as formal courses in bacteriology are concerned, inasmuch as present personnel cannot keep up with present safety requirements brought on by the new buildings and new programs.

3. Combined vaccines will be put in use as fast as possible.

4. Negotiations are underway to produce a combined bacterial vaccine by contract.

I. CONTROL OF BW AGENTS

1. ACCIDENTS INVOLVING BIOLOGICAL MATERIALS

a. INTRODUCTION

During the past half century in which bacteriology has developed, the equipment used by the bacteriologist has been borrowed almost entirely from the older science of chemistry. In many cases this is unfortunate, as often the tools used by the chemist are not safe for use with highly infectious microorganisms. It is well known that a fairly high percentage of all bacteriologists who work with pathogenic cultures sooner or later become infected with the organism with which they are working. In many laboratories this is a foregone conclusion; however, at Camp Detrick we do not adhere to this philosophy and believe that work with infectious agents can be carried out with a reasonable degree of safety without undue hardships and undue slowing of work. In the course of daily work with infectious material, and often due to pressure of work, accidents occur which expose personnel to these agents. A system of reporting these accidents was initiated at Camp Detrick with the start of studies with infectious agents. It is by means of these reports that steps can be taken to evaluate the causes of accidents and in turn reduce the number of infections. Also, reporting of an accident sets in motion necessary prophylactic medical procedures which may prevent or ameliorate the infection.

b. SUMMARY OF BIOLOGICAL ACCIDENTS

Table 1.1 summarizes the accidents reported to S Division from October 1943 to April 1953 (1,2). It must be remembered that supervisors vary greatly in their conscientiousness in reporting accidental exposures to agents. Therefore figures summarized in Table 1.1 are only indicative. Between 1944 and 1951 there was a total of 261 accidents involving 407 workers. In 1952 there were 52 accidents involving 119 workers while up to April 1953 there were 27 accidents involving 78 workers. Since October 1943 up to April 1953 there have been a total of 340 accidents in which 604 individuals were exposed to pathogenic agents.

c. DISCUSSION

With the advent of new safety devices especially designed to eliminate certain hazards in the Biological Laboratories, there have

been definite changes in the types of accidents. One of the most frequently occurring accidents in the early years was aspiration of infectious material when pipetting. This type of accident has been almost eliminated by the mandatory use of safety pipettors. Leaks in valves, joints and gaskets were common in the Pilot Plant. Use of all welded lines, Sanders diaphragm valves, and seating of valves in cups of disinfectant has done much to eliminate those sources of infection. The use of containers to carry bottles and flasks containing cultures has greatly reduced the dropping of the latter. The installation of ventilated cabinets has not eliminated accidents, but has prevented exposures to infectious agents by containment of the aerosols produced during accidents. Accidents due to mechanical failure are difficult to prevent and only constant safety checks and improvements in equipment will reduce this hazard.

In the prevention of accidents, and in the establishment of future preventive measures after an accident, the single most important factor is the attitude of the supervisor toward safety and his feeling of responsibility for the health of his helpers and associates. Many young men, newly elevated to supervisory responsibility, are careless in their attitude toward safety, simply because they are too inexperienced in life to realize the effect of acute illnesses, chronic illness, and death upon themselves and others.

d. REFERENCES

1. Safety Division Report No. 6, 11 Jan 1952.

2. Supervisor's Notification of Injury, Accident, or Exposure to Disease. CD 6-14. Agent Control Branch Files.

This section was prepared by: Morton Reitman, Chief
Laboratory Hazards Section

TABLE 1.1

ORGANISMS INVOLVED IN LABORATORY ACCIDENTS AT CAMP DETRICK
(Oct '43 to Apr '53)

Organism	1943 - 1951		1952		1953	
	No. of accidents	Persons exposed	No. of accidents	Persons exposed	No. of accidents	Persons exposed
B anthracis	96	151	6	9	1	1
Brucella sp.	60	104	21	46	9	27
P tularense	8	18	4	14	10	32
Shig. dysenteriae	3	4	0	0	0	0
P pestis	6	12	5	6	1	1
Ps aeruginosa	1	2	0	0	0	0
Sal enteritidis	1	2	0	0	0	0
Listerella monocytogenes	1	1	0	0	0	0
Coxiella burnetti	7	19	1	7	2	4
Psittacosis virus	8	13	3	7	2	10
Newcastle disease virus	1	1	0	0	0	0
Venezuelan equine encephalomyelitis virus	11	11	1	1	1	2
Botulinum toxin	18	6	0	0	0	0
Malleomyces mallei	2	2	0	0	0	0
Unknown	38	61	11	29	1	1
TOTAL	261	407	52	119	27	78

2. AEROBIOLOGY

The deliberate creation of concentrated aerosols of pathogenic agents demands the ultimate in safety precautions. Since the primary function of Aerobiology Branch of AS Division is the creation and study of such aerosols, the emphasis in a safety program must be on containment of agent. The two animal exposure chambers, "A" and "B", in T-524 were designed for operation as a closed system under reduced pressure, and each chamber is enclosed in a room which is itself under reduced pressure in relation to the rest of the building. Thus, if by accident, an agent is released into the chamber room, it is not spread to the rest of the building. The Aerobiology Building, chamber equipment and its operation, and safety precautions observed during World War II are fully described by Rosebury (1). The general lag in activity at Camp Detrick following World War II was reflected in Aerobiology Branch and in the activity of Safety Division there.

Equipment in Building T-524 and operating techniques have changed little since the inception of Aerobiology Branch, but increased activity there in the past few years has resulted in an increase in the number of safety tests and a few modifications of equipment and techniques.

a. MODIFICATION OF BUILDING AND EQUIPMENT AFTER 1946

(1) Ventilated transfer hoods have been installed in the 2 transfer rooms to provide better protection for personnel handling infectious agents, preparatory and subsequent to operation of the aerosol chamber itself.

(2) The decontamination room for chamber A now serves as an airlock and the original airlock has been converted to a laboratory room with a ventilated transfer cabinet.

(3) The decontamination room on chamber room B now serves as an airlock, and the original airlock has been converted to a walk-in incubator.

(4) A Class I ventilated cabinet has been installed in a room formerly used for animals. This cabinet is used for plating of samples and for animal autopsy. This cabinet is used as a Class I cabinet without the glove panel or on a Class III cabinet with the glove panel and attached gloves depending upon the agent and the work being carried out.

(5) An ultraviolet pass-through box has been installed in the inner utility room door and in the door between the office and

laboratory sections of the building. Ultraviolet lamps have been placed in the walk-in incubator and in the laboratory rooms.

(6) An ultraviclet barrier has been installed between the contaminated change room and the shower.

(7) An airlock has been added to the formerly closed end of each exposure chamber. This was done primarily to accommodate a large latex balloon used in the stabilization of aerosols. This balloon is enclosed in a gastight metal box which bolts onto the flange of one airlock.

(8) Exposure Chamber A has been replaced with a new chamber of similar design. Both original chambers are worn out and "A" in particular leaked to an extent beyond repair, causing 2 infections in 1953.

(9) An apparatus for sterilizing the "A" and "B" animal exposure chamber airlocks with formaldehyde and steam has been added to both chambers.

b. MODIFICATION OF TECHNIQUES

(1) Diluting and plating techniques have been modified somewhat by the installation of the ventilated safety cabinets. All transfer work with pathogens is carried out in these cabinets with operators wearing rubber gloves and using safety pipetting devices.

(2) Ventilated personnel hoods are worn by all personnel in chamber rooms during work with infectious agents.

(3) Autopsies are performed in the Class I cabinet when possible. Due to the press of work this cabinet is not always available and autopsies are performed in the open face ventilated cabinets in the transfer rooms.

c. SAFETY TESTS

(1) Biological Tests

Biological tests of exposure chambers and related activities using simulant agents have been continued at intervals through the years. These tests all follow a similar pattern. A simulant is nebulized inside the chamber and samples are taken and animals exposed in the same manner as with pathogens. Air and surface samples are taken to detect escape of the simulant agent during exposure of animals and subsequent plating activities (2,3,4,5,6). Similar tests have been conducted on 7 January, 22 and 23 January, and 2 April 1953.

These tests show that under normal operating conditions there is no escape of agent from the chambers. On one occasion, B globigii was recovered on a surface from the airlock of chamber "A" after removal of samples, and on another occasion, B globigii was recovered from the airline connection to the animal transfer box which is used to transfer animals from the chamber to ventilated animal cages. These airline connections have subsequently been fitted with filters.

On 15 April 1953, air and surface samples were taken in chamber "A" during an animal exposure test with B anthracis. A positive surface sample was obtained from the outlet airline on an animal holding box. These boxes have subsequently been provided with filters.

On 22 April 1953, surface samples were taken during animal exposure runs with B anthracis. No positive samples were recovered.

On 24 May and 12 July 1948, the ultraviolet barrier between the kitchen and the cage processing room was tested for bactericidal efficiency (7,8). This was done by nebulizing S marcescens on one side of the barrier and sampling air on the other. The first test showed that numerous organisms passed through the barrier. After modifications, it was capable of preventing passage of a concentrated aerosol.

On 10 October 1952, a safety test was conducted on a spinning disc apparatus for aerosol production. The present design of this apparatus was shown to be unsafe for use with infectious agents (9).

(2) Hydrostatic Tests

For approximately one year hydrostatic tests have been conducted on the chambers every 4 to 6 weeks. The chambers are filled with water at 42 lbs pressure and the chambers are observed for leaks.

(3) Freon Tests

Freon testing of the exposed chambers was begun in the first quarter of 1949 (10) and the frequency of these tests has increased. A routine of daily leak tests has been established since February 1953. After the chambers have been used and decontaminated, freon is admitted at 5 lbs pressure. Each chamber is then tested for leaks with the General Electric Halogen Leak Detector, Type H. Any leaks found are repaired before the next day's operations.

d. WEAK POINTS IN THE SYSTEM

(1) The many lines and valves connected to aerosol chambers "A" and "B" provide numerous possibilities of leakage. These are of the same type as on the original equipment and utilize screw and

compression fittings. The lines should be all welded and valves should be of the diaphragm and solenoid type. Proper filters and traps are also needed in these lines. This change-over is contemplated in the near future and should provide better safety.

(2) Animal cages are of the original type with a removable bottom. These should be replaced with ones which are light weight and gastight.

(3) Building T-524 is much too small for operations as presently conducted. Crowded conditions do not promote safety.

e. NEW AEROBIOLOGY BUILDING, BS-1 (P-376)

A new Aerobiology Building has been designed and constructed. This building incorporates many desirable safety features which are lacking in the old Aerobiology Building. It is expected to be in operation with infectious agents before the end of 1953.

f. REFERENCES

1. Rosebury, T. 1947 Experimental Airborne Infection. The Williams and Wilkins Company, Baltimore, pp. 2-60, 117-120

2. S Division QTR, April-June 1948, page 6
3. S Division QTR, July-Sept 1948, page 12
4. S Division QTR, Jan-Mar 1951, page 3
5. S Division QTR, Jan-Mar 1952, page 1
6. S Division QTR, Oct-Dec 1952, page 8
7. S Division QTR, Apr-June 1948, page 2
8. S Division QTR, July-Sept 1948, page 10
9. S Division QTR, Oct-Dec 1952, page 7
10. S Division QTR, Jan-Mar 1949, page 22

This section was prepared by: Gardner G. Gremillion, Chief
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3. AIR STERILIZATION STUDIES

a. AIR CLEANING

(1) Inlet Building Ventilation

The extent of air cleaning for inlet air depends upon the needs of the individual building operation. There are 3 degrees of air cleanliness that are obtained at Camp Detrick.

(a) Removal of large airborne particles is achieved by the use of 6 ply air mat filter media which is housed in type PL 24 filter frame. About 10 percent of organisms the size of B globigii are removed (1).

(b) For greater particulate removal (1 micron size), 1 layer of 100 FG spun glass filter material housed in deep-bed filter frames is used with a resultant efficiency of approximately 80 per cent.

(c) The highest particulate removal is achieved with 2 layers of the 50 FG spun glass housed in deep-bed frames. The efficiency of the latter system is 99 per cent when using S indica as a test simulant.

(2) Exhaust Building Ventilation

The degree of air purification is determined by the nature of the operation within the building. Prior to 1950, general building air was discharged through the electrostatic precipitators (2) to the outside atmosphere. Periodic inspections (3) and tests of the electrostatic precipitator showed that rated operational efficiencies (85 per cent) were not being obtained. Efficiencies varied from 65 to 78 per cent. For proper operation, electrostatic precipitators must be inspected every 2 weeks and cleaned at monthly intervals. The nature of the activity and the work load of the Research Engineering Division at Camp Detrick is such that maintenance personnel cannot service these filters at such frequent intervals. To be assured of a given bacterial removal efficiency, 2 layers of 50 FG Spun glass installed in deep-bed filter frames were placed behind the electrostatic precipitators (99 per cent efficiency against S indica) in buildings using rather highly infectious materials. Buildings such as T-428, 429, 430, 459, and 567 were so equipped.

(a) Bacterial Filtering Efficiency of the Spun Glass Filter

The effectiveness of 25, 50, and 100 FG spun glass filter material in removal of bacteria and bacteriophage from the air has been reported by H. M. Decker, F. A. Geile, H. E. Moorman, and C. A. Glick, in the American Society of Heating, Ventilating and Engineering,

Jl Section, Oct 1951 in an article entitled, "Removal of Bacteria and Bacteriophage from the Air by Electrostatic Precipitators and Spun Glass Filter Pads."

More detailed studies on an electrostatic precipitator and spun glass filter pads are reported in Special Report No. 124, dated 29 November 1949. Application of spun glass as filters for bacteriological safety cabinets is reported in Special Report No. 145, dated 4 June 1951. Tests were performed using S indica and E coli bacteriophage T-3 as bacterial and viral simulants to determine the filtration efficiencies of units containing 2, 3, and 4 layers of spun glass. Tests were also conducted on an experimental unit containing 4 layers of spun glass separated by a 1 inch space. The average minimum efficiency in all tests exceeded 99 per cent.

A series of tests conducted in 1951 (4) at air flows of 10, 20, 30, 40, and 50 fpm indicated that the filtration efficiency of spun glass does not change materially, in fact there is a slight increase in efficiency as the velocity increases.

Commercially available filter units using 50 FG spun glass are available. The American Air Filter Co., Louisville, Kentucky, manufactures a deep bed frame for housing spun glass filter pads for any desired airflow. The Dollinger Corp., Rochester, N.Y., had developed a 250 cfm cylindrical pipe line filter containing pleated 50 FG spun glass. The latter type of filter unit may be decontaminated with steam prior to filter removal. Both types of filter units have been evaluated with S indica as the test organism and have shown an efficiency of 99 per cent or greater (5). Modifications of the Dollinger type filter have been made at Camp Detrick for airflows of 10 cfm and are in use as part of the exhaust system from the Schwab type cabinets installed in Building T-459. (RE Design Drawing No. D-93-1-2145).

(b) The Spun Glass Paper Filter

Several paper mills have initiated programs to develop either an all-glass paper filter or else a combined filter medium of glass and asbestos fiber that has performance equal to that of the present Cml Corps asbestos cellulose type filter papers. The greatest progress in this field has been made by the Hurlbut Paper Co., South Lee, Mass. Mr. Kenneth J. Busse reported in November 1952 issue of the Wire and Wire Products Journal that the all-glass filter paper being made by Hurlbut Paper Company is actually superior to the asbestos type paper in its filtration properties. When conducting a test in smoke filled air, only 1 particle of smoke in 100,000 penetrated through the filter. A smoke particle averages .2 micron. He also reported that it is easily visualized that the use of all

glass paper will not be confined to gas mask filters alone. A wide usage is apparent in the medical, chemical, bacteriological, radiological and commercial industrial fields as well. This filter paper discussed by Mr. Busse was originally developed jointly by the Department of the Navy and the National Bureau of Standards. Fibers of this new type filter are known commercially as E type glass micro-fibers and possess an average diameter of 0.5 to 0.75 microns and a melting point of 1450 F.

Results of recent biological tests conducted at Camp Detrick on this type of paper filter are shown in Table 3.1. The efficiency of the filter paper was determined by sampling the cloud concentration of the simulant (S indica) before and after the filter. Penetration of S indica through the type E glass filter paper was .02 organisms, per million test organisms recovered before the filter, when the airflow was 20 linear feet per minute. The penetration at an airflow of 10 linear feet per minute was 0.01 organisms. The penetration through the asbestos glass was .28 organisms, per million test organisms recovered before the filter, at an airflow of 20 linear feet per minute. The penetration increased to 1.4 organisms per million when the airflow was reduced to 10 linear feet per minute.

The data obtained on the filtering efficiency of these filters is most promising. There is now available a highly efficient mineral filter than can be used at high temperatures, which is fire resistant, yet can be biologically decontaminated with heat prior to changing of the filter.

(c) The Asbestos-cellulose Paper Filter

Another effective filter media is the asbestos-cellulose material developed by the Chemical Corps. This filter media has a higher efficiency than the 50 FG spun glass had. However, it has certain recognized shortcomings. The progress report on high efficiency air filters with mineral fiber medium to Division of Engineering, U.S. Atomic Energy Commission, by the Arthur D. Little Inc., Cambridge, Mass., dated March 20, 1952, states that at the present time high efficiency air filters based on a cellulose and asbestos paper medium are in full commercial production. This type filter is being produced commercially by the Cambridge Corp., at Syracuse, New York, and by Mines Safety Appliance Co., at Pittsburgh, Pennsylvania. These filters have proved their value. They are entirely adequate for most normal temperature uses and are even desirable in those cases where the spent filter must be reduced in volume by incineration. However, it is recognized that the present filters cannot be exposed to water or air saturated with moisture and may be destroyed or damaged by certain chemical fumes. To provide a filter that will overcome these objections and retain the performance of the present asbestos filter, is the purpose of an all-mineral fiber paper medium described in the foregoing section (b).

b. AIR INCINERATION

(1) Oil-fired Incinerators

Experiments which include the liberation of highly infectious aerosols in exposure chambers require equipment for sterilization of air from the exposure chamber or cabinets subjected to a high degree of contamination. Since air incineration provides the greatest degree of decontamination, there are in use at Camp Detrick three 2500 and one 5000 cfm oil fired incinerators. Two 2500 and one 5000 cfm incinerators are in non-operating status. Tests (6) were conducted during April-June 1948 on the oil fired air incinerator at T-235 which at one time sterilized all air from the munitions test chamber in T-263. The results showed that the air incinerator effectively destroyed spores of B globigii when operated at normal operating temperature (530-560 F). At temperatures of 440 F or less killing of B globigii will not be complete at the time the air passes the center of the checker break stack. It was concluded that since the retention time through the air incinerator will vary with the amount of air being passed through the system and since it is desirable to maintain a large safety factor, recommendation was made that temperatures be maintained well above 440 F, at the center of the checker break stack.

(2) The Trent Electric Grid Incinerator

Construction and operation of an oil fired incinerator is costly. At times it becomes economically infeasible to install such a system where perhaps only small quantities of air need to be sterilized from a given operation. As a result an investigation was conducted to determine the efficiency of a Trent electric grid air sterilizer for decontaminating of air. This incinerator will process 100 cu ft air per minute. Data was collected relative to temperature and retention times required to kill micro-organisms in air. Results of this investigation recorded in detail in Special Report No. 140, dated 31 December 1950, indicate that virtual sterilization (99.9999 per cent or more corresponding to 0.001 per cent or less penetration) may be achieved by a Trent Sterilizer under the following conditions: (1) 425 F - 24 seconds retention time, (2) 475 F - 10 seconds retention time, (3) 525 F - 5 seconds retention time and (4) 575 F - 3 seconds retention time. A greater degree of safety can be obtained if the air is maintained at 625 F - 3 seconds retention time.

Knowing that 425 F and 24 seconds retention time gives the desired sterilization, an approximate rule that may be used to determine the desired temperature and retention time combination is that a 50 F increase in temperature is approximately as effective as tripling the retention time. For instance, if the control temperature was increased to 475 F the retention period could be cut to one-third of 24 (i.e. 8 seconds). When a safety factor of 2 seconds is added, this rule will check with the 4 conditions enumerated above.

c. LOW VOLUME AIR INCINERATOR (1 cfm)

An air sterilizer (7) consisting of glass tubing with an electric nichrome wire coil sealed within, developed by MV Division personnel, has been tested for efficiency of air sterilization of B globigii spores. Results of tests showed that the air sterilizer effectively destroys spores of B globigii when operated at flows of 1 cfm or less.

d. STEAM EJECTORS (8)

Small steam jets have been used in various locations, particularly the Pilot Plant. Tests were conducted in 1948 to determine the bactericidal efficiency of a steam jet against aerosolized B globigii and against suspended B globigii. Results of the tests indicated that the amount of organisms ordinarily passing through a steam jet, as from the fermentation tanks, will probably be killed. However, the steam jet has definite limitations as to the volume of liquid suspension or aerosol that can be sterilized. Retention time of aerosol or liquid suspension is of primary importance.

e. REFERENCES

1. S Division QTR, Jan-Mar 1949, page 22
2. Special Report No. 70
3. S Division QTR, Jan-Mar 1949, page 24 and Apr-Jun 1949, page 9
4. S Division QTR, Dec 1951, pages 13 and 14
5. S Division QTR, 30 Sept 1950, pages 4-11
6. S Division QTR, Apr-Jun 1948, pages 16 and 17
7. S Division QTR, Jan-Mar 1948, pages 9 and 10 and Oct-Dec 1952, page 21
8. S Division QTR, Jul-Sept 1948, pages 14-16 and Oct-Dec 1948, page 25

This section was prepared by: Herbert M. Decker, Chief
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TABLE 3.1

EFFICIENCY OF GLASS AND GLASS-ASBESTOS FILTERS IN REMOVAL OF S INDICA FROM AN AIR STREAM

Filter Material Evaluated	Air Flow linear feet per minute	Resistance (inches H_2O)	Number of filters* evaluated	Penetration per 100 million org. recovered before filter
Glass Type E Filter Paper	20	3.2	7	2
	10	1.5	5	1
Asbestos-Glass Paper	20	3.1	5	28
	10	1.5	5	140

* Each filter was tested a minimum of ten times

4. DECONTAMINATION OF SURFACES

The biological decontamination of surfaces seems to present as many problems as there are surfaces. Contact being a primary factor in decontamination, any factor which interferes with contact compounds the difficulty of decontamination. Roughness and/or porosity of a surface complicates decontamination with liquid disinfectants or ultraviolet light. An accumulation of dirt, grease, or oil interferes with decontamination by liquids, gas, heat, or ultraviolet. At the Biological Laboratories where entire buildings and all the equipment therein are considered potentially contaminated, all of the above methods of decontamination are utilized. The application of a sufficient degree of heat to a surface for a sufficient length of time is the most certain method of decontamination (i.e. sterilization). It is obvious, however, that all surfaces presented by a building and its equipment cannot be subjected to this treatment. It is then necessary to resort to one or more of the other methods, by which sterilization may or may not be achieved, but by which surface contamination at least will be reduced.

a. MISCELLANEOUS SURFACES

Surface decontamination is an integral part of bacteriological work. Routine decontamination is difficult to assay when original contamination is unknown. But when an accident occurs which releases bacteria, the effectiveness of decontamination may be judged from subsequent sampling. A few such examples may be cited.

In January 1950, Bacillus anthracis was being processed in a sonic vibrator in T-427. The suspension leaked into the cooling water, which drained into a stone sink. The area was decontaminated with sodium hypochlorite solution and surface samples were taken (1). The agent was present on the sink and drainboard. The area was again decontaminated and subsequent surface samples were negative.

In August 1951, a flask containing a broth culture of Brucella suis was dropped on the floor of a room in T-433. The area was flooded with 5 per cent phenol and an ultraviolet lamp was placed over the area. Surface samples revealed no agent (2).

In March 1952, a broth culture of an avirulent strain of Pasteurella pestis was being aerated in an incubator in T-429. A variation in pressure in the vacuum line caused the culture to foam up through an attached manometer. Surface samples from the shelves and floor of the incubator showed a heavy contamination with the agent. The incubator was decontaminated with 2 per cent Roccal and subsequent surface samples were negative (3).

In June 1952, the sonic vibrator in T-427 again leaked B anthracis into the cooling water. Positive samples were recovered from the sink, drainboard, an electric furnace, icebath, gas cylinders, centrifuge, and clothes of one of the operators. The room was closed to traffic and 1400 ml of 37 per cent formaldehyde was vaporized into the room and allowed to act overnight (4). Subsequent surface samples were negative.

In August 1952, an agar slant of a virulent culture of P pestis was broken on the floor in T-429. The area was flooded with 2 per cent Roccal and surface samples taken. Positive samples were recovered from fragments of the agar slant. The Roccal was ineffective in the presence of agar and these fragments were autoclaved (5).

In 1949 a test was conducted to determine the effectiveness of a procedure for sterilizing the neck of the 8B-E1 transfer cabinet used in T-527 (6). The neck of the cabinet was contaminated with a concentrated suspension of Bacillus globigii spores. A solution of sodium hypochlorite (30,000 ppm in available chlorine) was drained into the neck of the cabinet, allowed to stand for 5 minutes, and was then drained out. Filling and draining took 27 minutes. Surface samples were then taken from the neck of the cabinet. One portion of the neck with smooth surfaces was sterilized, while another portion containing many corners and crevices was still highly contaminated with B globigii spores.

In 1952 similar tests were conducted using S indica and B globigii. Incomplete kills were obtained when S indica was exposed to 0.1 per cent Roccal for 15 minutes and B globigii was exposed to hypochlorite (3000 ppm in available chlorine) for 20 minutes. B globigii was killed by 10 minutes exposure to formaldehyde and steam, but positive samples were found in the channels protected by the "O" ring gaskets(5).

After engineering changes were made in the neck of the 8B-E4 transfer cabinet, decontamination tests were again conducted. It was found that S marcescens could be eliminated from the neck with 0.1 per cent Roccal or hypochlorite (2000 ppm in available chlorine). Tests with B globigii required 60 minutes exposure to the same hypochlorite concentration for complete kill (7).

In 1952 tests were conducted to evaluate several bactericides for possible use as surface decontaminants in dunk baths through which spore contaminated items are passed (7). In preliminary tests, tubes of 0.5 per cent trisodium phosphate, 1.0 N sodium hydroxide, 5 N sodium hydroxide, and 10 N sodium hydroxide were inoculated with Bacillus globigii spores to a concentration of 1×10^6 mg/ml for the first two solutions and 5×10^6 for the other two. At intervals, aliquots were removed from the tubes, neutralized with sterile acid, and plated on corn steep agar. There was less than 99 per cent kill

in 0.5 per cent trisodium phosphate and 1.0 N sodium hydroxide after 10 minutes exposure at room temperature, and those were considered unsatisfactory. It was found that 5 N sodium hydroxide rendered a 99 per cent kill after 10 minutes exposure and 10 N sodium hydroxide rendered 99.9 per cent kill after 1 minute and complete kill after 5 minutes. It was concluded that these high concentrations were not satisfactory for practical use in large volumes due to the expense and toxic hazard involved.

Similar tests were then conducted using several different concentrations of peracetic acid and sodium hypochlorite (Clorox) plus 0.5 per cent Nacconal NF (8), a surface active agent. It was found that when 18×10^4 B globigii spores per ml were exposed to 1 per cent peracetic acid, complete killing was obtained in one minute; 0.4 per cent acid required 5 minutes and 0.2 per cent required 10 minutes to kill the same concentration. When 8×10^6 spores per ml were exposed to a 1:15 dilution of Clorox (approximately 2000 ppm available chlorine) 99.99 per cent kill was obtained in one minute. Complete kill was not obtained in 10 minutes, even with a 1:5 dilution.

A practical test was conducted to simulate the sporicidal activity of 1 per cent peracetic acid and 1:15 dilution of Clorox in a dunk bath. Acid cleaned impinger bottles were dipped into a suspension of B globigii spores (1×10^7 spores/ml) for 2 seconds. Each bottle was then dipped for 5 seconds into the sporicide. Swab surface samples were then taken from each bottle at 1, 5, and 10 minute intervals. Fewer positive samples were obtained from the bottles decontaminated with 1:15 Clorox, but neither solution gave complete decontamination. Peracetic acid was also found to be very unstable in solution and 1:15 Clorox was recommended as the sporicide of choice for dunk baths.

b. WALLS, FLOORS, FIXED EQUIPMENT

The reduction in activity at Camp Detrick immediately following World War II presented an ideal situation for testing the effectiveness of liquid disinfectants in decontaminating surfaces of various types on a large scale. During the war, experience had shown that the work carried on in the pilot plants resulted in continuous contamination of surfaces in the rooms with B anthracis, and after operations in these buildings had been concluded it was considered essential to decontaminate the buildings and all contained equipment as a whole. Walls, ceilings, floors and installed processing equipment were decontaminated by spraying with a water solution of calcium hypochlorite containing 15,000 ppm in available chlorine. Surface samples were then taken with cotton swabs, and surfaces found still contaminated

were again decontaminated. Some locations required several successive treatments before contamination was reduced to a level not detectable with swabs. Buildings closely associated with the pilot plants, i.e., the air incinerator and sewage decontamination system were treated in a like manner and were extensively sampled by S Division (9).

In 1947, Building T-263 was decontaminated by vaporizing formaldehyde into the building by means of a Chemical Corps smoke generator. Before decontamination, surface sampling showed the building to be highly contaminated with B globigii. Contamination was greatly reduced by treatment with formaldehyde vapors (10).

In 1948, an effort was made to decontaminate the simulants explosion room in T-263 with formaldehyde vapor. The effort resulted in great reduction in number of B globigii organisms detected by air and surface sampling.

In 1948, in Rooms 4, 6, and 7, T-433, surface samples were taken, after completion of laboratory work, with B anthracis. The agent was found on tables, benches, shelves, and sinks (12). The rooms were washed thoroughly with hypochlorite solution and on resampling were found to be still contaminated. Another thorough washdown was required before negative samples were obtained.

In August 1948, surface samples were taken following animal exposures to B anthracis in a Henderson apparatus. Benches, tables, and floor were found to be contaminated with the agent. Two thorough washdowns with hypochlorite solution were required before negative samples were obtained.

During the Summer Field Trials of 1950 at Dugway Proving Ground, a considerable amount of work was done on decontamination, by natural and artificial means, of surfaces and soil contaminated with vegetative organisms (11). This work included the decontamination of soil and various test surfaces by sunlight, vehicles by sunlight and liquid disinfectants, and surfaces and personnel by ultraviolet light. The ability of vegetative agents to survive under the decontaminating effect of sunlight and temperature was generally greater than had been expected, the type of surface being a major factor. Artificial ultraviolet light was found to be effective in decontaminating plywood, glass, rubber, canvas, and personnel protective clothing. It was also found that vehicles could be adequately decontaminated with a liquid disinfectant. After completion of the field trials, the laboratory building and all large equipment was decontaminated by washing down with 0.1 per cent Roccal. Subsequent sampling recovered no infectious agent (11).

In March 1952, several gallons of B anthracis culture was discharged to the sewage sterilization plant, T-314. Surface samples

showed the agent to be present on several valves and a pump shaft. The plant was sprayed with hypochlorite solution and subsequent surface samples showed B anthracis still present. The valve packings were tightened and the plant was again decontaminated. No further positive samples were recovered (4).

In December 1952, work with B anthracis was resumed in Pilot Plant Building T-431. Shortly thereafter, B anthracis was recovered from routine surface samples taken in the Sewage Decontamination Building, T-314. A thorough sampling of the building revealed that the contamination was widespread, B anthracis being recovered from the floor, work bench, valves, collection tanks, and wash basin (13). Daily washdowns of the entire building with hypochlorite solution were instigated. Subsequent sampling revealed that Building T-312 (lunchroom) and T-313 (incinerator room) were also contaminated and they were included in the daily washdowns. Building T-313 was the most heavily contaminated and contained instruments, motors, and burners which could not be sprayed but had to be washed down by hand. In spite of the large volumes of hypochlorite solution which were utilized in T-313, contamination persisted and consistent negative samples were not obtained until 3 weeks after decontamination procedures were begun. Formaldehyde could not be used in this building due to explosion hazard from the incinerators which are in constant use.

T-312 was not at any time heavily contaminated, and it is felt that the contamination here was carried by contact from T-313 and T-314. Besides the daily hypochlorite washdowns, T-312 was twice decontaminated with formaldehyde and steam. Contamination disappeared here when the other two buildings were decontaminated.

In three weeks the contamination in T-314 decreased to the point where only an occasional positive sample was obtained. This condition has continued until the present time (14). The equipment in T-314 is badly in need of modernization, because there are fittings, valves, and pumps which are liable to leakage. Incidents of the above type should be eliminated when the new decontamination plant is put into operation.

Since work with B anthracis was resumed in T-431, contamination of that building with the agent has been widespread and continuous. Date obtained there are included in the section concerned with safety in the Pilot Plant.

The decontamination of the Test Sphere, T-527, has provided much data on surface decontamination with formaldehyde and steam. These data are included in the section on safety in the Test Sphere, T-527.

c. VENTILATION AND DUCTWORK

Following the postwar decontamination of the pilot plants in 1946, it was discovered that surface sampling of ventilation ductworks revealed only slight contamination with B anthracis and very little contamination with organisms of other types (15). However, ventilation fans which were covered with grease were heavily contaminated. It was assumed that chlorine liberated during the decontamination of the buildings had been present in sufficient quantities to decontaminate the ductwork. In these few cases where B anthracis was found, effective decontamination was accomplished by vaporizing formaldehyde into the ductwork with a Chemical Corps smoke generator.

Intensive tests were conducted in 1952 (16) to determine the effectiveness of formaldehyde vaporization into exhaust ducts. A Chemical Corps portable smoke generator (55 gal/m cap.) was used to vaporize steam and formaldehyde through the building exhaust systems. The results of the tests showed considerable reduction in the bacterial count, but there was not complete decontamination of all surfaces. Present design requirements in the building program are that all new buildings in which spun glass filters are installed in the exhaust filtration system must be equipped with a fixed steam ejector system to supply a steam formaldehyde spray. The steam flowing through the ejector will draw formaldehyde from a carboy into the plenum chamber and filter area. To maintain a formaldehyde vaporization rate of 0.5 cc/cf on passing through the filter, it will be necessary to have an airflow not in excess of 500 cfm. Nebulization of formaldehyde should be for a minimum of 30 minutes. These requirements necessitate installation of dampers to reduce the airflow to 1/10 normal flow. At the present time, other more desirable methods of decontamination, such as the use of heat, are being investigated. Formaldehyde decontamination of exhaust systems generally necessitates absence of personnel and removal of animals from the building as vapors of formaldehyde have a tendency to penetrate back into the building.

To give additional protection to workers entering exhaust ducts, all temporary and new buildings are being equipped with an air supply manifold at the plenum chambers in the attic so that workers can wear ventilated personnel hoods when entering these potentially contaminated areas.

d. BACTERIOLOGICAL WORK CABINETS

In 1951, tests were conducted to determine the effectiveness of formaldehyde and steam in the decontamination of bacteriological safety cabinets (16). A 60-inch Blickman cabinet was contaminated with cultures of B globigii and E coli by swabbing a 2-inch square section of the inside surface of the cabinet at 10 different locations. These

cabinets involved a special problem in that it was necessary to leave the exhaust blower in operation during decontamination.

Formaldehyde was vaporized into the cabinet by boiling it from a beaker on a hot plate. Humidity, time, and airflow through the cabinet were varied, and throughout the tests surface samples were taken from the contaminated areas to determine viability.

It was found that at relative humidities between 36 and 43 per cent and formaldehyde vaporization rates of .54 to .57 ml per cu ft of air passing through the cabinet, E coli was killed within one hour. At relative humidities between 67 and 76 per cent and formaldehyde vaporization rate of .46 to .51 ml per cu ft of air passing through the cabinet, B globigii spores were killed in 2 hours.

An adaptation was then made of a Hydromist Vaporizer (17) to be used for decontaminating safety cabinets. The vaporizer is a portable steam ejector with a reservoir for water and one for the decontaminating solution. An electrical heating unit turns the water to steam which passes through a steam ejector drawing the formaldehyde from its reservoir. The nozzle was adapted to fit on the standard 8-inch gloveport. In tests similar to those above, it was found that with the apparatus the safety cabinet could be sterilized in 30 minutes at a relative humidity of 88 per cent when formaldehyde was vaporized at the rate of .51 ml per cu ft of air passing through the cabinet.

e. EQUIPMENT

(1) Fixed

Some mention has already been made concerning the decontamination of fixed equipment or equipment so large that it must be decontaminated in place. The decontamination of such equipment must be accomplished by methods similar to those used for buildings. In the pilot plants at the close of World War II, the exterior surfaces of large processing equipment were decontaminated by washing down with hypochlorite solution or by vaporizing formaldehyde inside rooms (15). The interior surfaces of processing tanks and lines were sterilized by steam under pressure. Large equipment such as refrigerators and incubators were decontaminated by washing with calcium hypochlorite. These same methods are still in use. Processing equipment in Pilot Plants and Decontamination Plants are routinely decontaminated by steam under pressure. External surfaces of large equipment are decontaminated by washing with a suitable disinfectant or by being exposed to formaldehyde vapors.

The exposure chambers in T-524 and T-459 are routinely sterilized with steam under pressure as are filters from the ventilated cabinets in T-459 (18).

(2) Transportable Equipment

(a) Steam sterilization: The application of steam under pressure is the method of choice for decontamination of equipment which will not be damaged by the process, and is equally applicable for items contaminated with bacteria or their toxins. The autoclaving of glassware is routine in any bacteriological laboratory, and during the pilot plant cleanup after World War II extensive use was made of this method. All glassware, burners, ringstands, pipette canisters, etc. were autoclaved at 20 psi for one hour (15). This method is used extensively today for cleaning items from contaminated buildings.

(b) Gas sterilization: The use of ethylene oxide as a disinfectant has been in use in the food storage industry for over 20 years, but has had only slight usage by bacteriologists in general. In 1945 an investigation was begun to explore its usage as a decontaminating agent at Camp Detrick (19). It was found to be an effective bactericide when tested against S marcescens, B globigii, and B anthracis, and was found effective in decontaminating cloth, leather, rubber, metals, and various laboratory instruments with no physical damage. During the postwar cleanup of the pilot plants, 865 pieces of laboratory equipment were decontaminated with ethylene oxide. Since ethylene oxide is highly explosive, it was used in the form of Carboxide, a 90 per cent carbon dioxide - 10 per cent ethylene oxide mixture. Subsequent examination of these items, exposed to approximately 266 m/l of ethylene oxide for 6 hours, failed to reveal the presence of any B anthracis contamination or physical damage.

Since that time, extensive use has been made of ethylene oxide and numerous tests have been conducted for its effectiveness as a bactericide. In 1948 tests were conducted to determine the time and concentrations required to kill Brucella organisms with ethylene oxide (20). Time required for killing increased with the decrease in concentration of ethylene oxide, and in lower concentrations of ethylene oxide, and increase in temperature decreased the time required for killing. With the highest concentrations used (442 mg/l) 2 hours were required to kill 1×10^8 organisms dried on twill cloth patches.

Brucella abortus A-19 cells were then dried on various surfaces and exposed to 100 mg/l of ethylene oxide at room temperature. Complete killing was obtained in 6 hours on the following surfaces: agar, stainless steel, glass, wood, guinea pig feces, cardboard. It was found, however, that incomplete killing was obtained when Brucella cells were inoculated into oil, oil soaked cloth, or a broth medium more than 0.5 cm in depth (21).

Work was also done (20) to determine the absorption of ethylene oxide by anhydrous activated alumina and anhydrous calcium chloride. It was found that both agents absorb ethylene oxide and thus interfere with sterilization in any chamber in which they are

present. Further experiments (21) showed that powdered egg albumin, gelatin, wet animal bedding and moist soil also absorbed considerable amounts of ethylene oxide.

In 1949, experiments were conducted to determine the effect of concentration of spores on concentrations of ethylene oxide and time and temperatures required for sterilization. Sterile penicillin assay disks were inoculated with various numbers of B anthracis spores and exposed to various concentrations of ethylene oxide at various times and temperatures (22). It was found that at 27 C an increase in the numbers of spores required an increase in time necessary for sterilization by each of the concentrations of ethylene oxide used. At 37 C, the time required for sterilization was not increased by an increase in the number of spores. Later experiments showed the action of ethylene oxide on B anthracis spores to be truly bactericidal and not merely bacteriostatic (22). Spores exposed to ethylene oxide were cultured on agar to determine viability and reproductive ability and injected into mice to determine viability and infectivity. It was found that spores exposed to sublethal doses, as determined by plating, possessed a lowered infectivity for mice; those exposed to lethal doses, as determined by plating, produced no mouse infections.

In 1952 lyophilized S indica cells and lyophilized particles of E coli bacteriophage T-3 were exposed for 16 hours to ethylene oxide in a concentration of 240 mg/l of air at room temperature. Subsequent plating failed to recover the test agents. However, when dried spores of Aspergillus fumigatus were exposed it was found necessary to increase the concentration of ethylene oxide to 310 mg/l in order to obtain complete kills under the same conditions (23).

Books: Tests were also conducted to determine the efficacy of ethylene oxide in the sterilization of the inside pages of books. Filter paper strips contaminated with B globigii spores and S indica cells were placed on interior pages of books, the books were closed and exposed for 16 hours at room temperature to ethylene oxide in a concentration of 330 mg/l. The strips were then removed and cultured in broth. An occasional positive sample was recovered when high concentrations of spores were used. However, books in normal use in laboratories are not likely to become contaminated to the extent encountered in the tests.

The use of ethylene oxide as a decontaminating agent has gained wide usage since its first trials in 1946. Autoclaves converted for use with carboxide are included in specifications for all new buildings which will house infectious agents, and conversions have been made in a number of the older buildings. Its use was introduced at Dugway Proving Ground during the Summer Field Trials of 1950 (11), and it has been in constant use there since.

Skin: Due to the occurrence of chemical burns from rubberized clothing exposed to ethylene oxide (24), studies were made to determine necessary precautions to prevent reoccurrence of this type of injury. Small patches of rubber were exposed to ethylene oxide and then taped to the depilated skin of rabbits. Severe burns resulted. Similarly treated patches were then exposed to the air for periods ranging from 0 minutes to 120 minutes before being taped to the rabbit's skin. It was found that the longer the airing period, the less severe the reaction(25). Simultaneous chemical assays run on duplicate patches also showed that ethylene oxide was dissipated into the air from the rubber. When the skin of rabbits was exposed to vapor of ethylene oxide alone, the same type of damage resulted as from the exposed rubber patches. It was concluded that burns from exposed rubber were due entirely to the ethylene oxide absorbed and not to a product of chemical combination (26), and that sufficient airing of exposed rubber articles will eliminate the danger of burns.

f. DETOXIFYING AGENTS

The decontamination of surfaces contamined with bacterial toxins has not been a major problem at Camp Detrick. Except for a few occasions, work with botulinum toxin has not been on a large scale, and necessary surface decontamination is readily achieved by autoclaving or by detoxifying with formaldehyde, alkali (27), or Lysol (28). Substances which have been tested for detoxifying properties and found inactive includes propylene and methylene glycol, ethyl alcohol, quaternary ammonium compounds, and ethylene oxide (27).

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5. DETECTION, BY BIOLOGICAL MEANS, OF BW AGENTS IN LABORATORIES AND PILOT PLANTS

a. SELECTIVE AND DIFFERENTIAL MEDIA

To evaluate the hazards resulting from work with infectious microorganisms, it is necessary to obtain air and surface samples from the work area. All new equipment and techniques are tested before use with infectious agents by carrying out the procedures with nonpathogenic simulants such as S marcescens, E coli T-3 bacteriophage and B globigii. During actual operations with infectious agents, samples are taken to determine whether the equipment or technique is allowing the escape of the infectious agent. Although leaks in equipment may be detected by use of gases such as freon, all agent detection methods are biological. These methods are slow and sensitivity is not great. The detection of specific microorganisms in contaminated samples is in large part dependent upon the type of medium used. Considerable work has been carried out to develop the following differential and selective media for the detection of bacterial agents.

(1) Bacillus anthracis

Animal inoculation tests were first used in the detection of B anthracis. Housewright (1) in 1944 tested concurrently 863 samples by animal and cultural methods. It was found that more than twice as many recoveries of B anthracis were made by culture as by animal inoculation. The method now employed for the detection of B anthracis consists in recognizing the colonies on blood agar (1). The colonial characteristics (nonhemolytic, grey, rough with feathery irregular margins) are quite consistent and reliable. Mouse virulence tests with recovery of the organisms at autopsy are used as confirmatory evidence. The addition of 1:75,000 potassium tellurite to the blood agar medium has been found to reduce the number of gram negative contaminants (2).

(2) Brucella

Several selective media have been proposed for the detection of Brucella. A medium consisting of a modified Difco tryptose agar

containing tyrothricin and sodium azide was proposed for the isolation of Brucella suis (3). It was not completely satisfactory in our experience. The inclusion of brilliant green in modified tryptose agar was recommended (4) and further work (5) demonstrated that a medium (albimi brucella broth) containing brilliant green, polymyxin, and agar would inhibit a large portion of the organisms found in soil, fecal material, milk, water and air, and still permit quantitative recovery of Brucella.

(3) Pasteurella pestis

A medium containing beef heart infusion, tryptose, sodium chloride, agar, whole blood, polymyxin, actidione and crystal violet was found to be satisfactory for the detection of P pestis (6).

(4) Pasteurella tularensis

The addition of brilliant green and penicillin to dextrose cystine blood agar resulted in a very satisfactory selective medium for P tularensis (7). The use of penicillin was later discontinued and actidione (8) was added to suppress growth of molds.

(5) Malleomyces mallei, Malleomyces pseudomallei

The detection of M mallei and M pseudomallei has been facilitated through use of a 1:200,000 crystal violet glycerol agar (bacto nutrient agar, sodium chloride, bacto peptone, glycerine, and crystal violet) recommended by B Division (9). This medium has proved satisfactory for the detection of small numbers of both agents in heavily contaminated samples (4, 10, 11, 12).

(6) Coccidioides immitis

A suitable medium for the detection of C immitis is a medium composed of dextrose, peptone, agar and 0.05 per cent copper sulfate (4). Many common fungi were inhibited with this medium. Potassium tellurite, sodium azide, and calcium propronate added separately to Sabouraud's maltose agar also showed some inhibition of contaminating fungi (4).

(7) Serratia marcescens and Bacillus globigii

The extensive use of S marcescens and B globigii as simulant agents in processing techniques and in safety testing of equipment

requires an inexpensive medium which is easily prepared and on which the agents are easily identified. A medium satisfactory in all respects is composed of 3.0 per cent treated corn steep liquor, 1.0 per cent black strap molasses, and 2.0 per cent agar, by weight. Sodium chloride may be added up to 3 per cent by weight to inhibit spreading of S marcescens, and actidione (0.1 mg/ml) may be added to inhibit growth of molds.

(8) Escherichia coli bacteriophage T-3

T-3 bacteriophage is also used as a simulant in many safety tests. The medium of choice for its detection is Difco Tryptose-phosphate broth plus 1.5 per cent agar (13).

b. STUDIES ON ACTIDIONE AS A MOLD INHIBITOR FOR SOLID MEDIA

In the isolation of specific bacteria from air, surfaces and other sources over growth by mold contaminants is commonplace. Actidione had been reported as having antifungal properties (14). Further tests (8) showed that actidione in a concentration of 0.1 mg per ml was inhibitory to 13 strains of molds and noninhibitory to 14 different genera of bacteria tested. It was recommended that actidione be included in all media used in routine sampling procedures where large numbers of mold contaminants are present.

c. MEDIA STUDIES WITH THE MEMBRANE FILTER

It was of interest to find selective media for the various agents being employed for use with the membrane filter (MF). It seemed desirable first to survey several of the commercially available media as to their growth enhancing abilities of the agents on the membrane filter. The following media were tested (15) using B anthracis: trypticase soy broth (BBL), albimi brucella broth (Albimi), brain heart infusion broth (Difco), tryptose phosphate broth (Difco), heart infusion broth (Difco) and tryptose broth (Difco). These various media were prepared in regular and double strength and added to the nutrient pads supplied with the membrane filters. Small petri dishes were used, the cover containing blotter pads moistened with water to insure sufficient moisture. The inoculum consisted of growth from 24 hour old slants of B anthracis on trypticase soy agar. Ten ml portions of distilled water containing various concentration (diluted ten-fold) of cells were passed through the membrane filters. The incubation period was ten hours. Membrane filters were examined using a binocular dissecting microscope.

Higher counts on the membrane filter were obtained with tryptose broth (regular and double strength) and with heart infusion broth (regular strength) than the other media employed.

In a study of US, streak plates were made as a comparison with the MF counts. The media used for the streak plates was the same as that for the MF's except 1.5 per cent agar was incorporated. In addition to the media used for B anthracis, infusion broth (BBL), AC broth (Difco), thioglycollate medium Brewer modified (BBL), eugon broth (BBL) and NIH thioglycollate broth (Difco) were employed. Stock cultures of US were maintained on tryptose phosphate agar plants to which thiamine hydrochloride and ferrous sulfate were added.

It was found that the glass petri dishes had to be discontinued in favor of the disposable plastic dishes manufactured by the Lovell Chemical Company due to drying out of the nutrient pads. Using the plastic dishes, the incubation time had to be increased to 72 hours. After this prolonged period considerable difficulty was experienced with contamination. In addition, the size of the colonies on the MF with all the media employed, was many times smaller than the size of colonies on the streak plates incubated for the same length of time. The colonies on the streak plates were visible to the eye while the colonies on the MF were completely invisible. Employing double and regular strength media, it was found that the colonies on the MF using infusion broth (BBL) were much larger than with any of the other media, although the colonies on the streak plate were many times larger. No growth was visible either on the MF with trypticase soy broth or on the trypticase soy broth agar plates.

A selective medium (5) used for the selection of Brucella was employed in double and single strength using the MF. Colonies were detected with the microscope on the MF after 70-72 hours incubation. The colonies on the streak plates were as with the other media visible to the eye.

Development of suitable methods for detection of pathogens by using the membrane filter has been transferred recently to the Biological Detection Branch of PD Division.

