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MILITARY BIOLOGY AND BIOLOGICAL WARFARE

By

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By authority of Chief Chemical Officer
by J. C. Greenlander Lt Col Cml C
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INTRODUCTION

Biological warfare may be defined as the organized cultivation and mass dissemination against an enemy of those same agents of disease which have plagued mankind throughout history — the sickness and death produced in men, animals and plants by the microscopic world of bacteria, fungi, viruses, rickettsiae, and by the toxic products of living organisms. What biological warfare might mean under modern conditions has been vividly foretold by popular scientists who envisaged an enemy directly infecting masses of people gathered in public places, contaminating food and water supplies, and inoculating animals with diseases contagious to man, thus loosing against whole populations hordes of the parasitic agents of disease.

The idea of deliberately using disease organisms in modern warfare is not new, nor has it been untried. Confirmed and undoubted use of certain disease agents was made by the Germans in the First World War. The Germans infected horses of the Roumanian cavalry with glanders, attempted to carry out the same activity in the French cavalry, and succeeded in infecting horses and cattle in the United States prior to their shipment to Europe. Still more recently, in 1934, it was charged that German scientists were studying offensive aspects of large-scale biological warfare. In 1940 unsubstantiated but convincing claims were made by the Chinese that Japanese planes over Chekiang province had released infected fleas wrapped in little cotton bags containing grain to attract rats. Again in 1941 the Japanese were accused of dropping rice infested with the short oval bacillus of bubonic plague.

Upon this country's entrance into World War II, the War Department took steps at once to determine the possibility and likelihood of such warfare. A committee of scientists was appointed by the Secretary of War to investigate the problem and make recommendations. Following the committee report in February 1942, which admitted the feasibility of biological warfare and urged that we begin its study at once, a War Research Service, attached to the Federal Security Agency, was created and given charge of all preparations for biological warfare. With the cooperation of the Medical Department of the Army, the Chemical Warfare Service and the Navy Bureau of Medicine and Surgery, measures were at once taken to protect the water and food supplies on the mainland and in all overseas areas controlled by the United States. However, despite the establishment of the War Research Service, there was still no single organization in the country fully capable of undertaking the research and development program necessary to ensure adequate preparation for biological warfare. To meet this need the War Department in November 1942 directed the Chemical Warfare Service to assume the responsibility.

In the summer of 1943 a Special Projects Division was created in the Chemical Warfare Service and both defensive and offensive aspects of the biological warfare program which were being investigated by a number of agencies in various parts of the country became subjects of investigation by the new organization. The Division, operating in great secrecy, expanded rapidly until its organization numbered almost 3,900, of which approximately 2,800 were Army personnel, 1,000 Navy and 100 civilian. To the Chemical Warfare Service had fallen the task of uncovering any means that an enemy might use to overcome our medical defenses, determining the agents he might cultivate to new extremes of virulence, the missiles he might develop in order to project these agents, and with this knowledge, the task of preparing for defense and retaliation. Its activities became far-reaching as it began the study of specific pathogenic agents, developing virulent strains and working on defenses against their high disease-producing properties. Manufacturing processes were devised for the mass production of certain biological agents. Field tests were conducted to determine the effectiveness of viable agents under simulated combat conditions. These activities of the Special Projects Division were coordinated with those of participating agencies in the program by the establishment of close liaison with the Medical, Ordnance and Intelligence services of the Army and Navy, with the Army Air Forces, the United States Public Health Service, the Department of Agriculture, and with British and Canadian biological warfare organizations. The Intelligence services of the Army and Navy, the Office of Strategic Services and the Federal Bureau of Investigation supplied information as to the progress of enemy plans and provided a gauge of the success of our own efforts.

The intensive efforts of the Special Projects Division of the Chemical Warfare Service in studies and production of biological agents resulted in the development of methods and facilities for the possible mass production of a number of pathogenic microorganisms and their products and the creation of means for accurate detection of minute quantities of these agents. As a result of the research program of defense, it became possible to devise protection for troops against the effects of a number of bacterial agents that might be employed in combat. In addition, the investigation of defenses against biological warfare resulted in significant contributions to our knowledge concerning the development of immunity in human beings and animals against many infectious diseases. It also resulted in the development of effective protective clothing against biological agents and equipment such as sampling and rapid detection devices. Information was gathered on the effects on living plants of more than 1,000 different chemical agents. The objective was attained; adequate defenses against a new and potentially devastating method of warfare were developed, and surprise from this quarter was forestalled.