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6. GERMICIDAL MATERIALS

a. GERMICIDAL CEMENT

(1) Pentachlorophenol Cement

An investigation was carried out in 1950 and 1951 to determine the effectiveness of an antibacterial cement produced by the North American Cement Corporation (1). As a result of tests carried out over a period of years at Alfred University, Alfred, N. Y., the antibacterial substance incorporated in the cement was established as pentachlorophenol with a final concentration of 0.7 per cent in the mix. This cement was tested at Camp Detrick for antibacterial activity against several organisms, using standard Portland Cement as a control. Both cements were made into blocks and tests were conducted under various conditions, e.g. various bacterial concentrations, vehicles of cell suspension, lengths of time of the exposure of bacteria on cement blocks, and various temperatures.

From the results obtained, there did not appear to be any significant difference between the bactericidal activities of Portland cement and pentachlorophenol cement. In some tests, more organisms could be recovered from Portland cement than from pentachlorophenol cements and in other tests the reverse was true. The alkalinity of unwashed blocks resulted in inhibition of the test organisms. Washed blocks with lessened alkalinity, tested with the same organisms, exhibited little or no inhibitory action. Even Portland Cement control blocks were found to give zones of inhibition in agar plates when the blocks were broken.

It was concluded that pentachlorophenol cement was no more effective in inhibiting bacteria than ordinary Portland Cement and therefore should not be recommended for use at Camp Detrick.

(2) Cupric Oxychloride Cement

An inorganic cement containing cupric oxychloride, Hubbellite, produced by the H. H. Robertson Company of Pittsburgh, Pa., had been reported to have antifungal and antibacterial properties not present in ordinary Portland cements. An investigation was made to determine the antibacterial activity of Hubbellite in comparison with Portland cement. It was found also that Hubbellite cement did not appear to have a consistently significant specific effect on the bacteria tested. Some slight evidence was obtained that it was active in repressing growth and survival of B globigii. It was not definitely established what role pH played in these tests. This product likewise was not recommended for use at Camp Detrick as far as its general antibacterial action was concerned. Because of its reported antifungal and cockroach repellent action, it was recommended for use in change rooms and

animal rooms.

b. GERMICIDAL SOAP

An effective bactericidal soap was long felt to be a desirable adjunct to the safety program at Camp Detrick. in 1949 Dial* soap and Armours 99* liquid soap containing hexachlorophene were purchased for use on a trial basis. Extensive research by competent workers (2,3,4,5) had shown that daily use of soap containing hexachlorophene adds efficient and lasting bactericidal properties to the skin. In February 1950, a survey of building personnel who had been using these germicidal soaps revealed few objections to their use as a standard item (6). Voluntary usage of the soaps was promoted by Safety Division, and in March 1951 a letter was issued directing the use of these or other soaps containing the active ingredient in all buildings using infectious organisms (7). In 1951 a cheaper rough-cut bar soap containing hexachlorophene was substituted for Dial, but numerous complaints of skin rash resulting from its daily use forced a return to the use of Dial (8).

Since that time, Jan-O** liquid soap containing hexachlorophene has been substituted for Armours 99 and has proved satisfactory in service.

It is impossible to determine whether any infections have been prevented by bactericidal soaps, but it is felt that their daily use in showers and numerous handwashings provides an additional barrier against bacterial invasion.

c. SELF-SANITIZING VINYL PLASTIC FILM

In March 1951, three samples of a "self-Sanitizing" vinyl film*** were received for evaluation by Agent Control Branch. These samples were submitted to a series of tests in which they were deliberately contaminated with known numbers of organisms and tested at various time intervals for evidence of bactericidal activity (9). It was found that the materials had considerable bactericidal activity against Serratia indica and Staphylococcus aureus but not against Bacillus globigii spores. It was also found that the bactericidal activity of one sample was removed by washing. No practical use has so far been made of these plastics at Detrick.

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* Armour & Co., Chicago, Ill.
** Janitor Supply House Inc., Baltimore, Md.
*** Mansanto Chemical Co., Springfield, Mass.

d. GERMICIDAL TALC

In 1951 a study was undertaken to determine whether the use of talcum powder containing hexachlorophene would provide continuous cutaneous disinfection when applied to the hands before putting on rubber gloves (10). The use of rubber gloves in handling infectious agents is widespread in the Biological Laboratories, and a secondary barrier for cutaneous protection was desired in cases of accidental puncture or tearing of the gloves while in use. Hands of test subjects were washed in a suspension of S indica and then coated with a talcum-hexachlorophene mixture. The hands were inserted into rubber gloves for one hour, the gloves were then removed and the hands rinsed thoroughly in water. Bacterial counts were then made on the rinse water. The rinse water showed a marked reduction in count when compared to that from control rinses of hands coated only with talcum. Talcum containing 10 per cent hexachlorophene showed greater bactericidal activity than 5 per cent hexachlorophene. It was also found that the regular use of a hexachlorophene soap enhanced the germicidal activity of the hands coated with the hexachlorophene talc.

Hexachlorophene-talc suspensions were found to be bactericidal against a number of infectious and noninfectious agents.

This compound is being used to a limited extent.

e. GERMICIDAL PAPER

In 1949-50, under contract No. DA-18-064-CML-403, an investigation was undertaken to develop methods of incorporation of germicides into paper so that they might be self-disinfecting when subjected to bacterial contamination by direct contact or infectious aerosols (11). This investigation utilized white slick writing paper, yellow typewriter paper, paper towels, blotting paper, and cleaning tissues. These papers were impregnated with solutions of phenyl mercuric acetate, hexachlorophene, silver nitrate, and cetyl trimethyl ammonium bromide, and were tested for bactericidal properties. When 0.5 per cent solutions of three of these compounds were used, the papers demonstrated considerable bactericidal activity against the test organism, S marcescens. Silver nitrate impregnation was not recommended.

In 1952, an investigation was made of an antibacterial tissue marketed under the trade name "Yes Tissues" (12). These tissues were found to contain a quaternary ammonium compound bacteriostatic for gram positive organisms. It was concluded that the quaternary ammonium compound used in these tissues is unsuitable for treatment of paper for use in the Biological Laboratories.

None of these papers are now in use.

f. GERMICIDAL ANIMAL BEDDING

Under Contract No. DA-18-064-CML-403, investigations were also made of methods of impregnating animal bedding (11) to render it germicidal. Of 23 germicides studied 3 were chosen for extensive testing. These three were phenyl mercuric acetate, hexachlorophene, and silver nitrate. Pinewood shavings impregnated with certain formulations of these three compounds were found to be germicidal for the test organisms, S marcescens, and these three compounds were recommended for practical use.

Phenyl mercuric acetate treated bedding was used in cages of infected animals at Dugway Proving Ground during the Summer Field Trials of 1950, and has been in limited use at Camp Detrick since that time. Problems of impregnating and drying the treated bedding on a large scale have prevented its extensive use, and these problems are under study at the present time. In 1951 and 1952 some further investigations were conducted at Camp Detrick (13).

The most practical formula was found to be: 0.1% phenyl mercuric acetate, 10% isopropyl alcohol (10% methyl alcohol may be used if cheaper). Two hundred pounds of solution are required for each 100 pounds of shavings. The cost is estimated to be $2.36 exclusive of labor and amortized cost of equipment.

g. GERMICIDAL PAINT

In 1950-51 tests were undertaken to determine possible germicidal activity of paint containing a quaternary ammonium compound (14). This paint had been supplied by the Glidden Company, Reading, Pa. and the exact formula was not revealed. Surfaces painted with the "germicidal paint" were contaminated with a variety of organisms, and surface samples taken at intervals to detect bactericidal action. No evidence was found to indicate that the "germicidal" paint contributed any bactericidal activity not present on painted and unpainted control surfaces.

The principal difficulty is that moisture is necessary to transport toxic chemicals to the bacterial cell, and if the toxic material in the paint is water-soluble, the paint is slowly leached by weather or washing.

Very few germicidal paints have ever been available commercially; this situation is reviewed in the reports by Lambert Pharmacal Company under contract DA-18-064-CML-403.

h. GERMICIDAL FIRE FIGHTING FOAM

Fighting fires in contaminated buildings involves the hazard of raising infectious aerosols, especially when glass containers of infectious materials are broken by the streams of water from fire hoses. Some studies on this safety problem have been carried out (19) and some preliminary standards for fighting fire in bacteriological laboratories have been formulated.

A germicidal foam to be used in fire fighting would greatly reduce the hazard, and in 1952 an effort was made to render fire fighting foam germicidal (15). The foam used is a protein compound manufactured by the Pyrene Company. A sample of the foam solution containing 0.1 per cent Roccal was tested for germicidal activity against Serratia indica, Staphylococcus aureus, Escherichia coli, and Bacillus globigii spores. Results were compared to those from a 0.1 per cent aqueous solution of Roccal. It was found that the foam solution inactivates Roccal so that the combination does not constitute an effective germicide.

On the new infectious disease buildings, water fire lines have been omitted and carbon dioxide extinguishers have been installed. The fire department has also been equipped with fog-type nozzles which provide maximum wetting with least force and least water.

i. CORROSION INHIBITOR

Processing tanks and pipe lines in Pilot Plant operations are sterilized with steam under pressure and the use of a corrosion inhibitor lengthens the life of such equipment. "Morlex" Corrosion Inhibitor A[1]*, was tested for germicidal activity to determine whether it would be suitable for use in Pilot Plant operations (16). In tests against 4 organisms, "morlex" was found to be bactericidal to B abortus and P pestis and bacteriostatic to S indica and P tularensis at a concentration of 1 per cent in trypticase soy broth. However, in 0.1 per cent concentrations, no bactericidal or bacteriostatic activity was observed. Since the inhibitor is generally used in concentration of 10 ppm, it was concluded that it would be satisfactory for use in bacteriological processing tanks and lines.

j. LIQUID SEAL FOR SHAKING MACHINE SAFETY CONTAINER

A safety container for holding cultures on shaking machines provides a cover which rests in a trough containing a liquid disinfectant seal (17). Present investigations are aimed at the discovery of an

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* Carbide and Carbon Chemical Corp.

active germicide which is nonvolatile, of low viscosity, and noncorrosive for use as the disinfectant seal. A two-thirds saturated solution of calcium chloride possessed the desired characteristics except for a lack of germicidal activity. To make the solution germicidal, mercuric chloride was added to a final concentration of 1:1000. When the solution was tested, it was found to be inhibitory for S marcescens but not for B globigii (18). The bactericidal properties of mercuric chloride were apparently inhibited by the presence of calcium chloride. Investigations along this line are continuing.

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7. IN-SERVICE TRAINING

a. SAFETY TRAINING DURING WORLD WAR II

During World War II Safety Division at Camp Detrick realized the need for a formal training safety. In February 1944 a training program was put into operation which consisted of orientation in Biological Warfare, Security, Fundamentals of Virology and Bacteriology, and Safety for the personnel, military and civilian, engaged in the study of pathogenic viruses and bacteria.

The program (7) was planned as an intensified three-weeks course with lectures, demonstrations, laboratory exercises, examinations, study periods and recognition of achievement through graduation exercises.

Attendance at this training school was limited to officers and civilians placed in a supervisory capacity, since it was assumed that all responsibility must rest primarily on supervisory personnel.

While the purpose back of this training program was to orient in the field of Biological Warfare, it is significant in that the need for organized training was recognized and that at least a token amount of that training was devoted to the safety of the individual actually engaged in research and of the persons who in any way aided the research man.

While the first efforts at training required the entire time of the trainee, later it was recognized that a coordinated work-training program might be more effective, since the individual was able to carry on his work at the same time that he received a background in BW and bacteriology.

A program in safety training was therefore begun which included all new military personnel brought into Camp Detrick and which was aimed almost exclusively at the safety of the individual. These sessions were relatively short, consisting of 3 meetings of 3 clock hours each. The groups were in charge of S Division supervisors, and the content included films, slides made of persons ill with diseases contracted while on the job, talks by supervisors and demonstrations of safety equipment. This type of safety orientation was very effective, even though it utilized the "scarce" technique rather than subjective approach.

During the World War II period of safety training a thorough publicity campaign was carried out on the subject of safety, utilizing bulletin boards and posters. The posters were developed by 1st Lt. Donald Gorlitz of Safety Division and were pertinent, timely and original.

b. UNIVERSITY OF MARYLAND CLASSES

Dr. Noel H. Gross of Agent Control Branch began formal training in Bacteriology within his own branch in 1948. All non-professional personnel were permitted to take an organized course in General Bacteriology. This course, although general in nature, was applied to the special problems of control in the Biological Laboratories. The work consisted of lectures, demonstrations, laboratory work discussions, and examinations. The course proved so popular and valuable that a general demand for such training developed outside Safety Division.

At the request of the Technical Director and Civilian Personnel a course in General Bacteriology (1) was organized. Classes for 85 students with laboratory work for 37 students began January 3, 1949 to last 12 weeks. The course was designed to be the equivalent of one carrying five academic credits in any standard accredited institution of higher learning. The classes were in charge of Dr. Noel H. Gross, Chief of Agent Control Branch, with laboratory assistant, Mr. Morton Reitman.

On-the-job training was continued in 1949-50 (2) with two innovations: (a) The classes were operated under a contract with the University of Maryland and (b) a course in Pathogenic Bacteriology was begun. Dr. Noel H. Gross continued in charge and taught General Bacteriology and Pathogenic with assistance in the laboratory from Mr. Bruce Harstad, Mr Marcus L. Moss and Mr. Henry Brown. The course in Pathogenic Bacteriology carried four hours credit.

The classes under Agent Control Branch continued popular in 1950-51 (3) with no significant change except that Mr. Gardner Gremillion with Mr. Henry Brown was in charge of the laboratory in T-605.

Demands for an increase in scope of the training courses in 1951-52 were met by the introduction of a course in Virology taught by Dr. O. N. Fellowes. Mr. Henry Brown, Dr. Richard Patty and Dr. Warren Iverson assisted in the laboratory. The couse received two hours university credit. Dr. Gross continued in charge of the program (4).

With September of 1952 came continued expansion in the In-Service Training Program. Lectures which had been given in a number of locations were now presented in Building T-707 where suitable furniture and blackboards had been installed. Bacterial Metabolism was placed in charge of Agent Control Branch and was taught by Dr. Garner Wessman. This course was continued as a lecture course only, carrving two hours credit. The course in Virology was expanded to one lecture and two laboratory sessions per week and received three hours credit. The course in Virology was taught by Dr. Francis R. Gordon of the Bacteriological Laboratories with Dr. Mary Louise Robbins of George

Washington University in charge of laboratory work. Dr. Noel H. Gross (5) continued in charge and lectured in General Bacteriology and Pathogenic Bacteriology, assisted by Dr. Warren P. Iverson, Mr. Henry Brown and Pfc. Edward Abraham. During the period 1949 to 1953 the order of subjects continued to be General Bacteriology and Bacterial Metabolism in the fall semester, while Virology and Pathogenic Bacteriology were taught in the spring semester.

In addition to the regular laboratory equipment and apparatus used in teaching, the instructors have made wide use of visual aids. Unclassified films (6) in regular use and owned by Agent Control Branch, in addition to the Laboratory Hazards Films made by S Division are:

(1) Body Defense Against Disease
(2) Basic Biology of Bacteria
(3) Bacteria
(4) Airborne Infectious Disease
(5) Housefly
(6) Molds and Yeasts
(7) Protozoa
(8) Safe Tuberculosis Nursing
(9) Winged Scourge

c. BIOLOGICAL SAFETY TRAINING

In September of 1951 a program was instituted (8) of training in biological safety, utilizing such visual aids as moving pictures and film strips accompanied by sound. The majority of the films used in this program were made under the direction of S Division. Research work on the hazards surrounding the worker in the Biological Laboratories was carried out by the Laboratory Hazards Section of Agent Control Branch under Mr. Morton Reitman, Section Chief. This data was made the basis for the script for a number of films on the general subject of the "Infectious Hazards of Bacteriological Techniques." The script for these films was arranged and written by the Chief of Safety Division, Dr. A. G. Wedum. In addition to the films made by S Division, a number of commercially available films was utilized. Actual filming was done by the Armed Forces Institute of Pathology and the Public Health Service at the Communicable Disease Center, Chamblee, Georgia.

A list of the films used is as follows:

(1) Basic Biology of Bacteria (9)
(2) Infectious Hazards of Bacteriological Techniques
(3) The Pipette
(4) The Lyophilizer
(5) The Inoculating Needle
(6) The Hypodermic Syringe
(7) The High Speed Mixer

All personnel working in the Limited Areas and those working in the Clinical Investigation Branch of S Division were invited to view the films at the T-426 Conference Room or at the T-707 classroom. In addition films selected from the above list were shown to the Firemen, Station Hospital personnel and Property personnel.

Showings were conducted on a division basis. Arrangements were made so that only part of the personnel viewed the film at a given time. This was done so that as little interference as possible was made with the activities of any given building. Persons who failed to attend the original showings for any reason were given the opportunity to see the films at a later date with new people brought into the Biological Laboratories. A summary of the films content is routinely passed around and read before the actual showing. This is done to focus the attention of the group on the film's pertinent features. Discussions are encouraged following each showing. Records are kept of the attendance at the film showings.

It is planned that the program will be continued by showing the entire list of films to new personnel as groups of sufficient size accumulate. Future film productions will also be shown. New films are in process of completion. Harvey A. Davis is in charge of the Biological Safety Training through the use of films.

d. ORIENTATION IN SAFETY

Classes in safety orientation were begun in November of 1951. The groups consist of new personnel and those being given Special Procedures for the first time. A list of these people for each week is prepared by the nurses in Special Procedures and given to Agent Control Branch. This group, or if the number is too small, an accumulation of persons over a two weeks period constitute an orientation group. These groups are segregated into professional and nonprofessional personnel. The content of the biological safety orientation varies for the two groups.

Safety orientation for nonprofessional personnel, i.e. laboratory workers, animal caretakers, and maintenance and construction personnel, consists of (1) a 40-minute talk on Biological Safety Regulations as they relate to clothing, showers, food, smoking, safety clearances of property for turn in or repair, illness, regulations that control the work of the custodian and animal caretaker, etc., (2) showing of two films: Basic Biology of Bacteria - 12 minutes, and Infectious Hazards of Bacteriological Techniques - 15 minutes.

For professional people Safety Orientation consists of a talk on the Biological Safety Regulations, 30 minutes. This includes responsibility for safety, clothing, showers, germicidal soap, food, use of the pipette, syringe, pipettor, signs, etc. Demonstration of

safety equipment, 30 minutes. Showing of the following films: (1) The Inoculating Needle, 12 minutes, (2) The Pipette, 12 minutes and (3) The Lyophilizer, 12 minutes.

e. SAFETY TRAINING FOR SPECIAL GROUPS

(1) Biological Safety Training During Field Trials

Training in Biological Safety was conducted during the Field Trials at Dugway Proving Ground in 1950 (13), by Agent Control Branch personnel. The training was conducted especially for those who had little or no experience in working with pathogenic agents. The training was limited to those techniques likely to be used in an infectious program. Actual laboratory techniques usually employed were studied. Sieve-type air samplers loaded with corn steep agar plates were placed around the area to pick up any contamination of the air that might occur during the study of the techniques. Serratia marcescens was the test organism utilized. Swab samples were taken of adjacent surfaces as well as the hands of the workers.

Techniques studied for their hazards included:

(a) Grinding guinea pig spleen in mortars
(b) Removal of cotton stoppers from agitated test tubes
(c) Removal of cotton stoppers from agitated sufficiently to wet stoppers
(d) Thrusting red hot inoculating loop into culture in test tube
(e) Thrusting red hot inoculating loop into 200 ml culture in 500 ml Erlenmeyer flask
(f) Inoculating loop dipped into a culture in a test tube and allowed to touch the side of tube as it is withdrawn
(g) Hypodermic needle withdrawn from rubber stopper on test tube full of culture
(h) Opening liquid impingers after vigorous shaking
(i) Liquid impingers left open after air had been drawn through them for 30 seconds
(j) Control samples both air and surface were taken before the demonstration

On-the-job instruction was also given by the Agent Control group by talks, demonstrations and guidance in the use of apparatus and techniques. Dr. Noel H. Gross, Mr. Gardner Gremillion, and Mr. Briggs Phillips were in charge of the training program.

(2) Detrick Guard Force

A training school (12) was conducted for the Camp Detrick Guard

Force, during September and October 1952. The training consisted of talks, demonstrations of laboratory and safety equipment, and training and informational films. The purpose of the school was orientation in both safety and bacteriology. S Division personnel in charge of the course were Mr. Everett Hanel, Jr., Mr. G. Briggs Phillips, Mr. Kenneth Hindman and Mr. Joseph Prestele.

(3) Escort Detachment Groups

Personnel from Safety Division conducted a training school (10) for the Escort Detachment Group, Army Chemical Center, during September and October 1952. The course consisted of 40 hours of lectures, demonstrations and discussions. Emphasis was placed on actual field work of a nature demanded of the Escort Detachment Group when in charge of BW agents during transport. Safety Division personnel were assisted in the training program by M and RE Division representatives. The group trained consisted of 6 CmlC enlisted men, 1 CmlC officer and 2 Air Force Officers. Dr. Noel H. Gross, Mr. Everett Hanel, Jr. and Mr. G. B. Phillips conducted the school.

(4) Air Proving Ground Command, Eglin Air Force Base

Since BW materials were to be stored, assessed and sent on from Eglin Air Force Base (11a), it was deemed necessary to conduct a special school for selected personnel who had been designated to handle such materials. Accordingly S Division conducted a training program for members of the Air Proving Ground Command. The course material consisted of General Bacteriology and special problems of handling BW munitions under field conditions. Bacteriological sampling, incubation, use of media, recognition and the use of safety equipment and protective clothing were taught. The course was conducted during August 1952. Mr. G. B. Phillips was in charge of the course.

The experience gained at Eglin Air Force Base was summarized by William H. Lee, Major, USAF, MSC, in an excellent manual which may serve as a guide in future field training of transport and surveillance crews (11b).

f. SAFETY PUBLICITY (BIOLOGICAL)

(1) Posters

Posters relating to biological safety have been a feature of the Safety Division training and information program since 1947. The post war efforts to popularize the practice of safety in the biological laboratories began with a poster featuring emergency telephone numbers that related to first aid, escape of agent, etc. This type of

information poster proved so popular and useful that it has been renewed repeatedly as individuals and telephone numbers changed.

In 1948 a series of posters was begun by PhM3 Walter Duensing, which was designed to make personnel safety conscious by the use of humorous art. These posters were placed on bulletin boards throughout the infectious units.

The use of posters has continued. The program now includes a change of the posters on the infectious units bulletin boards every three months.

(2) Safety Division Bulletin Boards

In December 1952 Safety Division bulletin boards (14) were placed in all buildings of the Limited Areas. These boards were designed for the sole use of Safety Division in making available pamphlets, posters, bulletins and the Biological Safety Regulations. A metal pamphlet holder is a feature of these boards. The Safety Division Bulletin Board is placed on the contaminated side of infectious units so that safety information will be available where most needed and without making it necessary to go to a clean office. The boards are serviced regularly by Agent Control personnel as new Safety Bulletins, posters and revisions to the Biological Safety Regulations are prepared.

(3) Bulletins

In 1948 Agent Control Branch began the publication of a Laboratory Hazards Bulletin (15). The bulletin was devoted to sketches of safety apparatus with information as to its fabrication, purchase of other means of procurement. This bulletin was profusely illustrated and was the work of PhM3 Walter Duensing.

With the transfer of Mr. Duensing the use of bulletins was reduced and they were published sporadically as the need for spreading safety information was apparent.

In 1952 at the request of the Chief of Agent Control Branch regular publication was begun of a Safety Bulletin (16) devoted to bacteriological, radiological and industrial safety. This bulletin is devoted to new developments in safety equipment, instructions and announcements to personnel on safety problems and policies. An attempt is made to publish this bulletin eight times per year. Illustration of the Safety Bulletin has been facilitated by the employment of Mr. Fred Hammell as illustrator in February 1953.

g. BIOLOGICAL SAFETY REGULATIONS

For a time during the postwar period safety rules and policies constituted the only formal training conducted by S Division. In August of 1948 the Chief of S Division, Dr. A. G. Wedum, collected these regulations and organized them into a pamphlet of mimeographed sheets of eleven pages. These regulations were later revised and expanded.

h. BIOLOGICAL AND RADIOLOGICAL SAFETY REGULATIONS FOR THE LIMITED AREAS

The General Biological Safety Regulations of August 1949 (17) prepared by Agent Control Branch were the minimum for normal operations and were supplemented from time to time by special regulations for different buildings and operations. They were intended to supplement the Post Safety Regulations.

The Biological Safety Regulations have undergone a number of revisions since they were first codified. It is important to note that the present revision (18) entitled "Biological and Radiological Safety Regulations for the Limited Areas" is a loose leaf book of 60 pages as compared to the original stapled pamphlet of 11 pages. Supplemental regulations may be added from time to time. The first such supplement was added 4 January 1953. The present edition is a guidebook of safety for the Biological Laboratories. It serves not only as a code of rules but also as a training handbook in orientation for new personnel. The book includes rules and information on (a) New Employees and Personnel, (b) Illness and Accident, (c) Equipment Supplies and New Agents, (d) Disinfection and Sterilization, (e) Laboratory Techniques, (f) Animals, (g) Radiological Safety Regulations.

i. LABORATORY SAFETY COUNCIL

Under the date of 8 January 1953, Administrative Order No. 3 (19) created a Laboratory Safety Council for the Biolggical Laboratories. Membership in the council is to include the safety officer from each organization designated by the Chief, Safety Division. The chairmanship of the council is vested in the Chief of Agent Control Branch. Administrative Order No. 3 also sets forth the duties of the council and established frequency of meetings and provides for keeping of minutes of the meetings and their submission to the Post Safety Director.

An organizational meeting of the Safety Council was held 26 February 1953. The scope of the work was discussed and a time and place of meeting agreed upon. Regular monthly meetings are to be held on the first Tuesday of each month in the Conference Room at T-501-C at 1300 hours.

Three regular sessions of the Safety Council have been held since the organizational meeting. Interest in the meetings on the part of the members attending has been good, as evidenced by free discussion of biological safety problems. Attendance at the council meetings has been only fair. At the 7 April session 73 per cent of the membership were present. Letters have been sent to the absentees by the Safety Director calling attention of these members to their absence.

Sample recommendations by the council approved by the Safety Director include: (a) The continued use of laundry bags as a container for decontaminating clothing, (b) Stocking of stainless steel pipette discard trays in the warehouse, and spot checks to learn of their proper use, (c) RE Division to be asked to provide welded safety test tube supports instead of soldered ones which become loosened during autoclaving, (d) Separating combustible from non-combustible wastes, and opening all closed containers placed in the discard before autoclaving, to prevent gaseous explosions, (e) Agent Control Branch to present a report on non-slipping rubber gloves.

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11. (a) S Division QTR, 30 Sep 1952, p. 31
    (b) Safety Procedures and Equipment in the Field, Relative to Control and Laboratory Assay of BW Munitions and Training of Personnel. Interim Report BLIR-19, 15 Nov 1952
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16. Safety Bulletin, Vol. 1, No. 2, 1952
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8. LABORATORY HAZARDS

The best of the wartime work on this subject was that with high speed photography which revealed that minor bacterial aerosols were created during common techniques such as blowing out a pipette or removing a stopper from a flask (1).

a. HAZARDS ASSOCIATED WITH CAGE DESIGN

The first research in the field of laboratory hazards after World War II appears in 1947 (2a). Guinea pigs were fed B globigii spores by stomach tube and placed in cages containing false bottom inserts and solid bottoms. Samples of the air showed that contamination of the air in animal rooms equipped with false bottom cages was lower than in animal rooms equipped with standard solid bottoms (2b, 3).

Other information relative to cages appears in the i (5) section of the report on ultraviolet.

b. STOPPERS, PLUGS, AND CAPS

Using Serratia marcescens and S indica as the test organism and the sieve type air sampler for air sampling and the cotton swab for surface sampling, Stein and Anderson found aerosols were inadvertently produced during removal of rubber stoppers, especially Escher stoppers (4). Removal of cotton plugs wetted with culture from test tubes, or removal of cotton plugs or plastic caps from centrifuge tubes after centrifuging, produced recoverable aerosolized bacteria (8), but loose fitting aluminum culture tube caps which covered test tubes were found to contain any aerosol inside the tubes which was produced by shaking tubes of culture for 10 seconds (18). Using a 250 ml Erlenmeyer flask with a circulatory motion to mix culture suspensions was found to be safer than the standard method of shaking an 8 oz square dilution bottle fitted with an Escher stopper (5,6).

c. PIPETTING

Pipetting and plating (4), pipetting operations with vaccine bottles (5), using a pipette to inoculate culture flasks (8), and mixing suspensions with a pipette by alternate suction and blowing produced aerosols.

d. THE HYPODERMIC SYRINGE AND THE VACCINE BOTTLE

The removal of aliquots from vaccine bottles by means of the syringe and needle was found to be hazardous (5). Larger amounts of

aerosols were produced when the vaccine bottles contained positive or atmospheric pressure than when the bottles contained negative pressure. Further work with the syringe and needle showed that aerosol production was greatly reduced by surrounding the needle with a cotton pledget soaked with CTAB (8). Dr. Milton Segalove showed that all syringe plungers became contaminated in 107 tests during the filling procedure. This was due to capillary attraction between the plunger and the barrel. Modifying the plunger by grinding down the distal two-thirds of the plunger destroyed this capillary attraction and contamination was reduced to only 6.6 per cent in 91 tests (5).

e. THE CENTRIFUGE

Very early, it was found that each step in the preparation of concentrated cultures by means of centrifugation produced aerosols (10). Reitman, Alg and Raymond reinvestigated the hazards associated with centrifugation of cultures and found that aerosols were produced by: (a) dispensing cultures into centrifuge tubes (directly proportional to the amount of culture dispensed, (b) removal of cotton plugs from centrifuged cultures, (c) removal of supernatant by siphoning, (d) resuspending the precipitate, (e) breakage of tubes during centrifugation. The use of rubber "Sani-Tab" caps for sealing centrifuge tubes greatly reduced the aerosol in (b) above (16).

f. THE LYOPHILIZER

An investigation into the use of the lyophilizer and lyophilized cultures by Reitman, Moss, Harstad, Bibler, Capt. Daume, Raymond and Alg revealed that the lyophilizer becomes heavily contaminated during the freeze-drying of bacteria and viruses (14). Insertion of a cotton filter between the condensor and the vacuum pump prevents contamination of the pump and attached McCloud vacuum gauge. The latter was tested with S indica (14,16) coliphage T-3 (17), and B globigii spores (18). Aerosols were produced (a) when ampoules sealed under vacuum are opened, (b) reconstitution and transfer of reconstituted cultures. Breakage of 1 ampoule containing dried culture created aerosols which remained airborne as long as 1 hour. Surrounding the ampoule with a 70 per cent ethanol soaked cotton pledget prevented dissemination of the aerosols when ampoules were opened (14). Aerosols produced by breakage of ampoules showed that disinfection procedures consisting of ultraviolet irradiation, spraying contaminated areas with 2 per cent lysol and air washing at the rate of 11 changes of air per hour reduced the aerosol 99 per cent in 10 minutes when the aerosol was produced with organisms suspended in a milk broth menstruum and 100 per cent when the organism was suspended in a sheep serum menstruum (15).

g. THE SONIC OSCILLATOR

A study of the hazards involved in the sonic oscillator by Reitman, Alg, and Raymond revealed that leakage of aerosols occurred when the O ring used to make a tight seal between the metal cover and rim of the sonic cup becomes distorted by repeated autoclaving. This was eliminated by modifying the cover to accommodate a large O ring which is readily availabe at the post and can be changed frequently. A modification by BN Branch of PS Division consisting of a rubber diaphragm insert in the cover through which one end of a U shaped needle can be pushed with the other end inserted into a filter flask, allows the removal of treated materials safely, provided the needle is surrounded by 70 per cent ethanol soaked pledgets (18).

h. VIRAL TECHNIQUES

An investigation into the hazards connected with viral techniques by Reitman, Alg, and William S. Miller has revealed that the vaccine bottle method commonly used by virologists for making dilutions of infectious viral suspensions produced slight aerosols (19). Sixty-two per cent of the rubber diaphragms were contaminated with the test virus T-3 phage. The use of a 70 per cent ethanol pledget around the needle (which was also used as a swab) completely eliminated the aerosol escape and reduced the number of contaminated diaphragms to 12 per cent. Intranasal contamination of mice produced large amounts of aerosols together with extensive contamination of the table top and hands of the technician. Intracerebral inoculation produced only slight aerosols but the fur of the head of the mice was found to be grossly contaminated. The safest method for intracerebral inoculation involved surrounding the hypodermic needle with an ethanol pledget and decontaminating the injection site with 2 per cent tincture of iodine (19). The allantoic and yolk sac methods of inoculating fertile eggs did not seem to create much of an aerosol but shell contamination by the latter route was considerable. The shell contamination was greatly reduced by employing ethanol soaked pledgets. No aerosol was detected when chick embryos were inoculated via the chorio-allantoic membrane using the window flap technique although egg shells were found to be contaminated on 3 out of 10 tests. Aerosols were also found to be produced during harvesting of allantoic and anmiotic fluid. Harvesting operations produced considerable contamination of the egg shells, egg trays and hands of the operator. Grinding of a mixture of mouse brain and phage in a ten Broeck grinder produced detectable aerosols together with contamination of the outside of the grinder and the operators hands (20).

i. MISCELLANEOUS TECHNIQUES

Investigation into the hazards associated with the Waring blendor revealed that the blendor leaks around the bottom bearing. Organisms

were recovered from the air when the blendor bowl was opened an hour after blending had ceased (7). It was recommended that all blending operations with the Waring blendor be carried out in a ventilated cabinet (8).

Stein and Anderson found that the addition of mineral oil to cultures in liquid and solid media resulted in splattering of contaminated material when loopfuls of this material is flamed (7). A small aerosol was produced by flaming a loopful of culture regardless of the method of flaming used or the type of suspending medium (11).

Vibration of the inoculating loop shook off droplets of culture and pouring cultures from one container to another set up aerosol, as did falling drops of culture into various surfaces such as steel, wood, paper and linen towels (9).

It was found that the routine method of stainless cultures on glass slides with Gram's stain, crystal violet, safranin and methylene blue does not kill all the vegetative organisms on the slide (10). It was also found that autopsies of infected guinea pigs produced aerosols as did grinding of infected tissued in a mortar and pestle (12).

Reinvestigation of the pour plate technique by Reitman, Alg, and Raymond revealed that pouring 15 ml melted agar into plates containing 1 ml of a broth culture (1.4×10^9/ml)did not produce any significant aerosol (17).

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9. PILOT PLANT SAFETY

a. PP-1 (in Bldg T-201); PP-2 (in T-201); Bldg T-263

One of the primary missions of the CmlC Biological Laboratories has been the development of BW production on a pilot plant scale. The Pilot Plant activities have received, through the years, close attention by Safety Division.

Early in 1943 the first Pilot Plant was constructed at Camp Detrick (1). This was a temporary, two story wooden structure, 30 ft sq and contained one 100 gallon reactor tank. Information gained during operation of this plant, referred to as the "Black Maria," was used in the design of PP-1 and PP-2. Studies were also being made at this time by Dr. Pappenheimer at Harvard University on methods for the production of botulinum toxin. The first organized safety group at Camp Detrick was established by Order No. 1, 17 May 1943 as the Defensive Development Branch, Operations Division. Order No. 2, 3 June 1943, appointed Gail M. Dack, M.D. as chief of this safety branch. On 21 July 1943, Order No. 8 appointed Dr. Dack as Safety Officer of the Operations Division. Safety Division was activated by General Order No. 7 on 27 September 1943, Dr. G. M. Dack, Chief. At this time Safety Division published and circulated among all personnel a General Statement of Safety Principles, and mimeographed forms were provided for the reporting of all accidents and exposures to infectious and toxic materials.

In October 1943, PP-1 (2), which was designed for the production of botulinum toxin was completed, and S Division submitted its first official report of safety activities. PP-1 was located in T-1 (T-201, the hanger) and at this time several fundamental safety problems had been met. All air from the fermentors and processing equipment was piped to oil fired incinerators where it was sterilized. These incinerators were well designed and are operating satisfactorily today. All liquid effluents from the plant were batch sterilized by heating with steam. Ventilation air from the plant was passed through electromatic filters and the plant area was maintained under a reduced pressure. Change rooms and clothing were provided for the Plant operators. No major safety difficulties were encountered in the production of botulinum toxin. In January 1944, production of B anthracis in PP-1 was started. In March 1944, PP-2, which was designed for the production of B anthracis, was completed. Instead of the 230 gal reactors found in PP-1, this plant had four 15 gal precatalyst tanks, four 230 gal catalyst tanks, two 3500 gal reactors and one 10,000 gal reactor. Plant T-63 (T-263) (3), a separation and processing plant for PP-2, was also completed at this time. This processing plant contained Dorr settling tanks, Sharples centrifuges, a vacuum evaporator, a Fletcher centrifuge, lyophilizer, drum drier and two spray driers. These Pilot Plants were originally designed to give great flexibility so that any system or part of a

system could be operated independently. However, this created many points of potential leakage through the huge numbers of pipes and valves, and as time went on the systems were greatly simplified. Welded pipe systems and diaphragm (Saunders type) valves were used wherever possible. Centrifugal pumps were eliminated and no insulation of piping was allowed in the plants. A safety clearance was required to remove an item from the plant area (4).

S Division followed the production of B anthracis in PP-1 and PP-2 by obtaining large numbers of surface and air samples. A high percentage of all samples taken during periods of activity contained B anthracis. In some cases, 70 per cent of all swab samples taken in a certain laboratory during a month's period were positive for B anthracis. The spore was often recovered from the nasal nares of workers in T-201. Swab samples from the hands of workers leaving T-201 at lunch time or after work showed a considerable number with contaminated hands.

Escape of agent during this period of Pilot Plant activity occurred from 5 primary locations: (1) Pilot Plant proper, (2) laboratories, (3) processing areas, (4) sewage system and (5) air incinerator. Leaks from sight glasses, flanges, valves, etc. in the Pilot Plant accounted for a considerable amount of the escape of agent during the early periods of activity. However, improvement in equipment in the Pilot Plant was rapid so that during the latter part of this period laboratories had a higher level of contamination than the plants. Many standard laboratory operations were shown to produce surface and air contamination. The use of processing equipment such as Sharples centrifuges also resulted in the escape of agent. A serious safety hazard in the PP-2 - T-263 system was the movement of culture from reactors in PP-2 to T-263 through the use of air pressure. This use of air pressure in the reactors resulted on several occasions in contaminating the air incinerators and surrounding area. Operators would forget to close the air outlet valve from the reactor and when air pressure was put on the reactor, several hundred gallons of culture was pushed into the air incinerator. Although the plants were routinely sprayed every 24 hours with calcium hypochlorite, it was found that complete elimination of this agent could not be accomplished through the use of hypochlorite solutions.

b. PP-4 (Bldg T-431)

Many of the principles of operation of a Pilot Plant learned from operation of Plants PP-1 and PP-2 were incorporated in the design of Plant T-431. This plant was designed for the production of vegetative agents, principally brucella, and was completed in January 1945. Standard Operating Procedures (5) were established in May 1945 and production of brucella (A-19 strain) was started. Although Plant T-431 was better from the safety standpoint than Plants PP-1 and PP-2, the plant still retained many bad safety features. For example, sight

glasses, flanges and numerous valves were retained in this system. Therefore T-431 was completely redesigned and rebuilt in 1948. This rebuilt plant is essentially the plant which is in use today. The large fermentors were replaced with 65 gal stainless steel, all welded tanks. There are no flanges or other openings on these tanks, except welded lines and sampling diaphragms. Aeration of the tanks is by critical orifices and air is withdrawn from the tank through a steam ejector into a small attached tank (hot well) that drains to the blowcase and discharges air to the incinerator line. Culture is moved from the fermentors to processing equipment through the use of vacuum only.

Prior to Feb 1947 much difficulty was encountered in the Pilot Plant in obtaining sterile cultures. At times, over 50 per cent of all tank cultures became contaminated. During this time the plant was engaged in the study of brucella (A-19 strain) and the purification of "X" toxin and also several tanks of P tularensis were produced. However, after the new type fermentors were installed the contamination level was somewhat less. All the air lines were replaced with all welded stainless steel tubing which also helped to eliminate much of the contamination problem.

Several new pieces of equipment, such as a vacuum evaporator, a spray drier and the filling machine were installed in 1947. Most of this equipment was designed and built to be used for concentrating cultures for munition fills. Much safety had to be designed in this equipment as it was potentially dangerous when large volumes of concentrated agents were to be processed. The operation of the vacuum evaporator (6) offered little or no possibilities for the escape of agent during the normal operations. It was suggested that the transfer of highly infectious material from the fermentors to the evaporator could be accomplished with fewer possibilities of escape of agent if an all welded line was used from the fermentors to the evaporator.

The operation of the vacuum evaporator was observed and several safety tests were made on this equipment (7), which indicated that a safer procedure of sampling from the evaporator should be developed before an infectious agent was used in this apparatus.

The spray drier has been modified and safety tested many times. The initial safety test was made in August 1946 (8) at which time the method of changing the collection bottles and removing the finished product constituted a serious hazard. These problems still exist with this piece of equipment although it has been modified many times. At the present time the spray drier is enclosed in a steel paneled room. The room is maintained under a reduced pressure at all times during the drying operation, but the system for collecting and removing the finished product is unsatisfactory. Much difficulty is being encountered in providing a water tight seal between the cement floor and the steel panel walls of the room which contain the drying

equipment. A more serious problem is to find some satisfactory method for decontaminating the collection jar prior to its removal from the collection chamber. Dunk baths containing disinfectants have not been satisfactory for this operation. A satisfactory method of decontaminating the room and the spray drier have been developed. The method is as follows: (1) the whole system is washed with a 5 per cent sodium hydroxide solution, (2) then a large volume of formaldehyde is vaporized with steam throughout the system for 30 minutes. It is hoped that in the immediate future these difficulties will be overcome as the demand for spray dried material is increasing.

(1) Munitions Filling Machine

Much effort has been devoted to the development of a safe and satisfactory munition filling machine. The filling machine now being used, is a modified version of the original which was safety tested in April 1947 (9). Previous safety tests (10) had shown that a light but consistent aerosol was being produced during operation of this equipment, and a recommendation was made that the filling head be enclosed in a ventilated cabinet to minimize the hazards which arise during the filling operation. On 28 Nov 1949 (11) the filling machine was approved for use with infectious agents. The machine was housed in a cabinet which was ventilated with a 200 cfm collective protector filter and blower. No escape of the test agent could be detected during the safety tests to which the filling machine was subjected. This machine is in use at the present time and is still considered safe. The existing hazards which are associated with this operation are that the rubber diaphragms of the containers are contaminated after filling, and the container is then moved through the open room from the filling machine to the disinfectant bath. It is also recognized that liquid disinfectants (dunk baths) are not adequate for quick and complete decontamination. Personnel operating the filling machine wear ventilated head hoods and rubber gloves so that they are adequately protected during normal filling operations. In the design of the new filling line the complete operation is enclosed in a series of connected ventilated cabinets which will eliminate even more of the potential hazards associated with this operation.

(2) Gas Masks and Ventilated Personnel Hoods

In the latter part of 1948 (12) the operating personnel wore hooded combat type gas masks in the fermentation room for protection against unknown leaks and accidents which might occur during the production of infectious agents. In 1949 (13) the piping system for the supply of clean air for ventilated personnel hoods were completed and soon afterwards the operating personnel used individual ventilated head hoods for aerosol protection and the gas masks were

discarded. The ventilated head hoods were much more comfortable and at the same time provided adequate protection to the personnel. The scope of the use of ventilated head hoods has been increased in the present day operations and is now used during any type of operation, in the fermentation plant or laboratories, which is deemed unusally hazardous.

At the present time, much emphasis is being placed on the development of a ventilated suit. It is believed that more protection can be provided and greater flexibility can be had with a protective suit. However, at present, no satisfactory ventilated suit is available, although the development of this safety item is being continued.

The Pilot Plant (T-431) was given final approval (14) on 20 Oct 1948 for the introduction and production of highly infectious bacterial agents. This approval was given after extensive safety testing of the equipment and thorough checking of the operating procedures.

The equipment and operating procedures had been carefully developed with a view toward safe operation, and the safety tests had indicated that there was little chance for the escape of the agent during normal operations. Nevertheless, it was recommended that all personnel entering the reactor room wear ventilated head hoods.

(3) Development of Sampling Needle

Safety tests had shown that the method of seeding and sampling was potentially hazardous. Numerous swabs taken from the surface of the rubber sampling adapters after they had been punctured with a needle and immediately after the needle had been withdrawn were contaminated with the agent. Even when the surface of the diaphragms were disinfected with formaldehyde it was possible to recover the agent if the surface was swabbed immediately after the needle was withdrawn. To help overcome this hazard, Pilot Plant personnel designed and built a hooded type needle (15) to be used for introducing and withdrawing samples from the fermentors. This sampling device has helped to minimize this hazard. At the present time many of the accidents which occur in the plant are the result of sampling and seeding. These accidents usually result from a leak or a faulty connection of the rubber tubing used on the hooded sampling needle. Flexible stainless steel tubing has been tested on the sampling needles and found to be satisfactory. However, all of the hooded needles have not been replaced with the flexible tubing. All of the needles will be converted as soon as this flexible tubing becomes available. Safety Division has tested this tubing and has found it to be freon tight. This tubing will withstand repeated heat sterilization, while rubber tubing does not, and therefore will help to eliminate some hazards. The hooded needles are screwed into the sampling adapters and are left in place during the entire period that the agent

is contained in the fermentors and the entire system is decontaminated with steam. They are withdrawn through the rubber diaphragm but the surface of the sampling adapter is protected by the sampling device.

Even with this hooded needle all of the hazards associated with sampling are not removed because the other end of the sampling device becomes contaminated from puncturing the rubber diaphragm of the sample bottle. Also, the surface of the diaphragm on the sample bottle is contaminated, therefore liquid decontaminants must still be used to sterilize these surfaces. A ventilated cabinet with a dunk bath, built around the sampling adapters would help to eliminate these hazards.

In the design and construction of one of the new Pilot Plant units (T-431 annex) all sampling and seeding manipulations will be carried out in an integrated ventilated cabinet system, but such an elaborate system as this will not be available in all the pilot plant units. Therefore, portions of this operation will remain a potential hazard unless better methods of surface decontamination are developed, breakage of sample collecting bottles is eliminated and a completely reliable sampling device is developed for removing infectious samples from fermentors and other processing equipment.

(4) Decontamination System

A pressurized decontamination system was installed in the fermentation rooms and the processing area of the Pilot Plant in the latter part of 1950 (16). The main decontamination system consists of a motor driven pump and a 400 gal tank (located under rear loading ramp). The system is piped throughout the areas where large quantities of infectious material is handled. In the fermentation room spray head nozzles are located above the fermentors. Decontaminant spray covers the entire outer surfaces of the fermentors when the nozzles are opened. Several other outlets are provided with rubber hose which can be used to wash the entire area. Electrical switches located throughout these areas are available to activate the system in an emergency or a general washdown. A smaller system consisting of a 50 gal stainless steel barrel with pump is available when hypochlorite solution is needed.

The entire fermentation area is routinely sprayed with a liquid disinfectant after each sampling or seeding operation. The whole area is immediately sprayed after a known accident has occurred.

The entire plant is periodically washed with water and disinfectant when a spore forming agent is being processed in the plant.

In addition to the pressurized decontamination system there are always the portable 3 gal hand operation decontaminating tanks available for use in case of an accident. The ventilated work cabinets have a decontaminating system available for routine or emergency use.

These units consist of a 9 gal oxygen tank for a reservoir which is connected to the pressure air supply of the building. With this system it is possible to spray the entire inner area of the cabinet before it is opened; or, to decontaminate equipment or containers before removal from the cabinet.

(5) Centrifugation

One of the most hazardous operations carried on in the Pilot Plant is the centrifugation of infectious agents and the handling of the packed cells after centrifugation. Approval for the centrifugation of infectious agents was given for the first time in August 1952 (17). Prior to this time centrifugation was confined to dead cells. The first approved centrifuge for infectious agents was a laboratory model Sharples contained in an all welded steel ventilated cabinet. The centrifuge is an air driven type with the spent air exhausted to the vent line which goes to the incinerator. The cabinet containing the centrifuge is exhausted by a steam jet which produces a negative pressure in excess of 2 inches of water pressure at all times. The drain lines from the centrifuge is connected to a collecting tank, which in turn is connected to the blowcase located in the fermentation room. The centrifuge cabinet also contains a dunk bath through which the centrifuge bowls are passed. The bowls are placed in a water tight container and passed through the bath which contains a liquid disinfectant. The centrifuge is decontaminated by flowing steam. The system is piped so that the feed line as well as all the drain lines can be decontaminated with flowing steam. The cabinet is decontaminated by vaporizing formaldehyde with steam throughout the cabinet, vent lines and drain lines. The drain from the cabinet is connected into the blowcase. The centrifuge is operated continuously (not batch-wise) and the feed system is piped from the fermentors.

This centrifuge and cabinet will soon be replaced, as the equipment has not been satisfactory. The new centrifuge will be contained in a Blickman type ventilated animal transfer cabinet. This new cabinet will provide more working space as well as be safer for performing such operations as are necessarily associated with centrifugation. The present venting and drainage system will be utilized on the new equipment.

The centrifugation operation itself is relatively safe as long as the centrifuge is contained in a tight cabinet and is maintained under a reduced pressure. The hazardous operation in this process is the removal of the centrifuge bowl, containing the packed cells, from the cabinet. The present system involves the use of a dunk bath for passing the container, which contains the bowl, out of the cabinet. Again it is necessary to depend on liquid decontaminants to sterilize this container. This is reasonably satisfactory for vegetative pathogens but not for spores. At the present time it is possible to recover the agent (spore former) from this container even

though the container has been wetted by passage through a strong hypochlorite solution. Occasionally the agent has been cultured directly from the contents of the disinfectant dunk bath. A better method of surface decontamination is needed, or some method of removal in an externalized sealed bag. This is under current study in Contract DA-18-064-CML-2378 with Battelle Memorial Institute.

It would be possible to contain the agent if a series of integrated ventilated cabinets were available for use. With the present system it is necessary to remove the bowl from the centrifuge cabinet and carry it from the fermentation room through a hallway to the laboratory where it is placed in a bacteriological cabinet and the cells are scraped out of the bowl and resuspended. This latter operation is potentially dangerous because the removing of the packed cells from the bowl requires the use of a spatula which sets up a heavy aerosol within the cabinet. The cells then are resuspended with a Waring Blendor which also increases the hazard. Another bad safety feature of the system is the removal of the slurry, equipment for autoclaving, and dilution blanks from this heavily contaminated work cabinet. For this operation it is highly desirable to have a system of integrated cabinets which contain an attached autoclave, incubator and refrigerator. At the present time it is possible to recover the spore forming agent in large numbers from the laboratory and incubator room. It would be desirable to have the cabinet containing the centrifuge attached to the other cabinets, therefore eliminating the necessity of having to expose the heavily contaminated centrifuge bowl to the atmosphere.

(6) Use of Freon Gas in Testing for Leaks

The use of freon for testing (Jan 1949) has been a big advancement in pilot plant safety. This method of detecting small leaks in a closed system has been very helpful in maintaining tight equipment. The present operating procedure calls for a weekly freon test of all the equipment and piping being used for the production of infectious agents, which will withstand a positive pressure. With the exception of the centrifuge, ventilated work cabinets and the spray drying equipment, all processing equipment can be freon tested.

The present Pilot Plant method of freon testing consists of introducing freon (12) gas into the closed system until a positive pressure of 5 lbs psi is reached; then air is introduced until a pressure of approximately 30 lbs psi is obtained. Operating personnel equipped with General Electric type H Freon Leak Detectors test each weld, sampling adapter, valve, etc., in the plant system. If a leak is found it is repaired before the plant is again put into operation. This method of leak detection is superior to the biological method using nonpathogenic bacteria, because it is quicker, requires much less preparation, and locates the leaks exactly.

Freon testing is also used in checking new equipment or piping which is installed in the process system of the pilot plants.

(7) Other Routine Pilot Plant Safety Tests

Other routine safety procedures are performed in the daily functioning of the pilot plants. These include the changing of the diaphragms of all valves and sampling adapters after each cycle of operation. A safety measure was introduced recently which requires the personnel to wear ventilated head hoods when changing the diaphragms on the Saunders type valves. This is to protect the individual from any condensate or unsterilized liquid which may have collected in a tank or a piece of equipment, and which might escape when the valve diaphragm is removed. Such accidents have occurred. A shower is taken including the use of germicidal soap before leaving the contaminated areas, and street clothes are changed to laboratory whites before entering contaminated areas. Special shoes are provided.

(8) Safety Problems Associated with the Drying Program

The production and handling of large quantities of dried viable infectious cells requires even greater safety precautions than are necessary when handling cells in a liquid form. When working with dried material it is very difficult to confine the finely powdered particles. An exceptionally tight cabinet and a highly efficient air filtering system are necessary. The entire drying process involves concentrated materials.

In addition to the drying process, a grinding operation is usually performed. Grinding also creates aerosols.

The decontamination of these cabinets, grinders, driers, etc. is difficult. At the present time, ethylene oxide gas is used to decontaminate one of the driers and it is proposed that steam and formaldehyde be used in other driers and work cabinets whenever possible. However, it has been found that steam-formaldehyde will not penetrate all globules of dried infectious material; therefore, there is an increasing probability that the drying apparatus must be enclosed in a shell which will permit steam sterilization under pressure (autoclaving). Two of the driers now in use are equipped with dunk baths for passing materials in and out of the series of cabinets. They are also equipped with attached autoclaves to steam sterilize equipment and glassware before removal.

On 17 May 1949 (18) Safety Division made a safety test of the drying operation, in preparation for the drying of highly pathogenic agents. At this state of the program all the work was carried out in the open laboratory.

Safety tests made during the preparation and lyophilization of S indica indicated that lyophilization apparatus (NRC Drier) itself offers little chance for the escape of the agent. However, the preparation procedures of centrifugation, resuspending of cells, diluting and plating, and the removal of the cotton stoppers from the vials after shell freezing are a safety hazard because they produce aerosols.

As a result of the safety test, S Division suggested that cabinets be designed and built to enclose completely all of the preparation procedures.

(a) Semi-continuous type lyophilizer: On 28 Oct 1949 (19) a semi-continuous type of lyophilizer was checked for safe operation. The results obtained from this survey showed that aerosols were produced at the time and place where the bacterial suspension was introduced into the holding tank. It was suggested that a safer method of filling the holding tank be developed and that the line connecting the holding tank to the spray head be an all welded line, as this part of the system is maintained under a positive pressure.

(b) Ventilated wood safety cabinet: The first ventilated cabinets built to contain lyophilization equipment were safety tested and approved in July 1951 (20). They consisted of two attached plywood cabinets. The larger one contained the lyophilizer with supporting units, and a freezing element in the floor of the cabinet. This cabinet is attached to a smaller cabinet which contains a freezing unit for freezing the materia before it is placed in the lyophilizer, and a dunk bath containing phenol solution for the passage of materials into and out of the cabinet. These cabinets are equipped with glove ports and the air is exhausted through a Chemical Corps type collective protector; the air is then exhausted into the building exhaust system. To maintain safe conditions in these cabinets, it is necessary that the air exhaust system maintain a negative pressure at all times.

These units, with the air exhaust system on, confined a heavy aerosol which was nebulized inside. The cabinets were approved for work with an infectious agent.

(c) Lyophilization of viruses: A group of cabinets like those described above were installed in the processing branch for lyophilization of virus. This group differs in that an autoclave is attached to one of the cabinets. This is preferable to detached autoclave. A number of retests have shown that these units are safe.

(d) Introduction of new equipment: The drying program has been expanded considerably since the first drier was put into operation. In view of the expansion of the program, and the fact that two individuals working with the old equipment were exposed to a sufficient amount of the agent to develop a clinical illness, it was recommended

by Safety Division (21) that safer equipment be secured for use in the accelerated program. The new drying and processing equipment was installed in the Pilot Plant Laboratory Branch. This area consists of two laboratory rooms which are completely isolated from the rest of the building. The interior walls of the rooms were covered with a strippable plastic (cocoon) and all air is exhausted through an efficient bacteriological filter before entering the building exhaust air system. These two rooms are also separate and can only be entered through an ultraviolet air-lock. This air-lock serves as a break between the laboratory rooms and the hallway of the building. These units contain an integrated system of ventilated safety cabinets which are exhausted to the air incinerator vent line. They are maintained under two inches of water reduced pressure at all times. One of the laboratories which is considered the most likely to be contaminated contains the Proctor Schwartz lyophilizer and freezing unit connected to a Blickman cabinet and a wood cabinet. This series of cabinets is extended through the wall (separated by an air-lock) and is attached to a large cabinet in the adjoining room, equipped with an attached autoclave and a dunk bath.

All work with dried powder, as well as the grinding and weighing operation is done in the cabinets which are attached to the lyophilizer. Diluting and plating is carried on in the unattached section. The great hazard of this operation cannot be over estimated.

(e) Ventilation: The air balance is adjusted so that the air flow is from the cabinets which contain less, to the area of heaviest contamination. This balance is controlled by adjusting the exhaust vents of the cabinets. All drain lines from these cabinets are connected to a blow-case and the contents is sterilized prior to discharge to the contaminated sewer.

(f) Decontamination: The lyophilizer is decontaminated by exhausting all the air within the drier and replacing it with ethylene oxide. All electrical appliances and power units are shut off prior to the introduction of the inflammable gas.

(g) Safety test of the integrated cabinets for the drying program: Safety Division (22) tested this equipment by creating aerosols with both liquid and powdered cells and found that the cabinets would retain and prevent any leakage of the agent as long as they were maintained at a reduced pressure. When these cabinets were tested at atmospheric pressure the agent escaped in several places. Approval (23) was given for the use of this equipment for drying infectious material, provided that the cabinets are maintained under a reduced pressure of 1 to 2 inches of water. As of the date of this report no infections have been traced to the operation of this unit.

(9) Gelatin Precipitation

Concentration of bacterial cells by gelatin precipitation has been an important process investigated by the Pilot Plant. The first piece of equipment was made of glass and was confined in a metal ventilated work cabinet. This cabinet and equipment was found to be safe (24), with certain limitations, after being safety tested in Nov 1949. From that time on, other types of equipment were developed and tested for use with gelatin precipitation of pathogenic agents. The first equipment used in pilot plant production was approved for use with infectious agents on 19 April 1950 (25). This apparatus was later replaced with a 60 gallon column. After adequate safety tests had been made, approval for concentrating pathogenic cultures was granted on 12 Feb 1951 (26). This column was later replaced by two tanks which contain a series of cones that collect the precipitated cells as the cells fall out of the culture. These tanks were safety tested by the freon test and were approved for use in Jan 1952 (27). This equipment is still in operation. During the spring and summer months of 1952 while the plant was steadily producing large volumes of concentrated cultures of Br suis for field trials, several pilot plant personnel had infections. One possible source of exposure was the withdrawal of samples from the gelatin precipitation tanks. This procedure is questioned because the sampling adapters are located so that hooded sampling needles cannot be used when samples are withdrawn. Safety tests have shown that when samples are withdrawn with the type of sampling apparatus used at that time an aerosol is produced. At the present time the gelatin precipitation method for concentrating cells is not being used. However, if in the future it is again used, the sampling adapters will be changed to facilitate the use of the hooded type needles.

This problem of removing liquid samples safely from any sort of pilot plant size container is one which has never been satisfactorily solved; accidents and infections occur at intervals during this operation. The best solution so far is to attach a safety cabinet to the container so that the technician can remove the sample while he is externalized by rubber gloves on the cabinet glove ports.

(10) Production of Simulants

Another objective of the pilot plant, in addition to the developmental work on production of pathogenic agents, is the production of simulants for use at Camp Detrick and other agencies. Prior to January 1953, simulants were produced in the same equipment used for the production of infectious agents. The policy covering the production of simulants and pathogenic agents in the same building is covered by Policy Letter No. 25, 1950 (28).

This letter states that, provided S Division is notified and safety standards are met, (1) simulants may be produced in the same building in which pathogens are produced, and with the same equipment; (2) when the simulant is to be used (a) in the same building where it is produced, (b) in another infectious or toxic unit, (c) in a clean building at Detrick, (d) as an aerosol or on an aerial carrier in field tests at an officially designated BW testing ground (not the Detrick Grid) or (e) on, or in, other officially designated, authorized, isolated, government lands and buildings. For example, in pilot plants it is required that, after use with pathogens: (a) the production tank and all service lines to tanks be sterilized with steam, (b) sterile media be incubated in the tank for 48 hours and remain sterile, (c) all processing equipment be sterilized by a method approved by S Division and (d) containers be removed from the building in a manner that meets requirements stated in "General Biological Safety Regulations for the Limited Areas."

Special permission must be obtained from Safety Division before a simulant may be produced or processed in an infectious or toxic unit for use in an inhabited civilian area.

(11) Isolation of A-Plant and B-Plant in Bldg T-431

In January 1953 (29) Safety Division approved a request of Pilot Plant Division for permission and plans for isolation of B-Plant and A-Plant for the production of simulants. B-Plant is isolated in such a manner that it is now possible to produce simulants while pathogenic agents are being produced simultaneously in A-Plant. The production of simulants then meets all the safety requirements necessary for use on or off the post. In addition to the production facilities now available, there is also processing equipment for drying and grinding available for simulant work. The demand for simulants was always greater then could be met by the production facilities at Camp Detrick; however, since facilities have been made available recently for full time simulant production (Simulant Branch, Pilot Plant Division), such demands are being met.

The greatest demand is for wet and dry cells of S marcescens and spores of B globigii/ Occasionally requests are received for other type simulants. In the event of such requests the Simulant Branch consults Safety Division to determine if additional safety requirements should be executed during the production and processing.

In viewof medical reports now appearing in the literature and also information being gathered by the Clinical Investigations Branch of Safety Division, the simulant S marcescens is being reviewed for possible toxicity. In January 1953 Policy Letter No. 5 (30) established the exposure tolerance for man to the organism S marcescens. In the past considerable amounts of S marcescens has been released throughout

Camp Detrick. No attempt was made to confine or protect personnel from exposure to heavy concentrations. One of the sources of excessive exposure to this organism was in the Pilot Plant where large quantities of S marcescens was produced and processed. Several tests have been made to determine which operations were responsible for producing large aerosols during the process. It has been established by air samples taken during these operations that the centrifugation process (31 & 32) and drying operations (33 & 34) were responsible for heavy aerosol production.

The heaviest aerosols produced during the centrifugation process were found during the time packed cells are scraped from the centrifuge bowl and when the bowl itself was being cleaned. Considerable aerosols are released whenever the door of the centrifuge is opened during operation. The actual centrifugation process does not produce excessive aerosols. Air samples taken at various locations in the building indicated that these aerosols were being disseminated to other parts of the building.

The heaviest aerosols produced during the operation of the large lyophilizer equipment were found to be created during the reconstitution of the packed centrifuged cells and upon removing the dried cells from the collection chamber. However, other activities in the area such as weighing and mixing of powdered cells contributed to the production of heavy aerosols.

Results of air samples taken at various locations about the building show that S marcescens was constantly present in the building and that during certain phases of operation of the freeze drier large aerosols are created which disseminate throughout the building. Personnel working in the immediate vicinity of the drier were exposed to the highest concentration of airborne S marcescens, but those working on the ground level of the building are also exposed.

At a later date air samples were again taken during the operation of the large lyophilizer. Previously several suggested changes had been made to confine aerosols. The results of these air samples showed that the present method of drying and the numerous improvements made on the drier and its accessories constituted a great improvement over conditions that existed during previous sampling. More care in the removing of the dried material from the collection chamber to avoid spills should reduce aerosol production to a minimum.

In the very near future the Simulant Branch of Pilot Plant Division will be moving into a new building (PP-6 or P-472). This building is equipped with the latest type of equipment and many safety factors have been incorporated into its design. It is hoped that the change will eliminate most of the excessive exposure to S marcescens and to other simulants.

(12) Experience with Production of Brucella

In April 1952 the Pilot Plant started a heavy production schedule of Br suis to be used for the 1952 summer field trials. The Bacterial Pilot Plant Branch has published a Special Report (38) on the operating procedures followed for the production of brucella in the plant. This report is complete and covers the various safety procedures for working with this agent as well as procedures for experimental production.

During this period of the accelerated program, to meet the demands for the summer field trials, there were serious accidents occurring in the pilot plant which resulted in the infection of Pilot Plant personnel. One of the accidents was due to the failure of a sampling diaphragm in a 65 gallon fermentation tank (39). After removing a sample from the fermentor and drawing the sampling needle back into the sampling apparatus, the sampling device is unscrewed from the sampling adapter. In this instance, the retaining ring which holds the rubber diaphragm in place was unscrewed with the sampling apparatus. The retaining ring came loose, and the rubber sampling diaphragm was loosened allowing several gallons of Br suis culture to run onto the floor. This accident was unforeseen, as the sampling apparatus had been used several years without any difficulty. A locking device was then placed on all the sampling adapters in the plant to prevent the recurrence of this type of accident.

In August 1952 the plant was engaged in filling munitions (M-114) with Br suis for a shipment to a BW field test. In preparing these munitions it was noticed that an unusually large number of the final closure caps had come loose on the individual bombs. In investigating this particular lot of munitions, it was found that at the time these munitions had been filled that occasionally one would leak at the diaphragm when the bomb was inverted. In view of this information Safety Division questioned the advisability of releasing this lot of munitions for field trial purposes. An inspection of each individual bomb was made and those munitions with a tight final closure were released and all other bombs with loose closures were sterilized and discarded.

On 19 August 1952 (40), six munitions from the above lot were removed from the cold room for assay purposes. The munitions were placed on a metal tray and shaken on the shaking machine in the incubator room of Laboratory A. One of the laboratory men removed the munitions from the shaker tray and carried them into the laboratory. The technician noticed that some of the contents of one of the munitions had leaked on his arm. Two other workers were present in the laboratory at the same time. The faulty bomb was placed in a container of Roccal and everyone left the room. The technician who had carried the bombs discarded his clothing and disinfected his hands and arms with Roccal. All three of the individuals exposed in the laboratory reported to the Clinical Investigation Branch, Safety Division. Other

workers donned ventilated head hoods and sprayed the laboratory and incubator room with Roccal solution.

Investigation determined that the neoprene diaphragms for use with the M-114 munition had been purchased without having been subjected to tests by the Biological Laboratories to determine suitability before they were used as closures in the munitions containing an infectious agent. As a result, the technician involved in the accident developed brucellosis.

A series of air and surface samples were taken while munitions were being filled with Br suis. Results of these tests showed that some of the diaphragms of the munitions were still contaminated after being withdrawn from the Roccal dunk bath. After the munitions were filled and weighed a solution of polymyxin and brilliant green was added as a preservative. This solution was being added by a technician using a syringe and needle. Br suis was recovered from swabs taken from the surface of this syringe and solution bottle. It was recommended that this solution be added to the final culture in the filling tank, thus eliminating the necessity of having to puncture the diaphragm of the munition again.

A second serious accident occurred during the 1952 summer program (41). At the time of the accident a tank of Br melitensis was being transferred from a fermentor to the centrifuge. A stainless steel line, with a needle brazed on each end of the line, was being used to transfer the contents of the fermentor. During this operation one of the needles broke and approximately 2000 mls of the brucella culture sprayed out before the flow was stopped. Two plant operators were at the scene of the accident. Their clothing was discarded in the area of the accident and the operators immediately showered and reported to the Station Hospital. The area of the accident as well as all the equipment in the room were sprayed with Roccal solution.

This method of transferring culture material had been used routinely in the pilot plant without any previous difficulty. However, to avoid any such recurrence, Safety Division recommended a permanent type all steel welded line be installed for transferring purposes. One worker has contracted brucellosis, probably as a result of this accident.

(13) Safety Problems Associated with Production of B anthracis

The production and control of B anthracis in the bacterial pilot plant has presented several problems to both Pilot Plant and Safety Divisions. In the early days of anthrax production the same difficulties existed in the control and confinement of this agent (4). In the interim period between the early work done on this agent to Jan 1952 when the work was reactivated, there was little advancement made in controlling spore forming agents in the bacterial pilot plant.

(a) January to April 1952: Prior to introducing anthrax in the plant a considerable amount of preliminary work was done in the Pre-Pilot Plant Branch. Most of this preliminary work was on the development of a method for concentration of spores by frothing or foaming. Safety tests were made at various times when different types of apparatus were being studied. Most of this work was confined to one laboratory in the building. This room was equipped with two attached ventilated work cabinets and most of the work was carried out in these cabinets. These ventilated cabinets had been safety tested several times before and after the foaming experiments had started. An extensive safety testing program to follow this project was initiated in July 1950. From that time, results from repeated testing showed that B anthracis was escaping and being disseminated throughout the building (35). At this time, there was a general clean-up of the entire building. The laboratory and all its apparatus were washed with hypochlorite and the whole building was decontaminated by vaporizing formaldehyde with steam.

In December 1951 (36) air and surface samples were taken in the laboratory where the foaming project was being done. Results of this test showed that considerable aerosols were being created by the floatation apparatus. The agent was probably escaping from the ventilated cabinets whenever they were opened for adjusting the equipment and when moving material in and out.

In December 1951 B anthracis was introduced into the bacterial pilot plant and this program has been followed very closely by Safety Division. The work in the pilot plant included the operation of a 50 gal foam floatation chamber. This piece of equipment required a considerable amount of work before it was made gastight and was approved for work with anthrax, with certain limitations, in Feb 1952 (37).

Anthrax was produced in the plant from Dec 1951 to April 1952. During this short period of time several hundred surface and air samples were taken. Results of these tests showed that the agent was not being confined in the closed system of the plant. The agent was escaping probably during the sampling and seeding operations, as the plant equipment was freon tested weekly to detect any leaks which might occur. During this time there were no major accidents reported.

Extensive surface sampling showed that the agent was spread throughout the building. It was probably being carried from the fermemtation room and spread from there throughout the plant. The agent was not only recovered from the fermentation room and laboratory but also in the air-lock between A-Plant and the hallway, the main hallway, in and on cabinets located in the hallway, rain coats that are worn in the plant, pedal of drinking fountain, tool cabinets and on the instrument control panel in the hallway.

All operations in the plant were stopped and a general clean-up of the building was made. The inside of the building was washed with hypochlorite (2000 ppm) three times and surface samples were again taken. These samples showed that the use of hypochlorite was not sufficient for complete decontamination, as many objects still retained the agent. Another general clean-up was attempted, this time everything that could be autoclaved or carboxoclaved was so treated and the other equipment was scrubbed with a strong hypochlorite solution. The most difficult areas to decontaminate were the walls of the blowcase pit. A thick paste of sodium hypochlorite was used to cover the surfaces, and even this did not sterilize the rough surface. It was decided that rain coats would no longer be worn in the plant by the operators, as they were heavily contaminated and standing under a disinfectant shower was not sufficient to decontaminate the surface of the coats.

After each seeding and sampling operation the entire area of the fermentation plant is routinely washed with hypochlorite solution, and when operators leave the room they wash their gloved hands in a basin containing hypochlorite, and walk through a foot bath which contains hypochlorite solution. All of these precautions were not able to prevent the agent from being carried out of the fermentation room.

(b) November 1952 to present (May 1, 1953): Work with anthrax was interrupted between April and November 1952 by production of brucella, and then the plant again turned to the anthrax problem. Work on this agent has been active and is still being pursued at the present time.

(1) Concentration of Spores by the foaming method: At the beginning of the current program much effort was devoted to the concentration of spores by the foaming method. A new type of foaming column had been designed and installed in the fermentation room for this project. This piece of equipment was made from a 10 inch pipe 16 ft long with all welded seams. Sampling adapters were welded into the column at various heights to permit selective sampling at any fixed location up or down the column. This equipment was installed as a permanent fixture, that is, all drains, feed, air lines are welded tight. This foaming column was made freon tight prior to use with B anthracis. The equipment was freon tested weekly along with all other processing equipment being used in the plant study of anthrax.

Approximately one week after the work on anthrax was started an extensive surface sampling program was started in the pilot plant by Safety Division. It was soon determined that anthrax was being disseminated throughout the building. The magnitude of contamination far exceeded the amount that had occurred previously when work on this agent was in progress.

The agent was recovered most frequently from the interior of the

fermentation plant, the hallway and air-lock entering the plant, hallway leading from the plant to the laboratory and inside the laboratory, and less frequently from the building, including the dirty change rooms, clothes, head hoods, shoes, work shop, floor drains and even from the air exhaust system of the building. The areas such as the hallways and air-locks were contaminated from the shoes of operators and laboratory men moving between the plant and laboratory. Work on the agent was halted and a general clean-up was ordered for the entire building. However, as soon as work was again started the agent was spread throughout the building. Repeated sampling showed that the area around the foaming column, and the sampling adapters, were heavily contaminated indicating the considerable escape of the agent was occurring during the operation of this equipment. The sampling adapters on the foaming column would not accommodate the use of the hooded type sampling device and therefore it was suspected that the agent was escaping during the sampling periods. It was the custom to determine the level of the liquid in the column by inserting probes through the sampling adapters. Swabbing had shown that the diaphragms containing these probes were heavily contaminated with the agent. It was shown by freon testing that it is possible to produce a leak around a needle when it is inserted through a diaphragm if the needle is vibrated while protruding through the diaphragm. Likewise this operation was also suspected as a source of escape of the agent. Evidence to support this idea was found when swabs showed that the agent could be recovered from the handles of the various valves used when operating this equipment, thus indicating that the plant operators hands were being contaminated at the time of sampling, and were therefore contaminating everything they touched.

Almost simultaneously anthrax was found to be appearing in large numbers on the floors and elsewhere inside the rooms of the sewage decontamination plant T-314 and Incinerator Building T-313. The agent could not be coming from the contaminated sewage because all liquid waste from the fermentation room and equipment is emptied into a blow-case and sterilized by steam before it is discharged to the sewage line leading to the decontamination plant. Surface and air samples were taken throughout the decontamination plant rooms and it was found that the floor drains in some of the buildings contained large numbers of anthrax. It was determined that the sewage drain lines for this building was connected to the drain lines from the liquid separators which are part of the air vent system from the foaming column in the pilot plant T-431. Whenever the foaming column in the pilot plant was operating, large quantities of foam were being discharged through this vent line from the liquid separators into the T-314 - T-313 building sewage line, and up through the T-313 - T-314 building floor drains into the decontamination plant. This was a possible explanation for the escape of the agent through the floor drains in buildings T-313 - T-314. Further information on the corrective measures taken to eliminate this condition in the decontamination plant is covered in another section of this report.

One of the corrective measures taken to prevent the excessive contamination of the vent line from the pilot plant to the decontamination plant was the installation of a liquid separator on the foaming column. The foam escaping from the column was collected in this separator, and the drain line from the separator was connected to the blowcase and all the liquid from the separator was sterilized in the blowcase.

Other corrective measures taken to eliminate and confine the escape of anthrax in the plant were made. All the sampling adapters on the foaming column were replaced with threaded adapters so that the hooded sampling device could be used for withdrawal of samples. The surface of all rubber diaphragms containing the probes were painted with a liquid neoprene paint which formed a tight seal. All personnel changed to rubber shoes before entering the plant and removed them before leaving the plant. The floor of the air-lock was kept flooded with a hypochlorite solution so that plant operators had to walk through this solution upon entering or leaving the fermentation room. Shoes were changed before entering or leaving the laboratory. The operating personnel when leaving the fermentation room or laboratory went directly to the dirty change room and changed into clean clothing and showered before going to any other section of the building.

These precautions, added to the existing precautions, helped confine the agent, but did not provide complete elimination of the agent. The work on the foaming method of concentration was soon terminated and the pilot plant is now investigating centrifugation as a method for spore concentration. (Note: One man contracted a fatal pulmonary infection in Dec 1951 during study of the foaming process involving B anthracis. See Section II, 2a).

(2) Centrifugation: To prepare for this process a steel ventilated cabinet was built to enclose the centrifuge. This cabinet was equipped with glove panels, a dunk bath, and maintained at a reduced pressure of two inches or more of water pressure. The drain lines were run to a separate collecting tank which in turn was discharged to the blowcase for sterilization. The exhaust from the air driven centrifuge was discharged into the incinerator vent line. The centrifuge was located in the fermentor room and the operators wore ventilated head hoods when the centrifuge was operated.

Various safety tests made during the centrifugation process have shown that the escape of aerosols is slight. The most hazardous part of the operation is in removing the centrifuge bowl from the centrifuge cabinet. The bowl is placed inside a water tight container and is passed through a hypochlorite solution dunk bath. The agent has been recovered from the surface of this container after having been passed through the dunk bath and occasionally from the hypochlorite bath. The concentration of the disinfectant in the bath varies from 12,000 ppm to 1000 ppm. Swabs taken from the surface

of the floor beneath the centrifuge are occasionally positive for anthrax. The floor is no doubt becoming contaminated by the splashing or overflowing of liquid from the dunk bath. This is probably one of the sources of contamination of the hands which in turn contaminate the ventilated head hoods worn by operating personnel; also, this is a source of organisms to be carried about on shoes.

(3) Contamination of ventilated head hoods: A hazardous situation is created by the presence of B anthracis on the inside and outside surfaces of the ventilated head hoods. The only way that the head hoods can become contaminated other than from a direct leak or spray is from the hands of the individual wearing the hood. Many positive swabs have been obtained from the inside or outside surfaces of the hoods worn by both the plant operators and laboratory personnel. It is doubtful that the hoods have been sprayed by a leak from a tank or other source because such a leak would be discovered when the freon test is made. It seems evident that the hoods are contaminated from the hands.

This condition can be partially corrected if the operators will rinse their hands in a solution of hypochlorite and then remove the rubber gloves before using their hands to remove the hood, but then it is possible that they would contaminate their hands again from the hood itself. There is, therefore, a safety problem as to what can be done to eliminate this hazard. The contamination of the inner surface of the hood can only be caused by contaminated hands as the hoods are always under positive pressure when worn in the plant or laboratory. Therefore the individuals wearing a ventilated head hood should avoid touching the inside surface of the hood in the process of removing from or placing the hood on the head. A number of the positive swabs from the surface of hoods have been taken from those hanging in an ultraviolet airlock. The possible explanation for this is that portions of the hoods are not irradiated by the ultraviolet rays because of shows. It has been suggested by Safety Division that an ultraviolet rack be constructed and installed in the hallway for supporting the ventilated head hoods worn by the laboratory personnel. A routine decontamination procedure has also been put into operation for sterilization of the head hoods with carboxide gas. These safety measures should help to eliminate much of the hood problem.

(4) Safety problems in the laboratory of the pilot plant: The anthrax work being carried on in the laboratory in conjunction with the plant program has also created safety problems. The laboratory removes and resuspends the packed cells from the centrifuged culture, makes dilution and plate counts of the cell suspensions and prepares the seed cultures used for inoculating the fermentors. It has been established that heavy aerosols are created when removing cells from a centrifuge bowl and resuspending them with an electric mixer. The laboratory technicians have also been hand filling some of the munitions for test purposes. At the present time there is only one ventilated work cabinet available for this work. It is practically

impossible to control the escape of anthrax under such conditions. The contamination level inside the one hood is exceedingly high due to the nature of the work being performed.

At the present time it is necessary to prepare all the dilutions and inoculate plates in this same cabinet. Pour plates are used in the assay methods which necessitates the removal of the plates from this contaminated cabinet to be poured and rotated on an open table top. Then the plates are placed in a walk-in incubator for incubation. Positive swabs can usually be obtained from this laboratory. The agent can be found on the table tops and in the incubator, and frequently on the floor. It usually can be recovered from the gloves, head hoods and clothes worn by the laboratory personnel.

(5) Effect of current demands for production: The plant and laboratory have had to strain their facilities beyond safe limits to meet the demand for B anthracis for field trials this summer, 1953. The plant is trying to meet a requirement for 10 gallons of concentrated anthrax suspension to supply one request. With the present equipment it is impossible to produce this amount of material except by producing and concentrating small batches at a time. The cultures are pooled in a 15 gal stainless steel seed tank. There is no cabinet available which will accommodate this 15 gas tank, therefore it has been necessary to introduce these small lots of cells into the tank in an open room as well as remove the many samples that have been required for assay purposes. The rubber diaphragm on this 15 gal tank has been punctured many times and it has been impossible to change the diaphragm because there is no other container available large enough to hold the heavy spore suspension while replacing the sampling adapter. Before a sample is withdrawn for assay the barrel is placed on a shaking machine for two hours to obtain a well mixed, homogeneous sample. If the diaphragm on the sampling adapter is no longer tight then an aerosol could be produced by leakage from the barrel during shaking. There is no refrigerator in the laboratory large enough to store this container so it has been necessary to carry the barrel along the hallway to the walk-in refrigerator for storage.

Such conditions as discussed above are undesirable. The complex and diversified activities of the pilot plant are always challenging safety, as they necessitate continuous development of new procedures and equipment to keep pace with such activities. The present accelerated program on drying of bacterial and viral agents and on methods of producing more highly concentrated suspensions of cells is demanding more and more safety. Any escape of the agent when working with concentrated material may result in personnel becoming infected. These problems are becoming more and more serious as new lethal agents are introduced into the plant.

(6) Seasonal increase in infections among personnel: During spring and summer of 1951 and 1952 the number of infections among the pilot plant personnel increased. The only logical explanation for this is that the pilot plant goes on a heavy production scale to meet the requirements for the summer field trials. It is believed that the present plant is not adequate to undertake such programs. In order to meet seasonal requirements the plant is operated beyond its safe limitations and therefore accidents occur and workers become infected. Most of these accidents can be traced to faulty equipment and piping. The equipment now in use has become worn and mechanical failures, as well as leaks in the old piping, occur frequently. When operating at an accelerated pace certain routine safety checks are omitted such as the weekly freon test of the entire plant. Other protective measures are also probably foresaken in favor of increased production.

It is recommended that the present pilot plant T-431 undertake no heavy production schedules until it has been completely redesigned. The plant T-431 is obsolete and its use for highly infectious agents should be discontinued at the earliest possible date.

c. T-434 (VIRUS PILOT PLANT)

(1) 1943 to 1949

In October 1943 a study of 35 viruses pathogenic for man was undertaken to determine which viruses should be developed for Pilot Plant production (42). Psittacosis virus was selected. Cal-10 strain of mouse meningopneumonitis virus was selected as the simulant for the psittacosis virus (43). Virus Pilot Plant, T-434, was designed in the Spring of 1944 to produce BW agents that require embryonated eggs as the culture medium. The plant was designed to process 5,000 eggs at a time in a batch process. Construction of T-434 was started in the Summer of 1944 and the building was completed in March of 1945. The building had the following 7 areas: (a) Receiving Section - a 52,000 egg capacity incubator was located in this area. (b) Inoculating Section - eggs were to be inoculated in an open front cabinet (44). Trays of eggs to be inoculated moved down a track to the end of the cabinet and trays of inoculated eggs moved along a second track to the air-lock of the cabinet. (c) Production Incubator Section - eggs in solid bottom trays were incubated and canled in this area. (d) Harvesting Section - eggs were harvested in a cabinet which was kept under a reduced pressure and had attached rubber gloves. This harvesting table is being used today. Two automatic harvesting machines were designed, but construction was not completed before the end of the program. A Charlotte colloid mill, a GGC Manton-Gaulin Homogenizer and 4 storage tanks were included in the system. (e) Storing and Shipping Section. (f) Control Laboratory Section. (g) Other - office, change rooms, etc.

Plant T-434 embodied many safety features which were found desirable from experiences gained in operation of the earlier Pilot Plants. A decontamination spray system was installed with outlets at various locations throughout the Plant. This system was subsequently abandoned, but a new spray system is to be reinstalled during changes in Plant T-434 during 1953. All lines were color coded. Cabinets were built for the egg inoculation and harvesting processes and cabinets were designed but construction never completed for the colloid mill, the homogenizer and for covering the sampling adapters on the holding tanks. All cabinets had an air exhaust system so that they could be operated under a slightly reduced pressure. A decontamination spray system was installed in all cabinets. The transfer of materials between pieces of equipment was by vacuum, which was a pronounced safety improvement over the older methods which used air pressure. Pressure of course contributes to leaks to the outside, but vacuum draws outside air into the contaminated area. As in plant T-431, triethylene glycol vaporizers were installed in certain hazardous locations. In July 1945, Standard Operational Procedures for operation of the plant were written and safety procedures were written and approved by Safety Division. Standard clothing changes were required of persons entering the plant. In addition, all women were required to wear surgical cloth caps. A list of personnel authorized to enter the building was maintained by a clothing checker who checked out clothing to persons entering the plant.

During the summer of 1945 eggs were inoculated with sterile media to study percentages of traumatic deaths and amount of contamination. S marcescens was inoculated into eggs and carried through the entire plant process. S Division made a test during egg harvesting and processing procedures at this time. One S marcescens colony per 10 cu ft of air was found at the harvesting table, and 14 S marcescens colonies per 10 cu ft of air sampled was found near the colloid mill during operation. It was found that eggs steamed for 30 minutes at 8 psi were not free of S marcescens contamination. Alkalies were used to clean plant equipment of egg materials. It was found that 1 per cent sodium hydroxide killed psittacosis virus in 5 minutes.

(2) 1950 to Present (May 1953)

Neither Cal-10 nor psittacosis virus was brought into plant T-434 because activities were suspended in the fall of 1945.

In 1949 a decision was made to reactivate the Virus Plant. Under the direction of Dr. O. N. Fellowes, the Virus Plant was greatly simplified. Two of the harvesting lines were removed as well as the homogenizer in the process line. An open front ventilated cabinet was used for inoculating eggs and the egg harvesting cabinet was tightly sealed before being put into use. Steam seals were placed around the shafts of mixers on the holding tanks.

Plant T-434 was officially opened 22 Jan 1950 with the delivery

of 2,000 embryonated eggs. During February and March 1950 safety studies were carried out in T-434. The tests were divided into three main parts: (a) egg inoculation process, (b) harvesting and processing procedures and (c) egg grinding procedures carried out in a Class I ventilated cabinet. These tests using E coli T-3 bacteriophage showed (45) that the plant processing operations were relatively safe, but the laboratory operations and the egg inoculation procedures needed further safety development. Many general and specific recommendations for the improvement of biological safety were made at this time (46). Some of the recommendations were carried out, but many other changes were not made as the Virus Pilot Plant workers felt that elaborate safety procedures were not required in the production of psittacosis and Q fever organisms. It was planned to rebuild the Virus Pilot Plant before more hazardous agents were produced.

In December 1950 (47) and in April 1951 (48) Virus Pilot Plant operations were studied by the use of E coli T-3 bacteriophage. These tests showed three basic points of weakness in plant operations (a) egg inoculation procedures, (b) removal of egg trays from the harvesting table and (c) withdrawing samples from the holding tanks. In all tests a high percentage of the egg shells were contaminated during the inoculation procedure. Neither liquid disinfectants nor ultraviolet were successful in eliminating all contamination on the egg shells (49). During the harvesting procedures pieces of embryo fall into the egg tray. The subsequent passage of these trays with bits of egg material through 5 to 10 per cent phenol solutions did not completely sterilize the embryonic debris. The cabinets designed and built in 1944 for covering the sampling adapters are not used, nor is the hooded needle (50) developed for use in the bacterial Pilot Plant. Therefore, it is not surprising that some escape of agent occurs during the withdrawal of samples using a large hypodermic needle attached with rubber tubing to a flask. In early 1952 a Class II type, egg inoculating cabinet was installed (51). This cabinet is used with rubber gloves attached and provides maximum safety during the inoculation procedures. However, no satisfactory method has been developed for sterilizing the surfaces of eggs after inoculation. These eggs are now handled in the open during incubation and candling procedures. This is unsafe and will result in infection of employees.

On 8 and 11 November and on 17 December 1952 (52) processing equipment such as tanks, lines, and the blowcase were checked for gas leakage by using freon gas and a GE Halogen Leak Detector. Several gas leaks were found during these tests. The leaks were repaired and retested and found to be freon tight (53). Virus Pilot Plant personnel have now secured a halogen leak detector of their own and tests for gas leakage from the processing equipment are routinely made by plant personnel.

(3) Present Concept of Virus Pilot Plant Design

Several Virus Pilot Plant operations are fundamentally unsafe. It is known that the surfaces of eggs may become contaminated during inoculation and no method is available for sterilizing these egg shells. These eggs at present are handled in the open during several operations. Also, accidents occur due to the breakage of infected eggs.

To provide adequate safety during pilot plant work with certain viruses, it will be necessary to carry out all the various pilot plant operations in integrated continuous closed ventilated cabinet systems. This includes a closed system for mechanical entry of eggs into and exit of eggs from the incubator, with provision for a man in a ventilated suit in the incubator. Such a system has been designed and is planned for installation in the Virus Pilot Plant during 1953.

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10. PROTECTIVE COATINGS

a. STRIPPABLE VINYL PLASTICS

An investigation of the characteristics and potential uses of strippable vinyl plastics in the laboratories and pilot plants is reported in S Division Report No. 1, dated 29 Oct 1951. It was concluded from this report that protective plastic coatings such as cocoon may be used for the following purposes:

(1) Packaging material for storage
(2) Sealing walls and ceilings in contaminated areas, particularly animal rooms
(3) Sealing test chambers and exposure chambers
(4) Inside or outside coatings for tanks and vats
(5) Sealing work cabinets and hoods
(6) Outside covering for asbestos installation
(7) Sealing walls around wall autoclaves
(8) Sealing ventilation duct piping
(9) Repairing broken plastic equipment
(10) Coating munitions and cultures of infectious materials for shipment
(11) Covering and sealing crash panels
(12) Coating centrifuge tubes and other glassware containing infectious agents

It was reported that the use of strippable vinyl plastic films in laboratories, plants, etc, is somewhat limited because of some of the physical and chemical properties of the plastic. These limitations are as follows:

(1) Plain cocoon films should not be exposed to 2537A ultraviolet in moderate or high intensities, or to sunlight.

(2) Cocoon films should not be subjected to temperatures higher than 75°C lest they melt nor should they be used in contact with solvents such as acetone and benzene.

(3) Dunk baths, vats, or tanks holding solutions of lysol or phenol should not be coated on the inside with cocoon.

In applying these films it is important in securing good adhesion to surfaces to follow the manufacturers directions carefully, particularly in regard to the pressure under which the liquid is sprayed.

b. PROTECTIVE PAINTS

An investigation was conducted using 16 paints of different base, application, color and manufacture. The paints were applied to black iron metal, Keene cement and plywood and exposed to various chemical and physical agents to determine the durability of the paints. The information collected is consolidated in Interim Report No. 23 prepared by S Division, dated 1 Feb 1953. All testing agents, procedures and results are given. Tygon TP 12 and 61 and Amercoat 23, 33, and 55 were the most durable paints tests.

c. PROTECTIVE COATINGS FOR LIQUID DISINFECTANT DUNK BATHS (1)

Four plastic protective coatings were applied to the inner side and bottom of stainless steel containers for evaluation of the effect of 3 disinfectant solutions on the plastic. The 4 compounds evaluated were: GE Cocoon, Chemcoat R, Amercoat, and Lankote. The disinfectants used were: clorox, (pH 9 and also 7) and peracetic acid. Under the conditions tested, Amercoat was the most satisfactory protective coating. No apparent change was caused by any of the 3 disinfectant solutions. The second best coating was GE cocoon. This coating apparently was unaffected by hypochlorite at a pH 9 or 7, but blistered and separated when in contact with peracetic acid. Lankote and Chemcoat R were completedly unsatisfactory under the test conditions.

d. EVALUATION OF LIQUID NEOPRENE

One of the newer developments in synthetic rubber compounds and in protective coating materials has been liquid neoprene. A variety of liquid neoprenes are available for different uses. In general, the neoprene is suspended in a volatile type base and the per cent solid contents varied to provide flexibility and adaptability of use. Some preparations require the addition of an accelerator before application and subsequent curing ranging from 190° F warm air overnight, to room temperature for several days. Others require no accelerator and the applied film cures at room temperature. Liquid neoprene may be applied by brush, spray or dip. Some are available in several colors. Several uses of liquid neoprene in biological laboratories have been proposed which are:

(1) Coating liquid dunk baths to prevent corrosion by liquids

(2) Coating animal rooms to provide bacteria-tight walls

(3) Coating various special ventilated bacteriological cabinets to provide an air tight liquid resistant surface

(4) The coating of animal transfer cages and experimental test tanks

(5) Coating contaminated air ducts and walk ways in dangerous locations to provide a non-skid surface

Since almost all of the proposed applications of liquid neoprene are of such a nature that biological safety is involved, evaluation tests are being conducted in S Division. These tests are almost completed at the present time. Three neoprene preparations are being tested:

(1) N-250, which is a neoprene putty for use in an expansion joint filter and in lining cabinets or other items of equipment (2).

(2) N-700, a general maintenance protective coating which cures at room temperature (2).

(3) N-600, a skid-proof safety coating that is brushed on (2). It is cured at room temperature.

The evaluation tests include exposure of coated metal and wood surfaces to (1) wet and dry heat, (2) ultraviolet light and ozone, (3) liquid disinfectants and several temperatures, (4) gaseous disinfectants, (5) acids and alkalies and (6) penetration tests with air and bacteria.

The results thus far have been very satisfactory. The liquid neoprene coatings tested are flexible, tough films which are resistant to autoclaving temperatures, ultraviolet light and ozone, oils, gaseous disinfectants and most liquid disinfectants, acids and alkalies. A few instances of failure in the tests have not been the failure of the neoprene film itself, but rather a failure of the films to bond to the test surface. It is felt that more careful attention to the recommended methods of application will eliminate this minor failure.

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11. RADIOLOGICAL SAFETY

a. RADIOLOGICAL STUDIES, 1947-1951

(1) Physics Branch

The first investigations by Physics Branch were conducted in October 1948 and consisted of studies of Rubidium-86 uptake by various plants. The inhibition of Rb-86 assimilation by 2,4-D was also investigated. Corn, among other plants, was examined. The radio material was administered in various ways:

(a) Rb-86
(b) Rb-86 + 2,4-D
(c) Rb-86 + idolacetic acid
(d) Rb-86 + onion juice

Rubidium-86 was allowed to enter leaves and roots which were subsequently crushed and analyzed with appropriate laboratory counters. Some radioautograms were made.(1) Plans were made to order radioactive strentium, iron, phosphorus and iodine, but were cancelled in August 1948. (2)

In April 1949 studies on the fixation of CO_2 by Escherichia coli were begun, using Carbon-14 as the radioactive isotope. Cells were incubated with $NaHCO_3$ containing C-14 and then killed and the radioactivity measured. One of the results of these investigations showed that the cells picked up a low degree of activity which was not variable with time (3).

In July 1949 carbon dioxide fixation by bacteria and the effect of dinitrophenol on CO_2 assimilation by bacteria were studied (4).

Respiration studies with KHC^*O_3 were also conducted.

In January 1950, NaAc assimilation was studied, using activities of carbon-14 in the order of 5.7×10^4 counts per minute (5).

Additional work of a similar nature was carried out during the summer of 1950, the results of which can be found in the respective Quarterly Reports of Physics Branch, PD Division.

During this period arrangements were being made by Physics Branch with Tracerlab, Ind., to prepare various C-14 labelings. Correspondence concerning these plans are on file (6).

(2) M Division

Some exploratory work was conducted in the Test Tank T-263 by

M Division in cellaberation with Nutrition Branch using phosphorus-32. Laboratory assays were conducted in T-433 by Dr. Brewer. He and Dr. Hesaki studied the tracer methods of brucella by means of carbon-14, but their work was seen interrupted and discontinued with little or no results (7).

It is to be noted that, during this period, the divisions cellaberated in their work so that the results are somewhat vague and appear collectively in the Quarterly Reports of M Division, Nutrition Branch and Physics Branch.

(3) C Division

C Division also conducted studies with C-14 in the summer of 1949. The entry, translocation and metabolism of 2,4,D in higher plants, and the decomposition of 2,4,D by soil bacteria were investigated (8,9,10). The contracts for labeled 2,4-D were made with Tracerlab, Inc.

(4) Entomology Branch

Entomology Branch did some work with radiomaterials in conjunction with the Army Chemical Center at Edgewood, Maryland.

(5) Summary

In general during this period, the studies by the above-mentioned organizations were preliminary and exploratory in nature and were not continued very far. As explained in Part b, most of the manipulations were accomplished by Physics Branch which had the dual responsibility of developing handling techniques and procedures and regulations for the safety of isotopic investigations.

b. RADIOLOGICAL SAFETY, 1947-1951

Prior to 5 May 1951 Physics Branch received and distributed isotopes for this installation. Dr. John Bateman and Miss Mary Davis were in charge of Radiological Safety, although a committee was set up in September 1947 to be the controlling group for radiological activities for Camp Detrick. The functions of the committee were to "direct and control safety procedures; to review and pass on all proposals for the use of radiomaterials; and to offer suggestions in the solution of these problems" (11).

The first meeting of this committee was held 4 November 1947, and a set of "Tentative Rules Governing the Handling of Radioisotopes at Camp Detrick" was sent to each member (11b).

The elusiveness of definite data and records, the awkwardness of the safety program and the ineffectual control of personnel engaged in studies with radiomaterials prompted the committee to inaugurate a Radiological Safety Group to operate on a full-time basis.

c. RADIOLOGICAL STUDIES, 1951-1953

Pvt Joseph A. Prestele, a graduate of Stevens Institute of Technology, was appointed the first Radiological Safety Inspector in January 1951 and was authorized to set up a complete Radiological Safety Program which included film badge service, laboratory, monitoring, waste disposal, isotope procurement and a filing system to maintain complete records of all isotope work. A committee on Use of Radioactive Materials was appointed by the Director, Biological Laboratories, with Chief, S Division as the Radiological Safety Officer (11c).

(1) M Division

M Division conducted activities in T-263 (Test Tank) with P-32 as a tracer in studies of the viability and material balance in bacterial aerosols (12). Sulfur-35 as H_2SO_4 was also used during this period as a tracer injected into bacteriological media. The organisms were disseminated as aerosols and material balance studies were made (13). Approximately seven persons per week were given film badge service in this division. All work was terminated in August 1952. In December 1952 (14) and again in March 1953 Battelle Memorial Institute collaborated with M Division for short projects in the Test Tank T-263. Temporary film badge service was administered during these tests. Mr. Robert B. Painter was named Radiological Area Supervisor for this area (No. 3). Periodic laboratory surveys were conducted and in a few instances above-maximum levels of contamination were found (200 cpm) (15). In one instance, two five-gallon radioactive liquid waste carboys were broken. The radiological safety group supervised the decontamination of the affected laboratory.

(2) Aerobiology Branch, T-524

Aerobiology Branch conducted studies on the Retention and Decay Rates and the Stability and Virulence of aerosols in the trachea and lungs of guinea pigs using P-32. Pvt Ephraim Fine and Mr. Gilbert Briggs, the area supervisor (Area 5) used B globigii and S marcescens in radiophosphate media for their work (16). Approximately eighteen persons per week were given film badge service. The work in this area concluded in May 1952.

In several instances heavy contamination was found in the T-524 area. One of the floor drains in particular was found to be radiating

at 80,000 cpm. This finding resulted in the establishment of specially designed waste containers for both solid and liquid waste. Decontamination was effected in every case of excessive environmental contamination.

(3) C Division

C Division continued its studies enumerated in Part a. of this report. Dr. Robert L. Weintraub was appointed Radiological Area Supervisor of Area 2. Approximately 15 persons per week were given film badge service. All isotope activities were located in building T-321.

Surveys revealed generally safe procedures in this area. On one occasion oral pipetting of radioactive solutions was observed. A letter to all division chiefs was sent by S Division forbidding this practice. It is interesting to note that this occurrence was in direct violation of one of the rules set up by the Isotopes Committee in 1947 and that insufficient control led to this practice. The Radiological Safety Group supplied manual pipettors as a safe substitute.