MILITARY BIOLOGY AND BIOLOGICAL WARFARE

Chapter 1

FUNDAMENTAL PRINCIPLES

Section I

GENERAL NATURE AND OCCURRENCE OF MICROORGANISMS

CHARACTERISTICS. Microorganisms are primitive living forms which are too small to be seen by the unaided eye. When magnified 500 to 1,000 times by a microscope, the individual is seen to be composed of a single cell which is capable of carrying on all the functions of life, including growth and reproduction. Microorganisms are in many ways dependent on their environment and are unable to modify it by the means available to larger forms of life. The parasites among the microorganisms feed on living plants and animals, the saprophytes feed on dead or decaying organic matter. Lacking a mouth and digestive tract, they must acquire food in soluble form which is taken in through moist membranes that surround the cell contents. Being without organs of sight, they do not differentiate between light and darkness. Having no circulatory system, they assume the temperature of their surroundings. In these and certain other characteristics they resemble plant life and are, therefore, usually regarded as primitive members of the vegetable kingdom, though some animal-like forms are known. They are the most elementary forms of life possessing the ability to grow and develop.

KINDS OF MICROORGANISMS. Microorganisms are commonly subdivided as follows:

Bacteria. Bacteria are minute, unicellular, plant-like, microscopic organisms which differ from true plants in that they lack chlorophyll. Their three typical forms are exhibited as spherical, rod-shaped or spiral threadlike cells and are widely distributed in soil, air, water, the bodies of living animals and plants, and dead organic matter.

Viruses. The viral organisms are too small to be seen with the compound microscope or to be retained by filters which prevent the passage of bacteria. They have been photographed with the electron microscope, yet they are commonly detected only by the disease symptoms which they produce. They are obligate parasites, that is, limited to a single life condition, and grow only in the presence of living susceptible tissue. They cannot be cultivated on artificial, lifeless media.

Rickettsia. The rickettsial bodies resemble bacteria in shape and are considered as intermediate between the bacteria and filterable viruses to which they are related. Eventually they may be included in the bacteria; at present they are in a special class because of their peculiarities of habitat, appearance, intracellular position and resistance to artificial cultivation. The rickettsia are originally insect parasites and the diseases they cause are all transmitted to man by intermediate hosts such as insects, ticks and lice. Rickettsia are distinctive in their apparent selective affinity for specific types of cells in the human and animal body.

Yeasts. Yeasts are unicellular fungi of somewhat larger forms than bacteria and are commonly spherical, oval or egg-shaped. They are noted for their ability to produce alcohol and carbon dioxide from sugar solutions such as fruit juices or molasses.

Fungi. The group of plants which include the molds, mildews, rusts, smuts, mushrooms, toadstools and puffballs are known as the fungi. The group as microorganisms is well-known for its ability to grow and cause spoilage under conditions which prevent development of most other microorganisms.

Protozoa. The most primitive and elemental of animal forms are found among the protozoa. They are unicellular animal-like organisms which are variable in size, shape and structure and have highly specialized functions of the protoplasm. They are mostly aquatic, abounding in the sea and in stagnant fresh water. Some are parasites and, like the malaria parasite, are highly pathogenic.

DISTRIBUTION IN NATURE. The distribution of microorganisms is universal. All surfaces exposed to dirt and dust are inevitably contaminated by a variety of organisms which normally occur in soil. Even virgin top soils provide the natural home for billions of microorganisms in every cubic foot. Certain types of microorganisms show a preference for plant materials, some prefer stagnant water, and others thrive best on and in the animal body. The skin, hair, nose, mouth and intestinal tract harbor a considerable variety of bacteria in astronomical numbers. Fortunately, most of the organisms commonly encountered in nature are harmless to man. Some forms are beneficial, performing such functions as decomposition of organic wastes and residues, making inert gaseous nitrogen from the air available to plants, or producing valuable foods, beverages, drugs and industrial chemicals. Only a few, perhaps five to ten per cent, of all the known species of microorganisms, produce disease. Though numerically small, the damage which they may do when proper control measures are lacking exceeds that of any other destructive influence to which living things are commonly subjected.

Section II

MORPHOLOGY AND REPRODUCTION

MICROSCOPIC APPEARANCE. The common microorganisms are so small that ordinary units of measurement cannot be applied. The unit usually employed is the micron, which is equivalent to approximately 1/25,000-inch or 1/1,000-millimeter. A micrometer is used to measure the apparent diameters of microorganisms which subtend minute angles.

Bacteria range in size between 0.5 and 10 microns across their greatest cell dimension. They are somewhat variable in form, but three general types are commonly encountered. As tiny balls or spheres they are called cocci (s., coccus); as straight rods they are called bacilli (s., bacillus), and as curved rods they may be either spiral-shaped and called spirilla (s., spirillum) or comma-shaped and termed vibrio.