(4) MM Division (MB Division)

MM Division, in T-427, began using radioisotopes in May 1951. $NaHCO_3$ labeled with C-14 was used in studying the pathways of biochemical processes in B anthracis. The mechanism of glutanyl polypeptides synthesis by this organism and the assimilation of uridine compounds in Staph. aureus treated with penicillin were studied by Dr. Curtis B. Thorne (Area supervisor, area 6) and later by Dr. James T. Park (17).

An average of 10 persons per week were given film badge service. Activities are continuing in this area.

(5) Electron Microscope Studies

Area 7, under Miss Mary Davis, was used as a storage area for Rb-86 until the Radiological Safety Group could move these materials to its laboratory. When surveying this area, it was found quite by accident that Mr. Charles Senseney and Mr. George Hess were using natural uranium to shadow specimens for the electron microscope in an adjoining laboratory (18). Radiological Safety immediately set up a safety control program for this laboratory. This area (No. 7) is periodically checked. From the time of this discovery it was decided that the Radiological Safety Group alone would have the authority to order any radiomaterials for this installation.

d. RADIOLOGICAL SAFETY, 1951-1953

Until April 1951, Pvt Prestele was setting up the Radiological Safety Program. He spent time studying with the Health Physics Group at Army Chemical Center. He also went to Ann Arbor, Michigan, for a conference. Twice during this period he spent a month at the AEC at Oak Ridge, Tennessee.

On 28 March 1951 a directive from S Division was sent to all division chiefs requesting physical examinations for all personnel engaged in work with radioactive materials. Now annual physical and semi-annual blood examinations are required of all persons exposed to radiations.

On 11 April 1951 all areas using or planning to use radiomaterials were requested to define the areas in which they would work so that a laboratory monitoring program could be set up. Also the appointment of an area supervisor for each radiological area was made mandatory in order to fix responsibility for individual laboratories.

In May 1951 film badge service was begun in collaboration with Army Chemical Center. T-605 was designated as area 1, the Radiological Safety Sub-section.

During the summer of 1951, the Radiological Safety Program got under way with laboratory monitoring, personnel dosimetry, periodic physical examinations, waste disposal facilities and isotope procurement service. A trench in the grid area was dug to buy solid radioactive waste. The Oak Ridge policy of diluting liquid radioactive waste to .1 uc per liter was followed. Approximately 94 gallons of solid waste and 80 gallons of liquid waste were disposed of during this period.

During the last quarter 1951 radiological first aid kits were assembled and distributed; lucite syringe shields were fabricated for use in T-263, and "radiac Wash," a decontaminating agent was obtained.

During this period, 54 gallons of solid waste and 110 gallons of liquid waste were removed from the areas (19).

In May 1952 four large tanks were constructed in Building T-270 and are now used to store liquid waste of a high activity until natural decay makes it feasible to dilute and dispose of the material (20). As of June 1952 the solid waste disposal system was altered in that a shaft rather than a trench was dug for solid radioactive waste disposal. The shaft is surrounded by a wire fence and is visibly marked with "Radioactive" signs. This new pit was found to be necessary since rain spread contamination throughout the trench used previously.

An inventory of all radiomaterials on the post was accomplished and monthly inventories of radiochemicals in storage by Radiological Safety are sent to the divisions concerned.

Metal carboy containers, marked with the Radioactivity symbol and painted in fuchsia and yellow, were made as were GI cans painted in the same colors for solid waste.

During the summer of 1952 and later, a replacement for Pfc Prestele was trained. Activities in the section were usual. A proportional counter was purchased to better survey for alpha contamination. It was decided that smears surveys would be instituted to determine any contamination.

At this time Pfc Prestele also began to formulate an SOP for Radiological Safety for Camp Detrick. This was completed in October 1952 and now supersedes the SOP of the Army Chemical Center which heretofore was used. This SOP (21) presents the requirements for the initiation of isotope activities, and sets up the standards by which isotope activities will be measured.

Radioactive hood design requirements are available (22) to persons wishing to operate a radioisotopes laboratory.

During the last quarter of 1952 (23) routine activities were maintained. Eighty-five gallons of solid waste were buried in the grid area and 85 gallons of liquid waste were stored in the tanks in T-270.

The Battelle Memorial Institute project mentioned earlier was closely supervised and proceeded without mishap.

The Radiological Safety Group also gave an orientation discussion to the Guard Force and a similar talk is planned for the firemen.

There is on file in the Radiological Safety Office, Room 202, T-201, in addition to progress reports, data on radiomaterials, hoods, laboratory equipment, instruments, laboratory facilities, and AEC regulations. Radiation exposure records of personnel, in accord with SR 40-1025-66, are maintained in the file of the Radiological Safety Inspector.

Since the institution of the Radiological Safety Group in January 1951, it has been found that most of the hazards prevalent in the period before were resolved. No major exposures have occurred. No personnel have been injured.

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This section was prepared by: Pfc Jesse F. Hunsberger
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12. SAFETY EQUIPMENT FOR THE LABORATORIES

a. MISCELLANEOUS EQUIPMENT

(1) Air Sampling Devices

(a) Sieve Sampler: The sieve type of bacterial air sampler has been extensively used at Camp Detrick during the past 8 years. The first sieve samplers were developed by Dr. DuBuy of the U. S. Public Health Service. The original design (DuBuy and Crisp, 1944) has been changed somewhat during its use at this installation.

The sieve sampler is constructed of aluminum and consists of a round metal box which encloses the standard petri dish. The sampling head is perforated with 340 evenly spaced holes, each .0145 inches in diameter (No. 79 drill). The sampling head is adjustable and should be adjusted by means of a center guide pin so that the sieve plate is 2 to 4 millimeters above the agar surface. This permits air to escape around the petri dish and out through a hole in the bottom of the sampling device. To obtain good impingements of particulates on the surface of the agar, the rate of airflow through the sampler should be about 1 cu ft per minute. (RE Division Drawing #D-93-1-1070)

The vacuum supply for the sampling device should be constant and at a known rate so that a definite amount of air is sampled in a given time. A suitable vacuum can be obtained by the use of a small vacuum pump or from laboratory vacuum lines. The airflow can be determined by use of flowmeters, standard orifices, or other air measuring devices.

The sieve type sampler is preferred for sampling low concentrations of vegetative organisms. Although the efficiency of this type of sampler is not as high as some of the other samplers in use at Camp Detrick, the sieve and the slit type samplers are the only ones that enable detection of the very low concentrations of the agent under consideration. The sampler costs less than the slit. Furthermore, fewer maintenance problems are involved and there is greater portability.

Other air samplers in use at Camp Detrick are as follows:

(a) Capillary flask, midget impingers
(b) Cotton collectors
(c) Lense bubbler sampler
(d) Shipe impinger
(e) Millipore filter impinger
(f) MSA midget impinger
(g) Porton all glass impinger

(h) Slit sampler-modified by S Division Drawing Nos. C-93-1-276, C-93-1-277, and D-93-1-300

(i) Electrostatic bacterial air sampler manufactured by General Electric Company (7)

(2) Ampoule Container

An ampoule container has been designed by Bacterial Nutrition Branch, AS Division, for opening ampoules containing lyophilized infectious cultures. Ampoules are placed in an aluminum container which is sealed by screw caps at either end fitted with rubber diaphragms. A hexagonal nut may be placed over the neck of the ampoule or a stainless steel ball bearing may be placed under the bottom of the ampoule. An adequate amount of the desired liquid is also placed in the container. (RE Division sketch #828)

(3) Autoclave Sign: Sterile - Contaminated

This sign is constructed of stainless steel (8" x 2") reading "Sterile" on one side and "Contaminated" on the other, and may be attached above the autoclave door. The sign operates on a hinge so that it reads "Contaminated" in the normal position and "Sterile" in the raised position. A spring clamp holds the sign in place in the raised position. This sign helps in preventing removal of contaminated material from the autoclave. (RE Division sketch #1287)

(4) Blender, High Speed

Results of tests conducted by Laboratory Hazards Section, S Division, (1) have shown that biological aerosols may be released from high speed mixing bowls by (a) a loose fitting cover, (b) lack of a gasket in a tight fitting cover, and (c) a worn bearing or a loosely fastened drive shaft. On the basis of this data, a mixing bowl was designed to eliminate the sources of aerosol. The bowl has a leakproof bearing and is equipped with a siphon and drain valve which allows the removal of fluid in a closed system. Leakage at the bottom bearing is prevented by (a) use of teflon washers between the cutting blade assembly and the bottom of the bowl, and (b) a keyhole fitting for the cutting blade assembly which allows the exterior locking nut to be tightened so that any possible loosening of the assembly by vibration is eliminated. Blended material cannot get into the bottom bearing, because any material that may rise up between the cutter shaft and the drive housing is expelled by centrifugal force through 4 openings at the top of the cutter shaft. (Drawings D-93-1-1771, D-93-1-1803 and D-93-1-1802)

(5) Cans for Acid and Alkali Transportation

A stainless steel bucket (7"x7"x8") has been developed with 2 conveniently placed handles which holds a gallon bottle. (RE Division sketch #1015)

(6) Cans, Discard

A discard can which holds discarded petri dishes and other dirty materials is in use. It is constructed of stainless steel. Dimensions of the can are 10" high, 10" wide and 20" long, and possesses handles at each end of the long axis. This discard can will conveniently hold 130 petri dishes stacked on their sides in 2 layers, and will fit in a small sized autoclave. (RE Division sketch #756)

(7) Cans for Shaking Machine Flasks

A container to house individual culture flasks while in motion on a shaking machine has been constructed. The container is cylindrical and equipped with a spring device which securely holds a 250 ml or 500 ml flask in place. The head is made of pyrex glass and aluminum. A circular opening in the center of the head houses a spun glass filter held in position by pyrex glass secured in a groove with a true arc retaining ring (2).

(8) Centrifuge Cups, International 1, 2 and Refrigerated Centrifuges

A review of infectious laboratory accidents at Camp Detrick has shown that between Jan 1944 and Feb 1953 five major exposures to infection occurred in connection with centrifugation. In addition, there have been many exposures which have not been formally recorded. These accidents are characterized by breakage of glass culture tubes and consequent shattering of the culture. At the request of S Division, the International Equipment Company has developed a #384 duraluminum slotted cup containing a dome-shaped screw-on cover. This is now commercially available. Normally the centrifuge cups are used with 250 ml bottles. To permit the use of the 10 ml, 15 ml and 50 ml tubes, adapters have been designed to house the various sized tubes in the centrifuge cup. Adapters for the 10 ml tubes will house 10 tubes, while the 15 ml tube adapter will take 7 tubes. Two 50 ml tubes may be used at one time in each slotted cup. These cups have been tested by the International Equipment Company at full speed with overloading test weights, and were found to be satisfactory. They may be run at full speed (3000 rpm) on the International size 1 and 2 reinforced centrifuges and at 2500 rpm on the Model PR1 refrigerated centrifuges. The speed is limited on the latter so as not to overload the motor.

The International Equipment Company pin-type centrifuge head #277

must be used for swinging the slotted cup on the size 1 and size 2 centrifuge and pin-type head #284 must be used on the Model PR1 refrigerated centrifuge. It is absolutely necessary that the centrifuge bowls be of the reinforced type, possessing all welded seams. Guard bowls which are riveted together cannot be used as they are the non-reinforced type and should be replaced by the all-welded steel type prior to the use of the modified centrifuge cups. In ordering steel guards, one should specify #798 for size 1, type SBR or SBV centrifuges and #799 for the size 2 Model R or V.

(9) Clothing Discard, Ultraviolet Rack

A discard clothing rack was designed which utilizes a protective barrier of ultraviolet light to isolate discarded articles in a canvas laundry bag. The entire unit consists of essentially two parts: (1) a metal bag holder and (2) a shielded box containing two 15-watt hot cathode ultraviolet bulbs. Both parts are mounted on the wall. The bag holder is mounted at such a height so that the bottom of the bag is held several inches off the floor. The ultraviolet bulbs in an aluminum frame are mounted above the bag holder. A nonultraviolet penetrating curtain hangs from the lower edge of the ultraviolet box, extends down past the top edge of the laundry bag. In this manner the ultraviolet radiations are confined and most of the intensity is directed into the open bag. (RE Division Drawing #D-93-1-2134)

(10) Compressor, Mobile, for Portable Air Supply Apparatus with Ventilated Head Hoods (3)

A portable and mobile compressed air system has been developed which will supply air through filters to 4 head hoods. This unit may be used in emergencies or for temporary operations. (RE Division Drawing #D-93-1-1157 and #F-93-1-1158)

(11) Filters for Personnel Head Hoods

E12R4 or M11 all purpose Chemical Corps canisters may be used where resistance of 2.3 inches H_2O at airflow of 3 cfm is not a problem. These are highly efficient filters with efficiency approaching close to 100 per cent.

Pie pan filters (4) containing 2 layers of 50 FG spun glass have been developed where high resistance is a problem. This type of unit has an efficiency in excess of 99 per cent. (RE Division sketch #1548)

(12) Leak Detector (5)

A leak detector manufactured by the General Electric Company has been in use since January 1949. The detection unit consists of a hand

held probe containing an element which is sensitive to halogen compound vapors. A blower draws air into the unit. When freon or other halogen containing compounds are placed in an enclosure to be tested, and the probe moved along the outside surface, leakage is indicated by an increase in current on a dial of a control unit. This instrument is used for safety tests of the Reynier chambers (T-524), the test sphere (T-527), vessels and lines in the Pilot Plants, and such cabinets as the Schwab type unit (T-459), and the General Electric Type H Leak Detector.

(13) Pipette Can Holder

This apparatus is constructed of aluminum with a stable wide base and holds a pipette can at approximately a 30° angle for easy withdrawal of pipettes. The potential danger of broken culture flasks caused by rolling pipette cans accidentally set in motion by the operator is thus eliminated. (Drawing No. C-93-1-1087)

(14) Pipette trays

A compact tray is constructed of stainless steel 16" long, 4" high, and 4" wide will easily hold 100 one ml pipettes. One end of the tray is slanted so that pipettes may be inserted into the tray without breaking the delicate tips. A handle is placed at one end to allow convenient handling of the tray. The tray should be filled with enough disinfectant such as 5 per cent phenol or 2 per cent lysol so that pipettes are covered completely. (RE Division Drawing No. C-93-1-1001)

(15) Pipettors, Safety (6)

The most common method massive exposure to infectious or toxic agents is the aspiration of material through a pipette. Infections have been traced directly to aspirations of infectious microorganisms.

A number of practical safe pipetting devices have been designed and constructed. Some of these have been successfully used in the various laboratories at Camp Detrick and are described as follows:

(a) Roberts-Munshour pipettor: This safety pipettor or modifications of it is the most common type used at Camp Detrick. It utilizes a soft rubber gasket for seating the pipette with the gasket held in a ring which fits over the index finger of the hand. A cotton air filter placed in the suction is attached to the arm with a metal clamp. The suction is obtained by the mouth. This type of pipettor has been used for some time in the Pilot Plant, Aerobiology and Bacterial Nutrition Branches for pipetting infectious bacterial suspensions. Workers in these buildings feel that this type of pipettor does not hamper them; in fact, it is felt that pipetting

with this type of apparatus is faster, more efficient, less tiring and decreases the hazards of the conventional methods. (Drawing No. RE Division D-93-1975).

(b) BN pipettor: This pipettor consists of the Roberts-Munshour gasket and holder attached to a movable arm which can be swung to the desired position over a work bench and can be pushed to the back of the bench when not in use. The vacuum supply obtained from a motor driven pump or a water vacuum pump is constant. The air is bubbled through a disinfectant trap (5 per cent phenol). The suspensions and the pipette are moved toward the pipettor until the pipette forms a firm seal with the soft rubber gasket. The BN pipettor is now a standard item of equipment that is used with the bacteriological safe cabinet. (RE Division Drawing #F-93-1-1562)

(c) O'Brien modification: This pipettor is so arranged that either vacuum or pressure can be applied. With slight pressure against the rubber gasket, the vacuum is maintained, and with still a greater pressure against it, a pressure is obtained. This latter feature is not so desirable when working with pathogens, because it has been shown that aerosols are created when the pipettes are blown out. Mercury traps regulate the height that the liquid can be drawn into the pipette. (RE Division Drawing #C-93-1-1159)

(d) Rosen pipettor: This pipettor consists of a metal tube with rubber gaskets for attaching the pipette and is activated by a rubber bulb attached to the side. The release of the contents of the pipette is controlled by a plunger on the top of the pipettor. (Commercially available from National Instrument Company, 5005 Queensbury Avenue, Baltimore 15, Md.)

(e) Frank pipettor: This pipettor is hand operated device which utilizes a rubber diaphragm creating a vacuum or a pressure. A special level winding screw (left and right thread) device enables either a left or right handed person equal ease in operating it. (M. Frank, SO Division)

(f) Rawson pipettor: This is a hand operated device which uses a piston to create a vacuum or pressure. The action of the piston is controlled by a geared arrangement, making it very sensitive to control. The pipette is held in the pipettor by rubber gaskets. (RE Division Drawing #C-93-1-1161 and D-93-1-1160)

(g) Schantz pipettor: This pipettor is a hand operated device which utilizes a rubber bulb creating a vacuum or pressure. (Sketch 1534)

(h) Wish pipettor: This pipettor is attached to a movable bracket provided with a spring that is attached to a vertical stand. A vacuum line is attached to the pipette holder. (RE Division Drawing D-93-1-1534)

(16) Sonic Vibrator - BN Sonic Cup Cover (8)

A safe procedure for removing the contents of a sonic cup was developed by Bacterial Nutrition Branch, AS Division. A siphoning device is used. It consists of a long stainless steel needle bent into a U shape with one end of the U inserted through a rubber stopper into a test tube large enough to contain all the material in the sonic cup; the tube in turn is contained in a suction flask. The sonic cup is fitted with a cover which has been modified by the insertion of a rubber diaphragm (Vaccine bottle stopper) through its center as well as the addition of a large size "O" ring (#24). The siphoning operation is accomplished by inserting the free end of the needle through the diaphragm until it reaches the bottom of the cup, and then applying suction to the filter flask. The vacuum line is protected by a cotton filter. The foregoing modification eliminates the need of opening the sonic cup after the suspension has been subjected to sonic vibration. However, it is recommended that the sonic oscillator be operated only in a bacteriological safety cabinet because of the aerosols produced by dispensing bacterial suspensions into the sonic cup prior to sonic bombardment, and the possibility of a leak developing through a faulty gasket.

(17) Syringe, Safety (9)

Plungers of normal and Luer Lok syringes have been modified by grinding the first 2/3 portion of the plungers to prevent capillary action. The result of tests indicated that the plungers of the safety syringe were sterile as compared to gross contamination of plunger of regular syringe.

Luer Lok syringes which prevent breakage of the syringe tip are available commercially from Becton, Dickenson Company, Rutherford, N.J.. and Amega Precision Instrument Company, Englewood, N. J.

(18) Test Tube Rack with Tray

An ordinary test tube rack has been modified by removing the wire bottom and replacing it with a stainless steel tray. This tray will confine the fluid from a broken test tube. When the rack is used to hold pathogenic cultures, the tray is lined with a disinfectant soaked in cotton. BN Branch has modified this test tube rack by application of neoprene to the entire rack and tray. (RE Division Drawing #C-93-1-1698)

(19) Trays for Shaking Machine Flasks

A rectangular shaking machine tray with overall dimensions

of 19" x 14-3/8" x 12-1/4" containing a spun glass filter and a liquid seal trough around the lip of the tray cover has been developed by Bacterial Nutrition Branch, AS Division. This tray has been placed in use this year. (RE Division Sketch #2930)

b. SAFETY EQUIPMENT UNDER DEVELOPMENT

(1) Rubber Gloves

Agent Control Branch, in cooperation with M Division, is actively engaged in the development of safe, durable and properly designed rubber gloves, for use with infectious agents and on special laboratory and plant equipment. The types of rubber gloves required in the laboratories are as follows:

(a) Arm length 8 inch diameter gloves for Class 1, 2, and 3 cabinets.

(b) Arm length 8 inch diameter gloves with a bead for cabinets using a liquid seal closure.

(c) Arm length 5 inch diameter gloves with a bead for cabinets and animal transfer boxes using a liquid seal closure.

(d) Surgical type gloves preferably nonslip to be used in ordinary laboratory procedure and for animal necropsy.

The gloves presently used for items 1, 2, and 3 are not satisfactory, mainly because of the failure of the rubber compounds to withstand the exposure in decontamination procedures employed. There is also some lack of standardization of glove design between various divisions, although a Glove Standardization Committee keeps matters from getting out of hand.

Progress to date in development of suitable rubber gloves has been satisfactory. A study was conducted on the relative suitability of various rubber and rubber type compounds. The tests included exposure to wet and dry heat, ultraviolet light, ozone, liquid disinfectants, gaseous disinfectants, acids and alkalies. As a result of these studies it was determined that a neoprene formulation was a most suitable material for the arm length rubber gloves required. Further investigation to simplify fabrication details has been completed. Standard material design and fabrication requirements have been written. Several glove designs have been proposed and the various commercial concerns are fabricating a limited number of neoprene gloves according to the proposed designs. These gloves will be tested under practical conditions before being accepted as the standard glove.

There is at present a critical shortage of arm length rubber gloves in all laboratories, because of the rapidity with which the present rubber gloves deteriorate and the limited commercial output available for purchase. Efforts are now being made by Purchasing Division to procure suitable temporary gloves.

(2) Personnel Hoods, Suits and Masks

(a) Mine Safety Applicances Company Personnel Hood: The MSA personnel hood was developed jointly by Mine Safety Appliances Company and Camp Detrick prior to 1946. This hood is an all plastic hood with a nonflexible visor. The hood served its purpose; but as increased need for its use became apparent, several objections arose. The chief objection to the hood was that when the visor became broken, the entire hood had to be discarded. This was wasteful, because each hood cost from $40.00 to $60.00. The second objection was that the airflow through the hood was directed onto the back of the neck of the laboratory worker. This was considered rather annoying and uncomfortable.

(b) 1953 Ventilated Personnel Head Hood: An aluminum plastic personnel hood has been designed by Mine Safety Appliances Company with the cooperation of Safety and M Divisions. The hood has the following features:

1. A crack resistant aluminum top
2. Removable front visor 10½" wide
3. A plastic cloth neck and body assembly which reaches to the waist and is provided with belt loops
4. An inner neck assembly of plastic cloth equipped with a draw string
5. Adjustable head band and cup type chin strap
6. Metal loop on the top for hanging
7. Large diameter corrugated rubber air inlet hose
8. An inside rubber channel to direct air across the visor rather than against the back of the neck
9. An alligator type steel clamp for attaching the canister filter to the belt

The cost of the new hood is about the same as the old. The main advantage is that the plastic visor in the new hood is replaceable, whereas in the old hood a cracked visor meant that the hood must be discarded.

(c) 1953 All-plastic Personnel Head Hood: An all plastic personnel head hood has been developed by the Standard Equipment Company, Chicago, Illinois, at the suggestion of Safety Division

personnel. The air supply to the hood is distributed to the front portion just over the visor which is an improvement over the rear type of air distribution (which was found objectionable) used in the Mine Safety Appliances all plastic hood. Another desirable feature of the hood is that the plastic portion of the head hood may be replaced in the event of damage at a cost of about $8.00.

(d) All Plastic M Division Diving Suits: An all plastic M Division diving suit has been developed for an individual to go through a dunk bath into a pathogenic area. Four suits have been manufactured by the Lobund Memorial Institute of the University of Notre Dame, South Bend, Indiana, under a research and development contract. This type of suit is used at Lobund for sterile life studies. It has been found that the suit is applicable for the purposes outlined above. However, it cannot be used for field or general use, because of the weight created by inlet and the outlet hose drag. Other objections are that a considerable length of time is required to get into and out of the suit. Furthermore, an electronic seal is required of the waist and pants each time an individual uses the suit. The suit must also be tested before each use. The cost would be relatively high for this type of suit, and there is still the problem of nondurability due to the nonsupporting nature of plastic material.

(e) Laboratory Ventilated Personnel Suit Under Contract: Development work on a laboratory personnel suit is being carried out by M Division by contract. This suit would be of a coated fabric. Effort is being devoted toward development of a suit which can be worn for a 4 hour period and one that is light and that will contain a fresh air supply. The need of such a type suit is apparent for usage in such places as Pilot Plant and other hazardous operations.

(f) Masks: Where ventilated personnel hoods or suits are not used in laboratories, and the need arises for respiratory protection, the following type masks may be worn:

1. Masks - Chemical Corps all purpose, type M9A-1 with M11 canister. This is the normal field type mask used in general warfare.

2. American Optical Company, R2000 respirator

3. Mine Safety Appliance Company paint spray respirator with M-11 aerosol filter and M-2 absorbent cartridge, or

4. Some other respirator which has been found by PD Division to have an equivalent efficiency in filtering out bacteria

c. SAFETY CABINETS

(1) Design Requirements for Safety Cabinets (10)

The design requirements have been categorized into 3 different classifications, class 1, 2, and 3. The classification has been established in accordance with the degree of hazardous operation conducted within the cabinet. Class 1 cabinets are used for pipetting of infectious material or for the autopsy of infected animals, whereas class 2 and 3 cabinets are used for operations involving the release of higher aerosol concentrations.

(a) Class 1 cabinets: These cabinets are subdivided into different types, type 1 through type 5. Type 1 is a 5 ft long cabinet with 2 sets of glove ports on the removable front panel, with a hinged vision panel on the sloping top. Type 3 is the same as type 1 except that the cabinet is 6 ft long. Type 2 is a 5 ft long cabinet in which the glove port panel in front is replaced by a blank panel and the glove ports installed on the sloping top. Type 4 is the same as type 2 except that the cabinet is 6 ft long. Type 5 is a cabinet 20 inches by 90 inches long which is used as an animal transfer cabinet.

Type 1 and type 3 cabinets are used in laboratories in which test tubes of infectious materials are handled, or pipetting of infectious materials is performed, or in which infected animals are autopsied. The length of the cabinet depends upon the space limitations and the nature of the work performed. Type 2 and 4 cabinets are used in laboratories in which manual transfers of infectious agents are made. All class 1 cabinets are to be arranged so that material may be introduced or accessory equipment attached at either end if need be. Drawings of this accessory equipment may be found in plates 21 - 26 in the Design Requirements for Technical Facilities, Camp Detrick, Md., 1 Oct 1952.

(b) Class 2 cabinets: This type of cabinet is tested for water tightness. It is fabricated in sections of 5 or 6 in length where possible, and is flanged and bolted together. A refrigerator and autoclave connections to the cabinet are also bolted together. The glove port panel and the individual sections, fluurescent light housings, ultraviolet light housings, and vision panel frames are all gasketed and bolted to the cabinets. All equipment or parts of equipment exposed in the cabinet are to be of noncorrosive material. In the event that a conveyor is installed in the cabinet, the drive motor should be located outside of the cabinet and a suitable water tight liquid seal must be provided for the shaft. The bottom of the cabinet should be reinforced with mild steel plates so that floor of the cabinet will support a load of 500 lbs per square foot. All services entering the cabinet must be sealed, electrical service included.

(c) Class 3 cabinets: Class 3 cabinets should be freon tested gas tight hoods. Incubators or refrigerators connected to these type cabinets originally were attached to the cabinets by welding in the inner shell of the incubator or refrigerator to the cabinet. Autoclaves and airlocks were attached to the cabinet by stud welding and a liquid seal ring around the opening. All vision panels and fluorescent lighting panels are connected to the cabinets with studs and liquid seals similar to the autoclave connections. The incubators and the refrigerators have outer shells removable so that the cooling coils wrapped around the inner shell are readily serviceable if necessary. Due to the explosion hazard, no pyrofax gas service is used in these cabinets, although it now seems possible that limited use may be made of a burner which has an automatic spring activated gas cut-off. All services entering the cabinet are sealed, electrical service included. Shaft seals and floor loading specifications are the same as in class 2 cabinets.

These cabinets are now in Buildings T-459 (SO Division), 431 addition (Pilot Plant), P470 (Pilot Plant), and P376 (Aerobiology). This is the only type which will contain a highly persistent agent.

(d) Modular type cabinets: A contract has been awarded to the S. Blickman Company, Weehawken, N. J., by RE Division to prepare preliminary drawings of 24 inch modular type cabinets. This type cabinet will be standardized in length and width. The cabinet will be designed so that it will be made in sections. Some sections will contain incubators, refrigerators and other laboratory equipment. The modular type cabinet will be assembled with bolted and flanged joints, and it is hoped that after testing, they may be accepted in lieu of the class 3 type cabinets discussed above. This new type cabinet provides flexibility of hood arrangements and permits alteration of hoods as required by future policy changes.

(2) Design Requirements for Cabinet Exhaust Systems

(a) Bacterial filters: All filter installations except class 1 involving a hundred to a thousand cfm shall be the deep-bed spun glass pocket type filter unit or approved equal. All class 1 cabinets utilize a deep-bed spun glass filter unit into which is incorporated strip heaters so that the entire unit may be decontaminated in place prior to removal of the spun glass. There is no objection in employing this filter unit for other than class 1 cabinets, requiring 100-300 cfm of air.

Installation of filters involving less than 100 cfm can be determined by the respective division with approval of the Safety Director.

The standard design for sizing this type filter will be

an air velocity of 20 ft per minute through the filter medium. Generally, all filters on equipment located in contaminated areas will require an integral means of sterilization. Bacterial filters handling 100-1000 cfm will utilize electricity. A temperature of 400 F for 2 hours is recommended for decontamination prior to the changing of the filter.

Design criteria specifies that exhaust air from class 1 and 2 cabinets be discharged through a bacterial filter, then into the building exhaust system prior to the bacterial filter of that system in such a manner as not to upset the air balance in the room whether the cabinet fan is operating or not. It is also specified in class 2 and 3 cabinets that the pressure requirements be such to afford manually controlled operations from -3/8 inch water to -1 inch water. The static pressure and rate of air flow will be adjusted by means of dampers in each supply and exhaust branch. Damper shafts should be provided with a positive seal against leakage of air into or out of the ducts.

Requirements for class 3 cabinets differ slightly and are more severe than those for class 2. These limitations are as follows:

1. Glove panels are not removable

2. Exhaust ductwork shall be 18 gauge sheet metal welded and tested for leaks as required for a gastight cabinet

3. The exhaust air after leaving the cabinet must go to an incinerator

(3) Types of Safety Cabinets

There are in use or under design at Camp Detrick several types of safety cabinets. These cabinets are designed primarily to protect the worker rather than to protect the products. Cabinets are made so that they can be used for many different operations to afford standardization for production in quantity. Due to the high cost of design, and of stainless steel construction, it has been deemed desirable to keep construction of a variety of cabinets to a minimum.

The most important features of these cabinets are: (1) the interposition of glass between the operator and the operation, (2) the flow of air away from the operator into the cabinet and (3) a filter to remove infectious organisms from the outgoing air. It is then up to the operator to determine the level of risk he can assume in relation to whether the cabinet is used with (a) the rectangular opening, (b) open portholes or (c) gloves attached to the portholes.

(a) Cabinet, bacteriological safety: There are presently in use 62 bacteriological safety cabinets. The bacteriological safety

cabinets are 32 inches wide and are available in 3 sizes, 45 inch, 60 inch, and 72 inch long. They are composed of stainless steel, 12 gauge, with a pyrex window. The removable glove port panel and hinged window allow them to be converted to chemical hoods or ordinary laboratory tables. They are equipped with hot and cold water, drain, vacuum, air, gas, 110V AC ultraviolet light, fluorescent lighting, air exhaust through a superimposed filter and 2 BN pipettors. (RE Division drawings for 60 inch cabinet - F-93-1-2332 through F-93-1-2348). (For the 72 inch cabinet - F-93-1-2265 to F-93-1-2281)

A blower with a capacity of 300 cfm provides a minimum airflow of 60 linear ft per minute across the face of the 72 inch cabinet opening when the glove panel is not in use. A damper is provided on the effluent side of the cabinet to vary the airflow when the glove panel is used.

A 300 cfm Owens Corning spun glass filter unit has been developed which contains interior strip heaters. When it is necessary to replace the filter, the unit can first be decontaminated in place by turning on three 200V, 750 watt strip heaters which heat the inside of the filter cabinet to more than 400 F. Time temperature studies have shown that after the filter has reached a temperature of 400 F, an exposure of 20 minutes is sufficient to destroy B globigii spores. (RE Division design drawing F-93-1-2324 to F-93-1-2327)

The following adapters can be used with the bacteriological safety cabinets

Autoclaves - A double door autoclave can be attached to either end of the standard bacteriological safety cabinet by the attachment of an autoclave adapter. The autoclave adapter is a 2 porthole cabinet which contains ultraviolet light and fluorescent lighting, water, air, and a hydraulic elevator. The autoclave can be attached either to the end or back of the adapter. The adapter for the side attached autoclave is 37-1/4" high, 30" long, and 39" wide. The adapter for the back attached autoclave is 37-1/4" high, 37" long, and 26" wide. The autoclave is 20 inches in diameter and 36 inches deep. The hinged door and the front panel in the adapter can be opened out to allow access for servicing of the autoclave (11).

Airlock, Straight through type A - This is a rectangular airlock 20" x 24" with sliding interlocking doors at both ends. Only one door can be opened at a time. A sight glass and porthole is provided for the maneuvering of objects in and out of the safety cabinet. Facilities are ultraviolet and fluorescent light (12).

Straight through, type B - This airlock has only one swinging door on the side. There is no door between the safety cabinet and the ultraviolet lock. Facilities are 2 ultraviolet lamps (13).

Right Angle, type A - Dimensions are 20" x 23". The entrance to the air lock is at the front instead of the side. Other features are similar to the straight through air lock (14).

TEE - The T-shaped air lock can be attached between 2 safety cabinets. The air lock has 3 sliding interlocking doors, one at each side and one at the front for removal of materials from either cabinet. Utilities are ultraviolet and fluorescent light (15).

(b) Cabinet, weighing, gastight: This cabinet is constructed of stainless steel and is a modification of a weighing cabinet now in use by the Atomic Energy Commission. It is sometimes referred to as a balance box or as a Fairchild Balance Dry Box. This cabinet can be used either to hold a balance or as a bacteriological work cabinet. It has been adapted for use with infectious microorganisms by the addition of an exhaust system, spun glass filter and air lock. It is equipped with ultraviolet light, externalized fluorescent lighting, gas, 110V AC current, vacuum and water. There are 2 stopcocks for introduction and exhaust of a gas which permits sterilization of the interior provided the interior pressure does not exceed 10 inches of water. An outlet is also provided for a manometer to measure the interior pressure. The Rawson type glove rings provide tightly sealed gloves. There is a steel cover plate for each glove port which is used when the interior of the cabinet is to be sterilized by gas under pressure. The exterior overall dimensions are 51 inches long, 26 inches high, and 30 inches deep, without the standard filter but including the air lock. The cabinet inside has a clear space of 33-1/2" long, 20-1/2" high and 23-1/2" deep. (RE Division drawings F-93-1-1581, F-93-1-1665, F-93-1-1666).

(c) Cabinet, shaking machine (incubators)(16): A Victor milk cooling box has been modified into a shaking machine cabinet. The dimensions of the cabinet are as follows: exterior, 5 ft 5 inches long, 3 ft 9 inches wide, and 4 ft high; interior, 4 ft 1 inch long, 2 ft 4 inches wide, and 2 ft 2 inches high. This cabinet is provided with a sight panel for observation, and is equipped with 2 decontamination troughs with immersion heaters for vaporization of formaldehyde. It is also equipped with heating and cooling units to maintain a constant temperature. Fluorescent ultraviolet lights are also included in this cabinet as well as an exhaust filter. The temperature of the cabinet may be set from 20° C to 55° C and has a variation of plus or minus 1° C for any given temperature setting. The control panel is provided on the outside of the cabinet which allows complete operation of the shaker, including lights and control of the temperature from the outside. The cabinet has been designed to accommodate such shakers as those manufactured by International, Precision Scientific, Will, Aloe, Eberbach and Fisher.

(d) Cabinet, animal transfer (17): The animal transfer cabinet is 32 inches wide and is available in 2 sizes; 60 inches and 72 inches long, and is composed of stainless steel, 12 gauge. This cabinet differs from the bacteriological safety cabinet in that the glove port panel in front is replaced by a blank panel and the glove ports installed on the sloping top. It comes equipped with hot and cold water, drain, 110V AC ultraviolet light, fluorescent lighting, and air exhaust system by means of a super-imposed filter; it may be used in conjunction with a bacteriological safety cabinet.

This cabinet has not been suitable and is being used to house special apparatus such as the Sharples centrifuge. A work-up of a better design for use in transferring animals in and out of cages is now being tried out in building P-567.

(e) Cabinet, receiving or animal type (18): This cabinet is 20 inches wide by 90 inches long. It likewise is composed of stainless steel, 12 gauge, with pyrex sight glass windows. It is equipped with pipe connections for any utilities desired, 6 glove ports, and a light window located at the end of the cabinet. It is equipped with hot and cold water, drain, vacuum, air, 110V AC to which ultraviolet portable lamps may be attached, fluorescent lighting, and an air exhaust through a superimposed filter.

(f) Cabinets in use for individual operations:

Bell-jar Dryer (wooden) - a 3/4 inch plywood cabinet to house a double bell-jar dryer and pelletizer is being fabricated for use in Building T-428. When completed, the cabinet will contain ultraviolet light, air lock, dunk bath, and bacteriological air filters.

A similar cabinet has been in use in Building T-434 for a single bell-jar dryer and pelletizer as well as for weighing, grinding, and for routine diluting and plating operations. This cabinet is likewise equipped with glove ports, ultraviolet light, dunk bath and filter.

Dryer, NRC - An enclosed room with a walk-in air lock is being constructed to enclose an NRC dryer. Ultraviolet lights, air lock, and personnel head hoods are being provided for work in this area. (RE Division drawing D-9[illegible]-1-2288).

Dryer, Proctor and Schwartz - A stainless steel and plywood system of cabinets house a Proctor and Schwartz dryer in Building T-432. Equipment for weighing, grinding and centrifuging is included. There is sufficient room for diluting and plating. The cabinet is equipped with dunk bath, freezing element, glove ports, ultraviolet light and suitable air filtration equipment. (Design sketch #2369-70-72).

Egg Harvesting Cabinet - A stainless steel circular egg harvesting cabinet containing dunk bath, glove ports and ultraviolet air lock is in use in Building T-434. The dunk bath is the weak point in this arrangement. It is desirable that means be found to send the discarded egg trays into an autoclave through a closed ventilated cabinet system. (RE Division Drawing D-93-1-1141 and F-93-1-963. Ferguson Drawing B-491-60-134 53 thru 57).

Egg inoculating cabinet - A rectangular shaped stainless steel egg inoculating cabinet is in use in Building T-434. This cabinet is also equipped with glove ports and has an ultraviolet air lock. (Drawings F-93-1-1585, D-93-1-1586 to 1589).

Sharples centrifuge cabinet - An animal transfer cabinet similar in design to a 72 inch bacteriological safety cabinet is being modified so that a sharples centrifuge can be placed in the cabinet.

(g) Cabinets under design:

Centrifuge cabinets - Contract DA-18-064-CML-2378 has been awarded to the Battelle Memorial Institute to prepare design prints and engineering specifications and to fabricate and furnish a developmental model of a safety cabinet for a refrigerated centrifuge based upon CmlC Biological Laboratories preliminary design prints and specifications. In addition, the contractor will fabricate and furnish a scale model of the safety cabinet developed.

Cabinet, shaking machine - Another phase of the contract awarded to the Battelle Memorial Institute relates to the preparations of design prints and engineering specifications as well as fabrication and the furnishing of developmental model of a safety cabinet for a shaking machine as large as 51"x26"x33" based upon CmlC Biological Laboratories preliminary design prints and specifications. The contractor will also fabricate scale models of the safety cabinet development.

Modification (BN) safety cabinet - Bacterial Nutrition Branch has tentatively designed a cabinet which is a modification of the standard bacteriological safety cabinet. It is now planned that it will be 72" long and 48" wide. It will contain 4 portholes on each side.

d. CAGES, ANIMALS

(1) Cages, Ventilated Animal, (Temporary)

Ventilated animal cages containing inlet and exhaust spun glass filters and sight panel have been developed and are in use by M Division in P-567. The filters, sight panel, water bottle and feed hoppers are

part of the cage lid. Cage sizes are 18"x19"x10" which will accommodate 4 guinea pigs. (RE Division sketch 2520, 2538, 2553, 2610).

(2) Cages, Monkey

Ventilated monkey cage containing inlet and exhaust spun glass filters have been developed by RE Division and are in use by M Division in P-567. The cages are approximately 20½ inches wide, 42 inches high and 25½ inches in depth. The filters, water bottle, feed hopper entrance door and manometer are part of the front portion of the cage. RE Division is now completing final drawings of the standardized monkey cage. These drawings will be numbers 72971-74, 2980-83, 2990-91-95-99, 3004-6-7-8.

(3) Cage Rack, Ultraviolet (19)

This cage rack (48-1/4" wide, 71-3/4" high, 22" deep) consists of four shelves. Each shelf of the rack is equipped with two 15 watt, 18 inch hot cathode ultraviolet bulbs, and 2 aluminum reflectors. Each fixture is attached to the rack in such a manner that it can be easily lowered or raised to provide a curtain of ultraviolet across the top of the cage. (RE Division Drawing C-99-1-2219).

e. REFERENCES

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3. S Division QTR, Oct-Dec 1948, page 11
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7. S Division QTR, Oct-Dec 1948, page 11
8. S Division QTR, Sept 1952, pages 33-34
9. S Division QTR, Jan-Mar 1948, page 20
10. Design Requirements for Technical Facilities, Camp Detrick, Md. 1 Oct 1952

11. Design Requirements for Technical Facilities, Camp Detrick, Md. Plate 22

12. Design Requirements for Technical Facilities, Camp Detrick, Md. Plate 23

13. Design Requirements for Technical Facilities, Camp Detrick, Md. Plate 26

14. Design Requirements for Technical Facilities, Camp Detrick, Md. Plate 23

15. Design Requirements for Technical Facilities, Camp Detrick, Md. Plate 24

16. S Division sketch dated 6-6-50 and S Division QTR, Dec 1951, page 14.

17. Design Requirements for Technical Facilities, Camp Detrick, Md. Plate 21 and RE Division sketch 1968; also F-93-1-2332 to 2348

18. Design Requirements for Technical Facilities, Camp Detrick, Md. Plate 33 and RE Division design drawings F-93-1-1906, D-93-1-1934, 1907, 1918, 1922, 1924, 1983, and 1984

19. Design Requirements for Technical Facilities, Camp Detrick, Md. Plate 19

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13. ROUTINE SAFETY CONTROL ACTIVITIES

The Agent Control Branch, S Division, is a service, advisory and control group on biological safety, and consequently, its personnel participate in some manner or another in practically all activities of the Biological Laboratories. Some of the routine control activities of Agent Control Branch are as follows:

a. CLASSIFICATION OF BUILDINGS

At intervals the various laboratories are classified as to the potential biological hazard in the building due to the type of agent and the type of work being conducted. This classification is used to determine the type of restrictive signs (Class I, II, or III) placed on the entrance of the building, the need for sterilization of sewage, the type of air treatment needed, and the type of refuse disposal (post Engineer or RE Division) required. (19)

b. INVESTIGATION OF ACCIDENTS

Each accident that is reported to S Division is investigated by personnel of Agent Control Branch. Often members of this Branch participate in clean-up and supply equipment for decontamination of the accident areas. Accidents of major significance are written up in some detail and filed for future reference in Agent Control Branch files.

c. REPORTS ON PRACTICAL CONTROL PROBLEMS

Many short term, practical type safety problems are worked on in Agent Control Branch. As a result of this work eight (see references 2-9) S Division Intradivisional Reports have been published as well as several Special Reports (10-17) and an Interim Report (18).

d. SAFETY REVIEW AND DEVELOPMENT OF EQUIPMENT AND BUILDINGS

Chief, S Division, in cooperation with personnel of RE Division, has formulated and published a compendium entitled "Design Requirements for Technical Facilities, Camp Detrick, Maryland." Since the original publication in May 1951 a number of revisions have been added. This publication covers the technical and safety requirements for the unique facilities required for BW studies. All designs for new buildings or equipment to be used in infectious disease studies are submitted to S Division for study, comments and revision (21). Personnel of S Division work closely with RE Division personnel in the design of new equipment or buildings or in the modification of existing equipment

or buildings (20). A number of pieces of safety equipment have been developed by personnel of S Division.

e. SEWAGE

Several times daily samples are taken of treated sewage by RE Division personnel. These samples are checked for sterility by S Division personnel. Samples are taken routinely from potential sources of leakage (valves, flanges, pump shafts) in the sewage sterilization plant to determine tightness of the equipment.

f. SUPPLIES OF SAFETY EQUIPMENT

Experience has shown that it is often difficult to introduce and have new safety equipment accepted. An announcement that certain items of safety equipment are available does not necessarily mean that the items will be used. Therefore, in a number of cases, personnel from S Division have visited the various laboratories and gathered together all old items and replaced them with new items.

All signs are processed through S Division to insure that the signs are standard as to size, color and wording.

Clothing and shoes provided for reasons of safety are authorized through S Division (22).

g. SURVEYS

(1) Ultraviolet

Whenever an ultraviolet source is installed, the electricians notify personnel of S Division so that the installation can be checked for ultraviolet intensity and personnel safety, before the installation is put into service. Each ultraviolet installation and each lamp in the installation have been numbered for easy identification. Each 3 months all bulbs are checked for ultraviolet intensity and those bulbs having an intensity below a set standard are marked and RE Division is notified that the bulbs should be replaced.

(2) Air

S Division is notified whenever a new ventilated cabinet is installed. Personnel from the Engineering Section of Agent Control Branch then check the cabinet for adequacy of air flow, etc., before the cabinet is released for use. Also, this section makes a quarterly check of all ventilated cabinets to determine whether the air evacuation system of the cabinet is operating properly. Each quarter

this group, with members from RE Division, inspect all electrostatic precipitators treating exhaust air from buildings to determine whether the electrostatic precipitators are operating properly.

(3) Other Surveys

Numerous surveys of the laboratories are made by Agent Control Branch to determine techniques or safety apparatus in use. For example, surveys have been made to determine the types of pipettes in use, types of centrifuge apparatus used, methods used in handling discarded clothing, and buildings in which work with powdered infectious agents is being conducted.

h. TRANSFER OF PROPERTY

The removal of equipment or materials from potentially contaminated areas is under the control of S Division. The first regulation governing the removal of items was established by Lt. Lloyd R. Smith, Chief, Operations Control Branch, in 1944 (1). The procedure used for the Safety Clearance of all property until 1948 was for the person desiring clearance of property to notify S Division and personnel from S Division would sample the item and attempt to isolate the infectious agent that was being worked on in the building. This procedure entailed considerable delay and consequently the present system was adopted. The person desiring a clearance fills out a "Decontamination Certificate" (Form 6-72) giving catalog number, nomenclature, serial number, quantity and unit of the material to be cleared. The Safety Officer and the Building Chief sign the certificate stating the method of sterilization used. This form is sent to Agent Control Branch where a Biological Safety Clearance (CD-6-5L) is made out and sent to the receiving agency.

i. REFERENCES

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4. S Division Intradivisional Report No. 3, 23 Oct 1951 "Animal Isolation Procedures used during a Field Trial with an Agent Infective for Hogs (Operation Green)"

5. S Division Intradivisional Report No. 4, 19 May 1952 "Mercury Vapor as a Laboratory Hazard"

6. S Division Intradivisional Report No. 5, 17 Dec 1951 "Glycols as Liquid Disinfectants"

7. S Division Intradivisional Report No. 6, 11 Jan 1952 "Laboratory Accidents at Camp Detrick (Jan 44 to Sept 51)"

8. S Division Intradivisional Report No. 7, 4 Jun 1952 "Effectiveness of Various Quarternary Ammonium Compounds as Possible Substitutes for Roccal with Brucella suis"

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11. Special Report No. 122, 5 Dec 1949, "Human Infections in the Microbiological Laboratory"

12. Special Report No. 124, 29 Nov 1949, "A Study of Efficiencies of Electrostatic Filter and Spun Glass Filter Pads in Removing Serratia indica and E coli Bacteriophage T-3 from an Air Stream"

13. Special Report No. 130, 31 Dec 1952, "Trent Electric Grid Air Sterilizer"

14. Special Report No. 134, 27 Apr 1951, "Biological Safety During Field Trials, 1950"

15. Special Report No. 145, 4 June 1951, "Spun Glass Filters"

16. Special Report No. 146, 1 May 1951, "Thermophilic Bacteria as Simulants"

17. Special Report No. 147, 1 Jun 1951, "Germicidal Cement"

18. Special Interim Report, BLIR No. 23, 1 Feb 1953, "Resistance of Special Paints to Various Chemical and Physical Agents"

19. (a) CMLRE-CD-CO-400.93, Post Memorandum No. 5, Subject: Collection and Disposal of Waste, Refuse and Salvage, dated 19 Jan 1952

(b) CMLRE-CD-BL, Policy Letter No. 3, Subject: Chemical Waste Containers, dated 1 Feb 1952

20. CMLRE-BL, Policy Letter No. 24, Subject: Engineering Limitations and Responsibilities in Laboratory and Pilot Plant Buildings, dated 10 Apr 1953

21. CMLCD-CO-400.1144, Subject: Designs and Drawings, to Chief Engineering Division, dated 21 Aug 1951

22. CMLRE-CD-CO-420, Subject: Clothing for Locker Room Service, dated 5 March 1952

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14. SEWAGE SYSTEM

a. INTRODUCTION

The nature of the sewage at Camp Detrick is such that it is necessary to maintain three complete and independent sewer systems. One system is a storm sewer system that drains to Carroll Creek. Considerable water used for cooling compressors, spray towers, etc. in infectious units is drained to the storm sewer system. A second system carries the sanitary sewage from those buildings which do not work with pathogenic organisms. The sewage is prechlorinated at Building T-101 and then conveyed to the standard trickling filter type treatment plant. The third sewer system services those buildings which do work with pathogenic organisms. It is this sewage which must be rendered completely sterile before it enters the sanitary sewer line and proceeds to the biological treatment plant on the Monocacy River. Continual vigilance is necessary to insure that sewage or other water is not unnecessarily sent to the sterilization plant.

To insure complete sterilization of contaminated sewage, there has been initiated two lines of defense: (1) Sewage from certain contaminated buildings such as the pilot plant must first drain into a tank called a blowcase installed in or near that building. The function of this tank is to sterilize the outgoing sewage by means of steam heat either with or without pressure greater than atmospheric. (2) Sewage which has been supposedly sterilized in the blowcase, as well as sewage from other buildings using pathogens, which do not tie into a blowcase flows to a decontamination plant where it is subjected to heat and pressure to insure complete sterility before being discharged to the sanitary sewer (See Table 14.1). The functional design of both the blowcases and decontamination stations will be discussed later in this summary.

Figure 14.1 shows the contaminated sewer system. Decontamination stations in operation at present are: T-314 and P-375. Building T-280 is on a standby basis.

At present, there are 2 types of decontamination plants in operation at Camp Detrick. Decontamination Plants T-314 and T-280 are of the "Counter-flow" type, while that in Building 375 (U1,U2) is of the "Recirculating" type. The general principle of the continuous flow decontamination system is basically the same; i.e., the incoming sewage gains some of its heat from the outgoing sterile sewage and is then heated to the proper temperature by means of a steam heater and held at that temperature for a predetermined time, after which it loses a portion of its heat to the incoming sewage and then is discharged to the sanitary sewer as sterilized sewage.

The major difference between the 2 types of systems is the method in which the heat from the outgoing sterile sewage is conveyed to the incoming raw sewage. The schematic diagram of the counter-flow system (Figure 14.3) shows that the outgoing sterile sewage returns to the shell side of the heat exchanger containing the raw sewage to give up a portion of its heat directly to the raw sewage. This method is undesirable, as any leak in the heat exchanger tubes will tend to contaminate the outgoing sewage by creating a "short circuit." In the recirculating system (Figure 14.2), a recirculating fluid is used to convey the heat from the outgoing sewage to the incoming sewage and at no time is the raw and sterile sewage in the same heat exchanger. In this system, one leak or even a series of leaks in one bank of heat exchangers would not contaminate the outgoing sewage. However, cross-contamination is remotely possible if leaks occur in both the heating and cooling banks of the heat exchangers with the differential pressures through the system, tending to cause flow through the leaks in an adverse direction. Proper control of the pressures in the system can eliminate this danger to a great extent. Although the recirculating system is safer, it is comparatively expensive in that it requires 4 times the number of heat exchangers as the counter-flow system in order to transfer the same amount of heat.

In 1945, there were 3 sewage decontamination plants to serve the contaminated sewer systems. Buildings T-314, T-280 and T-509 (these buildings were then numbered T-314, T-280 and D-19, respectively).

Engineering specifications for plumbing in the sewage system are detailed in another manual (29).

b. DECONTAMINATION PLANT T-314 (Located back of the pilot plant area)

In 1946 decontamination plant T-314 was basically the same as today. (Figure 14.4). Minor modifications and relocation of certain equipment constitute the only changes since World War II.

On 15 August 1946, contamination was noted in the outgoing treated sewage of plant T-314. A series of tests showed that at least 4 out of 8 heat exchangers were leaking resulting in cross-contamination. The one good 3 tube heat exchanger bank from plant T-280 was removed and installed on the concrete pad between holding tanks 1 and 2 at T-314. This exchanger was connected to the system bypassing the existing exchangers. Building T-314 heat exchangers were removed from the building, tubes removed and shells cleaned. The exchangers were then re-tubed and installed outside Building T-314 as a permanent installation. In installing the heat exchangers, a closed cooling water circulation system was installed to prevent any chance of recontamination of the treated sewage. Meanwhile sewage was treated in T-314 using the counter-flow process with the temporary set-up utilizing the heat exchanger from plant T-280 (1).

(1) June 1947 (2)

On the 8th of June 1947 the temporary heat exchangers between the holding tanks 1 and 2 became clogged and failed to operate. This heat exchanger had been in operation since 16 Sept 1946. A batch system of sewage decontamination of all liquid effluent was put into operation. One-quarter of a 75 lb barrel of calcium hypochlorite was added to each 17,000 gallon tank when the tank was about 1/4 full. During the filling of the tank (to 12,000-14,000 gal), an injection of steam gave a thorough mixing of the hypochlorite with the sewage. Steam was injected into the tank until a temperature of 200 F was reached. A sample was then taken from the tank and the contents of the tank cooled by adding water before being discharged into the sewer line. A residual chlorine test and a bacterial culture was made on each sample. All samples tested in the above manner seemed to have been sterile, even though the sample showed little or no residual chlorine. The hypochlorite is probably effective before the steam is injected into the tank, but the high temperature reached by the contents of the tank quickly drives off the chlorine.

Two heat exchangers having 4 banks each, which are outside T-314, were put into operation using recirculating water from holding tank No. 5 to heat the raw sewage and cool the treated sewage. On 16 June 1947 the effectiveness of sterilization by this system was tested by using B globigii. Complete sterilization of the sewage was effected, but the temperature of the recirculating water could not be kept low enough for the system to operate satisfactorily. (Insufficient heat exchanger area was permitted for the recirculating water to lose its heat, thus it boiled and made it necessary to add cooling water). For this reason, the system was temporarily abandoned and a counter-flow system reinstated.

(2) 1 July 1948 - 30 Sept 1948 (3)

On 24 August 1948 the sewage decontamination system failed completely; batch decontamination was started. Water in the 400 and 500 area had to be shut off for about 5 hours. During this period water from the refrigerator compressors was run to the storm sewers. With the water turned on again in the 400 and 500 area flow of sewage to the decontamination plant was only 80,000 gal compared to the previous days flow of 165,000 gal (shown by the drop for the month of August 1948 on Figure 14.5). Two maintenance crews were assigned the work of making repairs. The crews operated on a continuous 24 hour basis. The repairmen cut four 180° turns on the retention tubes to determine their condition. The 2 top turns were almost completely closed with scale. The remaining tubes had about 1/4" of scale. As an emergency measure, the engineers decided to replace the first four lengths of tubes totaling 332 ft of 6 inch wrough-iron pipe. On the 26th of August new retention tubes were

installed and the system was ready for testing. Loose scale from the old tubes continued to come through with the result that from the 26th of August to the 15th of September the sterilization system was put into operation 10 different times, but each time the system failed after a few hours of operation. Altogether several thousand lbs of boiler scale, which had broken loose from the retention tubes, caught in the restriction just before the strainer and had to be removed. From 29 Sept 1948 to 12 June 1949 the system operated normally.

A survey of scale conditions in the retention tubes in the decontamination systems T-314 and T-280 was made. There was some study of chemical methods of removing scale, but the conclusion was that a change to a better water supply with less calcium salts would be the best solution to the difficulty.

As a result of this survey the use of Montevue spring water in the Camp was discontinued and all water was taken from the Monocacy water treatment plant.

(3) 1 Oct - 31 Dec 1948 (4)

The breakdown of T-314 precipitated action on a sewage connection line between T-314 and T 280.

(4) 1 Jan - 31 Mar 1949 (5)

Tests of the Biological Oxygen Demand (BOD) were run on the sterilized effluent of station T-314 in January and the first part of February 1949. It was noted that there was a wide variation of results between samples taken on different days. However, this variation was undoubtedly due either to substances that have inhibiting or destructive effects upon the living organisms in the inoculum, or to the daily plant operational fluctuations in buildings such as the Pilot Plant where fermentative media is dumped into the decontamination system.

Overall results showed that the pollutional load was not excessive and could easily be treated by the Monocacy sewage treatment plant.

(5) 1 Apr - 30 Jun 1949 (6)

Routine examination of the treated sewage from plant T-314 on 12 June showed that a number of samples were contaminated. Among 25 samples, 14 contained viable organisms and 11 were sterile. Only 2 contained E coli. Leakage of raw sewage was thought to occur within

the heat exchangers. The general absence of E coli in the samples was probably due to the high temperature which is reached in the heat exchangers. E coli is rather heat labile, whereas other more resistant organisms such as B subtilis would survive passage through the exchangers. To determine whether leakage was occurring a test using B globigii as a tracer organism was conducted by E and S Divisions on 13 June.

Test Procedure: Holding tank No. 1 (capacity 17,000 gal) was filled approximately 1/3 full with water from a fire hydrant. Five lbs of dried B globigii spores were poured into the tank and sufficient water added to make a total volume of about 12,000 gallons. The sterilization system was operating when the test was started. At the start of the test, all sewage passing into the system was stopped and the contents of tank No. 1 started into the system. A control sample was then taken from tank No. 1 and samples of the treated sewage withdrawn at 5 or 10 minute intervals. Tank No. 1 became empty 55 minutes after the contents were started into the system. This tank contained about 12,000 gal at the start of the test so that a flow rate of about 200 gpm through the system was obtained during the test. When the tank was empty, the sewage in the other holding tank was started through the system and sampling was continued for an additional 20 minutes. The B globigii count in tank No. 1 was 4.2×10^3 per ml. The B globigii counts from the treated sewage varied from 20 to 310 per ml. Therefore, leakage was occurring at a rate that caused 1 gal of raw sewage to contaminate between 13 and 210 gal of sterilized sewage, or 1 gal into an approximate average of 100 gal of sterilized sewage. The flow rate through the system was 200 gpm; therefore, the flow of the raw sewage into the treated sewage was an average rate of 2 gal per minute. The fact that B globigii was recovered from the sample taken 5 minutes after the start of the experiment proved that the leakage must have occurred in the heat exchangers since 20 minutes is required for the sewage to flow through the entire sterilization system. The E coli recovered from the first and last samples is not surprising since it is during these periods that cold raw sewage was present in the counter-flow heat exchanger system. In view of the possible serious consequence of allowing unsterilized liquid effluent to leave the Post, it was recommended that no more sewage be processed in plant T-314, until repairs were complete. The alternate sewage sterilization plant T-280 was put into operation and was used for processing all sewage which was ordinarily treated at plant T-314. Numerous samples were taken during the first few days of operation of T-280. A number of these samples contained viable organisms although no E coli was present the sterilization system was studied thoroughly by personnel of E and S Divisions and it was noted that several "pockets" were present in the outgoing sewage line. In particular, a bypass line was not being sterilized during the steaming process. A sampling adapter was relocated, dead ends eliminated and the bypass line operated so that it was flushed with steam during the sterilization process. All samples taken subsequent to these changes were sterile.

(6) 1 Jul - 30 Sept 1949 (7)

Building T-314 had to be shut down due to leaks in the old heat exchangers at the rate of about 10 gal. per minute. New heat exchangers were installed in this building and this system was put into temporary operation while operational changes were made in plant T-280. The old heat exchangers were tested and leaks repaired. These exchangers were used for testing and then scrapped for they were deemed no longer safe.

(7) 1 Oct - 31 Dec 1949 (8)

Final tests were made on the interconnecting line which was completed between buildings T-280 and T-314. With this line installed decontamination facilities served the Limited Area more effectively, since either station was able to handle the decontamination in the event of breakdown of one station.

(8) 1 Jul - 30 Sept 1950 (9)

Study of corrosion and scaling problems of sewage decontamination equipment were continued. A recirculating system was installed at Building T-314 which was believed would reduce scaling in the heat exchanger tubes. Research in the use of recirculating system for heat exchangers at decontamination plant T-314 showed that this method had good possibilities of reducing the hazards as well as the high maintenance now encountered.

(9) 1 Oct - 30 Dec 1950 (10)

Scaling, corrosion, and flow systems for sewage treatment were continually being studied in this period. Manufacturer's representatives were contacted to secure information that would lead to the installation of automatic acidity and alkalinity control.

Heat exchangers in decontamination system T-314 were cleaned and placed in operation.

(10) 1 Oct - 31 Dec 1951 (11)

In this period a severe leak developed in one of the heat exchangers of the recirculating sewage decontamination station at Building T-314. Since the effluent sewage proved sterile, the safety of the recirculating system seemed to be established. However, further study to determine the optimum materials and operating procedures for such a system were deemed necessary. A preliminary survey of available tube materials indicated copper as first choice, with chrome iron following. Test

tags of various possible materials were inserted in the existing heat exchangers. The observed variation of pH (daily range from 3.0 to 10.0) of the raw sewage exceeds the corrosion resistance of available tube materials. It was evident that pH control was necessary.

(11) 1 Apr - 30 Jun 1952 (12)

Active study of the problems of liquid waste decontamination was continued throughout the year. A recirculating system which prevents leaks between raw and treated sides of the systems was designed and operated. Tests confirmed the original estimates that this system requires approximately 4 times the exchanger surface required by a simple counter-flow system. The recirculating system showed less scaling as a result of reducing the rate of heat transfer over a period of 3 months' operation. pH adjustment equipment was designed and installed in T-314 at that time.

New copper tube bundles for the heat exchangers at T-314 decontamination unit were installed and the system revised to operate on a counter-flow basis. This increased the capacity from 100 gpm to 200 gpm.

The system was equipped with instruments for a detailed study of operations. This study was used as a guide in the design of the new decontamination station, U-1 and U-2. The counter-flow operation of T-314 was satisfactory. A study of alteration of U-1 and U-2 to operate on the counter-flow system was and is under consideration for increasing its capacity.

On two occasions several gallons of viable B anthracis cultures were discharged to the sewage sterilization plants. Subsequent sampling in plants T-314 and T-280 revealed a contaminated pump shaft, contaminated air filter, and several contaminated valves. The contamination persisted for 2 months in spite of several washings with hypochlorite solution. Several methods for preventing biological leakage from gate valves were being investigated by E Division.

(12) 1 Jul - 30 Sept 1952 (13)

From 24 August to 10 September the heat exchangers at Building T-314 were leaking around the tube sheets. The tubes were rerolled and the system tested. No leaks were detected after this repair.

(13) 1 Oct - 31 Dec 1952 (14)

Continued washings with hypochlorite solution of decontamination plant equipment did not succeed in complete removal of B anthracis. A

number of preventative measures were taken such as placing a well around all pump shafts and valve stems and maintaining 5 per cent NaOH in these liquid seals.

c. DECONTAMINATION PLANT T-280 (Located back of the hangar T-201)

(1) Period before 1946 (15)

Building T-280 was originally designed to batch decontaminate with 4 small 2500 gal. tanks (the same principle as a pressure blow-case). It could not decontaminate all the sewage flowing into it in the 8 hour working period, so storage tanks were placed in the system and it was operated on a 24 hour basis. Disadvantages to the Batch Decontamination System are : (See Figure 14.6)

(1) Small quantity can be treated.
(2) Cost in steam consumption is high.
(3) Great volumes of cooling water are necessary.

When the volume of sewage became so great it could no longer adequately decontaminate all the sewage in 24 hours, a continuous method such as is in existence now had to be designed.

From August 1944 to January 1945, positive samples were detected. B anthracis contamination was detected on 29 January 1945 at which time action to correct the difficulty was started. It was first thought that the system needed complete steaming out. This measure had reduced the nonsterile samples in T-314 from 64 per cent to 10 per cent and in T-509 from 80 per cent to 10 per cent with 2 hours of steaming. The above mentioned remedy was based on the assumption that contamination was through faulty operation, introduced to the clean side of the system, and not by faulty equipment. When positive samples were obtained after the first steaming, it was decided to take measures to be certain the system was sterile. Ten thousand gallons of 2 per cent NaOH was circulated through the system with the usual heating process in use. This treatment was continued for 3 hours. On 9 February 1945 samples were again positive and confirmation tests showed the presence of B anthracis. The system was completely shut down and the "Batch System" put into operation. Subsequent sample testing of the process while in action at various points along the line provided evidence that the last bank of heat exchangers was giving the trouble. The piping of the system was modified to utilize the first bank of heat exchangers while the other bank was dismantled and repaired. By 20 February the heat exchanger bank had been repaired and put back into operation after complete sterilization of the system by steam (310° F for 3 hours). Subsequent results showed that the difficulty was eliminated. As an added safety precaution, basins capable of holding the volume of sewage in the storage tanks were built under the tanks at T-280 so as to prevent loss of any agent should a valve break under any of the

tanks. Basins for the same purpose were constructed later under the storage tanks at T-314 and T-509.

(2) 1 Jan - 30 Mar 1946 (16)

In this period S Division carried on some studies relating to the most efficient method of decontamination of sewage sterilization plants. Sludge and solid material from sumps or sewage tanks have a high chlorine demand and thus a hypochlorite solution is not effective. Treatment of such material with a 5 per cent aqueous solution of NaOH was found to be an effective means of decontamination. A total of 1593 swab samples were taken from the sewage treatment system and B anthracis contamination was detected in 271 of these samples. In all cases the active agent was detected, the location involved was decontaminated, tested, and this treatment continued until no detectable agent remained. Formaldehyde was used as a decontaminant for storage tanks. In testing the storage tanks, the operator went into the tank and took swabs from the various portions of the tank where scale or material had collected. This was scraped off so that samples could be taken from the area underneath such deposits. It was necessary to clean the tanks thoroughly before the germicidal agent (formalin vapor) was effective against the spores of the agent. After thorough decontamination no agent was detected on 115 swab samples taken from the storage tank. Swab samples were collected from the inner surface of the sump tank. In order to take these samples, the operator entered the tank and was thus able to thoroughly sample all surfaces within. Test results showed that all detectable B anthracis contamination was removed from this equipment.

Valves and small pieces of equipment were removed from this system and sterilized in the autoclave at 240 F for 4 hours. Only equipment found to be free of B anthracis was released for reinstallation into the system. All equipment contaminated with active agent was returned to the autoclave and given a second sterilization.

The sludge from the storage tanks was collected in metal cans and mixed with water and NaOH. The final concentration of NaOH was approximately 10 per cent. In sampling this material, the contents of the can was mixed thoroughly and a 20 cc sample was collected with a metal dipper. This sample was transferred into a test tube and taken to the laboratory. Sufficient HCl was added to adjust the H ion concentration to pH 6.6 - 7.0. A portion of the material was then streaked over the surface of a blood agar plate with a transfer loop. At this point the incubation and examination of the plates was carried out in the same manner as that employed with swab samples.

Tests of the moat and the wooden structures around the storage tanks revealed B anthracis contamination. These areas were decontaminated with calcium hypochlorite until swab samples failed to reveal presence of any active agent.

Sixteen soil samples were collected from the area near the storage tanks. These samples were mixed with a saturated solution of sodium thiosulfate and a small portion of the liquid was transferred to a blood agar plate. The inoculated plates were incubated at 37 C for 18 - 24 hours and examined for typical colonies. No B anthracis contamination was detected. Swab samples from buildings of the sewage treatment system revealed some B anthracis contamination. Decontamination of the locations involved removed all detectable agent.

(3) 1 Jun - 30 Sept 1946

By 13 September 1945 treatment plant T-280 had been closed and decontaminated since it was no longer required for the treatment of liquid effluent. A total of 70 swab samples were taken on the inner surfaces of the equipment and no B anthracis contamination was detected.

(4) 1 Oct 1945 - 30 Mar 1948

Due to the constant use of equipment from decontamination station T-280 to replace worn out equipment in T-314, T-280 was no longer equipped to sterilize sewage.

(5) 1 Apr - 30 Jun 1948 (17)

During this period, work was started to replace the equipment which had been taken from T-280 so that T-280 could be placed on a standby basis.

(6) 1 Apr - 30 Jun 1949 (18)

Station T-280 was completed and successful test runs were made beginning 2 June 1949 and ending 8 June 1949. A few minor operational changes were made to insure continuous operation of this station.

(7) 1 Jul - 30 Sept 1949 (19)

Laboratory tests showed that certain undesirable growths were taking place in the sampling adapters at T-280 causing false samples. This was due to improper sterilization before resuming normal sewage decontamination runs.

(8) 1 Oct - 30 Dec 1950

Leaks were detected in the heat exchangers at decontamination plant T-280. New bundles of wrought iron were placed in the heat exchangers instead of the steel which had ordinarily been used. These heat exchanger tubes are still in use today.

d. DECONTAMINATION PLANT T-509 (NNE of the Hospital T-600 to 608)

This station was designed to decontaminate sewage from the hospital area. This flow was small enough that it could be stored in the four 10,000 gallon storage tanks overnight and decontaminated in a 12 hour period. This station had a 40 gpm capacity.

On 29 August 1945, this station was placed on a standby basis. This could be done because of a connecting line between T-509 and T-314. Plant T-509 could drain sewage to plant T-314 in case of failure; however, T-314 could not pump to T-509. In June of 1952 the tanks of T-509 were removed and the station was abandoned. However, a by-pass from the hospital was installed which could be closed to send hospital sewage to the sanitary sewer, or opened to send hospital sewage to decontamination station T-314.

e. DECONTAMINATION PLANT P-375 (began operations in 1953)

Station P-375 (U-1 and U-2) are recirculating type systems with a capacity of 200 gpm each. These plants utilize six 50,000 gallon reinforced storage tanks capable of batch decontamination by heat and pressure. It is specified that sewage in the tanks be brought up to 250 F within 6 hours after the turning on of the steam heaters, but at present it requires 24 hours to raise the temperature of the sewage up to that specified. It has been proposed that larger steam lines be installed to facilitate more rapid heating of sewage in the sewage tanks. This heating of the storage tanks is not only for emergency batch decontamination, but also to facilitate decontamination of the empty tanks when cleaning or repairing is necessary. To date, U-1 and U-2 have not been operated under actual "hot" conditions. Test runs on this station have been completed and temperatures and pressures throughout the system have been recorded (Figure 14.7).

Plant P-375 was tested by personnel of S Division on 27 March 1953. The procedure and results are as follows:

(1) Test of U-1 (20)

Ten liters of a suspension of Bacillus globigii spores (1×10^8 orgs/ml) was poured into a holding tank which contained

30,000 gallons of untreated sewage. The sewage was circulated through a portion of the system for 24 hours to effect mixing, and was then passed through the system in the regular sterilization procedure. Samples were taken in sterile syringes from the treated sewage at half hour intervals from 1000-1430, inclusive, from recirculating water at one hour intervals from 1015 - 1415, inclusive, and from the holding tank at 1000, 1200 and 1400. All samples from treated sewage and recirculating water were inoculated into lactose broth fermentation tubes (10 ml in 20 ml broth) and incubated at 37 C for 3 days. As soon as growth was apparent, subcultures were made onto corn steep agar. 0.1 ml of each sample from the holding tank was inoculated onto corn steep agar plates and incubated at 37 C.

Cultures from holding tank showed B globigii colonies too numerous to count (TNTC) on all plates. The 1000 sample from treated sewage remained sterile. All others showed growth after 24 hours and gas at 48. Subcultures showed no B globigii but a great variety of other organisms. No effort was made at identification.

The 1315 sample of recirculating water was sterile. Samples 1015 and 1115 showed growth at 24 hours. Samples 1215 and 1415 showed growth at 48 hours. No B globigii was isolated.

It is felt that the positive samples obtained were due to a previous incomplete sterilization of the system. If B globigii was present in any of the samples it was masked by extraneous contaminants. The sterility of the first sewage sample is not fully understandable unless it was sterile fluid left over from a previous run. The recirculating water was evidently not highly contaminated.

Sampling adapters on outgoing sewage line have been moved to the supply side of the cutoff valve so that it may be sterilized under pressure in the future. Subsequent samples from U-1 have been sterile and similar tests on U-2 are contemplated.

f. BLOWCASES

A blowcase, as was explained previously, is a small batch decontamination unit which serves to sterilize the sewage effluent from a building or from a section of a building. The blowcase can be operated in one of two ways, depending upon the resistance of the organism in the liquid to be sterilized. (1) For vegetative cells, a temperature of 200 F for one minute is sufficient to produce sterility. (2) For spore forming organisms, a temperature of 250 F for 30 minutes to 1 hour is necessary.

All blowcases can be pressurized to obtain more complete sterilization. Many blowcases, when operated to sterilize effluent containing vegetative cells, may perform this operation automatically.

However, no blowcase at Camp Detrick when operated under pressure is automatic. (See Table 14.1)

(1) Policy for Installation, and Construction Requirements (29)

When a large amount of contaminated material, as from a 60 gallon tank, or from a chamber in which infectious aerosols are regularly and deliberatedly created, is to be dumped into a sewage line, a blowcase shall be installed at that building to reduce the number of viable organisms. Complete sterility need not be achieved.

Blowcases shall be designed for 40 psig working pressure and 300 F.

Blowcases, generally, shall be designed to operate at a temperature of 200 F and one minute retention time. Provision shall be made for batch sterilization.

No blowcase is needed in a building in which the largest unit container is 5 gallons, or in buildings handling plant pathogens.

Blowcases shall be constructed so that the room or pit may be sprayed with decontaminant in event of leakage.

Lines to blowcases shall be all welded steel lines. Diaphragm valves shall be used in such lines.

A highly contaminated, all welded line running to a blowcase shall be suitably marked for easy identification.

Drain lines from blowcases shall have a heat resistant diaphragm valve, such as a "KEL-F," which shall be tested with Freon leak detector prior to installation. The drain line from a blowcase shall not have any other lines tying in except other blowcases. The line shall be all welded steel line and shall go directly to the decontamination station. This line must be able to withstand 40 lbs steam sterilization.

Vent lines from blowcases shall discharge into a vent head which discharges into an incinerator. If no header is available, the vent shall discharge to outside air through 2 layers of 50 FG "Fiberglas," or its equivalent, with the prior approval of the Safety Director.

Sewage holding tanks will vent through 2 layers of 50 FG filters to outside air.

(2) Period before 1946 (21)

One common trouble which occurred in the operation of blowcases was caused by the drainage of hot liquid into the blowcase which

hinders the operation. Ejector efficiency is based upon a thermal difference between the steam and the liquid being removed. Therefore, it was essential that some cooling device be employed to cool the liquid in the blowcase. Provisions for cooling the blowcase liquid were made by the installation of a water spray above the tank.

(3) Dec 1947 (22)

The batch sterilization system which was used to treat all liquid effluent from the cloud testing chamber in T-263 was tested by using B globigii as a simulant. Results of these tests showed that B globigii could be effectively sterilized at 280 F and 30 lbs steam pressure for 30 minutes in the batch sterilization tank.

(4) 1 Oct - 30 Dec 1951 (23)

On 28 December 1951 a test was conducted to determine the sterilizing effectiveness of the waste collector (blowcase) in the reactor room of T-431. Nine gallons of water were run into a reactor tank and approximately 300 gms of dried B globigii spores were added. The tank was then dumped into the waste collector which contained 200 gallons of water. The temperature in the collector was then raised to 250 F and held for 30 minutes. The collector was cooled and 2 samples were taken from the lower sampler adapter by sterile syringes. These samples were inoculated into sterile broth and incubated at 37 C. All samples collected were sterile. On 29 December 1951 the waste collector was tested for leaks. Freon gas was introduced into the collector up to a pressure of 5 psi. Air was then added until the pressure reached 25 psi. All valves, joints, and welds were tested with a halogen leak detector.

Conclusion: The waste collector is considered suitable for sterilization of large batches of waste materials containing spore forming organisms at the temperature and time utilized in the test. It was recommended that it be tested frequently for leaks.

(5) 1 Jan - 31 Mar 1952 (24)

On 14 March 1952, in Building T-431, the reactors and processing tanks containing B anthracis were discharged to the liquid collection tank. The normal operational procedure for sterilizing the contents of the tank was followed. A routine sample was taken from the blowcase and the contents discharged to the sewage sterilization plant. The next day a heavy growth of B anthracis on sample plates indicated that little or no sterilization of the contents of the blowcase had been accomplished. It was not known that the contents of the blowcase had not been sterilized because the automatic temperature recorder had failed to operate. Samples were taken throughout the sewage

sterilization plant and heavy contamination of B anthracis was found in Building T-314 on the valve in the line between the Pilot Plant blowcase and the collection tank. The sewage sterilization plant was operating satisfactory at this time and all sewage passing through the system was sterilized.

Conclusion: The sewage sterilization plant in Building T-314 has pumps, flange connections, and many large gate valves. It is difficult to keep this sytem completely tight. The escape of agent at a valve illustrates why the sterilization plant is used as a second line of defense after an attempt is made to have all liquid effluents sterilized before they reach the decontamination plant.

g. DISPOSAL OF RADIOACTIVE LIQUID WASTES

(1) 1 Apr - 30 Jun 1952

Radiological safety was improved during this period by the installation of four 100 gallon storage tanks for radiological liquid wastes in Building T-270. Also steel carboy containers have been put in use throughout the laboratories to reduce the hazard of storing the radioactive wastes in unprotected carboys (25). The reason for storing these liquids in metal containers is to permit the radioactive material with a relatively short half-life to decompose. Radioactive wastes may be discharged to the sewer when the radiation is less that of 0.1 μc/l. This limit is set up by AEC. After decomposition, it is disposed of in the usual manner.

Further details are given in Section II of this Status Report, "Radiological Safety."

h. WATER SURVEY

(1) 1 Apr - 30 Jun 1952 (26)

In an attempt to decrease the amount of water sent to the sewage decontamination plant, a water survey was made to determine what uncontaminated water from clean building refrigeration units, etc. was being discharged to the contaminated system. It is permissible to discharge such water to the sanitary sewer, thus saving the cost of sterilization. It was estimated that an ultimate reduction amounting to 60,000 gallons per day was accomplished. The steam savings alone from the reduction would amount to $18,250 per year. Periodic surveys of water use are necessary to prevent unnecessary discharge of water to the decontamination plant.

i. TESTS ON SHAFT SEALS

(1) Period before 1946 (27)

Some work was done to determine the most effective type shaft seals for both pumps and valve stems. In this period, 3 main types were investigated. First, the steam insert to the stuffing box was designed not only to control the direction of leakage but also to decontaminate any agent that escaped from the stuffing box via the packing. This method was deemed not too satisfactory because of mechanical difficulty and packing failure. The second method tried was hydraulic water pressure inserted into the stuffing box. It was noted that this water seal reduced contaminated drippage by 50 per cent in decontamination station T-280. The third method considered was hydraulic roccal pressure inserted into the stuffing box. Comparison of the water pressure seal and roccal pressure seal showed roccal to be 50 to 80 per cent effective in reducing contaminated drippage. Later, a carbon seal compound under the trade name of Dura-Seal proved to be superior to any previous packings used. It could endure the steam decontamination where the other packings failed under such treatment.

In the new decontamination plant U-1 and U-2, the pumps have both stuffing boxes and Dura-Seal in which the cooling liquid for the Dura-Seal is roccal thus killing any organism which may leak out. The comminuters in this station have a stuffing box with a sheet metal well which holds a decontaminant that slowly sterilizes any small amount of sewage which may leak out around the shaft.

j. TESTS FOR CROSS CONNECTIONS (28)

Tests for cross connections between sanitary sewers and storm sewers were conducted in July 1950. The results of these tests showed that sanitary sewage conducted into the storm sewer in 2 places. These tests for cross connections in July 1950 indicated the need for conducting additional surveys with dyes to determine whether any cross connection exists between the decontamination plant lines, the sanitary sewer lines and the storm sewer lines.

Personnel in all buildings working with pathogenic material were instructed to pour one bottle of prepared crystal violet dye solution down the floor drain, one down the sink, and one into a toilet, then to flush water down the drain for at least 10 minutes to introduce the dye into the sewer. The results indicate that no cross contaminations existed between the sewer lines of the 29 buildings studied in the Limited Area and the sanitary or storm sewers.

k. DISCUSSION

Since the continuous decontamination of liquid sewage was initiated at Camp Detrick, corrosion and scaling has been the cause

of well over 90 per cent of equipment failure. The equipment affected most severely by corrosion and scale is the heat exchangers.

The tube bundles in the heat exchangers were originally fabricated from steel. The first attempt to reduce scaling and corrosion in the heat exchangers by using a more corrosion resistant material was the installation of wrought iron tube bundles in the heat exchangers at T-280 in 1950. These tubes are still in use today and consequently their value has not been determined as yet.

After testing many metals in the decontamination plant lines at T-314 it was concluded that copper showed much promise in resisting corrosion and scaling. In 1952 copper tube bundles were installed in the counter-flow system at T-314 and have been operating satisfactorily to date.

As a result of scaling in the tubes of the heat exchangers which ultimately developed leaks between the tube side and shell side of the exchangers, research was conducted for a method of heat transfer superior to the counter-flow system. (In the counter-flow system the raw incoming sewage gains a portion of its heat directly from the outgoing sterile sewage). For many years it was believed that the answer to the heat exchanger difficulty was the installation of a re-circulating system. The sewage in a recirculating system gains a portion of its heat from a separate water system. The water system in turn gains its heat from the outgoing sterile sewage (30).

With the building of U-1 and U-2 (P-375) decontamination stations, of the recirculating type, general opinion has reversed itself and it is now established that the counter-flow system has certain economical advantages over the recirculating system. This reversal of opinion is due mainly to the fact that it is necessary to maintain 4 times as many heat exchangers in the recirculating system as in the counter-flow system. (U-1 and U-2 have 24 heat exchangers which cost $250,000.00)

Future decontamination stations will be of the counter-flow type with corrosion-scale resistant tubes such as copper. They will be modified to the extent that a booster pump will be installed near the discharge section of the sterile sewage line, which will permit rigid control of the pressure differential throughout the system. In the event of leakage in the heat exchanger tubes the pressure differential will maintain flow from the sterile side to the contaminated side.

U-1 and U-2 is to be modified into counter-flow systems and the new additions to this station (U1-A and U2-A) are to be of the counter-flow design.

l. RECOMMENDATIONS

(1) Research be instituted to develop a heat sensitive dye which will change color by undergoing an irreversible chemical reaction when subjected to the proper temperature and retention time required to sterile sewage. This would enable rapid detection of leaks in heat exchangers.

(2) Experimental heat exchangers be installed at decontamination plant T-314 which can operate in conjunction with and be isolated from the rest of the system. This would facilitate experimentation with different metals and alloys under actual operating temperatures, pressures, and retention times to secure vital information on materials that will lengthen heat exchanger tube life.

(3) The counter-flow system of decontamination be adopted as the system of choice for the following reasons:

a. It requires only one-fourth the number of heat exchangers as the recirculating system. (Heat exchangers are one of the most costly pieces of equipment in the plant; barring storage tanks).

b. The installation of a booster pump on the discharge line, to maintain higher pressure on the contaminated side of the heat exchangers, may possibly eliminate previous objections to this system.

c. It will tend to standardize this system and thus concentrate the efforts of the personnel to develop and improve this one system instead of dividing efforts between two possible systems.

m. REFERENCES

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(2) S Div and E Div Monthly Report, Jun 47
(3) S Div and E Div QTR, 1 Jul-30 Sep 48
(4) E Div QTR 1 Oct-31 Dec 48
(5) S Div and E Div QTR 1 Jan-31 Mar 49
(6) S Div and E Div QTR 1 Apr-30 Jun 49
(7) E Div QTR 1 Jul-30 Sep 49
(8) E Div QTR 1 Oct-31 Dec 49
(9) E Div QTR 1 Jul-30 Sep 50
(10) E Div QTR 1 Oct-31 Dec 50
(11) E Div QTR 1 Oct-31 Dec 51
(12) E Div and S Div QTR 1 Apr-30 Jun 52
(13) E Div and S Div QTR 1 Jul-30 Sep 52
(14) S Div QTR 1 Oct-31 Dec 52
(15) "Special Report of Leaks and Heat Exchangers in Sewage Treatment Systems T-80." (T-280) Report #42 (E Div)

(16) S Div QTR 1 Jan-31 Mar 46
(17) E Div QTR 1 Apr-30 Jun 48
(18) E Div QTR 1 Apr-30 Jun 49
(19) E Div QTR 1 Jul-30 Sep 49
(20) Test performed by G. G. Gremillion
(21) Special Report #42 (E Div)
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(23) S Div QTR 1 Oct-30 Dec 51
(24) S Div QTR 1 Jan-31 Mar 52
(25) E Div QTR 1 Apr-30 Jun 52
(26) E Div QTR 1 Apr-30 Jun 52
(27) Special Report #42 (E Div)
(28) S Div QTR 1 Jul-30 Sep 50, 1 Oct-1 Dec 50
(29) Design Requirements for Technical Facilities. Camp Detrick. 1 Oct 52
(30) C.D.B.L., S Div Special Report 81, Sewage Decontamination at Camp Detrick - The Process, Its Failure, Detection of Failure, and Recommendations

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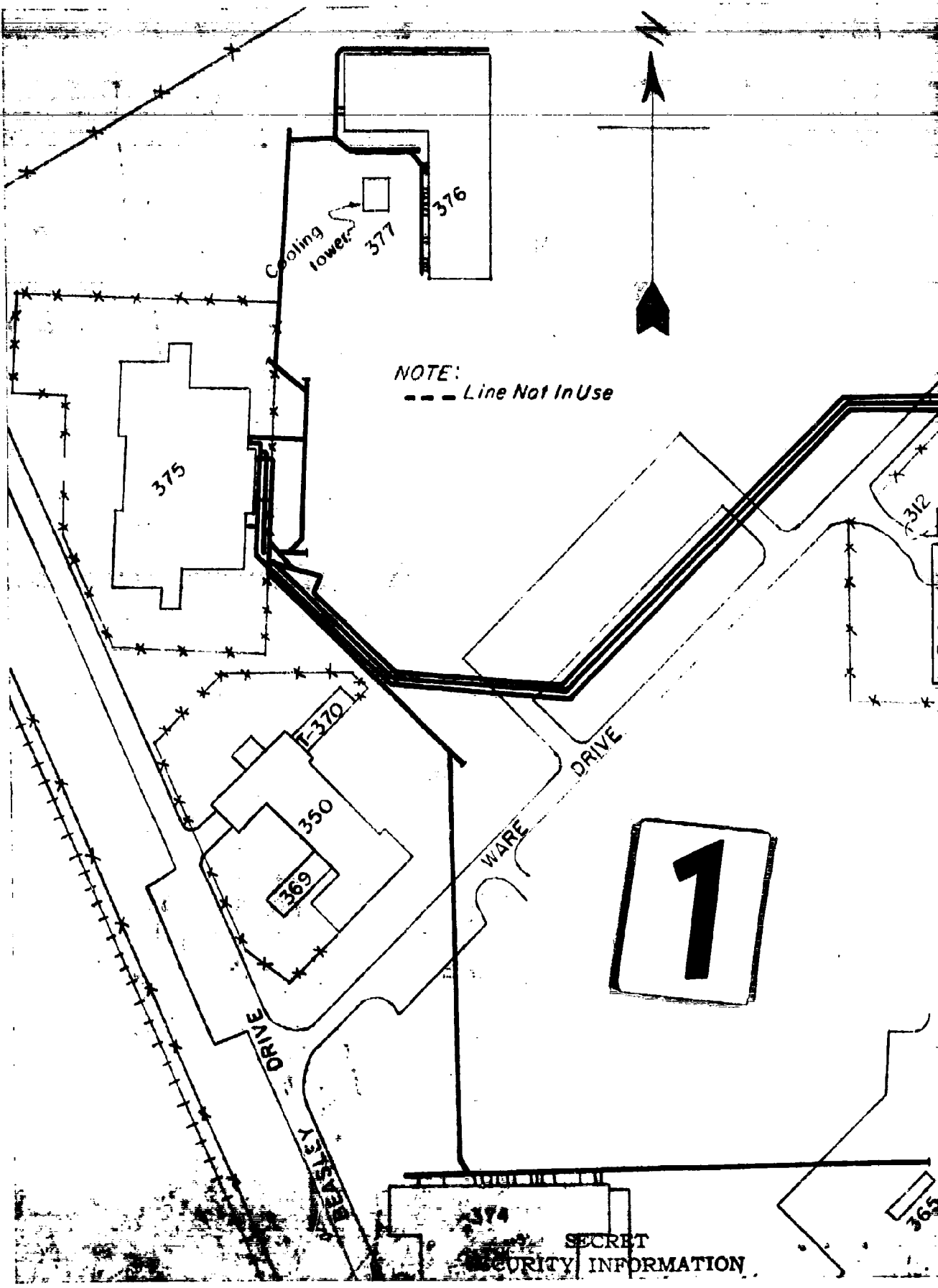
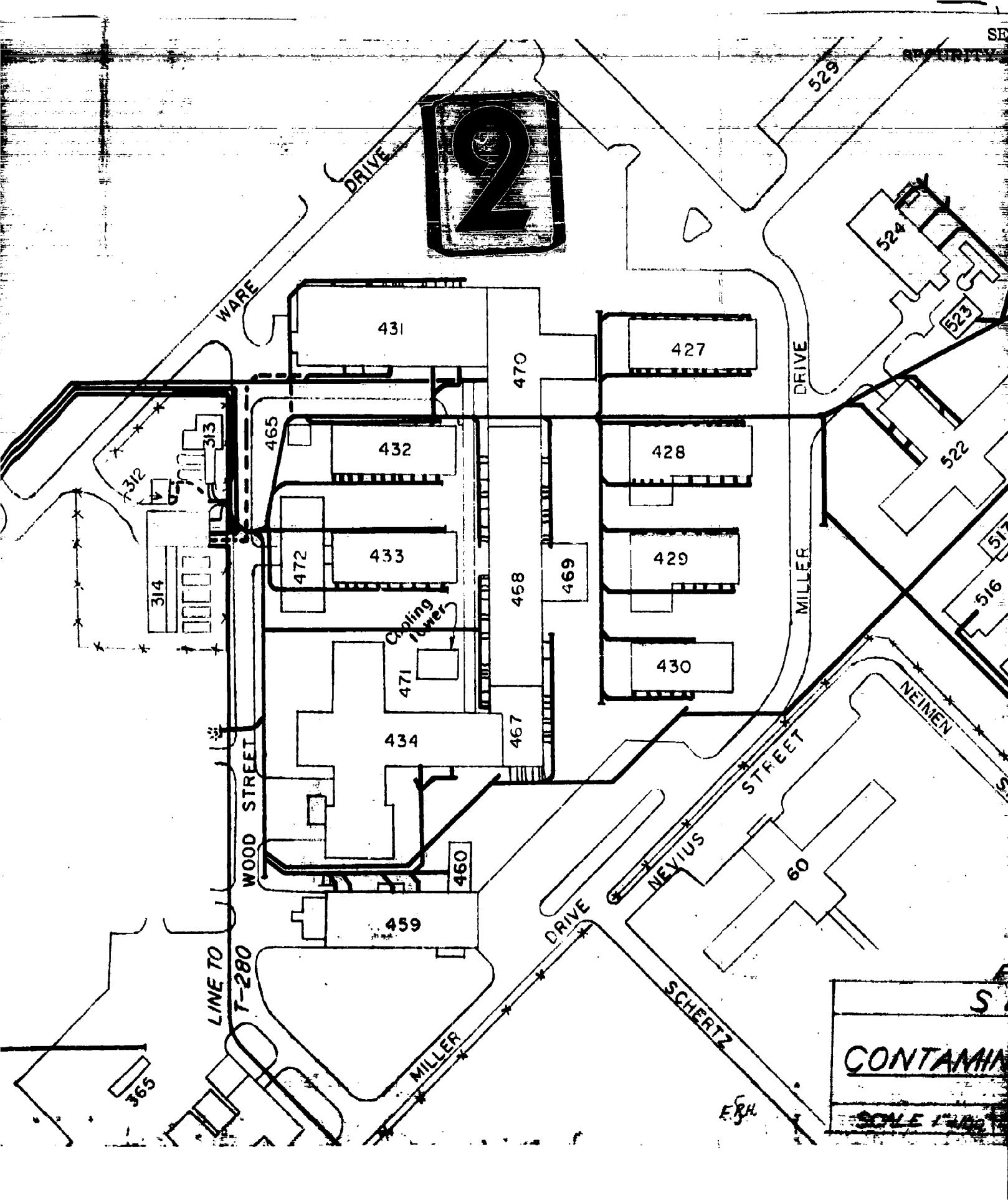
Safety Engineering Section
Agent Control Branch, S Div

TABLE 14.1 LOCATION AND SIZES OF BLOWCASES

Building Number	Number of blowcases	Capacity (gal)	RE Division Drawing Number
T-201 *	1	1000	
T-263 *	1	750	
T-431	3	750	
		400	D-93-1-1428
		2500	
T-431-A	2	500	431-711
		500	"
T-434	1	2500	D-93-1-1403
T-459	1	500	F-93-1-1662
PP-1	2	5000	PP-1-714
		5000	"
PP-2	18	30	B-93-1-2124

* Not used for intended purposes


376
Cooling tower
377
NOTE:
Line Not In Use
375
E-370
350
369
WARE
DRIVE
DRIVE
1
374
365
SECRET
SECURITY INFORMATION


2
DRIVE
WARE
529
524
523
522
516
DRIVE
MILLER
431
427
470
432
428
465
313
312
314
472
433
429
468
469
Cooling Tower
471
430
434
467
WOOD STREET
460
459
NEIMEN
STREET
NEVIUS
60
DRIVE
SCHERTZ
LINE TO T-280
MILLER
365
CONTAMIN

SECRET
SECURITY INFORMATION

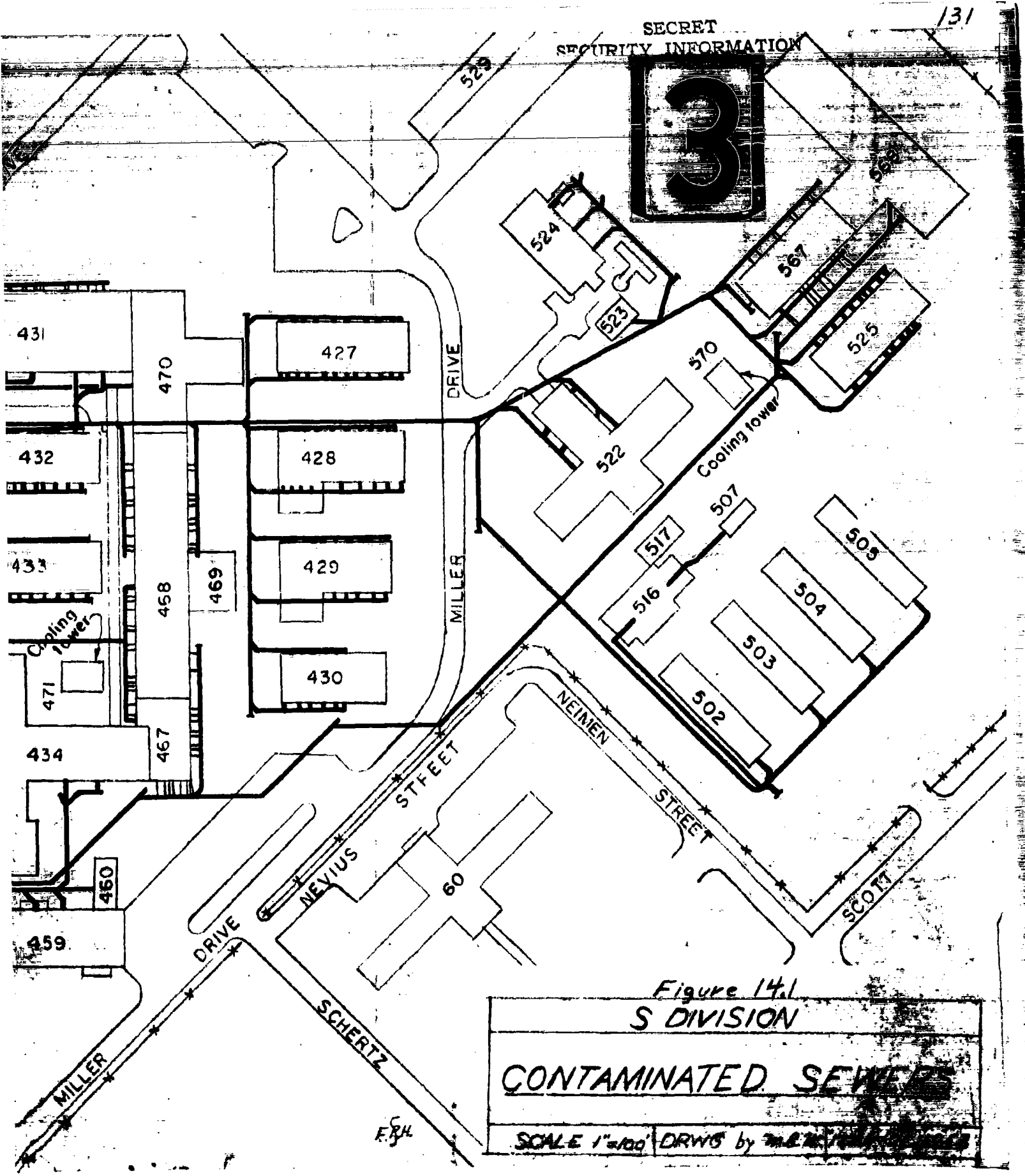


Figure 14.1

S DIVISION

CONTAMINATED SEWERS

SCALE 1"=100' | DRWG by [redacted]