Viruses are invisible in the microscope, measuring less than 0.4 micron across their greatest dimension. Because they are invisible as individuals, they are of doubtful form. Globules, crystal-like needles and other forms have been described. There is some doubt as to whether these represent organized cells, though they undoubtedly are composed of virus substances at least in part.

Rickettsia commonly measure between 0.4 and 1 micron in size. They resemble bacteria in shape and may be cocci, diplococci or short bacilli. As diplococci they are pairs of spherical cells, adhering together. Little detail of appearance can be observed because of their small size.

Yeasts may measure between 2 and 30 microns and are commonly round, oval or rod-like. The common beer or bread yeast is more or less egg-shaped.

Fungi or molds range between 3 and 50 microns in size and show a greater diversity of forms than any of the preceding groups. They range in size from the microscopic cells of the yeast plant to the highly organized fruiting body of mushrooms. Commonly the cells of the fungi are rod-shaped and arranged end to end in strands or filaments known as hyphae. A mass of these hyphae are termed mycelium. A compact mass of hardened mycelium, stored with reserve food material, is termed a sclerotium and is found among the higher forms of fungi. When a sclerotium opens, it sends out hyphae or produces spore fruits.

Protozoa may be as small as 1 micron in diameter or as large as 100 microns. They present such a variety of shapes that no general description can encompass them. They include the irregular and changeable masses of protoplasm known as amoeba and the regular oval shapes with hair-like outer covering called paramecium. Not only do different species vary in appearance, but the same protozoan may pass through a complex cycle of development in which one stage bears no outward resemblance to any other. This is especially true of the malaria parasite which develops in different ways in the mosquito and in man.

Microorganisms are usually composed of the fundamental structures which make up any living cell. There is a protective outer covering or cell wall which in the bacteria is rigid and in protozoa may be quite flexible. Next, there is the semi-fluid jelly-like contents or cytoplasm in which are dispersed a variety of granules and vacuoles. The granules and vacuoles are grains and pockets in the cytoplasm which contain saps or secretions for the metabolic processes necessary to the cell. The central mass or substance in the cells which controls the hereditary characters is called the nucleus. The entire cell contents, including cytoplasm and nucleus, are termed protoplasm. Among the few notable exceptions to this structural organization is the absence of a distinct nucleus in the bacteria and the lack of a well-defined cell wall in amoebae.

Special structures are elaborated by certain microorganisms which are not characteristic of all species. Such are the organs of locomotion of certain microorganisms such as the protozoa, which are usually hairlike structures that protrude through the cell wall and by waving in the surrounding liquid permit movement. These structures are called _flagella_ when they are flexible and whip-like or _cilia_ when rigid and oar-like. Microorganisms which exhibit or are capable of spontaneous movement are said to be _motile_.

Another special structure is the _capsule_, a broad colorless outer wall of gelatinous or gummy substance which may be formed around the outside of the cell. It is exhibited by many bacteria, particularly virulent pneumococci and the agent of anthrax. It is a protective envelope which interferes with the destruction of the cell by defense mechanisms of the animal body. As such, its presence is usually evidence of high virulence.

Another structural development is the _spore_, a heavy-walled and highly refractive body formed in the cell which is capable of resisting adverse conditions such as starvation, excessive heat or cold or drying that destroys the more delicate vegetative cell. Many rod-shaped bacteria produce a single spore within each cell. When the spore has matured, the surrounding cell autolyzes or disintegrates, leaving the free spore. This resistant form may remain dormant without food or water for years and then give rise to an actively growing cell when conditions again become favorable. It is a basis of persistency in the biological agents.

GROWTH OF MICROORGANISMS. Growth may be determined by several methods, some of which involve determination of the products of growth while others depend upon counting the number of cells formed. By such means it has been proved that microorganisms respond in definite ways to various environmental factors such as moisture, food, oxygen, temperature, light, reaction and time.

Moisture. Perhaps nine-tenths of the cell substance is water. It is essential for all microorganisms. If even a part of the water is removed it interferes with the normal functions of the organism and may eventually cause its death. Moisture in the immediate surroundings of the organism is necessary to prevent loss of water from the cell and to permit the acquisition of food in soluble form. Generally speaking, the more water present the more favorable is the environment for microbial growth.