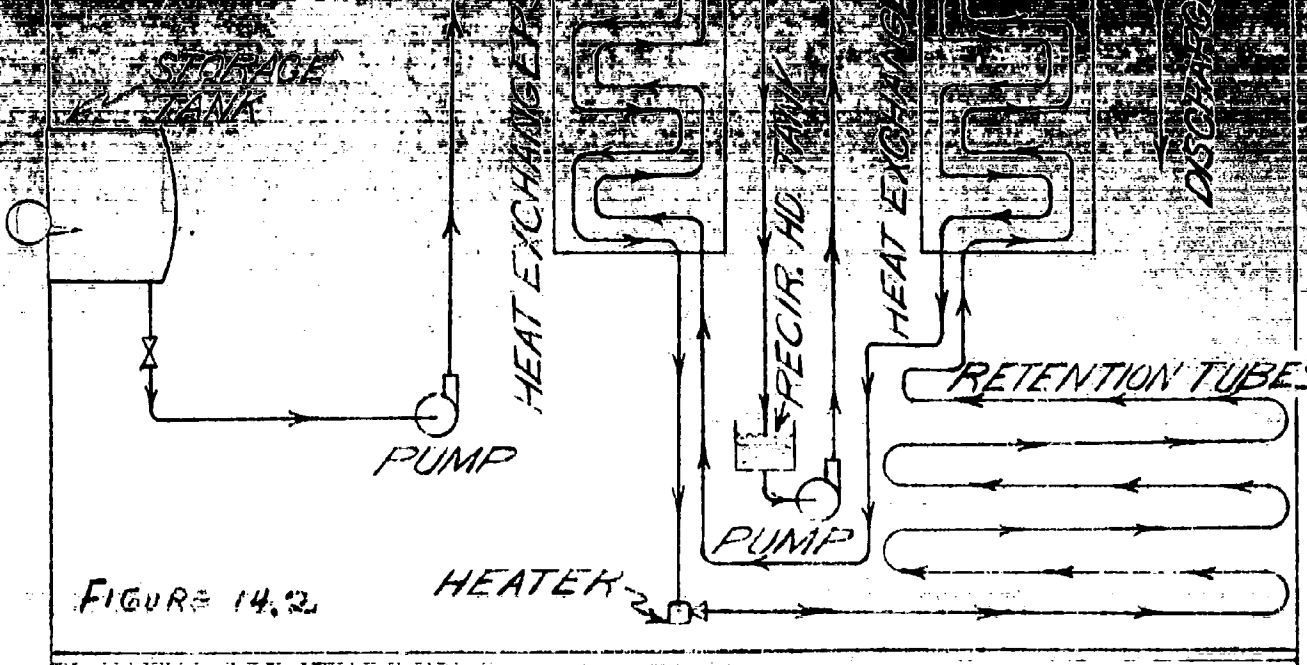


FIGURE 14.2

COUNTER-FLOW SYSTEM

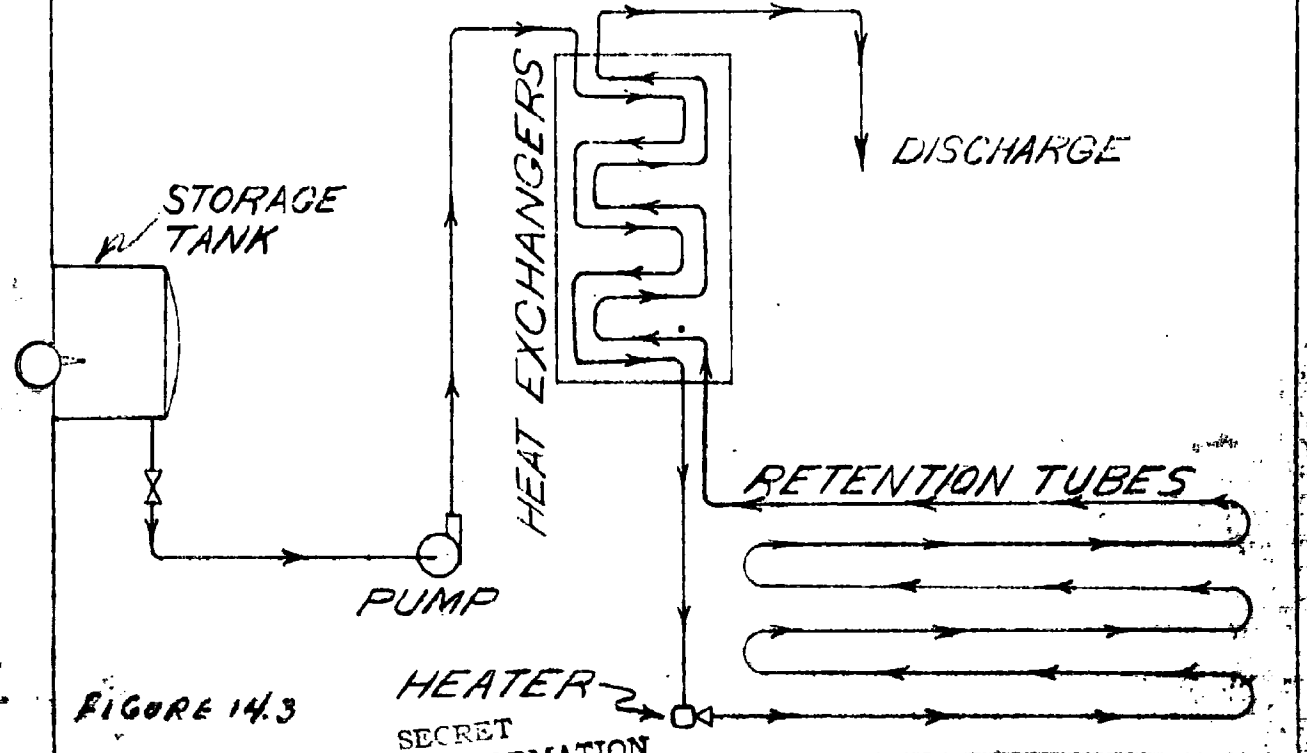


FIGURE 14.3

SECRET

SECURITY INFORMATION

SCALE: NONE	DRWG. by MEC 2d.	20 APR. 1953

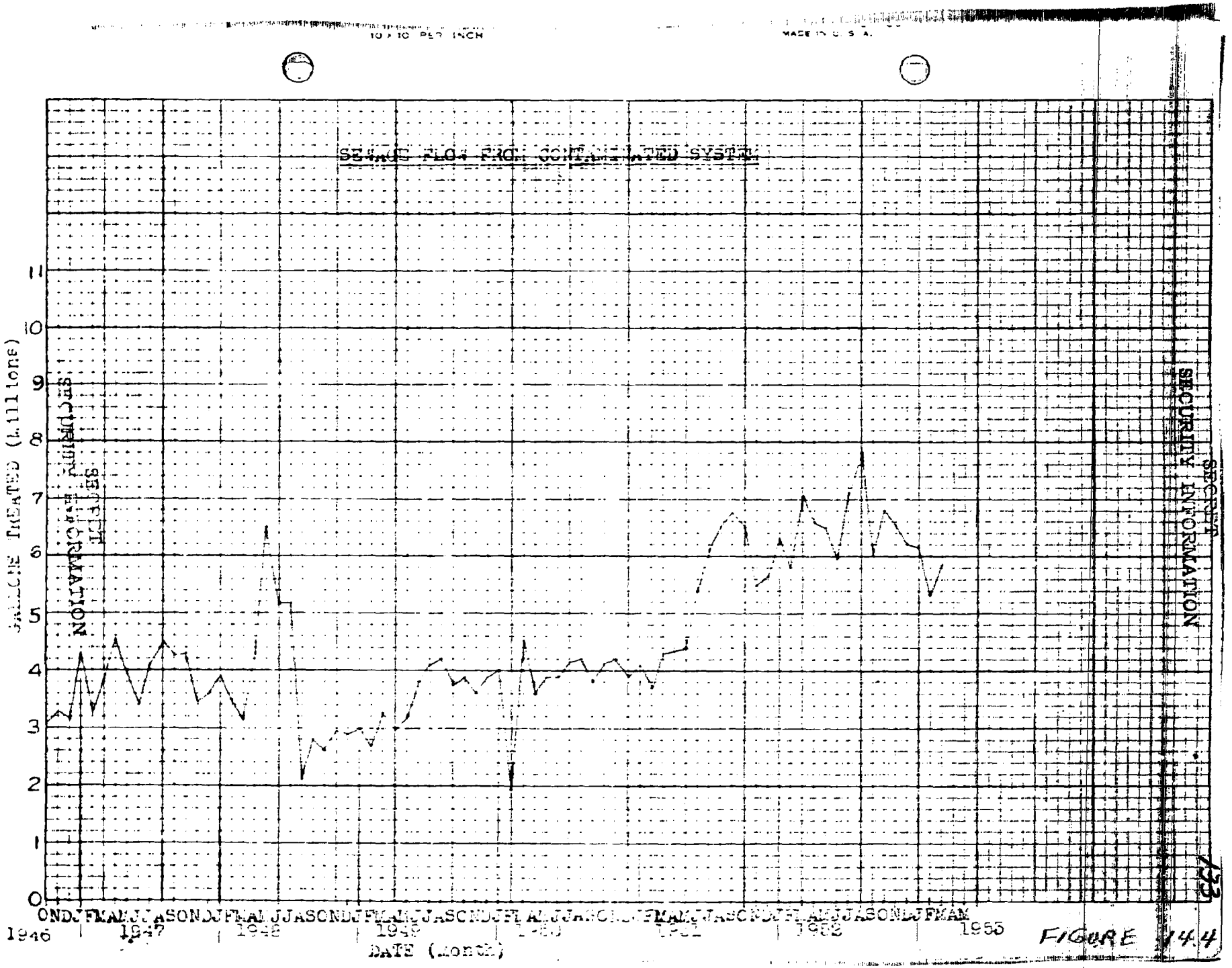
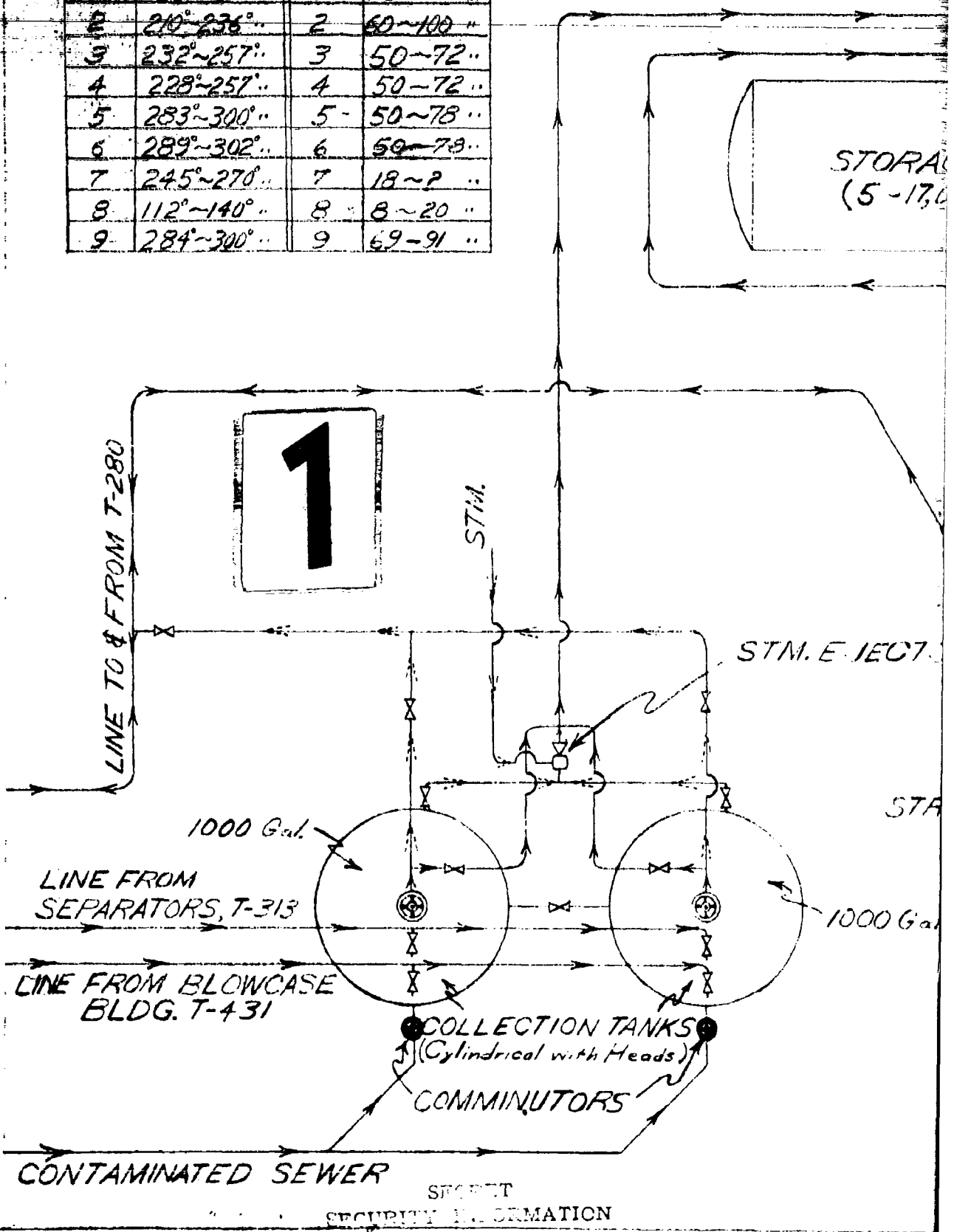

SEEPAGE FLOW FROM CONTAMINATED SYSTEM
GALLONS TREATED (1,111 tons)
0
1
2
3
4
5
6
7
8
9
10
11
SECRET
SECURITY INFORMATION
SECRET
SECURITY INFORMATION
ONDJFMAMJJASONDJFMAMJJASONDJFMAMJJASONDJFMAMJJASONDJFMAMJJASONDJFMAMJJASONDJFMAMJJASONDJFMAM
1946
1947
1948
1949
1950
1951
1952
1953
DATE (Month)

FIGURE 14.4

Operating Temps. and Pressures at Gage Points Shown on Drawing. (Nos. 1 Thru 9)

Gage No.	Temp. Range	Gage No.	Press. Range
1	56°~89°F	1	78~121 lb/in²
2	210°~236° "	2	60~100 "
3	232°~257° "	3	50~72 "
4	228°~257° "	4	50~72 "
5	283°~300° "	5	50~78 "
6	289°~302° "	6	59~79 "
7	245°~270° "	7	18~2 "
8	112°~140° "	8	8~20 "
9	284°~300° "	9	69~91 "



SECRET
SECURITY INFORMATION

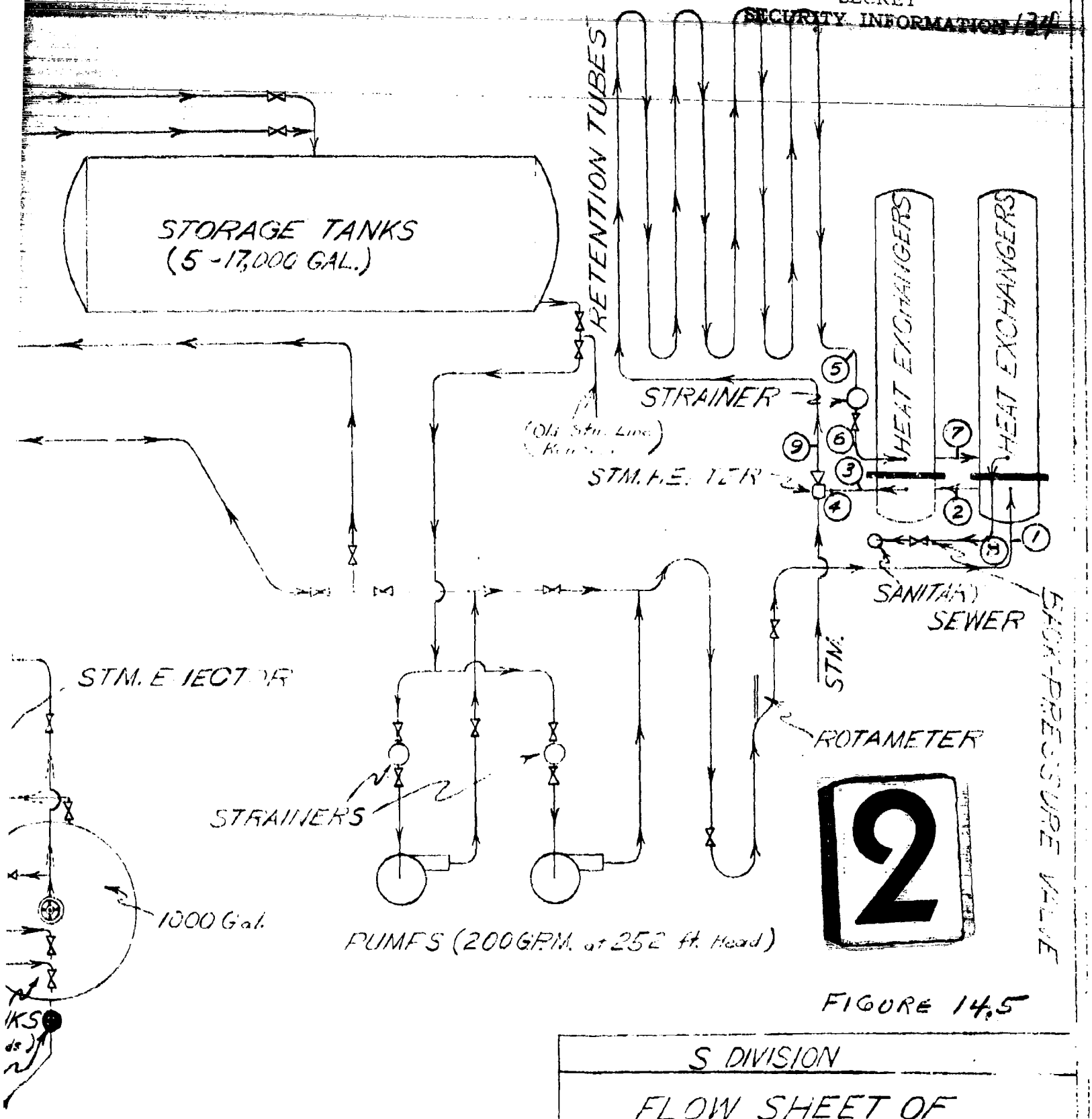


FIGURE 14.5

S DIVISION

FLOW SHEET OF
DECON. SYSTEM T-314

SCALE: NONE	DRWG. by M.E.W.	3 MAR 1953

Operating Temps. & Pressures at Gage Points Shown on Drawing.

Gage No.	Temp. Range (°F)	Gage No.	Pressure Range #/in²
1	285~296°F	5	90~103 #/in²
2	160~180 "	6	25~36 "
3	282~291 "	7	47~58 "
4	207~232 "	8	40~49 "
5	175~190 "	10	45~65 "
6	238~250 "	12	20~? "
9*	90~112 "	13*	132~149 "
		15*	124~144 "
		16*	Used in conjunction with Gage No. 3 to determine Press. Loss across Strainer.

Note:

* Numbers assigned by the drawer + is not that used for identification at the dewax plant.

(1)

(9) BACK PRESSURE VALVE

1

HEAT EXCHANGERS

(5) (6)

HEAT EXCHANGERS

STRAINER

RETENTION TUBES

(16)

(3) (8)

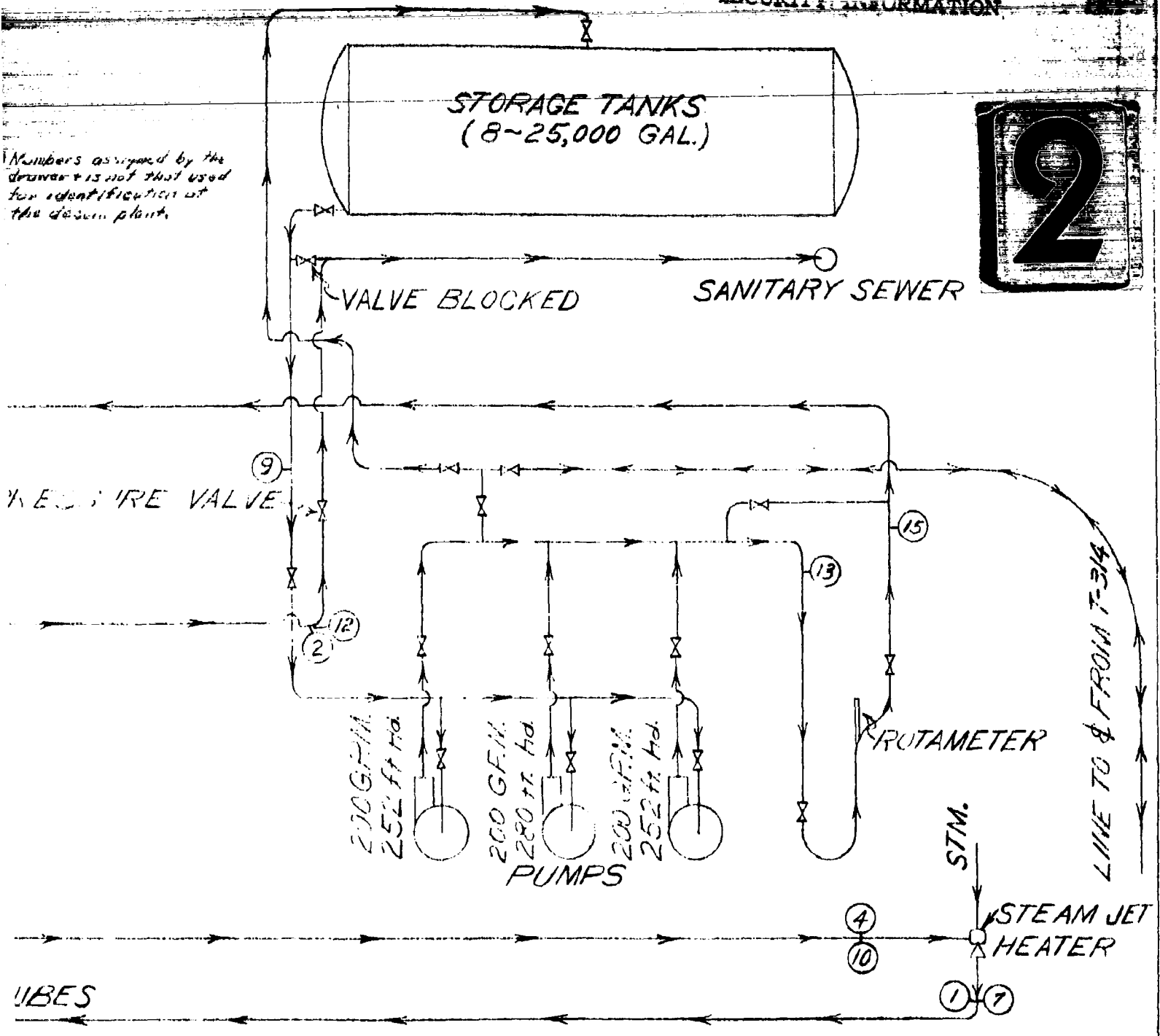


FIGURE 14.6

S DIVISION

FLOW SHEET OF DECON. SYSTEM T-280

SCALE: NONE	DRWG. 6, Mc #	12 MAR. 1953

Operating Temps. and Pressures at Gage Points - As shown on Drawing (Nos. E-1 Thru E-2)

Gage No.	Temperature Range	Gage No.	Pressure Range
T-1	44-49	P-1	125-132
T-2	269-274	P-2	76-82
T-3	297-310	P-3	64-71
T-4	296-303	P-4	62-67
T-5	295-302	P-5	62-66
T-6	76-84	P-6	59-64
T-7	64-68	P-7	35-37
T-8	274-286	P-8	114-115
T-9	62-68	P-9	50-52

Sewage Flow = 100-200 GPM.
Water Flow = 100-200 GPM
Note: Above data secured from trial runs on plant.

STORAGE TANKS (50,000 GAL. EA.)

STM. JET HEATER

RETENTION TUBES

HEAT EXCHANGERS (HEATING BANK)

(COOLING BANK)

CAUSTIC TANK

CAUSTIC FEED

ROTAMETERS

RECIR. PUMPS WASTE PUMPS (200 gpm at 240' Hd. & 230 gpm at 230' Hd.)

WASTE HEAD TANK

RECIR. HEAD TANK

VENT

VENT

OVERFLOW

C.O.

COMMINUTO

LLP #1

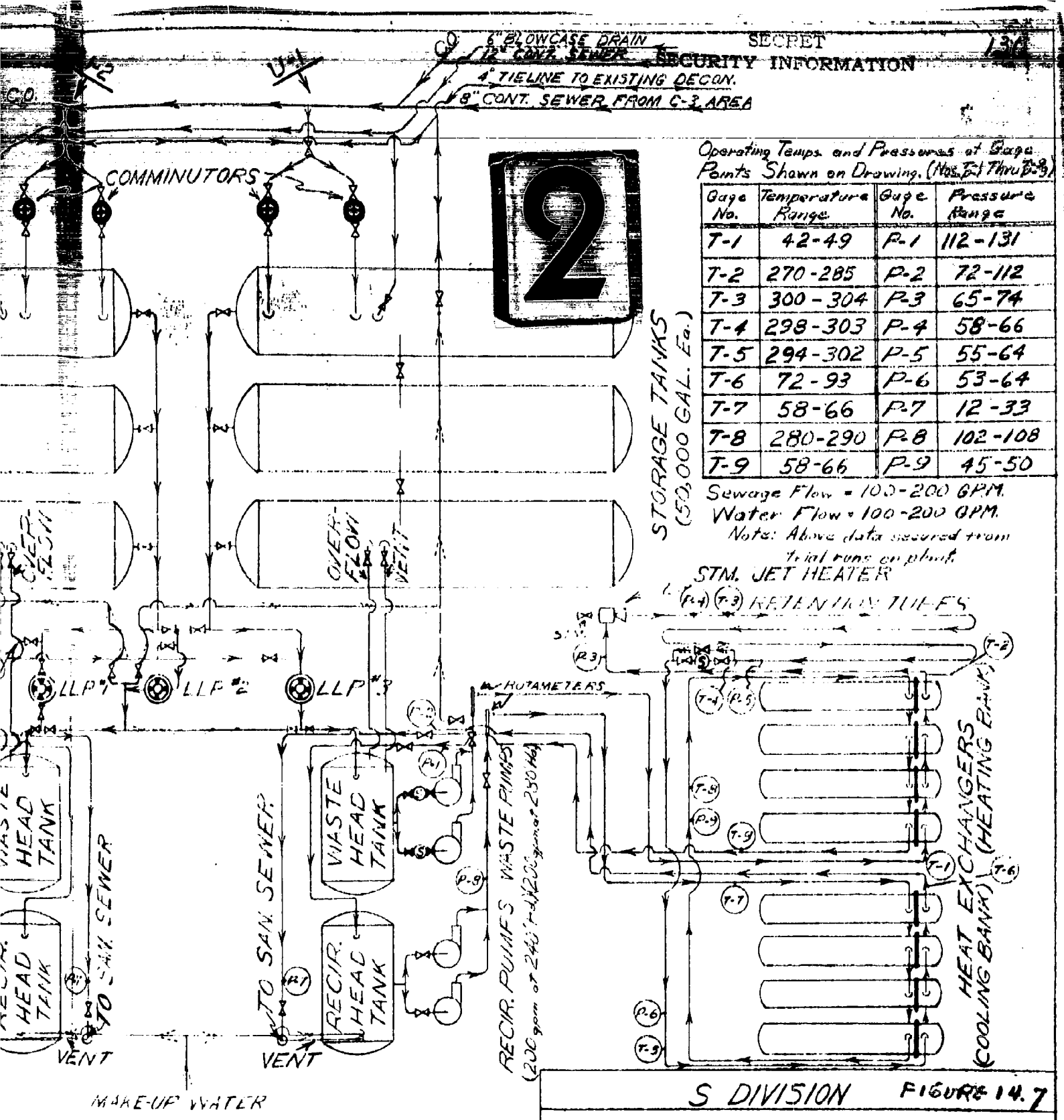
LLP

TO SAN. SEWER

MAKE-UP WAT

NOTES:
S - STRAINER
LLP #1 & LLP #3 - 200 GPM at 40 ft. Hd.
LLP #2 - 200 GPM at 100 ft. Hd.

SECRET
SECURITY INFORMATION



Operating Temps. and Pressures at Gage Points Shown on Drawing. (Nos. T-1 Thru T-9)

Gage No.	Temperature Range	Gage No.	Pressure Range
T-1	42-49	P-1	112-131
T-2	270-285	P-2	72-112
T-3	300-304	P-3	65-74
T-4	298-303	P-4	58-66
T-5	294-302	P-5	55-64
T-6	72-93	P-6	53-64
T-7	58-66	P-7	12-33
T-8	280-290	P-8	102-108
T-9	58-66	P-9	45-50

Sewage Flow = 100-200 GPM.
Water Flow = 100-200 GPM.
Note: Above data secured from trial runs on plant.

S DIVISION FIGURE 14.7

FLOW SHEET OF DECON. SYSTEM T-375

SCALE: NONE | DRWG. by M.E.M. | 15 APR. 1953

15. TEST SPHERE SAFETY

a. INTRODUCTION

(1) Historical

The need for larger chambers for the study of aerosols became apparent as the BW program progressed. Evaluation of munitions and the effect of munitions on the agent aerosol, and other important relationships, could not be studied in the smaller chambers available in Aerobiology Building T-524. A large converted culture tank in Building T-263 was tried out briefly as an aerosol tank in 1948 but was unsatisfactory and its use abandoned. Construction of the Test Sphere, commonly known as the "8-Ball", located in Building T-527 was started about March 1947 and completed on 4 June 1948. From June 1948 until April 1951 only simulant agents were used in the sphere.

(2) Description of the Test Sphere in Building T-527

The sphere is constructed of 1-1/2 inch all welded steel armor plate. It is approximately 40 ft in diameter and has a volume of about 1,000,000 liters, or approximately 36,000 cu ft.

Since its first use with infectious agents the Test Sphere and its auxiliary equipment has been continually modified and improved. Further modifications and additions are presently being planned. The changes have been primarily an effort to mechanize the test facilities as much as possible, to eliminate certain operational variables and to improve safety.

The Test Sphere is equipped with 4 stainless steel work cabinets at the equator level. These cabinets are provided with reach-through, arm-length, rubber gloves and are used for taking aerosol samples and the exposure of test animals. These operations, as well as the firing of test munitions, are directed from a central control room located at this level. A human disinfectant dunk bath for use with the plastic diving suit and an ultraviolet entrance air-lock are also located at this level. A specially designed work cabinet complete with refrigerator, munitions preparation chamber and autoclave is attached to the sphere at the ground level. From this cabinet test devices are assembled, filled and placed in a holder to be raised to the center of the sphere for firing.

A separate air conditioning system for the Test Sphere is used to obtain the desired temperatures and relative humidities. However, this is not a gastight system and the air conditioning must be done while the sphere is "clean". For example, there can be no control of temperature and humidity after the first shoot even when a series of 50 tests is being carried out over a 10 day period. A separation between the sphere and the conditioning ducts is made by automatically controlled steam chests. The

sphere recirculating air conditioning system compressors and blowers are located in the refrigeration room. All air chambers and ducts in this room are sealed with strippable vinyl plastic to prevent cross contamination with the sphere building T-527. In addition, the air in the ducts is treated with high intensity ultraviolet. The office and the change room area are equipped with a separate air conditioning system.

When the inside of the sphere is contaminated, exhaust air from the sphere is taken to the incinerator by means of an axilvane blower which also maintains the sphere under a reduced air pressure. Air can be drawn through the sphere to the incinerator at the rate of 300 cfm by opening an inlet air deep bed fiber glass filter box located at the three-quarter level. After the sphere has been sterilized, air washing can be carried out by means of a 3200 cfm blower which draws the exit air through a deep bed filter and discharges it to the outside. A removable "Top-hat" is provided on the top of the sphere to allow air to enter through a filter during this operation.

An air conditioning system for Building T-527 housing the Test Sphere is located in the utility room and is coated with cocoon to prevent air leakage from the duct to the utility room and is equipped with ultraviolet lights to sterilize the air passing through the recirculating air conditioning system.

Several large steel tanks are located on the ground level in the Sphere Building T-527. These are filled with water or disinfectant solution and, by means of high pressure pumps and water hoses, are used to wash-down the building or the inside of the sphere. A steam-formaldehyde generator is also located on the ground level. Formaldehyde vapor can be discharged into the sphere or, in case of emergency, can be used to decontaminate the entire building.

(3) Description of Building P-567

The construction of Building P-567 was started in March 1951. It is designed to provide laboratory facilities for the Test Sphere in Building T-527. Except for the test tank room and test tanks, which are as yet not completed, the construction was completed in Sept. 1952. Two of the tanks will have a volume of approximately 1790 cu ft and the third a volume of approximately 4060 cu ft. These tanks will provide accessory aerosol test facilities. Since Sept. 1952 some changes in the clean office area and the air conditioning system have been made by Ralph M. Parsons Co. in P-567. This is a two story building with a clean attic and a partial basement. The change rooms and utility rooms are located in the basement. This building is connected to Building T-527 by three ultraviolet air-lock corridors. The first floor of the building is devoted to animal rooms, autopsy room, virology laboratories, and facilities for washing clean animal cages. The main bacteriological laboratories, the safety laboratory, walk-in refrigerators and incubators, the dishwashing room, and the clean office area is located on the second floor. Separation of areas within the

building is maintained through the use of air pressure balance and ultraviolet door barriers and air-locks. Ultraviolet is also provided in all incubators and refrigerators. Twenty-two bacteriological safety cabinets and several special animal transfer cabinets are utilized in the building. All laboratories are provided with compressed air for ventilated personnel hoods.

(4) General Scope and Functions of the Safety Program in Test Sphere Operations

The scope of safety in Test Sphere operations includes supervision of laboratory assessment facilities and munitions trials. Taken together these two comprise a general functional group whose safety direction must be closely coordinated to accomplish a satisfactory biological safety program. There are two basic principles in Test Sphere and assessment facility operations that are important: agent containment, and agent decontamination.

(a) Containment of agent: Agent containment is particularly important in the Test Sphere. Operation of the sphere under contaminated conditions includes: generation of aerosols of infectious agents within the sphere, sampling the air within the sphere, exposing test animals to the aerosol and delivering samples and animals to the laboratory for assessment. During these operations three safety "walls of defense" are used to prevent the escape of agent.

(1) The Test Sphere is held continuously under a reduced pressure equivalent to 1 inch of water. This insures that any leakage will be inward.

(2) The Test Sphere, including all cabinets, gloves, valves, lines, etc., is tested and made Freon tight every 50 trials (1 or 2 weeks). The system must be sterilized before the Freon test.

(3) All personnel in the Test Sphere building wear ventilated personnel hoods or BW gas masks during operations with infectious agents.

(b) Decontamination: Decontamination of the Test Sphere after the use of infectious agents is done with steam and vaporized formaldehyde. Other decontamination safety features used during infectious trials are as follows:

(1) Liberal use of ultraviolet light in air-locks and door barriers.

(2) Treatment of the recirculating building air with ultraviolet.

(3) Use of liquid disinfectant in pass-through dunk baths.

(c) Other measures: Aerosol samples and exposed animals are delivered to Building P-567 for assessment. The main safety practices employed in this building are as follows:

(1) Delivery of samples is made in closed containers.

(2) Delivery of exposed animals is made in Freon tight metal animal transfer cages (8BE-4).

(3) All diluting and plating operations are done in closed ventilated cabinets.

(4) All plate counting is done in open panel ventilated cabinets.

(5) All exposed animals are housed in ventilated animal cages on ultraviolet cage racks.

(6) All autopsy operations are performed in closed ventilated cabinets.

(7) Ultraviolet lights are used in all incubators and refrigerators and in air-locks and door barriers.

The functional scope of safety in Test Sphere operations is all-inclusive. Safety is involved in nearly every phase of work. Engineering changes in existing equipment and the design of new equipment must be coordinated with Safety. Changes in procedure must be tested and approved. Newly installed equipment must be tested. The frequency with which leaks occur in the Test Sphere occurs in proportion to the number and type of trials conducted. For this reason the Safety Representative must be consulted when planning future work schedules. The periodic inspection and testing of ventilated cabinets, air systems and ultraviolet bulbs is an assigned function of Safety Division. In addition, Safety Division is called upon to furnish information concerning proper selection of disinfectants, proper use of carboxoclaves, ventilated personnel hoods, ventilated personnel suits, resistance of various protective coatings to disinfectants, ozone, and ultraviolet and selection of proper types of rubber gloves. Safety training for new personnel is also a vital function of the Safety Representative. Proper safety orientation in the form of Safety films and lectures are particularly important for the non-technical test unit operators employed in the Test Sphere.

In general, the functions of Safety in Test Sphere operations fall into the following broad headings:

(1) Freon testing.

(2) Bacteriological Safety Testing.

(3) Enforcement.

(4) Training.

(5) Inspection and Testing of Safety Equipment.

(6) Miscellaneous Experimental Projects.

The Test Sphere in Building T-527 has been operating intermittently with infectious agents since 10 April 1951. Building P-567 first used infectious agents on 2 December 1953. The effectiveness of the measures employed is attested to by a safety record of only 1 case of tularemia, in a man who accidentally stuck a hypodermic needle into his hand - despite a large volume of work, much of it on a 16-hour a day basis, with agents of brucellosis, tularemia, anthrax, psittacosis, venezuelan equine encephalomyelitis, Q fever and plague.

b. SAFETY TESTING PROGRAM

(1) Safety Testing In Building T-433, Room 3

From April 1951 until the completion of the P-567 building in December 1952 the laboratory assessment work for infectious samples taken in the Test Sphere was done in Room 3 of Building T-433 by a small group of M Division technicians under the supervision of Mr. John Ray and later Miss Rose Lieberman. Exposed animals from the Test Sphere were housed and autopsied in one of several buildings in the Limited Areas according to the infectious agent involved.

During this time periodic safety tests of methods and techniques were conducted (1). A ventilated cabinet was used for diluting operations and the tests showed that all work was done in a safe manner.

(2) Safety Testing in P-567

During July, August and September 1952, bacteriological safety tests were conducted of diluting, plating, and plate counting procedures using S marcescens and B globigii spores as test organisms (2). The results showed that vegetative organisms can be safely contained in open panel ventilated cabinets, provided that certain procedures are followed.

Spore-forming organisms, however, required the use of closed ventilated cabinets for diluting and plating operations. This is true because it is virtually impossible to sterilize a spore contaminated surface with liquid disinfectants. The tests showed that a completely closed system for the handling and delivery of impinger sample bottles is necessary to contain bacterial spores. Open panel ventilated cabinets were shown to be acceptable for the counting of the petri plates. Tests also showed that surfaces such as walls and floors which are moderately contaminated with spores can be thoroughly washed down, first with 0.1 per cent Roccal and then with tap water to effect a 95 per cent reduction in the number of spores present.

Other observations made and results obtained during these tests with the spore simulant were:

(1) The outside of petri dishes taken from the diluting and plating cabinets are usually contaminated unless there is exposure of all surfaces to ultraviolet light in the ultraviolet pass-out box.

(2) The inside surface of ventilated cabinets becomes heavily contaminated when spore diluting and plating is done.

(3) Treatment of the inside of ventilated cabinets with ultraviolet effectively sterilized all exposed surfaces.

(4) Decontamination of the hands of personnel wearing surgeon's gloves reduces but does not eliminate spore contaminants.

(5) Ventilated cabinets can be effectively sterilized by formaldehyde vapors.

Further safety studies with B globigii spores were done in P-567 during the first 3 months of 1953. Routine diluting, plating and animal handling operations were carried out by R.M. Parsons Company personnel, and air and surfaces were sampled to detect any escape of spores. These tests were conducted prior to the introduction of B anthracis into the building on 16 April 1953 and showed that the procedures used were adequate for containment of the spore agent. The installation of ultraviolet lights in the plating laboratories was recommended before full scale operation began with B anthracis.

(3) Animal Handling Methods in Building P-567

Before December 1952, all infectious animals were housed in other buildings in the Limited Areas. Since that date most exposed animals have been housed and autopsied in the animal rooms in P-567. The animal handling methods and equipment used offer little chance for escape of agent.

Exposed animals are received from T-527, via the contaminated corridor and housed in Freon tight ventilated animal transfer cages (8BE-4). No ventilating of the cage is done during the transfer. A special cabinet is employed to facilitate the transfer of animals to ventilated animal holding cages. These cages are placed on racks equipped with ultraviolet light and ventilated continually. Another special cabinet is used to transfer animals to fresh ventilated cages about once a week. Dirty animal cages are placed into a large stainless steel box which is attached to the cabinet. Subsequently, the box is disconnected, closed, rolled down the hall and sterilized in a large autoclave. Ventilated personnel hoods are worn in the animal holding rooms at all times. In the animal autopsy room all operations including animal sacrifice, tissue grinding, plating, etc. are done in closed ventilated cabinets.

Numerous safety tests of procedures and equipment have failed to detect any unsafe equipment or practices. However, as the animal handling capacity increases and a variety of test animals and infectious agents are processed at the same time, certain safety problems are expected to arise. The undesirable features of the all-metal ventilated small animal cages as compared to reinforced plastic ventilated cages are already apparent. The animal transfer cages (8BE-4) are becoming increasingly difficult to keep in proper working order and will probably have to be replaced eventually.

(4) Safety Testing in T-527

Safety testing of the procedures employed in T-527 using S marcescens or B globigii has been done periodically since January 1951. In addition, safety checks have been made from time to time while an infectious agent was in use. Changes in operational procedure, installation of new equipment, revision of existing equipment or the introduction of new agents usually requires approval which is based on the results of these tests. Air and surfaces are bacteriologically sampled, using the sieve sampler and swab technique, to detect the escape of bacteria. Tightness tests on equipment which can be pressurized are usually done using the Freon test technique which is discussed elsewhere.

Safety testing was extensive during January, February and March of 1951, before the first trials with P tularensis in April 1951. Together with numerous Freon tests they constituted the basis on which the Test Sphere was first approved for use with infectious agents (3). Assessment sampling techniques, animal exposure methods and munition filling techniques were approved after suitable safety testing. The liquid disinfectants for vegetative pathogens, all carboxoclaves, autoclaves, and ultraviolet light installations were tested. Sterilization of the Test Sphere with formaldehyde and steam is discussed in a separate section. Five bacteriological leak tests of the Test Sphere were conducted. These aided in correlating the relative accuracy of the bacteriological leak

test and the Freon test. Two complete dress rehearsals using S marcescens with the testing of all procedures were completed before introduction of infectious agents in T-527.

Undoubtedly the tightness of the Test Sphere and its operation under a reduced pressure account for the excellent agent containment. Weak points in the procedure, however, occur when samplers, animals or other materials are introduced into or removed from the sphere. There is the possibility of leaks occurring in the arm-length gloves used in the work cabinets. Nevertheless, safety tests conducted during 1951 and 1952 with S marcescens and vegetative infectious agents have failed to detect escape of bacteria from the sphere or its auxiliary equipment. Occasional failure of rubber gloves has necessitated replacement while an infectious cloud is in the sphere. Using proper precautions, this can be done with a minimum hazard, and when a reduced pressure is maintained, no airborne organisms escape.

During the latter part of 1952 and the first 3 months of 1953, extensive safety testing was done using B globigii spores. These tests were preparatory to the B anthracis trials scheduled to begin in May 1953. The normal presence of B globigii spores in the air and on most surfaces greatly complicated the interpretation of the safety tests. As a result of these simulant tests, the procedures required for B anthracis trials were established. These procedures include:

(1) The use of calcium hypochlorite in all dunk baths.

(2) Placement of articles removed through the dunk baths in carrying cases containing disinfectant.

Dress rehearsal trials with B globigii spores conducted in March 1953 showed that the procedures used were adequate for the containment of spores (4).

(5) Sterilization of the Test Sphere

After assessment of infectious agents in the Test Sphere it is necessary to sterilize the air and all surfaces within the sphere to permit subsequent entrance of personnel for cleaning, maintenance, etc. Although the use of steam-formaldehyde mixtures for sterilization is objectionable because it is difficult to remove all residual traces, no other method has been found. Studies (5) conducted during November 1952 showed that although the number of vegetative organisms in the Test Sphere could be greatly reduced by steaming, air washing, and washing with 0.1 per cent Roccal solution, complete sterility was not obtained.

Numerous tests have been conducted to determine the exposure time required, and the amount of liquid formaldehyde needed to be vaporized to obtain sterility with vegetative and spore-forming organisms. The original formaldehyde vaporizing apparatus attached to the Test Sphere has been replaced with a larger and more efficient vaporizer. It has been shown that deterioration of stored drums of 37 per cent formalin solution occurs and that this may affect the amounts that must be vaporized. Constant supervision and biological safety tests have been employed in Test Sphere sterilization. As far as can be determined no failure in the sterilization of the contaminated Test Sphere has occurred.

S marcescens and B globigii spores have been used as test agents to determine the conditions necessary for sterility. During the first infectious trials in 1951, 3 gallons of 37 per cent formaldehyde were vaporized and an exposure time of 2 hours was found satisfactory for vegetative pathogens (6). Later tests (7) showed that the addition of certain equipment to the Test Sphere and the use of the formalin solution available necessitated the vaporization of larger quantities of formaldehyde. In these tests, with an exposure time of 2 hours, 3 gallons of formaldehyde failed to sterilize S marcescens and B globigii spores, and 6 to 9 gallons effectively killed all S marcescens but failed to kill all B globigii spores. Assay tests on stored 55 gallon drums of 37 per cent formaldehyde showed that deterioration as high as 27 per cent may occur.

On 30 June 1952 further tests were reported (8) which established as a standard the use of 6 gallons of formaldehyde and an exposure time of one hour for vegetative organisms. Tests also reported on this date showed that vaporization of 6 gallons of 37 per cent formalin gave an initial concentration within the sphere of from 4.5 to 7.0 mg of formaldehyde per liter of air followed by a linear type decay which resulted in a concentration of 2.0 mg per liter at the end of one hour.

Subsequent unreported studies with B globigii have shown that spore sterilization can be accomplished with steam and formaldehyde. These studies show that when 320 ml of 20×10^9 spore per ml slurry was disseminated from an exploding type munition, no spores could be recovered from the surfaces or air after treatment with 10 gallons of formaldehyde for 2 hours. Contamination with 640 ml of spore slurry required the use of 15 gallons for 4 hours or 20 gallons for 2 hours. Preliminary tests conducted in April 1953 show that 20 gallons for 2 hours did not eliminate spore contaminants when 3840 ml of slurry was disseminated over a 10 hour period. Further tests are being conducted to determine the conditions necessary for the sterilization of the Test Sphere when contaminated with B anthracis.

(6) Freon Testing of Equipment

The routine tightness test of the Test Sphere and its auxiliary equipment is one of the most important safety control measures used in T-527. Freon 12 gas is injected into the Test Sphere until a pressure equivalent to one inch of water is obtained. Freon 12 is desired rather than Freon 22 because of the reported solubility of neoprene compounds in

Freon 22 (9). Compressed air is then added until a final pressure of 3 inches is reached. Auxiliary lines to be tested may be filled from the Freon mixture in the sphere. If there are no large leaks in the sphere and if all openings have been properly closed the pressure in the Test Sphere should hold constant, showing only slight variations due to temperature changes.

General Electric Type H Halogen leak detectors are used to test all outer surfaces of the sphere for leaks. The sampling unit is held about 1/2 inch from the surface to be tested and is moved along at the rate of several inches per second. This instrument is extremely sensitive and will detect leaks that are not found by other commonly used methods. A leak at the rate of 0.01 oz of Freon per year can be detected (10). The Freon testing method offers definite advantages over a bacteriological leak testing method. Leaks can be spotted immediately, thus eliminating the time required for incubation by the bacteriological method; the exact location of each leak can be determined; and the retesting of a leak after it has been repaired is more easily accomplished.

The frequency with which Freon tests of the sphere are made should be determined according to the frequency with which leaks are expected, the infectious agent being used and the particular munition and trial test procedure being employed. Complete sterilization of the Test Sphere with formaldehyde and steam is required prior to each Freon test. The tests are now conducted by members of Safety Division with the assistance of the Ralph M. Parsons Co. Safety group. Safety Division requires that a Freon test be conducted after each 50 trials with infectious agent or before. In order to facilitate test program scheduling the tests are usually done at night or during weekends.

When Freon leaks are found in the Test Sphere it does not necessarily mean that an escape of agent has occurred. As long as the negative pressure within the sphere is maintained there is very little danger of outward leakage from small pin-hole leaks. However, when the number of pipes, valves, lines, gloves, doors and other equipment involved are considered it is apparent that adequate control can be maintained only by routine leak tests. The effect of all Test Sphere operations is to wear the equipment and cause leaks; liquid disinfectants, water, steam and formaldehyde corrode and deteriorate metal, rubber, and plastic parts. Vibrations caused by the explosion of munitions cause leaks in equipment. Shrapnel from munitions probably has an effect.

The most frequent source of leaks found during Freon tests is the rubber arm-length gloves attached with a liquid seal to the sphere work cabinets. Most leaks are caused by the puncture of the gloves by sharp edges on equipment being handled. Efforts are being made to eliminate such hazardous equipment. The leaking gloves detected are replaced with tested gloves.

The remaining leaks are found in valves, soldered or welded lines, joints, doors, etc. When these occur it is usually necessary to have the leak repaired by a welder or pipe-fitter. After the repair job the leaking equipment must be rechecked. Sometimes several attempts are made before the leak is stopped. The Freon test must have been completed and all leaks repaired before permission to continue work with infectious agents is granted.

Past records of activities in T-527 include the number of Freon tests conducted and the number of leaks found (See Table 15.1). The records show that, on the average, almost 1 mechanical leak and 3 glove leaks are detected per Freon test. Over the 25 month's period since April 1951, the average number of infectious trials per Freon test was 23. Since 1951, the number of leaks has been steadily increasing per infectious trial, per Freon test, and per day the sphere is used in infectious work (Table 15.1). It is to be expected, therefore, that as more and more auxiliary equipment is placed on the Test Sphere, the leak frequency will continue to rise and safety control measures will have to be adjusted accordingly. Sooner or later a major overhaul may be required. This may shut-down infectious operations for sometime.

(7) Freon Testing Diving Suits

Commercially produced Freon gases are fluorinated hydrocarbon derivatives of methane and ethane. They are unusually inert and non-flammable under ordinary conditions. The use of a Freon gas-air mixture has been suggested for use in the ventilated diving suit to provide a means of detecting leaks. This suit was designed and fabricated for M Division by Lobund Memorial Institute to use in conjunction with a human disinfectant dunk bath to provide a means of entering the Test Sphere while it is contaminated. A survey of the available literature concerning the physiological characteristics of Freons was conducted and recommendations governing their use in ventilated suits were made (11).

Only those Freons (Freon 12 and Freon 114) classified under group 6 in the toxicity classification system established by Underwriters' Laboratories were recommended (12). It was further recommended that the Freon concentration used not exceed 0.742 per cent by volume for Freon 12 and 0.662 per cent by volume for Freon 114 at S.T.P.

(8) Sterilization of Impinger Bottles Passed Through a Germicidal Dunk Bath (13)

Liquid impinger bottles used within the Test Sphere become heavily contaminated on the outer surfaces with the organism in use. Tests were conducted during July, August, and September 1952 to determine if spore contaminated bottles from within the Test Sphere could be sterilized by passage through a dunk bath containing calcium hypochlorite. Chlorine concentrations of between 3000 and 800 ppm were maintained in the dunk

baths. Bottles contaminated in the normal manner with B globigii spores were held in the dunk bath from 3 seconds to 5 minutes. After the 5 minute exposure 43 per cent of the bottles were still contaminated. These tests showed that the use of such a germicidal dunk bath was not an acceptable method of decontamination for the removal of articles from an enclosure contaminated with a spore-forming agent.

These tests have subsequently been repeated by Ralph M. Parsons Co. The use of 3000 ppm chlorine solutions and an exposure time of 20 minutes sterilized glass surfaces while 2000 ppm solutions failed. The conclusion reached as a result of these studies is that bottles to be removed from the Test Sphere when contaminated with spore-forming agents should be placed in a separate carrying container which is filled with a calcium hypochlorite solution. An alternative method is needed; one employing disposable plastic bags is under study.

(9) Decontamination of 8BE-4 Animal Transfer Cabinet Necks

Studies were conducted to determine satisfactory methods for the decontamination of the neck of the 8BE-4 animal transfer cabinet. This ventilated animal cabinet is attached to the contaminated Test Sphere to expose animals. After decontamination of the neck area, which is a small closed air-lock, the cabinet is disconnected and used to transfer the exposed animals to animal holding rooms in P-567. The data given here was summarized from S Division QTR, Oct - Dec 1952 (page 50) and Jul - Sept 1952 (page 59).

During October, November, and December 1952 decontamination of the neck area was attempted by Roccal and Clorox solutions, flowing steam and steam with formaldehyde. The tests showed that slight modifications of the neck flange were necessary to permit liquid disinfectants to reach all inner surfaces. Flowing steam for 10 or 15 minute periods successfully sterilized the neck when contaminated with S marcescens. The use of flowing steam against B globigii spores was unsuccessful. The introduction of steam and 1, 4, or 10 ml of 37 per cent formaldehyde sterilized the inner surfaces in the neck when contaminated with B globigii spores, but failed to sterilize the surfaces in the neck channel. When steam is used in the 8BE-4 neck, temperature rises of from 25 to 30 F occur in the adjoining ventilated cabinet section.

Necessary modifications were made and further tests conducted during November 1952. As a result of these tests, the following disinfectants and exposure times were suggested for decontaminating the 8BE-4 neck.

(a) For vegetative agents - 0.1 per cent Roccal or 2 per cent Lysol for 15 minutes.

(b) For spore-forming agents - Hypochlorite solutions (2000 ppm chlorine) plus 0.1 per cent Dupanol C for 1 hour.

c. TRAINING PROGRAM

The introduction of a large scale safety training and orientation program for personnel connected with Test Sphere operations was not necessary until the operations were taken over by Ralph M. Parsons Co. The slow infiltration of new personnel before the work was taken over by this contractor permitted individual training of new personnel. The safety training program was begun during December 1952. Because of the large number and diverse types of persons involved and because of the normal turn-over in personnel, it is desirable to continue this program indefinitely. Such a continued program will provide a means whereby new safety research, equipment and regulations may be brought to the attention of all personnel.

The safety training and orientation program may be outlined as follows:

(1) Reading and explanation of all safety regulations, including the General Biological and Radiological Safety Regulations for the Limited Areas.

(2) Showing and explanation of safety training films.

(3) Lectures on various subjects including ultraviolet light, carboxide, liquid disinfectants, etc.

One handicap in the training program has been that the work schedule in Parsons Company has not permitted large attendance at the lectures and film showings. In addition, personnel work on a 2 shift basis. To facilitate the training program it may be necessary to request attendance on a regularly scheduled basis.

d. ACCIDENTS

During the period October 1952 through March 1953, there were 19 accidents reported in Buildings T-527 and P-567, 15 of which involved possible exposure to infectious agents. Both Ralph M. Parsons and M Division personnel were involved. The accidents involved the following:

Glove ruptures in T-527	5
Breakage of bottles or jars	4
Breakage of contaminated petri plates	3
Punctures of skin with needles	3

On 4 December 1953 an accidental spill of a concentrated slurry of Br suis occurred in Room 206 in Building P-567. A 50 ml serum bottle fitted with a 20×10^9 cell per ml slurry was dropped on the concrete floor. Three persons were in the room. To determine the extent of the aerosol created,

the accident was repeated using a culture of S marcescens. Air samples were taken at different locations in the room. The results (14) showed that the accident produced an aerosol which spread to all parts of the room within 2 minutes and lasted for approximately 17 minutes. The three persons who were exposed left the room immediately and no infections resulted.

Since the introduction of infectious agents in the Test Sphere only one accident resulting in an infection has occurred (15). On 2 February 1953, R.H. an M Division employee inoculated himself accidently in the heel of the left hand with a virulent culture of Pasturella tularensis. The accident occurred at one of the Test Sphere cabinets while R. H. was working through rubber gloves and using a needle and hypodermic syringe to remove an aliquot of culture from a serum bottle. The use of needles and syringes in Test Sphere operations was immediately stopped.

e. SAFETY PERSONNEL

Before December 1952, when operations in T-527 and P-567 were taken over by the contractor, Safety Division directed the M Division safety program in T-527, advised on matters concerning safety and conducted safety tests and Freon tests. One member of Agent Control Branch, Mr. G. Briggs Phillips, was assigned these duties and other personnel in the branch were utilized as needed.

As a result of the Ralph M. Parsons Co. Contract No. DA-18-064-CML-2283 and other subsequent agreements a reorganization of the safety program for T-527 and P-567 occurred (16). According to Army Regulations (17) there can be only one installation-wide safety program. Other regulations (18) require the reporting, and define the status, of "accidents resulting in injury to contractor's employees when the injured is on the installation, or off the installation but on an activity directly associated with the installation . . . . . ". Accordingly, a safety group has been employed by the Ralph M. Parsons Co. to carry on the safety operations of the contractor under the direction and guidance of Safety Division. Mr. G. Briggs Phillips has been appointed as the representative of the Agent Control Branch, S Division, to direct and coordinate the activities of the Parsons Co. safety group and to provide liaison with S Division. Pfc. Herbert Pless has been assigned to assist in these activities.

Mr. Philip C. Harvey has been employed by Parsons Co. as the responsible Safety Officer. Mr. Harvey directs 6 other Parsons Co. safety employees as follows:

1. Bacteriologist
2. Industrial Safety Officer
3. Safety Technicians
1. Clerk-typist

The duties and responsibilities of the Parsons' Safety Officer and the Safety Representative have been defined (16). One laboratory in P-567 and one office in P-568 are shared by the two groups. One clerk-typist (Parsons Co.) has been assigned to Mr. Harvey for use by both groups. The Industrial Safety Officer, Mr. Edward Lawry, is being trained on the job by Mr. Kenneth Hindman, Camp Detrick Safety Inspector. Industrial safety inspections are made jointly by Mr. Hindman and Mr. Lawry.

f. DISCUSSION

The unique nature of the Test Sphere and the operations conducted therein have resulted in a safety program which is all-inclusive. Since nearly all operations present certain hazards there has been a sincere effort on the part of M Division and Ralph M. Parsons Co. to obtain the best possible protection for their workers. Safety is usually considered first in planning new operations or designing new equipment. Full use has been made of the available facilities and personnel of Safety Division and the Safety Division Representative has been kept well informed of all operations.

Records have been kept of all activities in T-527 since April 1951. Five agents have been used on the dates indicated in the following list.

USE OF AGENTS IN T-527

Dates Inclusive	Agent in Use
1951	
10 Apr - 4 May	Pasturella tularensis
7 May - 16 June	Brucella suis
16 Jul - 17 Jul	Pasturella tularensis
18 Jul - 19 Jul	Coxiella burnetii
20 Jul - 3 Aug	Pasturella tularensis
6 Aug - 4 Oct	Brucella suis
5 Oct	Psittacosis
10 Oct	Pasturella pestis, strain A-2
11 Oct	Brucella suis
5 Nov - 30 Nov	Brucella suis
1 Dec	Coxiella burnetii
3 Dec - 7 Dec	Brucella suis
8 Dec	Coxiella burnetii
10 Dec - 14 Dec	Brucella suis

USE OF AGENTS IN T-527 - Cont.

Dates Inclusive	Agent in Use
1952	
19 Jan	Coxiella burnetii
22 Jan - 12 Feb	Brucella suis
13 Feb	Pasturella pestis, Strain A-2
18 Feb - 5 Mar	Brucella suis
6 Mar	Pasturella tularensis
10 Mar - 13 Mar	Brucella suis
14 Mar	Pasturella tularensis
17 Mar - 19 Mar	Brucella suis
20 Mar	Pasturella tularensis
21 Mar - 14 Apr	Brucella suis
15 Apr	Pasturella tularensis
16 Apr - 18 Apr	Brucella suis
21 Apr	Coxiella burnetii
22 Apr	Brucella suis
23 Apr - 25 Apr	Coxiella burnetii
28 Apr - 29 Apr	Psittacosis
30 Apr	Pasturella pestis, Strain A-2
1 May - 2 May	Psittacosis
5 May	Coxiella burnetii
7 May - 16 May	Brucella suis
19 May	Coxiella burnetii
21 May - 2 June	Brucella suis
4 June - 7 June	Coxiella burnetii
13 June - 20 June	Brucella suis
11 Jul - 16 Jul	Pasturella tularensis
17 Jul - 18 Jul	Coxiella burnetii
28 Jul - 1 Aug	Brucella suis
4 Aug - 8 Aug	Pasturella tularensis
18 Aug - 22 Aug	Coxiella burnetii
20 Oct - 24 Oct	Pasturella tularensis
27 Oct - 28 Oct	Coxiella burnetii
2 Dec - 22 Dec	Brucella suis
1953	
7 Jan - 11 Jan	Brucella suis
19 Jan - 13 Mar	Pasturella tularensis

Table 15.1 shows the complete past performance record of activities in T-527 from April 1951 to May 1953. A total of 1511 infectious trials have been conducted on 263 days. A total of 15,289 impinger air samples have been taken and processed and 9114 animals have been exposed to infectious aerosols. Records such as these are helpful in many ways. In case of accidental infection of personnel, past records can be examined to determine what agent was in use and what types of operations were being

performed. The frequency of leaks in the Test Sphere and the type of munition being used help to determine how often the sphere should be sterilized and leak tested.

Br suis has been the agent most frequently used. Seventy-two per cent of all trials were conducted with this agent. Twenty-one per cent of the trials were conducted with P tularensis. The number of trials per decontamination of the sphere, the days of infectious activity per decontamination, and the number of mechanical leaks per day of infectious operations are shown in Table 15.1 to be increasing.

Although the safety control program for the Test Sphere and assessment laboratories has been successful, many problems still exist and future activities will bring forth many new problems.

One of the current problems concerns the cumbersome and time consuming nature of the laboratory techniques employed. These are, in part, the result of the safety equipment and the safety precautions required for such hazardous operations. A recent communication (19) from Safety Division has alluded to the "remarkable safety record" and commended the Division Chief, Branch Chiefs and supervisors for this achievement. Only one laboratory infection has occurred during 25 months of operations. It was pointed out that, "When the number of persons and the time involved in the T-527 and P-567 operations is considered, it seems apparent that any significant facilitation of operations, which can be made without decreasing the safety protection provided, would pay for itself." Detailed time, motion and equipment studies by Ralph M. Parsons Company of all biological procedures were recommended. It will be the duty of the contractor's Safety Officer and the Safety Division Representative to evaluate and approve all changes that are recommended as the result of these studies.

Approximately 279 (1 May 1953) persons in Ralph M. Parsons Co. receive protective immunizations. With an organization as large as this, certain biological safety control problems arise which are not found in smaller organizations. Laboratory and Test Sphere operations are done on a 2-shift basis, thus safety supervision is required 16 hours per day. Often several operations which require safety testing are going on simultaneously. Personal contact with individual workers is lost and the enforcement of safety regulations becomes more difficult.

The present safety control program conducted jointly by the Parsons Safety Officers under the direction of the Safety Division Representative, has been in existence since December 1952. Most of the necessary routine functions have been established. These include:

(1) Safety approval of all work orders
(2) Weekly inspections of T-527 and P-567
(3) Daily records of operations in T-527
(4) Attending supervisor's and foremen's meetings
(5) Showing safety training films and giving safety lectures
(6) Holding weekly meetings of the safety group
(7) Clearance of property

Many future problems regarding safety in P-567 and T-527 are known. To maintain a high level of safety, an aggressive and dynamic type of safety program will be required. Future additions to the Test Sphere are scheduled for 1953 which will require extensive Freon testing and safety testing during construction and before final use. The additions include a sphere air handling system which can be operated under hot conditions, and which includes air washing and filtering mechanisms, a sphere purging system and a blowcase for treatment of contaminated effluents.

The three test tanks in P-567 are scheduled for completion in 1953. In addition to the considerable amount of Freon testing required, other problems involving air handling and tank sterilization are expected to arise. Inadequate change room facilities in T-527 and P-567 are expected to become more acute as operational facilities are expanded. The lack of facilities for storage of clean animals must be solved. The planned use of certain viral agents presents problems in control because of the inadequacy of safety sampling methods.

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6. S Division QTR, Jan-Mar 1951, page 17
7. S Division QTR, Oct-Dec 1951, page 27
8. S Division QTR, Apr-June 1952, page 39
9. The Neoprene Notebook, No. 43, Jul 1949, E.I. DuPont de Nemours & Co. page 251.
10. Type H Leak Detector, Bulletin GEJ-1188B General Electric Co., page 3
11. The Use of Freon Gas in Ventilated Protective Suits, 11 Dec 1951. Letter in S Division files.
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15. Report of Accident in T-527 on 2 Feb 1953. Letter dated 18 Feb 1953. S Division files.
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17. AR-385-10, page 3, section 3.
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TABLE 15.1 PAST PERFORMANCE RECORD OF ACTIVITIES IN T-527
April 1951 to May 1953

Feature	Year 1951	1952	1953	Totals or averages
Days of infectious operation	115	117	31	263
Total number infectious trials	534	781	196	1511
Number of trials with P tularensis	43	113	149	305
Number of trials with Br suis	474	596	47	1117
Number of trials with Coxiella burnetti	12	40	0	52
Number of trials with Psittacosis	5	16	0	21
Number of trials with P pestis, strain A2	0	16	0	16
Per cent of total trials - P tularensis	8	15	76	21
Per cent of total trials - Br suis	89	76	24	72
Per cent of total trials - Coxiella burnetti	2	5	0	4
Per cent of total trials - Psittacosis	1	2	0	2
Per cent of total trials - P pestis strain A2	0	2	0	1
Number of impinger air samples taken	3680	9079	2530	15289
Number of white mice exposed	236	800	0	1036
Number of guinea pigs exposed	1012	5476	1590	8078
Total number of animals exposed	1248	6276	1590	9114
Number of Test Sphere decons	40	33	4	77
Decons per infectious day	0.35	0.28	0.13	0.29
Infectious days per decontamination	2.87	3.54	7.72	3.41
Decontamination per trial	0.075	0.042	0.021	0.05
Trials per decontamination	13.3	23.6	49.0	19.6
Number of Freon tests	29	25	10	64
Freon tests per infectious day	0.25	0.21	0.32	0.24
Infectious days per Freon test	3.9	4.6	3.1	4.1
Freon tests per trial	0.054	0.032	0.051	0.042
Trials per Freon test	18.4	31.2	19.6	23.6
Number of leaking gloves	49	139	11	199
Number of leaking gloves per Freon test	1.7	5.6	1.1	3.1
Number of other leaks (Mechanical)	10	34	15	59
Total number of leaks detected	59	173	26	258
Mechanical leaks per infectious day	0.087	0.29	0.48	0.22
Mechanical leaks per trial	0.019	0.044	0.077	0.039
Mechanical leaks per Freon test	0.34	1.36	1.50	0.92

16. TRANSPORTATION OF INFECTIOUS MATERIALS

a. OFF POST

The interstate shipment of toxic and infectious substances is governed by regulations of the U.S. Department of Agriculture (applicable to diseases of animals) (4), U.S. Public Health Service (applicable to diseases of man) (5), Interstate Commerce Commission (6), Departments of the Army, Navy, and the Air Force (7), Office of the Chief Chemical Officer (8), and Chemical Corps Biological Laboratories (9). Many of these regulations are not entirely applicable to the quantities of materials which are regularly transported from Camp Detrick to various destinations in the United States and abroad. For instance, in the 18 page "List of Explosives and Other Dangerous Articles" in the ICC Regulations, there is no mention of bacteria, biological products, germs, microbes, serums, toxins, or viruses.

Biological products might be considered to be included in ICC Regulations Section 73.326: ".....extremely dangerous poisons, Class A, are poisonous gases or liquids of such nature that a very small amount of gas, vapor of the liquid, mixed with air is dangerous to life," and Section 73.328 "Poisonous gases and liquids not specifically provided for" and Section 73.330 "Chemical Ammunition. (a) Projectiles, shells, bombs, and grenades containing poisonous gases or liquids, Class A, paragraph 73.326 of this part, but not equipped or packed with ignition elements, bursting charges, detonating fuzes, or explosive components, may only be shipped by, for, or to the Departments of the Army, Navy, and Air Force of the United States Government" ....."(b) Chemical ammunition containing poisonous liquids or gases, Class A, must not be offered for transportation by rail express."

Requirements for shipping containers are particularly vague everywhere except in the ICC Regulations. The BAI Regulations require the shipper to submit an outline of proposed procedures; presumably the packaging requirements are then reviewed for adequate safety. Material infectious for man is covered in scant detail and only from the viewpoint of shipping small amounts of specimens of blood, tissue, and stock cultures, in SR 40-410-10, section 9g(3); TM 8-227, pages 323, 329, 593, 633, and paragraph 513 in Chapter 16; TB Med 237.

In 1952 an Operational Procedure for Biological Agent Shipments by Military vehicles and Aircraft (1) was drawn up for the particular use of the Air Force. An effort is now underway to analyze all these directives on transportation and packaging instructions (2) with a view toward simplifying the now cumbersome and extremely expensive procedure outlined in Reference 1.

b. ON POST

The transportation of toxic and infectious substances on the post is governed by Camp Detrick Biological Safety Regulations (3) which state that such substances will be carried in solid bottom containers with tight covers. The transfer of inoculated animals from one building to another is accomplished in the same manner. Animals are transported in closed metal cages or containers which are modified with a bacterial filter to allow access of air to the animals.

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(b) CMLWS (SD-9) dated March 1953, Safety Guide for Transporting Infectious and Toxic Materials.

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17. ULTRAVIOLET LIGHT

a. APPLICATION OF ULTRAVIOLET LIGHT IN THE BIOLOGICAL LABORATORIES

Experiments on the use and application of ultraviolet light as a biological control mechanism for the Biological Laboratories were begun in 1946. This program has been carried on intermittently up to the present time. A special report containing detailed accounts of the entire program to date is now being compiled. The information presented in this report is taken abstractively from the forthcoming special report. Some of the information has also been presented in the S Division QTRs from 1946 to the present.

The ultraviolet developmental program has been divided into 4 main parts:

(1) Collection of pertinent data from existing sources; publications, pamphlets, books and information from bulb manufacturing concerns.

(2) Laboratory experiments to determine the effectiveness of UV in killing microorganisms under a variety of conditions.

(3) Design, development and testing of practical UV installations for infectious units.

(4) Maintenance of UV installations; periodic cleaning and testing of bulbs.

History of Use of Ultraviolet Light

During the past several decades, an enormous amount of experimental evidence showing the bactericidal, viricidal, and fungicidal properties of UV light has accumulated. The most recent information has been the result of the development of better types of artificial UV sources. Many of the earlier UV studies were found to be of little value because of the lack of accurate intensity measurements and the use of non-monochromatic UV. This failure to determine accurate UV intensities during the course of experiments is still a common shortcoming in many experiments reported even now.

The bactericidal nature of sunlight was first observed in 1877. A few years later it was discovered that the bactericidal action was due primarily to the invisible UV component of sunlight. The sun, however, proved to be a rather unstable source of sunlight and arc lamps, glow lamps, and spark lamps for the artificial production of UV were developed. Monochromatic light was sometimes obtained by the use of special filters, but in general these lamps produced radiations in all parts of the spectrum.

It is generally accepted that the UV spectrum includes those radiations in the range of 150 to 4000 angstrom units (150 A to 4000 A). Further arbitrary subdivisions have been made including the Schumann region-wavelengths shorter than 2000 A, the abiotic region-wavelengths which kill or injure cells readily, the antirachitic region-wavelengths which aid in the activation of vitamin D, and the fluorescent region-wavelengths used in photography, etc. to produce fluorescent effects.

Mechanism of Biological Action

Many theories have been advanced to explain the mechanisms of the destructive action of UV on cells. Some have been based on the formation of formaldehyde, hydrogen peroxide, and other germicidal substances. Others have attributed the action to a drying process. Still others have spoken of the production of thermo-labile and thermo-stable poisons. None of these theories has proved to be entirely satisfactory.

Although the exact mode of action of UV is as yet unknown, many of the factors involved have been thoroughly investigated. It appears that the action of UV is photochemical in nature and that oxygen is not required. Also it is significant that the UV wavelengths of maximum absorption by nucleic acid are in the same general range as those wavelengths which exert maximum killing against bacteria, tobacco mosaic virus, paramecia and pin worms eggs. In addition, it is known that an individual organism may differ in its resistivity to UV radiations at different stages in its life cycle and that in a given species, different bacteria at the same stage of the life cycle may vary in their resistivity.

Characteristics of Various Wave Lengths

UV rays in the range of 2000 A are not efficient in their germicidal action because they lack the penetration properties of longer rays and are absorbed to a great extent by air. Experimental evidence conclusively demonstrates that the most effective range for bacteria and most virus strains are the wavelengths from 2250 A to 2650 A. The wavelength of 2600 A is probably the most effective against bacteria, while wavelengths closer to 2250 A are more efficient against the viruses. The effectiveness of 2537 A UV is about 4000 times that of wavelength 3650 A and 10,000 times that of 4047 A. .

Germicidal, low pressure, mercury vapor lamps emit most of their radiation (95 per cent) in the region of 2537 A. Since this wavelength is very close to the most efficient for bacteria and is within the most effective range for viruses, these lamps have almost entirely replaced other artificial UV sources for use against microorganisms.

b. GERMICIDAL EFFECTS OF ULTRAVIOLET

(1) Bacteria

All bacteria appear to be susceptible to the lethal action of ultraviolet. Three types of UV germicidal tests are usually employed; bacteria in air, bacteria on surfaces and bacteria in liquids. Bacteria in air are most susceptible and bacteria in liquid suspensions are the most resistant. Other factors such as clumping and particle size are important.

Various genera of bacteria show differences in resistivity to ultraviolet. The presence of pigment or fat globules generally increases resistance. It is desirable to know what dosage of ultraviolet are required to inactivate various bacteria under various conditions. Detailed information has been gathered from available sources and studies have been conducted in Safety Division (14, page 2). The latter studies were made principally with S marcescens, E coli and spores of B globigii under a variety of conditions. Composite lists show the ultraviolet intensities required for inactivation of many bacteria, including many pathogens.

The ET (intensity x time) value necessary to kill 99.99 per cent of 4.9×10^7 S marcescens cells suspended in distilled water 0.5 cm deep was about 250. Complete inactivation of around 200 E coli or S marcescens cells on an agar surface required an ET of approximately 150. Spore forming bacteria, B globigii and B cereus, required from 3.3 to 6.1 times as much energy for inactivation as did vegetative organisms. An ET of 3200 was necessary for the inactivation of B globigii spores in distilled water 0.5 cm deep. When the spores were suspended on surfaces such as agar, glass and stainless steel ET values from 532 to 800 were required for complete kill.

Tests in laboratory rooms equipped with ceiling ultraviolet lamps showed that irradiation of the room for one hour affected an average reduction in the bacterial air flora of 86 per cent. Some workers believe that airborne pathogenic bacteria can be killed with 1/10 the radiation necessary to inactivate the same bacteria on agar plates.

(2) Viruses

The susceptibility of the T-3 E coli bacteriophage was experimentally established in the S Division laboratories. On agar surfaces an ET value of 320 gave 99.99 per cent kill of the particles. Airborne phage were found to be inactivated quickly and completely in an experimental test chamber (9, page 15). The susceptibility of a variety of viruses has been reported in the literature. These include cholera bacteriophage, staphylocacci phage, dysentery phage, vaccinia virus, foot and mouth disease virus, herpes virus, influenza virus, tobacco mosaic virus, tomato bushy stunt virus, and encephalitis.

(3) Molds and Yeasts

Molds and yeasts are from 10 to 1000 times as resistant to the lethal action of ultraviolet as are vegetative bacteria. At certain stages in the life cycle some fungi appear to be more susceptible to ultraviolet rays. Fungus spores containing cellular pigments are more resistant than those without pigment. Molds spores are probably about 2 to 10 times as resistant to ultraviolet as are bacterial spores. A total ET of approximately 440 is required to inhibit completely the growth of Penicillium roqueforti spores (green), on agar, while the same figures of Aspergillus niger spores (black), Rhizopus nigricans spores (black), and Oospora lactis (white) are 5500, 3666, and 183.

(4) Bacterial Toxins

The use of ultraviolet to inactivate or neutralize bacterial toxins has not been very successful. Inactivation occurs only after long exposures to high intensity ultraviolet. Shigella dysentery toxin, for instance, is 120 times more resistant to the inactivation by ultraviolet than vegetative bacteria.

c. FACTORS AFFECTING THE BIOLOGICAL ACTION OF ULTRAVIOLET

(1) Temperature

The average temperature coefficient of the bactericidal reaction of ultraviolet is around 1.0, indicating that the reaction is physical or photo-chemical rather than chemical in nature. Within the range of temperatures commonly encountered in laboratories, the temperature of the air does not seem to affect the resistivity of microorganism.

(2) pH

Bacteria suspended in a medium of pH 4.5 to 7.5 appear to be more resistant to ultraviolet than those suspended at pH 9 to 10 or at pH 2.

(3) Age of Culture

Resistance to destruction by ultraviolet radiations increase with the age of the culture.

(4) Relative Humidity

Low relative humidities favor the germicidal action of ultraviolet. There is no appreciable change in the resistivity of bacteria below 35 per cent relative humidity. At relative humidities above 60 per cent the bactericidal effectiveness of ultraviolet diminishes.

(5) Irradiation of Media

Irradiation of the agar surface of petri plates to an intensity of 85 microwatts per sq cm for as long as 4 hours has no effect on the subsequent ability of the agar to support the growth of test bacteria.

(6) Heat Sensitivity

Cells which have been exposed to sublethal doses of ultraviolet are more sensitive to heat treatment than unirradiated cells. This phenomenon has been noted with protein solutions, bacteria, yeasts and tobacco mosaic virus. The thermal death of irradiated bacteria occurs at a lower temperature or upon a shorter exposure than unirradiated cells of the same strain.

(7) Photoreactivation

Under certain conditions cells which have been made nonviable by exposure to ultraviolet can be reactivated by exposure to white light. The capacity for photoreactivation disappears after a 2 to 3 hour storage in the dark. Cells exposed to high concentrations of ultraviolet cannot be reactivated. It is doubtful if this phenomenon has any real application in the practical use of ultraviolet where generous dosage of germicidal energy are applied in the presence of visible light.

d. TYPES OF LOW PRESSURE ULTRAVIOLET LAMPS

Three types of low pressure, mercury vapor germicidal, ultraviolet lamps are in general use today. These lamps were made possible through the introduction of low cost ultraviolet transmitting glass (Vycor 970).

(1) Hot Cathode Lamp

The operation of this lamp is similar to that of the standard fluorescent lamp. It operates at low voltage from a ballast and requires a glow switch to preheat the electrodes in order to start the lamp. The life of the lamp is governed by the life of the electrodes, whose life in turn depends on the frequency of starts. The lamp should not be used in refrigerators. At one meter from the 30 watt bulb the ultraviolet output is 72 - 80 microwatts per sq cm. The rated life is 2500 hours.

(2) Cold Cathode Lamp

This lamp utilizes sturdy cylindrical electrodes and operates from a transformer at a high voltage. No preheating of the electrodes is required and instantaneous starting is accomplished. The electrodes

operate "cold" and seldom wear out, consequently the life of the lamp depends mainly upon the ability of the glass tube to transmit the ultraviolet. These lamps can be operated at refrigerator temperatures. Lamps designed for high or low ozone production are available. The cold cathode 17 watt bulb has a rated life of 12,000 hours and the ultraviolet output is approximately 46 microwatts per sq cm at 1 meter from the bulb.

(3) High Intensity Lamp

The so-called "Slimline" lamp has characteristics in common with both the hot and cold cathode lamps. The electrodes start "cold" and become "hot" after the start. The life of the lamp depends upon the life of the electrodes and the frequency of starts. A high ultraviolet output is obtained and the lamps may be operated at several levels of amperage consumption. Per bulb watt, the rated ultraviolet output is 137 per cent of that obtained with hot cathode lamps. The rated life is 2500 hours and when the operating current is 300 milliampers the ultraviolet intensity at 1 meter is 110 microwatts per sq cm. This bulb is used mainly for the treatment of air in ducts and in air conditioning systems.

Explosion proof ultraviolet fixtures are available for use in areas which are considered hazardous due to the presence of flammable vapors, gases, or combustible dusts. These fixtures should not be used unless it is absolutely necessary. In addition to the high initial cost of the fixtures, the ultraviolet output of the bulbs is lowered because of absorbtion by the Vycor glass housing.

Although it is not generally known, most artificial sources of "white" light are weak sources of ultraviolet. The glass walls of incandescent lamps permit the passage of ultraviolet in the range of 2800 A to 3800 A. The total ultraviolet radiation of less than 3800 A emitted by a 1000-watt photoflood lamp is approximately 3 watts. The fluorescent lamp is nothing more than an ultraviolet lamp which utilizes a special glass envelope coated on the inside with fluorescent powders. Ultraviolet is produced inside the lamp and used to excite the fluorescent powders which produce the "white" light. The ultraviolet output of fluorescent lamps per foot candle of white light appears to be proportionally more than that from direct sunlight. It is obvious that unless all culture work is done entirely in the dark or unless special visible light sources are used, small amounts of ultraviolet radiation are apt to be present in any biological laboratory.

e. TRANSMISSION OF ULTRAVIOLET

In general, most surfaces including glass, plastics, paper, etc. are incapable of transmitting ultraviolet of the 2527A wavelength band. Laboratory glassware (soft glass or pyrex) does not transmit any of the

2527A band. The longer wavelengths, whose bactericidal effectiveness is greatly reduced, will pass through glass and plastics. A sheet of pyrex glass 3 mm thick will transmit 21 per cent of wavelength 3022A and 89 per cent of wavelength 3650A.

Although it does not transmit ultraviolet, some plastics such as Plexiglass (Rohm and Haas Co.), an acrylic type thermoplast, used for windows in some special safety cabinets, are discolored and made brittle enough to form fine surface cracks under tension, when subjected to ultraviolet for a long time (9, page 14).

f. REFLECTANCE OF ULTRAVIOLET

All radiant energy is reflected to some extent from surfaces and the amount reflected depends upon the type of surface as well as the wavelength of the rays. Aluminum metals and paints are the best reflectors of ultraviolet (40 to 88 per cent). Stainless steel is a relatively poor reflector of ultraviolet (20 to 30 per cent) while oil paints, water soluble paints and white procelain enamel give even lower values (4.7 to 12 per cent). White wall plaster has reflectance values on the order of 40 to 60 per cent for 2537A ultraviolet. In the use of ultraviolet for the control of infectious hazards, aluminum paint or metal should be used if a high degree of reflectance is desired (air lock, door barriers, etc) and oil or water paints should be used if the reflectance should be minimized.

g. PRODUCTION OF OZONE

The principal portion of the ultraviolet spectrum responsible for ozone production lies between 1000A and 2000A, with a maximum at 1850A. The maximum allowable limit of ozone in air for continuous breathing is between 10 and 50 parts per 100 million parts of air (0.1 to 0.5 ppm).

Oxone is present in the earth's atmosphere in a concentration of about 1 part in 100 million parts of air (0.01 ppm).

All low pressure, mercury vapor ultraviolet lamps produce some ozone. Only low ozone producing bulbs should be used in biological laboratories. The amount of ozone produced by ultraviolet lamps decreases after the first 50 to 100 hours of use. If there is the slightest degree of ventilation there is usually no difficulty in keeping the ozone concentration in the air adjacent to ultraviolet installations to less than 0.1 ppm, particularly since the ozone decomposes rapidly anyway.

A person with a good sense of smell can detect as little as 0.01 ppm of ozone by volume in air. There is some disagreement concerning the effects of concentrations of ozone of 0.1 ppm and higher on man,

since it has been found that many of the earlier toxicity tests were done using ozone containing high concentrations of nitrogen oxides. Later tests have indicated that concentrations as high as 2.5 ppm can be tolerated for long periods of time.

It is significant that ozone is decomposed by ultraviolet. Also the production of ozone by ultraviolet bulbs decreases as the relative humidity increases. The use of ozone as a germicidal gas probably requires the presence of moisture. The concentrations of ozone produced by ultraviolet lamps do not kill dry airborne organisms.

h. MEASUREMENT OF ULTRAVIOLET

The intensity of germicidal ultraviolet is most conveniently expressed in terms of microwatts per sq cm. This is a measurement of power. Total ultraviolet dosage is calculated as the product of the power, the exposure surface (1 sq cm) and the exposure time, thus giving microwatt minutes per sq cm. This term represents the total energy incident upon a sq cm of surface and for simplicity may be designated as the ET (intensity x time) value.

The measurement of germicidal ultraviolet is simplified because most of the rays are concentrated in the 2537A band. Any radiant energy measuring device that will compensate for visible radiations and for ultraviolet longer than about 3000A can be used. The simplest type of meter consists of a foot candle meter with an attachment made by placing a thin layer of fluorescent material between a sheet of quartz and a sheet of glass. The glass is unable to pass the ultraviolet rays; therefore, the difference in the fluorescence produced when the radiant energy passes through the quartz and through the glass is a relative measure of the ultraviolet. Such meters are standardized so as to give readings in microwatts per sq cm. The Westinghouse SM-600 meter is of this general type and is made so that the meter can be held directly against an ultraviolet bulb to obtain an intensity reading. This is the meter of choice for routine testing of ultraviolet bulbs.

Other types of meters are made which utilize sensitive phototubes. The Westinghouse WL-775 ultraviolet phototube, for instance, has a maximum response at 2400A and the upper and lower limits are 3000A and 2000A respectively. A suitable amplification system is employed to measure the current produced by the ultraviolet in the phototube. Meters of this type should be used when accurate intensity measurements are desired.

i. DEVELOPMENT OF PRACTICAL ULTRAVIOLET INSTALLATIONS

Most of the effort in the ultraviolet experimental program has been directed toward the design and testing of practical and usable ultraviolet installations for use in infectious units. The improper use of ultraviolet must be avoided because it leads to a false sense

of safety. The proper use of ultraviolet involves knowing how much ultraviolet to use, where it should be applied, how long it should be applied, what the personnel hazards are, and how the ultraviolet bulbs should be maintained.

Each device or use of ultraviolet has been termed an installation, e.g. ultraviolet door barriers, ultraviolet discard clothing bag holder, ultraviolet air lock, etc. Each type of installation has been tested and standardized. Practical bacteriological tests using simulant test organisms were done. Ultraviolet intensity measurements were made and the ultraviolet intensities of the installation adjusted to obtain the maximum germicidal effectiveness. Finally the standard conditions such as the intensities required, the types of ultraviolet bulbs required, and the types of reflectors and switches required were set forth.

Germicidal ultraviolet has been used in industry, hospitals, schools, etc. in a number of different ways. Louvered ultraviolet fixtures may be placed on the walls of occupied rooms to irradiate the upper strata. Bare ceiling bulbs may be used in areas when the areas are not occupied. Ultraviolet is used to some extent in hospital operating rooms. The industrial applications in bakeries, distilleries, bottling plants, pharmeceutical houses, etc. are many. Many special devices using ultraviolet have been reported. Some have gained wide usage while others have proved to be impractical. These devices include:

(1) Devices for irradiating vaccines
(2) Devices for sterilizing milk and water
(3) Devices for irradiating the blood of persons with bacterial infections
(4) Devices irradiating the fundus of deep cavities
(5) Devices for sterilizing tissue sections and sugar solutions

A total of 14 types of ultraviolet installations have been tested, approved and standardized for use in infectious units. These installations are now in general use in most buildings handling infectious agents.

(1) Ultraviolet Door Barriers

This installation may be used around doorways where it is desired to separate a contaminated area from a clean area (4a). The barrier utilizes five 17-watt, cold cathode ultraviolet bulbs mounted in a double walled cubical which extends around the sides and over the top of the doorway. Each fixture is equipped with an aluminum metal reflector. The curvature of the reflectors is such that the rays from the ultraviolet lamps are directed in a narrow bank across the doorway opening. The inside of the cubical is painted aluminum. The lamps are operated from a manual switch with a blue indicator light and should remain on continuously except when cleaning or changing bulbs. The ultraviolet intensity obtained should be equal to a total flux of 250 microwatts per sq cm at a point 3 feet above floor level in the center of the barrier. The total flux is obtained by adding the intensities falling

upon a surface from 3 directions (from above, from the left, and from the right).

The biological effectiveness of this type of door barrier was tested against airborne clouds of S marcescens and S indica (5, page 22). Under conditions of actual use the barrier is from 92.5 to 100 per cent effective in preventing the passage of airborne bacteria. Bacterial cloud concentrations of approximately 200,000 organisms per cu ft were used for the tests.

(2) Ultraviolet Air Locks

This installation is also used to separate contaminated areas from clean areas (4a). Cold cathode ultraviolet bulbs are installed in the ceilings of double doored air lock rooms. No reflectors are required. Each lock should have an outside switch with a blue indicator light. The number of bulbs required depend upon the size of the air lock room. The doors of the air lock room are equipped with pneumatic door closing devices. The walls and ceiling of the room are painted aluminum to increase reflectance (4b). The ultraviolet intensities required vary with the length of the air lock and the rate of air movement in the lock. However, it is desirable to have an intensity of approximately 20 microwatts per sq cm reaching the floor level of the lock. This installation is usually used in conjunction with ultraviolet door barriers. For example: the air lock at the service entrance to a building may be an ultraviolet air lock and an ultraviolet door barrier may be installed around the door leading into the air lock from the contaminated side.

A variety of ultraviolet air locks have been tested using airborne clouds of S indica. When properly designed and maintained ultraviolet air locks are approximately 99 per cent effective in preventing the passage of airborne bacteria (11, page 10; 12, page 2).

(3) Direct Ultraviolet Irradiation of Laboratory Rooms

This type of installation is recommended for laboratories handling infectious agents where there is danger of accidents or spills. It is also useful for the control of non-specific fungal and bacterial contaminants. Bare cold cathode lamps are installed in the ceiling and the entire room is irradiated if a spill occurs or during periods when the room is unoccupied. A control switch, blue indicator light and warning sign must be located on the outside of the room, near the entrance. The number of ultraviolet bulbs required for each room will vary with the room size, ceiling height, etc. In general, an ultraviolet intensity of 10 microwatts per sq cm at the floor level is satisfactory.

Tests have shown that this type of ultraviolet installation is very effective in eliminating airborne organisms and organisms on exposed surfaces (13, page 2). Clouds of S marcescens generated in a room are

rapidly killed when the lamps are turned on. When the lamps are routinely burned overnight, the air and most surfaces are found to be almost completely free of bacteria and the loss of sterile culture materials due to the growth of contaminants is greatly reduced. Of course, shadowed areas are not affected.

(4) Indirect Ultraviolet Irradiation of Laboratory Rooms

This type of installation is used to reduce the bacterial flora of room air. It is a means of hygenic control and personnel protection. Hot or cold cathode germicidal bulbs are installed in fixtures equipped with reflectors and louvers and mounted near the top of room walls. The design of the fixture is such that the upper strata of the room is evenly irradiated but direct ultraviolet rays are prevented from reaching the eye level of the occupants of the room. Control switches and indicator lights are placed on the wall near each fixture. The lamps should burn continuously except when being cleaned or replaced. The ceiling of the room should be painted with an oil paint to reduce reflectance. Ultraviolet intensities of 20 to 30 microwatts per sq cm in the upper air are required.

The reduction in the number of airborne bacteria by this type of installation per hour of operation is roughly equivalent to the dilution caused by 11 changes of air. There is no surface decontamination at the working level. The use of indirect irradiation is only recommended in instances where it is impractical to use direct irradiation.

(5) Ultraviolet Animal Cage Racks

This installation is used to prevent the escape of infectious agents from animal cages housing infected animals. It also prevents cross contamination between cages. Each shelf of the cage rack is equipped with two 15-watt, 18-inch hot cathode ultraviolet bulbs, two fixtures and two reflectors. Each fixture is attached to the rack in such a manner that it can be easily lowered or raised. When the cages are placed on the shelf the fixtures are adjusted so that the ultraviolet bulb is on a level with or slightly higher than the top edge of the cage. The reflectors are shaped so as to direct most of the rays across the top edge of the cage. In this manner the entire top opening of each cage is completely screened with ultraviolet. (Design Drawing No. C-99-1-2219).

Considerable amounts of stray radiation are emitted from this type of ultraviolet installation. For this reason workers must wear eye and face protection when in the animal room.

Bacteriological tests with S indica proved this type of installation to be 97.32 to 100 per cent effective (10, page 9). Bacterial clouds generated in an animal cage were prevented from getting out and aerosols

generated in the animal room were prevented from entering the cage. The ultraviolet intensity on each shelf should be such that a point at the center of the shelf parallel with the bulbs will receive a total flux of at least 190 microwatts per sq cm. To what extent bacteria on or in dust particles are affected by ultraviolet is unknown. This requires further study.

(6) Ultraviolet in Walk-in Refrigerators and Incubators

The installation of ultraviolet bulbs in walk-in refrigerators and incubators is effective in reducing air and surface contamination. Studies have shown that sometimes the air in incubators equipped with ultraviolet was completely free of viable bacteria. Bare cold cathode ultraviolet bulbs are mounted in the ceilings and switches, indicator lights and signs are located outside near the door. Ultraviolet intensities of about 10 microwatts per sq cm at the floor level are desired (4b,d).

A series of studies were conducted to determine the limitations necessary for the use of ultraviolet in incubators and refrigerators and other enclosed spaces where viable culture materials are kept (7, page 23). Brucella abortus strain A-19 and Serratia indica were used as test organisms. Although there is no transmission of 2537A ultraviolet through pyrex or soft glass, it was found that if high intensities of ultraviolet were directed against cultures stored under glass, the penetration of a small percentage of longer rays could have a detrimental effect. No inhibition was found when the intensity directed against the glass was less than 100 microwatts per sq cm. For this reason all glass enclosed cultures in incubators or refrigerators equipped with ultraviolet should be stored at least 2 feet away from cold ultraviolet bulbs, 3 feet away from 30 watt cathode bulbs, and 18 inches away from 15 watt hot cathode bulbs. The use of ultraviolet in non-walk-in incubators and refrigerators is not recommended.

Although care must be exercised in the use of ultraviolet in walk-in refrigerators and incubators, the advantages warrant its use in most infectious units. Refrigerators and incubators are not subject to frequent washdowns with liquid disinfectants as are other areas and are often found to be contaminated with infectious agents. Objectionable odors and mold contamination are often found in incubators and refrigerators. Accidental spills of infectious agents from shaking machines, etc. often occur during the absence of the operating personnel. The continual presence of germicidal ultraviolet is helpful in all of these situations. The small amount of ozone produced has been found to eliminate successfully certain odors, but no bactericidal action against cultures stored under glass has been noted.

Ultraviolet in walk-in refrigerators and incubators has been used in some infectious units for over a year with good results. Certain laboratories have found that sterile agar plates may be dried without

excessive contamination in incubators equipped with ultraviolet by removing the petri plate tops for 24 hours.

(7) Ultraviolet Paper Decontamination Chambers

The purpose of this chamber is to provide a mechanism by which small articles can be transferred from clean to hot areas and by which sheets of paper and similar small articles may be sterilized and passed from contaminated to clean areas. The chamber is a 2 doored, rectangular shaped, stainless steel box (38"x22"x16") containing 4 30-watt hot cathode ultraviolet bulbs, designed to be installed in a wall or door separating a contaminated area from a clean area. An activating switch with a blue indicator light is located on each door. The doors may be interlocked so that only one will open at a time. Ultraviolet intensities of from 500 to 1500 microwatts per sq cm are present inside the chamber and the temperature with the bulbs burning is approximately 44 C. Eleven horizontal shelves made of thin stainless steel wire are provided.

Tests were conducted in the chamber using several types of paper contaminated with S indica and B globigii spores (6, page 22; 8, page 16). The chamber was shown to be effective in sterilizing single sheets of paper moderately contaminated with vegetative bacteria (S indica). The recommended exposure time is 10 minutes. Unsuccessful results were obtained when paper highly contaminated with S indica (e.g. dipped in a liquid culture) or paper moderately or lightly contaminated with B globigii spores were placed in the chamber.

Installation of ultraviolet paper decontamination chambers between clean and dirty office area has proved very successful in several buildings.

(8) Ultraviolet Clothing Discard Rack

This unit holds the canvas laundry bag which receives discard clothing in contaminated change rooms. The unit consists of a bag holding device and an inverted box containing two 15-watt hot cathode ultraviolet bulbs which is mounted on a wall over the open laundry bag. The inside and opening of the bag is continually bathed with germicidal radiations to prevent the escape of airborne organisms from contaminated clothing. An ultraviolet intensity of approximately 650 microwatts per sq cm is obtained at the bag opening while the inside bottom of the laundry bag receives 45 microwatts per sq cm. Using aerosols of S indica (200,000 cells per cu ft of air) a test test installation was 99.99 per cent efficient in preventing the escape of airborne bacteria (5, page 28). (RE Division Drawing D-93-1-2134).

(9) Miscellaneous Installations

In addition to the installations listed above, other applications of the germicidal rays have been successfully used in infectious units. The number of ultraviolet bulbs required, the desired intensities, etc. vary with the type of installation as well as the infectious agent in use (4d). The miscellaneous uses include:

(a) Decontaminating Station for personnel in protective clothing (used at Dugway Proving Ground).
(b) Elevator shafts (4c)
(c) Entrance air locks to ventilated cabinets.
(d) Recirculating air conditioning systems.
(e) Stair wells (4c)
(f) Ventilated animal transfer cabinets
(g) Ventilated safety cabinets

j. MAINTENANCE OF ULTRAVIOLET INSTALLATIONS

Ultraviolet bulbs usually continue to burn and emit a few lumens of blue light after the 2537A ultraviolet output has ceased. This means that visual inspection of the bulbs cannot be used to judge ultraviolet output. Special meters must be used. The most useful life of an ultraviolet bulb occurs during that period when the bulb is generating between 100 and 70 per cent of its rated ultraviolet output. Therefore, bulbs are discarded when meter readings show that the ultraviolet output has fallen below 70 per cent.

The 2537A ultraviolet wavelength is not particularly penetrating. The smallest film of water, grease, oil, or dust on an ultraviolet bulb will seriously reduce the ultraviolet output. In addition, the special ultraviolet transmitting glass used for the bulbs is easily scratched. This also will reduce the output.

The systematic use of ultraviolet in the Biological Laboratories necessitates a regular maintenance program for cleaning, testing and disposal of ultraviolet bulbs. Routine cleaning of all bulbs should be at two week intervals or more often if the bulbs in the particular installation become dirty. Bulbs and reflectors should be wiped off without being removed from the fixtures with a soft cloth pad which has been wet with absolute alcohol. Bulbs must be turned off while cleaning. Cleaning should be done by janitors or laboratory technicians in each building as a part of regular scheduled duties.

The routine testing of ultraviolet bulbs is the responsibility of the Agent Control Branch, Safety Division. Bulbs normally should be tested every 3 months except in special locations such as animal rooms where more frequent testing is recommended. In general, it is advisable to test the hot cathode bulbs more frequently than the cold cathode because of their shorter life expectancy. Quarterly surveys of all ultraviolet installations, including the testing of bulbs, were

conducted up until June 1952 (6, page 5; 9, page 4). Since that time the routine testing has been inadequate because of (a) a large increase in the total number of bulbs in use and (b) the shortage of personnel in Agent Control Branch. Approximately 1500 ultraviolet bulbs are presently in use in the Limited Area. The completion of several new buildings in the near future will probably double the number of bulbs in use. It is imperative that some satisfactory system for routinely checking ultraviolet bulbs be established.

The replacement of worn out ultraviolet bulbs is a function of M&O Branch, RE Division. Lists of bulbs requiring replacement are forwarded to this branch after each ultraviolet survey. The types of bulbs most generally in use are as follows:

17 watt, 34 inch cold cathode bulbs
15 watt, 18 inch hot cathode bulbs
30 watt, 36 inch hot cathode bulbs

Ultraviolet bulbs contain mercury vapor, metalic mercury and argon or some other inert gas; therefore, methods for disposal of used bulbs must satisfy the following requirements:

(a) Intentional breaking of bulbs must be done in the open so the mercury vapor can be quickly removed.

(b) Care must be taken that liquid mercury does not enter the sewage system.

Used bulbs are usually deposited in the disposal pit at the Grid Area.

k. HAZARDS OF ULTRAVIOLET AND PERSONNEL PROTECTION

The problem of ultraviolet radiation as a health hazard can be divided into three resulting types of acute injuries: inflammation of the eye, inflammation of the skin and metabolic disturbances. Because of the absence of the strongly absorbing horny layer in the conjunctiva, radiations of less than 2800Å are more effective in the production of conjunctivitis of the eye than in the production of erythema. Sufficient exposure will produce a very painful conjunctivitis which appears after a latent period. There is a very unpleasant foreign body sensation and blepharitis, lid edema and even blepharospasm may develop. Symptoms usually disappear after a day or two. In recent medical literature no case of permanent blindness due to ultraviolet burns has been found, although there were many cases of conjunctivitis which were quite serious for several days. One reported case resulted in the temporary partial blindness of the patient for 11 days. There is some disagreement concerning the relative resistance of the outer eye membrane and the skin to ultraviolet radiations. No erythemal dose for conjunctivitis has been established. With radiation in the 2537Å range it appears certain, however, that the eyes are very

sensitive. In reports of human injury due to 2537A ultraviolet the eyes are by far the most frequently involved.

Prolonged exposures of the skin to ultraviolet radiations may produce, after a latent period of a few hours, severe erythema up to blister formation. In extreme cases hyperpigmentation may persist for years. Without secondary infection the prognosis for restoration of the skin is generally good. The most effective wavelength for erythemal production is 2967A and 2537A is 80 per cent as effective. A minimum perceptible erythema (MPE) on the untanned skin can be produced by 2537A ultraviolet rays if an ET value of 400 is applied. A person standing about a foot from a 30 watt hot cathode lamp would receive an ET of 400 in a little over a minute. Standing 3 feet from the source, the same amount of radiation would be received in 5 minutes. Individual susceptibility is known to vary over a wide range. The Council of Physical Therapy of the American Medical Association has established the maximum allowable radiation level for persons exposed to ultraviolet for 8 hours daily at 0.5 microwatts per sq cm.

Exposures to ultraviolet (not necessarily 2537A) have been known to produce thyrotoxic symptoms. It is thought that the exposure causes the release in the body of histamine-like substances. These substances increase the blood supply of the thyroid gland, thereby increasing its activity.

Tests were conducted in S Division to determine the effect of 2537A radiations on the untanned skin. In one test, using a patch exposure method, an ET of 500 produced an MPE on the untanned forearm skin and skin patches receiving an ET of 8000 showed moderate erythema but no blister formation. In a further test individuals with bare arms and shoulders received no skin erythema or eye burns as a result of walking slowly through an 8 foot ultraviolet air lock. The exposure represented up to 10 times the normal time required for passage through the air lock (10, page 10).

The protection of personnel against injurious ultraviolet rays is accomplished in two ways: the use of visual aids such as warning signs and indicator lights, and the use of protective devices for eyes and skin. Appropriate warning signs and blue indicator lights are required with each ultraviolet installation. The wording of the sign should coincide to the safety regulations recommended for the particular installation. Examples are:

(a) Caution - ultraviolet, protect your eyes
(b) Caution - ultraviolet lights in use, do not enter
(c) Caution - turn off ultraviolet lamps before entering

Eye protection for persons working in the continual presence of ultraviolet is obtained by the use of tinted safety glasses or goggles. These are supplied upon request by S Division. Ordinary laboratory clothing and gloves give adequate protection for skin areas except for

the face. Face protection is usually obtained by wearing ventilated personnel hoods or plastic face shields. It is recommended that safety glasses be worn in addition to the hoods or face shields.

l. CONCLUSION

The use of ultraviolet has been found to be highly effective as a biological control mechanism for the Biological Laboratories. Fourteen types of installations employing approximately 1500 bulbs are presently in use. Although little is known concerning the mode of action of ultraviolet, the conditions affecting the reaction and the relative effectiveness against many types of microorganisms have been established. Specific data concerning the intensities and exposure times necessary to inactivate a number of organisms under a variety of conditions are available.

The information embodied in the forthcoming Special Report represents a complete coverage of all aspects of the practical application of ultraviolet light for germicidal purposes and demonstrates conclusively the usefulness of ultraviolet in the Biological Laboratories.

m. REFERENCES

Approximately 60 technical references are cited in the forthcoming Special Report. Although these references are not included in the present report, three general reference sources are worthy of note. The books listed below contain valuable discussions and reviews on various phases of past studies with ultraviolet. The work of Ellis, Wells and Heyroth is a standard reference and contains summaries of much of the earlier work with ultraviolet. The physical aspects of ultraviolet and some of the more recent bacteriological studies are presented by Luckiesh and by Koller.

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18. WATER SYSTEM

The water supply to Camp Detrick consists of two and in some special buildings three separate systems. They are: (1) water used for drinking, fire hydrants, and the installations outside the Restricted Area; (2) process water, which is water that passes through a breaktank at the southwest end of the Camp. The function of a breaktank is to prevent back siphonage of any water in case of pressure failure in the main; (3) in highly contaminated buildings there is sometimes water which has passed through 2 breaktanks. This is deemed necessary in some cases to insure against contamination of the primary breaktank water system in the event of pressure failure resulting in back siphonage. All process water is considered contaminated. This water is used to supply sinks, dishwashers, and all other pieces of equipment in potentially contaminated areas.

A better understanding of the water system can be had by briefly reviewing the history of the water supply at Camp Detrick.

a. PERIOD BEFORE 1946

From April 1943 to April 1945 the City of Frederick, Fishing Creek Reservoir supplied all the water used at Camp Detrick for drinking, fire hydrants and to buildings outside the Restricted Area (which was called "primary" or "main" water). The breaktank or process water was secured from Montevue Spring.

Early in 1945, a water treatment plant was built on the Monocacy River. By 1 May 1945, this plant was put into operation and supplied both the primary and process water until 27 December 1945. World War II had just ended and the rapid discharge of military personnel assigned to Camp Detrick inflicted a critical shortage of help. This made it necessary to shut down the Monocacy water treatment plant and return to using the city and Montevue Spring water supplies (4).

b. PERIOD 1946 - 1948

From 27 December 1945 to May 1946 the City of Frederick supplied all the primary water while the Montevue Spring was reactivated to supply the process water. In June 1946, the Monocacy water treatment plant began operating on a part-time basis. It did not treat sufficient water to supply all of the required primary water so the system was supplemented with city water until October 1948. During this time Montevue Spring still supplied the process water (4).

c. CROSS-CONNECTION SURVEY (1)

In 1947, bacteriological analysis of samples of water from showers and sinks of all buildings in the "400" area were positive for E coli. Since water contaminated with fecal matter is not desirable for use in washrooms, immediate steps were taken to correct the situation. The Post Engineer reactivated the chlorinator in the water line leading to the showers and sinks of the area.

Several cross-connections between the process water line and the shower water line were found to exist. At a meeting of representatives of the Post Engineer, PP-E Division and Safety Division, a satisfactory solution leading to the elimination of cross-connections and of possible back siphonage of process water into the shower water lines was evolved.

d. PERIOD 1949 - 1952

Prior to August 1948, the extreme hardness and instability of the Montevue Spring water, which was being used for process water, caused severe corrosion of the retention tubes in the Sewage Decontamination Plant T-314, which on 24 August 1948 had become sufficiently encrusted to cause complete failure of the plant. Stills in individual laboratory buildings, which prepared distilled water, were also subject to rapid deposition of a hard cake of calcium inside the still, which soon reduced the capacity of the still.

As an immediate solution to the problem, the water system was modified to utilize water from the Monocacy water treatment plant in the breaktank system. In November 1948, the Monocacy water treatment plant, which was presently supplying primary water, started supplying process water as well.

On 24 November 1948, a mutual agreement with the City of Frederick made it possible, in case of an emergency, for the Camp to use the city's water supply or sewage facilities without cost, and by the same token the city is permitted to use the Camp's facilities (4).

In January 1949, it was necessary to increase the operating period of the treatment plant from 8 hours to 16 hours to keep sufficient water in the storage tanks. In the Summer of 1952, because of the new buildings which were introduced into the water system, it was necessary to operate the Monocacy water treatment plant 24 hours a day. The Monocacy plant treats about one million gallons a day as of May 1953 (4).

e. BREAKTANK SYSTEM

Breaktank water is used by nearly all buildings in the restricted area. As mentioned previously, most laboratory buildings are supplied

with water from secondary breaktank water to eliminate the possibility of contaminating the primary breaktank system.

Originally (approximately 1943 and 1944) two secondary breaktanks were initiated into the process water system. They were T-436 and T-515.

Building T-436 was installed to supply secondary breaktank water to T-431, T-434, and T-459. This system was in operation until the summer of 1951. At that time T-459 was disconnected from the breaktank in T-436 and a breaktank installed in building T-459. Early in July in 1951 buildings T-431 and T-434 were disconnected from the breaktank in building T-436 and backflow preventers were installed in the process water lines to these buildings. Some question was raised as to the reliability of the backflow preventer devices. Tests on these devices were authorized.

On 25 July tests on 3 types of backflow preventing devices installed in building T-436 were started. The devices were installed in parallel on a 6-inch water main having a pressure of 62 psi on the supply side. The 3 backflow preventing devices were:

(a) Clayton Backflow Protection Unit No. 2, manufactured by Clayton Mfg. Company, Alhambia, California.

(b) Grove Voidsiator, manufactured by Grove Regulator Company, 65th and Hallis Street, Oakland, California.

(c) E. C. Model 6 Backflow Preventer, manufactured by Backflow Engineering and Equipment Company, 5725 Alcoa Avenue, Los Angeles, California.

These were tested in cooperation with SO Division and the U. S. Public Health Service Liaison Officer. The first tests were carried out after the valves had been in coninuous operation for 37 days. Each of the backflow devices consists essentially of the following parts in the order named: (a) vacuum breaker valve, (b) check valve, (c) differential pressure relief valve, and (d) check valve. Each is also provided with 4 teet cocks for hydrostatic pressure testing. On 31 August, the backflow preventing valves were subjected to a hydrostatic pressure test and it was found that only the E.C. Model 6 valve was working satisfactorily. The E.C. 6 valve had a pressure drop across the valve of 28 psi; this is approximately double the pressure loss found with the 2 other valves (2).

Recently with the building of PP-1 and the extension to T-431 (T-431-A), it was necessary to utilize the secondary breaktank in T-436 for these buildings. New buildings such as MIA and BS-1 have a secondary breaktank installed within the building.

Building T-515 secondary breaktank which can be fed by either the primary breaktank system or, in time of high demand, directly

from the "main" line. This tank has been in operation since the Camp was constructed and serves Buildings T-501, 502, 503, 504, 505, 507, 516, 517, 520, 521, 522, 524, 525, 527 and 700 with process water.

WATER

f. USE OF PHENOLPHTHALEIN FOR TRACING/LINES (3)

The water system in Building T-459 was connected to a water line that passes through Building T-434. It was not known whether this was a process water line or a primary water line. Objections to putting crystal violet or other dyes in these lines were raised. Therefore, a solution of phenolphthalein indicator was added to the breaktank water at T-436. Water samples were taken at 1 minute intervals in T-431, 434 and 459. A few drops of 20 per cent sodium hydroxide were added to the water sample, and when and if phenolphthalein came through the line a deep red color developed in the water sample. In this way the water lines from the breaktank in T-436 to T-431, 434 and 459 were clearly differentiated. The concentration of phenolphthalein in the water was probably below the concentration that would cause physiological disturbance in men.

g. DISCUSSION

Little trouble has been encountered in maintaining an adequate supply of water for the Camp's water systems. It is true that the extreme hardness of the Montevue Spring water caused some difficulty in the sewage decontamination plant, but the Monocacy water treatment plant has been capable of supplying both process and "main" water since 1948.

In some cases the secondary breaktanks have been replaced by Backflow Preventers. The main objection to the Backflow Preventer is that it cuts the pressure after the preventer to about one-half. Today, in the newly constructed buildings, contractors are installing both secondary breaktanks (as in MIA and BS-1) and Backflow Preventers (as in PP-4). The choosing of a back-siphon prevention device depends mostly on (1) the space available for the equipment and (2) the cost of the equipment; that is, the Backflow Preventer costs relatively little and occupies less space than the breaktank which costs considerably more and occupies considerable space but is the more reliable of the two.

h. REFERENCES

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II. PROPHYLAXIS, DIAGNOSIS, TREATMENT AND CLINICAL INVESTIGATION OF OCCUPATIONAL ILLNESS

1. PROPHYLACTIC VACCINES, TOXOIDS AND SKIN TESTS

a. SCHEDULES OF ADMINISTRATION

(1) Anthrax antigen
Source: Immunology Branch, S Division, Dr. George G. Wright

(a) This vaccine is an experimental one which has been administered to more than 500 persons. Studies on its clinical acceptability, possible schedules of administration, and immunologic effects in man are in progress. In the one group the schedule of immunization has consisted of 0.5 ml given at 2-week intervals for a total of 2 injections; in the second group 0.5 ml was administered every 2 weeks for a total of 3 injections.

(b) Booster injections of 0.25 ml every 6 months are given.

(c) Serologic testing is being performed in cooperation with Immunology Branch, S Division, using a complement fixation method which is in a developmental stage (36).

(2) Botulinum Toxoid Type A, alum precipitated
Source: Parke, Davis and Company, Detroit, Michigan
Obtained through contract with Immunology Branch

(a) Schedule consists of 3 injections of 0.5 ml given every 2 weeks.

(b) Effective date for admission to potentially dangerous areas is one week after the last injection.

(c) Booster injections of 0.25 ml are given yearly.

(3) Blastomycin and Histoplasmin Skin Test antigen
Source: Parke, Davis and Company, Detroit, Michigan

(a) Blastomycin, 1:1000, and Histoplasmin, 1:100, are used in 0.1 ml amounts for skin testing. The test is interpreted in the same manner as the brucellergen skin test and if negative is repeated yearly.

(4) Brucellergen
Source: Sharp and Dohme Company, Philadelphia, Pa.

(a) All new employees within the limited area are given an initial brucella skin test with 0.1 ml. A positive test is one in which there is 0.5 cm of edema and 2 cm of erythema. If negative, the test is repeated if the person develops an illness suggestive of brucellosis.

(b) Serum samples for agglutination test are obtained prior to the first skin test and 3 and 6 months later.

(5) Coccidioidin
Source: Dr. C. E. Smith, School of Public Health
University of California, Berkeley, California

(a) Skin test with 0.1 ml of a 1:100 dilution is given to all persons in infectious units where this agent is in use. The test is positive when there is present 0.5 cm edema and 2.0 cm erythema at any time after the skin test.

(b) Skin test, if negative originally, is repeated once a year until positive.

(6) Mallein antigen
Source: Veterinary Division, Armed Forces Medical Service
Graduate School, Washington, D. C.

(a) 0.1 ml of 1:1000 dilution of the material is used in skin testing. A positive test consists of at least 5 mm of edema and 20 mm of erythema.

(7) Plague vaccine
Source: Cutter Laboratories, Berkeley, California

(a) Initial series consists of 3 injections at 1 week intervals, as follows: 0.5 ml, 1.0 ml and 1.0 ml.

(b) Booster injections of 0.5 ml are given to laboratory personnel every 3 months and to others every 6 months.

(c) Admission to potentially infectious areas is allowed 1 week after last injection.

(8) Psittacosis vaccine, Borg strain
Source: VR Division, Dr. John Wagner

(a) The initial series consists of weekly injections of 1 cc for a total of three injections.

(b) Admission to potentially infectious areas is granted 1 week after the third injection.

(c) Booster injection for laboratory personnel is given every 6 months and consists of 0.5 ml. Other personnel receive the same quantity every 9 months.

(9) Q Fever vaccine
Source: Lederle Laboratories, Pearl River, New York

(a) The inital series consists of 1 ml injection given at weekly intervals for a total of 3 injections.

(b) Admission to potentially infectious areas is granted 1 week after the third injection.

(c) Booster injections for all personnel are given every 6 months and consists of 0.5 ml.

(10) Tuberculin, P. P. D.
Source: Sharp and Dohme Company, Philadelphia, Pa.

(a) First strength, 0.00002 mgm is used in the usual manner and, if negative, the second strength, 0.025, is given.

(11) Tularemia vaccine
Source: Dr. Lee Foshay, Cincinnati General Hospital

(a) New employees who are to receive this vaccine are first skin tested with 0.1 ml of 1:1000 dilution of the vaccine. A positive skin test is indicated by at least 10 mm of erythema 48 hours later. If accompanied by an agglutination titer of 1:40 or higher, this amount of skin reaction is considered to denote significant immunity.

(b) If the skin test is negative, the worker is given 0.25 ml vaccine on the first day, 0.5 ml on the second day, and 0.5 ml on the third day.

(c) Admission to the potentially dangerous areas is allowed one week after the initial injection.

(d) Booster injections are given to laboratory workers every 8 months and to other personnel every 12 months. In both instances a skin test is given first and, if negative, 2 injections, 0.25 ml and then 0.5 ml, are given on consecutive days.

If skin test is positive, no booster injections are given at that time.

(12) Typhus, epidemic, vaccine
Source: Lederle Laboratories

(a) Initial series consists of weekly injections of 1.0 ml for 3 injections.

(b) Booster injections of 0.5 ml are given every 6 months to all exposed personnel.

(c) Admission to potentially infectious areas is granted one week after the third injection.

(13) Venezuelan Equine Encephalomyelitis vaccine
Source: VR Division, Dr. John Wagner

(a) Initial series consists of two 1 ml injections given 7 days apart, followed by a third injection of 0.5 ml given 6 weeks after the inital injection.

(b) Booster injections of 0.5 ml are given every 6 months to all exposed personnel.

b. COMBINED VACCINES

(1) Botulinum Toxoids A, B, C, D, E and Anthrax

(a) Standardization of antitoxins: This was necessary to insure standard toxoids. The task of standardizing types A, B, C, D, E antitoxins was assumed by the British by agreement with Dr. D. W. Henderson 4 October 1951, as recorded in CDBL document S-1278. This job is nearly completed and a proposal for an international standard is under consideration by the British, according to oral information gained by Dr. George Wright during a visit to England in April 1953.

Preliminary serological examinations at Detrick reveal no confusion in types A and B, but European and American strains of types C and D are sometimes reversed in antitoxic response. The strains called type C in America, England, Australia and South Africa are apparently immunologically similar to those called type D in France, Germany and Belgium. (Ref 1, page 74; 2, page 77).

(b) Serological measurement of antitoxin in human serum (Miss Dorothy L. Farley): To avoid the need to use mice in a toxin-antitoxin measurement of serum antitoxin, and thereby facilitate the evaluation of the toxoid, a hemagglutination method is under study. Sensitization of the tannic acid treated sheep red blood cells with toxin is carried out at 37 C for 30 minutes and the hemagglutination tests are incubated at room temperature for 4 hours. The tests can be interpreted easily and the results are reproducible. This method will be used hence forth. (Ref. 1, page 78; 2, page 86). Further details are given in the 1 June 1953 Annual Report on Botulinum Toxin.

(c) Prophylactic toxoid

1. 1943 to 1951: During World War II an excellent combined types A and B toxoid was developed (4,5,11) which gave high persistent antitoxic levels in man (3,4,5,6,12) which were sufficient to prevent any laboratory illnesses despite significant accidental exposures by aspiration and inhalation (12). This combined toxoid was used until 1952. To this date, no botulism has been encountered in Detrick personnel although work has been continuous since 1943. However, during 1944-45 and during 1951-53, work on types C, D, E has been done on a lesser scale, without immunization, and also without untoward results; titers of toxin produced have been much lower for the E and slightly lower for C and D than with type A, so this has probably been of assistance in avoiding illness.

2. 1952 to 1953: Although the above combined A B toxoid gave good immunization, it also produced more local and systemic effects than were clinically desirable (Ref 4, pages 27-30; 12, page 92). Therefore efforts were begun in 1949 to prepare a toxoid which was clinically more acceptable (7). This work culminated in a procedure for type A toxoid which has been summarized (8, page 81-82) and now has been adapted to commercial methods by Parke, Davis & Company, Detroit, Michigan, under Contract DA-18-064-CML-1445. This aluminum phosphate precipitate type A toxoid has been under clinical trial since 1952. More than 500 persons have received series of injections without any serious local or systemic reactions. Studies of the antitoxic response to different schedules of immunization are in progress (1, page 79).

The method for the type B has also been summarized (9, page 82,103) but is not yet finalized, although initial lots are also being produced by Parke, Davis & Company.

Work on type C has been done by Dr. Carl Lamanna at Johns Hopkins University under Contract DA-18-064-CML-470 and also at Detrick. This, along with some work on D (also at Parke, Davis)

and E at Detrick is summarized in current S Div QTRS. (Supervisor: Mr. James Duff).

3. Current Immunization Schedule for Botulinum Toxoid: The present immunization schedule is as follows - using Parke, Davis & Company botulinum toxoid type A, alum precipitated, an injection of 0.5 ml is given at 2 week intervals for 3 times. Admission to potentially dangerous areas is permitted one week after the last of the 3 injections. Booster injections are 0.25 ml given yearly.

4. Combined Toxoids: The above Parke, Davis & Company contract provides for combination of types A, B, C, D, E, and another Parke, Davis & Company contract DA-18-064-CML-2339 provides for further combination with the anthrax antigen which has been described (10).

5. Details of Methods of Production and Titers of Toxin to be used for Toxoid: Details of above work on botulinum toxins and toxoids, with reference to original notebooks, is given in 1 June 1953 Annual Status Report of Agent X, II Basic Laboratory Development, 5. Immunology Section.

(2) Combined Epidemic Typhus, Rocky Mountain Spotted Fever and Q Fever Vaccines: Lederle Laboratories at Pearl River, New York, has agreed to supply this vaccine in May 1953. Clinical trial will begin, using the combined vaccine as a booster for persons previously immunized with the single typhus and Q vaccine. If adequate personnel are made available, laboratory assessment of titers will be made.

(3) VEE, Eastern and Western Equine Encephalomyelitis vaccine: This is prepared by the Army Veterinary School, Washington, D. C. (13) and will be used, as available, as booster injections for persons first immunized to the separate vaccines. Lack of personnel prevents laboratory assessment of immunological response.

(4) Multiple Viral-Rickettsial Combined Vaccine: A contract with Parke, Davis & Company of Detroit, to combine as many of these potential agents as possible, has been in the process of negotiation for 6 months. The company has experience in this field.

(5) Multiple Bacterial Combined Vaccine: A commercial contractor is being sought, to determine how many bacterial antigens and toxoids may feasibly be put together, preparatory to selecting the most useful combination. It is desired the final process be susceptible to large scale production.

(6) Some of the potentialities for combining immunizing antigens are illustrated in Table 1.1.

c. WARTIME EXPERIENCE WITH EFFECTIVENESS OF VACCINES

This has been given in detail elsewhere (4,21,22). To summarize briefly: (1) a vaccine of killed anthrax organisms was tried and discontinued, (2) a vaccine of killed brucella organisms was used during the war but discontinued soon after, (3) the tularemia vaccine is still being used; the degree of immunity it produces ameliorates the disease but does not often prevent it in the laboratory, (4) the botulinum vaccine has always been excellent.

TABLE 1.1 SOME POTENTIALITIES FOR COMBINED VACCINES

Department of Defense now uses 11 single vaccines for 13 diseases:

Diphtheria, tetanus, smallpox, typhoid-paratyphoids A and B, epidemic typhus, cholera, yellow fever, Rocky Mountain spotted fever, plague, influenza, Japanese B encephalitis

Potential Combinations -- 4 vaccines, 29 diseases:

1. Botulism A, B, C, D, E, anthrax, tetanus, diphtheria

2. Epidemic typhus, Rocky Mountain spotted fever, Q fever, endemic typhus, Venezuelan equine encephalomyelitis, eastern E.E., western E.E., psittacosis, Russian spring-summer encephalitis, louping ill, Russian autumn 4 encephalitis, St. Louis encephalitis, Japanese B encephalitis

3. Cholera, plague, typhoid-paratyphoids A and B

4. Smallpox, yellow fever, Rift Valley fever

Six potential BW agents for which there is treatment but no good vaccine:

Brucellosis, tularemia, glanders, melioidosis, scrub typhus, dysenteries

Three potential BW agents for which there is neither adequate treatment nor adequate vaccine:

Coccidioidomycosis, tuberculosis, rabies (?)

Potential BW agents of uncertain value:

Gas gangrene, dengue, Colorado tick fever, boutonneuse fever, Murray Valley fever, Bwamba fever and other exotics, drug resistant strains

2. SIGNIFICANT CLINICAL, IMMUNOLOGICAL AND EPIDEMIOLOGICAL ASPECTS OF LABORATORY INFECTIONS

a. ANTHRAX

During the wartime period 26 cases of anthrax were diagnosed and a published report on 25 of these cases was made (34). Three of the 25 had positive blood cultures and all had positive cultures from the cutaneous lesion. Organisms could be recovered during less than the first 24 hours after initiation of penicillin treatment in 22 persons by culture, and in 17 by stained smear from the lesion. It was noted that in all cases the local lesion "continued to advance through a well-defined and typical cycle in spite of treatment and in spite of the absence of viable organisms." It was concluded that penicillin did modify the ultimate course of the infection.

Since the war there have been three cases of anthrax. The first cutaneous anthrax case responded in the typical way to 100,000 units of penicillin every 6 hours for five days (25a, page 15). The second, very recent, infection was a lesion on a previously abraded site on the hand, in a man who 5 months previously had received two injections of the new vaccine previously mentioned (10). Response to antibiotic treatment in the one case seemed to be accelerated over that which had occurred in the non-vaccinated.

The third infection was a fatal one, diagnosed clinically and at autopsy as anthrax with systemic, pulmonic and meningitic involvement. It has been reported in detail (35). It was concluded that the disease was contracted by the respiratory route and that the dose was relatively small. The failure to isolate B anthracis subsequent to antibiotic therapy was considered to be due to the vigorous antibiotic therapy employed. The treatment used consisted of oxygen, cortisone, vitamin K, anti-anthrax serum, serum albumin, whole blood, and antibiotics. The antibiotics used included penicillin, terramycin, and dihydrostreptomycin.

b. BRUCELLOSIS

Eighteen cases of brucellosis occurred at Camp Detrick during World War II, May 1944 to 1 October 1946. These cases have been reported in both classified (4,14) and open reports (15). A summary and evaluation (16) of this experience showed that 15 per cent of 113 persons working in any way with the agent, and 27 to 44 per cent of those in the principal laboratory, developed the acute disease after an average of 7.3 months in the brucella laboratories. The time loss per case for 16 cases was estimated to be 177 days. None of the patients received what is now considered effective specific therapy. One of these patients had more than 10 relapses and has suffered a disabling degree of organic brain damage (17).

During the postwar period up to 16 May 1953 there have been 63 cases of brucella infection. Sixteen of these were mild or completely inapparent, while 47 were sufficiently ill to require hospitalization.

The incubation period has been determined with a high degree of certainty in five cases (16b,2). In this group it averaged 29 days. In five additional cases in which the evidence was good, the incubation period averaged 25 days.

Among the 81 infections 1943 to 1953, there were 4 persons twice infected (20) and 41 from whom the organism was recovered by culture.

Five hospitalized and 2 nonhospitalized persons were presumably infected by Br abortus A19. This organism is the vaccine strain used to vaccinate cattle, usually considered to be non-pathogenic for man.

The fact that 8 hospitalized persons had a known positive skin test prior to illness, and 4 known prior brucella cases were re-infected indicates that the amount of immunity represented by a positive skin test is no bar to illness.

An estimate of the inhaled dose in two cases of acute disease and one inapparent infection has been made on the basis of counts made of the air at the time of the accident. Assuming the retention of 25 per cent of the inhaled aerosol, the range of dosage was estimated to be 8 to 66 organisms (16). The circumstances of these cases were used as a basis for calculating an ID_{50} for man (18).

Certain conclusions regarding the effectiveness of antibiotic therapy can be drawn from our experience (19). Observations on a limited number of cases indicated a high relapse rate and this has been confirmed by Magill who found the rate to be 80 per cent. Subsequent experience with a therapeutic regime of streptomycin administered parenterally in daily doses of 2 gm concomittantly with 2 to 3 gm of aureomycin or terramycin orally has been much more impressive. Using this schedule, we have observed only two relapses in 32 treated cases.

During the calender year 1952 only six cases were observed. Four were due to Br suis, one to Br melitensis and one was not culturally proved. One of the two relapses was of interest in that in addition to the usual systemic complaint the patient developed a large ulcer over the anterior aspect of each ankle from which Br suis was isolated on several occasions. In two instances, brucellosis occurred after an accidental exposure to Br suis despite immediate prophylactic use of 3 or 6 gm aureomycin for 5 or 6 days (1, page 55; 2, page 66).

c. COCCIDIOIDOMYCOSIS

During World War II, within a time equal to 14 man years, there was one pulmonary and one cutaneous case from both of which the

organism was recovered (4, page 86; 14). The former was hospitalized in 1945 for 30 days and the latter 48; recovery was uneventful. However, inquiry in 1953 revealed that the cutaneous infection had not healed completely at the end of the 48 day hospitalization.

There was a later 5 weeks' hospitalization at another Army hospital in 1946, two other non-hospitalized recurrences in that year, and minor recurrences until about 1950. The patient received 10 per cent veteran's disability compensation until about 1949.

d. GLANDERS

Six of the 13 persons working with this agent in Building T-430 at Camp Detrick were infected within one year. The organism was never recovered. Diagnosis was clinical, epidemiological and serological. All six were hospitalized after about a 10 to 14 day incubation period. Five had definite pulmonary involvement. Treatment was with sulfadiazine; there were no complications (4,23).

e. PSITTACOSIS

Twelve cases of psittacosis have been contracted at Camp Detrick. All were sufficiently ill to require hospitalization. The first, which was in a vaccinated person, was probably of greatest interest in that the accident was duplicated using simulants and the infective respiratory dose for man was calculated to be 40 to 100 mouse intracerebral LD_{50} units of 6 BC psittacosis virus (24). A second case (25), this one in a non-vaccinated person, was contracted as the result of opening a small box containing a broken tube of lyophilized 6 BC vrius. In this instance duplication of the accident using E coli bacteriophage as a simulant yielded data indicating a high order of infectiousness.

The remaining 10 cases have occurred since 1951 and are thought to be the result of increased work with the Borg strain. Unfortunately efforts to recover the virus have been uniformly unsuccessful. Bacteriological safety cabinets necessary to confine the agent have been lacking in the buildings where work is being done on this organism.

Ten cases have occurred in persons immunized with vaccine prepared from 6 BC strain. The two most recent cases had received a series and many boosters of 6 BC vaccine and then either one or two booster injections of the Borg strain. In one case (26) it was concluded that reinfection with Borg strain had occurred. An unimmunized wife, not employed in the Virus building, apparently contracted it from her husband who was discharged from the hospital, with a diagnosis of psittacosis, 5 days before she was admitted for the same disease.

In all instances there has been a satisfactory clinical response to penicillin, aureomycin or terramycin. However, in two cases

relapses have occurred after cessation of aureomycin or terramycin therapy. These relapses responded promptly to a repeat course of the same drug.

No real conclusions can be drawn concernign the value of the psittacosis vaccine except that cases have occurred in immunized persons. It is presumed that these were caused by the Borg strain in persons immunized against 6 BC strain.

f. Q FEVER

Research on this agent has been in progress since 1949. Between 1950 and 15 May 1953 thirteen cases have occurred. Ten have required hospitalization. Proper safety equipment has been lacking in this building. Six of the illnesses have been in non-immunized persons and in most instances there has been only a history of contact with persons or apparatus from the Q fever laboratories. All six were ill enough to require hospitalization. One was an unimmunized wife, not employed at Camp Detrick, of a worker in the virus laboratory; he himself had not been ill (26, page 61). Seven of the cases were in immunized persons. Four of these required hospitalization. Three other immunized persons had no fever over 100 degrees and remained ambulatory. Two required no antibiotic therapy. The average duration of fever in the non-immunized group was 4.1 days and in the immunized 1.5 days, suggesting that immunization is of value in modifying the disease.

The average period of total disability for the entire group of 13 patients was 12.7 days per patient.

One case was proved by isolation of Coxiella burnetti (1, page 62), the remainder being diagnosed by a significant rise in complement fixation titer.

Chemoprophylaxis was ineffective in one patient who was given 3 gm of aureomycin daily for three days immediately after an accident. Twenty-one days after the accident he developed Q fever.

Treatment with aureomycin or terramycin has been uniformly effective. The dosage schedule recommended is 2 gm daily for 10 to 14 days.

g. TULAREMIA

Twenty-three cases of tularemia, acquired as a laboratory infection, were reported during the year 1943 to 1945 (4). It was estimated that the attack rate for tularemia among laboratory workers was 48 per cent (14). Illnesses were moderate to mild in all instances, probably because all had been vaccinated. Only 14 of the

23 were hospitalized. In four of the hospitalized cases no therapy was given. The effects of streptomycin were studied in seven cases during this period and reported by Howe et al (27). In general, it was found that streptomycin was effective during the early, acute phase of the disease but of little benefit when administered in the late convalescent stage.

Since 1945 there have been 28 additional cases of which 18 have been hospitalized (28). Four were in persons who entered or worked in the building devoted to the study of tularemia, but who were not engaged in research on tularemia - a situation which testifies to the high infectivity of this organism. Of the 18, two were of the ulcero-glandular type and 16 were typhoidal. Four of the typhoidal cases, or 25 per cent, had pneumonia. In all, the disease was minimized by prompt chemotherapy. The average period of hospitalization of this group of cases was approximately two weeks. The total period of disability in most instances rarely exceeded one month. This compares favorably with the duration of the naturally occurring disease of 3.8 months in untreated cases in non-immunized persons, and 2.05 months in early-treated, non-immunized cases (29).

The symptoms of tularemia, as experienced by immunized individuals, are quite non-specific. Myalgia, headache, easy fatigability, malaise and chills are among the most common. Fever is the most constant sign of the disease and is frequently the only physical sign. The ulcero-glandular type is characterized by a small ulceration, usually at the site of accidental auto-inoculation, and regional lymphadenopathy.

The white blood cell count is usually in the normal range with only occasional lymphocytosis. The blood sedimentation rate is increased. No positive blood cultures were obtained in this series of cases. Material from cutaneous or mucous membrane lesions did infect guinea pigs on three occasions. The diagnosis is alternately made by demonstrating a rise in titer of agglutinating antibodies. This rise most commonly occurs about three weeks after the onset of symptoms. When the diagnosis of tularemia is strongly suspected clinically, the patient may be treated and released from the hospital before the antibody rise occurs confirming the clinical impression.

An attempt to hasten the laboratory diagnosis by using a hem-agglutination inhibition reaction to detect B tularense polysaccharide in the patient's serum was unsuccessful (8, page 78; 2, page 64). However, successful use was made of the patient's serum as an antibody system to agglutinate polysaccharide treated erythrocytes; this can often give a positive agglutination test 3 to 10 days before the conventional agglutination test shows a significant rise (30).

Streptomycin is the treatment of choice in tularemia (31). When given intramuscularly in doses of 0.125 grams every six hours for six days, defervescence and symptomatic improvement almost always occurs within the first 48 hours of therapy. This pertains only to the acute phase of the disease. Relapses are rarely seen.

Illness with tularemia does not afford complete immunity from re-infection. At least 4 cases of reinfection have been observed and two proved by recovery of the organism (32). Inasmuch as all 52 cases had been vaccinated, it is also concluded that immunization with tularemia vaccine does not prevent infection, although the mildness of the illnesses indicates that it mitigates the disease.

h. VENEZUELAN EQUINE ENCEPHALOMYELITIS

Three hospitalized cases resulting from the injection of the VEE vaccine occurred in 1952 and were included in the 14 cases reported previously (26, page 73).

The most significant conclusion from this episode is that the infectious subcutaneous dose of VEE for man is less than that for the mouse, rat, guinea pig, rabbit, or monkey, inasmuch as it has been impossible to cause illness in these animals by injectiong the same lot of vaccine which infected man.

No cases have occurred in the first 500 persons who have received a new vaccine prepared by V&R Division (33). The essential difference in the vaccine is the use of 4 per cent formalin for virus inactivation instead of 0.2 per cent.

3. SUMMARY OF LABORATORY INFECTIONS, BY YEARS

When one considers only hospitalized persons, and excludes the 14 cases of Venezuelan equine encephalomyelitis caused by vaccination, there remains (Table 3.1):

1947-48	1949	1950	1951	1952	1953 (4½ mo.)
10 cases	14	15	28	22	7 cases

This reflects the rising tide of amount of work with infectious agents, and consequent rise of infections, until a maximum was reached in 1951. In that year, installation of Class I bacteriological safety cabinets (often referred to as "Blickmans," from the name of the first fabricator) became common in the laboratories. Buildings T-430 and T-433 were safety equipped, and infections ceased in these buildings. 1952 was the first year in the history of Camp Detrick in which all the personnel of a building (T-430) spent the entire year handling brucella, without a single case of brucellosis. During 1952 and 1953 (to 20 May), there were buildings, well equipped safety-wise, which have made excellent safety records in handling the most infectious agents:

T-429 - plague - no cases

T-432 - pilot plant lab - tularemia, anthrax, brucella - no cases

T-459 - all agents - 1 case brucellosis

T-527 - test sphere - brucella, tularemia, anthrax, Q, VEE - one case tularemia due to a hypodermic needle stuck in the skin

By way of contrast, infections occur with monotonous regularity in T-434 (virus pilot plant), T-522 (viruses) and T-525 (tularemia), all of which have little or no safety equipment of the ventilated cabinet type. Only the fact that both buildings house agents whose course of illness is modified by available vaccines makes it possible to continue operations at the comparatively primitive level of safety. Even then, each infection carries a risk of death.

In 1953 the increased emphasis upon the more infectious and more seriously disabling diseases seriously threatens our safety program. A great effort is being made to provide all infectious research units with adequate safety equipment so that the revised research programs may progress. However, in all safety practices the building supervisor is the key to a good safety record. Although certain basic safety apparatus is essential, there is no equipment which will substitute for conscientious safety supervision.

TABLE 3.1 INFECTIONS* CAUSED BY AGENTS

Dates	Treatment group(a)	Brucellosis	Tularemia	Anthrax	Glanders	Psittacosis	Q Fever	Coccidioidomycosis	Miscellaneous	Total
Oct 1943 to Oct 1945	Hosp	18 b,c	14	26	6	1	No research	2		67
	Not hosp	0	9	0	0	0		0		9
	Total	18	23	26	6	1(o)		2		76
1947 1948	Hosp	7 b,z	3 g							10
	Not hosp	12	3 h							15
	Total	19	6							25
1949	Hosp	8	4 i	1	No Research	1		No Research		14
	Not hosp	4	3	0		0				7
	Total	12	7	1		1 (p)				21
1950	Hosp	6 b	4				3		5	18
	Not hosp	0	0				0		3	3
	Total	6	4 j				3 t		8 w	21
1951	Hosp	18 d	3 g	1		4	2 u		8	36
	Not hosp	0	2	0		0	1		5	8
	Total	18 z	5 j	1 m		4(q)	3		13 x	44
1952	Hosp	6 e	3 g			5	4		7	25
	Not hosp	0	1		n	0	2	n	1	4
	Total	6	4 k			5 r	6(v)		8 y	29
1953 to 16 May	Hosp	2	2	1		1	1		0	7
	Not hosp	0	1	0	n	0	0	n	1	2
	Total	2	3	1##		1	1		1 **	9
Summary	Hosp	65	33	29	6	12	10 #	2	20	177
	Not hosp	16	19	0	0	0	3	0	10	48
	Total	81 f	52	29	6	12	13	2	30	225

* Includes reinfections but not relapses.

a Hospitalized or not hospitalized.

b Brucella skin test was positive in one of these persons before illness.

c Including one person in July 1946, possibly originating in 1945.

	Hosp									
Summary	Not hosp	16	19	0	0	0	3	0	15	225
	Total	81 f	52	29	6	12	13	2	30	

e Includes reinfections but not relapses.

a Hospitalized or not hospitalized.

b Brucella skin test was positive in one of these persons before illness.

c Including one person in July 1946, possibly originating in 1945.

d There are 2 relapsed persons from 1948 and 1949 not included here; 5 others of the 18 had positive skin tests before illness; 2 others were reinfected persons who had had brucellosis at Detrick in 1948 or 1950. One man included in prior summaries is omitted because he turned out to have tuberculosis.

e There are 2 relapsed persons from 1948 and 1951 not included here; 2 others are re-infections.

f Eight of these had positive skin tests before illness and 4 others are duplicates representing four persons twice infected (not relapsed). 4 had positive cultures. 5 hospitalized and 2 non-hospitalized were probably caused by Br abortus A19.

g One of these persons worked only on Dys shiga in the tularemia building T-525 where all the other cases occurred. (In the 1947-48 total, there was only 1 in 1947).

h One reinfection in a person having had tularemia before coming to Detrick and one reinfection in a person hospitalized in 1947 for a pneumonia later diagnosed serologically as tularemia.

i These were 1 painter, 1 secretary, and 1 laboratory worker, all employed in the tularemia building T-525, and one habitual visitor to the building.

j One case in Bacterial Nutrition Bldg T-433; the others in Building T-525.

k Two of these are believed to be reinfections of persons having non-hospitalized mild tularemia at Detrick in 1948 and 1949. All cases were in Building T-525.

l There are 3 duplicate persons here. See notes k and h. All were immunized.

m Fatal pulmonary anthrax; a detailed discussion is in S Div QTR 31 Dec 51. All other cases were cutaneous. The organism was recovered in culture in all except the pulmonary case.

n Some research begun under rigid safety precautions.

o Given as 2 in Detrick Special Report 72, but one was probably mouse pneumonitis. Vaccinated.

p Unimmunized, contact with broken tube of lyophilized culture.

q One unimmunized wife infected by her husband who was one of the 3 cases immunized with the 6 BC strain and probably infected with the Borg strain in virus pilot plant.

r All immunized with 6 BC strain; one is a reinfection of a man ill with the same disease 1 year previously.

s There is one duplicate person here. See note (r). Two were unimmunized.

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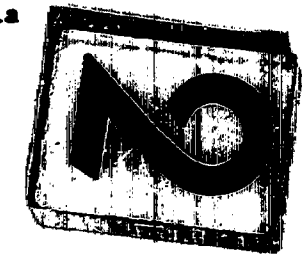

h One reinfection in a person having had tularemia before coming to Detrick and one reinfection in a person hospitalized in 1947 for a pneumonia later diagnosed serologically as tularemia.

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k Two of these are believed to be reinfections of persons having non-hospitalized mild tularemia at Detrick in 1948 and 1949. All cases were in Building T-525.

l There are 3 duplicate persons here. See notes k and h. All were immunized.

m Fatal pulmonary anthrax; a detailed discussion is in S Div QTR 31 Dec 51. All other cases were cutaneous. The organism was recovered in culture in all except the pulmonary case.

n Some research begun under rigid safety precautions.

o Given as 2 in Detrick Special Report 72, but one was probably mouse pneumonitis. Vaccinated.

p Unimmunized, contact with broken tube of lyophilized culture.

q One unimmunized wife infected by her husband who was one of the 3 cases immunized with the 6 BC strain and probably infected with the Borg strain in virus pilot plant.

r All immunized with 6 BC strain; one is a reinfection of a man ill with the same disease 1 year previously.

s There is one duplicate person here. See note (r). Two were unimmunized.

t Unimmunized and not working with Q; one probably infected by unsterilized equipment and one possibly by association with virologist, one unknown source.

u 2 Unimmunized probably infected by contact with unsterilized equipment.

v One unimmunized, unexposed wife of a worker in the virus laboratory, 2 designers and a carpenter briefly in the building.

w Three Venezuelan equine encephalomyelitis after injection of vaccine; 5 caused by Salmonella typhi murium.

x Eight Venezuelan equine encephalomyelitis after injection of vaccine; 2 caused by Shigella flexneri, 3 Newcastle conjunctivitis.

y Three Venezuelan equine encephalomyelitis after injection of vaccine; 4 caused by Shigella flexneri; one Salmonella sonnei (one S flexner reinfection also in 1950).

z One caused by Brucella abortus strain A 19

Six unimmunized, indirectly exposed; not working in the virus building.

** Serratia marcescens infection of finger with lymphangitis, probably complicated by Staphylococcus or Strep.

had been immunized, 2 injections 5 months before; cutaneous at prior injury; seemed to heal more rapidly than unimmunized.

4. SUMMARY OF LABORATORY INFECTIONS BY BUILDINGS

See section 3 above, and Table 4.1.

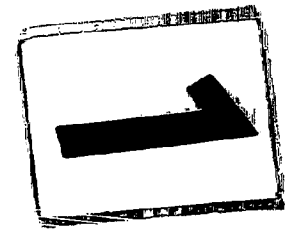

TABLE 4.1 INFECTIONS* CAUSED BY AGENTS, BY BUILDINGS
1947 - 16 May 1953

Building	Disease	1947-48	1949	1950	1951	1952	1953 to 16 May	Total Non-Hosp	Total Hosp	Total Bldg Total
T-263 Aerosol Tank	Brucellosis	1(b)	---------No pathogens used---------					0	1	1
T-427	Diarrhea	--Mostly anthrax work--			1	0	0	1	0	1
T-428	Diarrhea			5	--No pathogens used--			3	2	5
T-429	Brucellosis	3	2	2	(m)Plague			3	4	7
T-430	Brucellosis	-No pathogens-		1	2	(m)0	0	0	3	3
T-431	Brucellosis	No R	0	0	6	4	1	0	11	
Pilot	Anthrax	No R	No R	No R	0	0	1	0	1	13(o)
Plant	S marcescens	0	0	0	0	0	1	1	0	
T-432	Brucellosis	4	6	1	3	(m)0	0	6	8	
Pilot	Anthrax	0	0	0	1	No R	0	0	1(f)	16
Plant Lab.	Tularemia	No R	No R	No R	1	0	0	0	1	
T-433	Brucellosis	7	1	1	3	0(k)	0	4	8	
Lab	Tularemia	No R	0	1	1	0	0	0	2	14
T-434	Psittacosis	Bldg not		0(p)	4(g)	4	0		8	
Pilot	Q	in use			1	3(j)	0	2	2	12
Plant										

T-433 Lab	Brucellosis	7	1	1	3	0(k)	0	4	8	
	Tularemia	No R	0	1	1	0	0	0	2	14
T-434 Pilot Plant	Psittacosis	Bldg not		0(p)	4(g)	4	0		8	
	Q	in use			1	3(j)	0	2	2	12
T-459	Brucellosis	Not in use-------			0(q)	(m)1	0	0	1	1(s)
T-502	Brucellosis	No work on brucella			1			0	1	1
T-522 Lab	Psittacosis	0	1(c)	0	0	1	1	0	3	
	Newcastle				3			3	0	14
	Q	0	0	3(d)	2(h)	2(g)	1	1	7	
T-524 Aerobiology	Brucellosis	2	2	1	0	0	1	2	4	
	Q	No R	No R	0	0	1	0		1	8(r)
	Tularemia	No R	0	0	0	0	1		1	
T-525 Lab	Tularemia	6	7(e)	3	3	4	1	10	14	
	Diarrhea				1	5		2	4	30
T-527	Tularemia	Bldg no in use ----------				(m)0	1	0	1	1
Engineering (a)	Brucellosis	3	0	0	3(i)	1		1	6	
	Anthrax		1	0	0			0	1	8
Total		26	20	18	36	26	9	39	96	135

(a) Placed here only when the building in which the disease was acquired was not determinable; includes 1 in 1948 from Technical Director's office.

(b) Two other mild cases did not get in the early statistics. See T.S. Report CD Biological Laboratories YF 1654. WSEQ Report No. 8 on Brucella, and CD ?Z S Div QTR 30 Jun 49, page 27.

(c) Case N.D.R. caused in Bldg T-501-S by a broken lyophile tube sent there from T-522 for mailing.

(d) All 3 persons were unimmunized and did not work in the T-522 laboratory which, however, was the only place on the Post where the agent was handled.

(e) Three hospitalized cases were in the buildings; immunized secretary, an immunized painter worked in the "clean" change room, and an unimmunized habitual visitor.

(f) The only death 1943-1953.

(g) Includes unimmunized wife of an employee.

(h) Unimmunized person handling unsterilized equipment from T-522.

Lab	Diarrhea				1	5		2	4	
T-527	Tularemia	Bldg no in use ----------				(m)0	1	0	1	1
Engineering (a)	Brucellosis	3	0	0	3(i)	1		1	6	
	Anthrax		1	0	0			0	1	8
Total		26	20	18	36	26	9	39	96	135

(a) Placed here only when the building in which the disease was acquired was not determinable; includes 1 in 1948 from Technical Director's office.

(b) Two other mild cases did not get in the early statistics. See T.S. Report CD Biological Laboratories YF 1654. WSEQ Report No. 8 on Brucella, and CD BL S Div QTR 30 Jun 49, page 27.

(c) Case N.D.R. caused in Bldg T-501-S by a broken lycphile tube sent there from T-522 for mailing.

(d) All 3 persons were unimmunized and did not work in the T-522 laboratory which, however, was the only place on the Post where the agent was handled.

(e) Three hospitalized cases were in the buildings; immunized secretary, an immunized painter worked in the "clean" change room, and an unimmunized habitual visitor.

(f) The only death 1943-1953.

(g) Includes unimmunized wife of an employee.

(h) Unimmunized person handling unsterilized equipment from T-522.

(i) Possibly during remodeling of T-429 and T-430.

(j) All 3 were immunized but not regular employees of T-434 (2 design engineers and a carpenter).

(k) Well equipped with bacteriological safety cabinets late in 1951, and since then has safely handled Coccidioides immitis, brucella, and B tularense.

(m) Installation and use of bacteriological safety cabinets deserves a major credit for absence of infections. This record is in marked contrast to that in Bldgs 525, 522, and 434 where safety cabinets are either entirely absent or few in number and not well used.

(No R) No research on this agent during this year in this building.

(o) Botulinum A has been in T-431 and also in pilot plant laboratory T-431 continuously since their erection; B tularense in T-431 since 1952.

(p) Cal 10 psittacosis strain to April 1950 when 6 BC was started; Borg strain was started 4 Dec 50.

(q) Began work with pathogens in June 1950.

(r) T-524 has been handling psittacosis virus since 1948, VEE since 1950, and Botulinum toxin since 1945.

(s) T-459 has handled almost every highly infectious agent known.

* Includes reinfections but not relapses; does not include 14 cases of Venezuelan Equine Encephalomyelitis caused by living virus in a vaccine.

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