Food. Food is required to provide energy and to supply the materials from which cell substance is made. Microorganisms can utilize a wide variety of substances, including mineral salts, sugars, proteins and vitamins. They can use not only all the foods available to man and other animals, but also a great many other materials, especially the residues and wastes from plant and animal life. No one species utilizes all foods; in fact, some are extremely fastidious about the foods upon which they grow. Among these are the disease-producing organisms which have a predilection for the central nervous system, the skin or the respiratory tract, although the predilection may not be based entirely on the special foods of these environments.

Oxygen. All microorganisms require oxygen in order to live, but they differ markedly in the sources from which they obtain it. Aerobes are microorganisms which must obtain free oxygen from the air. The organisms causing pneumonia, tuberculosis, tularemia and typhoid are aerobes, or aerobic. Anaerobes cannot use atmospheric oxygen and may even be harmed by it. They obtain chemically combined oxygen from their food. The organisms causing botulism, tetanus and gas gangrene are anaerobes. Some microorganisms may reduce nitrates or sulphates to obtain oxygen, but many disease-producing anaerobes require complex organic substances for this purpose. Faculative microorganisms can obtain oxygen both from the air and from chemical compounds. Some prefer to use atmospheric oxygen and are called faculative aerobes, while others prefer oxygen in some chemically combined form and are therefore called faculative anaerobes.

Temperature. Temperature is a varying factor, each species of organism developing most abundantly at a particular or optimum growth temperature for that species. As variations in temperature occur either above or below this point, the organism functions less and less effectively until points are reached both above and below the optimum at which growth no longer occurs. The highest temperature at which growth takes place is termed the <u>maximum growth temperature</u> and the lowest which permits growth is the <u>minimum growth temperature</u>. The microorganisms which produce diseases among the higher animals and those which attack plants differ in their ranges of growth temperature as follows:

	In degrees Centigrade		
	Minimum	Optimum	Maximum
Animal parasites	15-20	32-37	40-45
Plant parasites	5-15	20-30	32-42

<u>Light</u>. Microorganisms do not require light for growth. While they are not affected by visible light, they are destroyed by ultra-violet rays from the sun or from artificial sources when exposed directly. Consequently, growth occurs best in the dark, or at least in areas protected from sunlight. The protection need only be a film of water or the shadow from a particle of dust.

<u>Reaction</u>. Reaction or pH factor is a term that may be used to indicate the degree of acidity or alkalinity of the substance in which growth of the organism is taking place. Most organisms associated with the animal body commonly grow best in environments with neutral or slightly alkaline reactions. Microorganisms associated with plants often prefer a slightly acid environment. Growth is inhibited whenever the reaction becomes markedly acid or alkaline. Yeasts or molds, however, tolerate much greater degrees of acidity

than do the bacteria.

The growth of microorganisms requires time. When microorganisms are deposited in any new environment there is a period of adjustment or lag phase during which the number of cells does not increase appreciably. If the essential factors such as moisture, food, temperature and oxygen are favorable, and there is no great opposition from the cells of the host body, a period of rapid multiplication follows during which the number of organisms doubles every 15 to 60 minutes. This may continue until lack of food, accumulation of wastes or other inhibitory influences slow down the processes. Within a relatively few hours, or, at most, days, an insignificant number of microorganisms may develop into numbers almost beyond comprehension.

BIOCHEMICAL ASPECTS OF GROWTH. The utilization of food by microorganisms involves a number of complicated chemical reactions, including processes of hydrolysis, oxidation, reduction and synthesis of new compounds. In the living organism these reactions are made possible by the presence of enzymes. Specific enzyme systems are required for each kind of food and each process going on within the cell. The enzymes are peculiar in that they initiate or enhance chemical reactions without themselves being used up in the process. The results of the biochemical activities of microorganisms are two-fold. Analytic processes cause the breakdown of food to less complex substances, some of which are used by the cell, while others accumulate as byproducts. Common byproducts include acids, gases, alcohols, aldehydes and salts. Synthetic processes cause the formation of new substances from the decomposed food. Some of the materials produced are new cell substances or protoplasm, material for the construction of capsules, pigments which give the characteristic color to some species, and toxins. The latter are among the most potent poisons to animal tissues in nature.

Conditions of growth often alter the capacity of microorganisms to perform certain of their normal functions. For example, cultivation at high temperatures in the absence of air or in the presence of certain chemicals may prevent bacteria from forming spores. Growth under one set of conditions, such as the environment of an animal body, may enhance the ability of an organism to produce disease, while growth on artificial media may decrease this power. Specific chemical substances may be essential for the formation of certain products of growth, such as toxins or pigments, even though the organism can grow without these substances.

METHODS OF REPRODUCTION. Asexual. Reproduction without sexual union is common among microorganisms. The bacteria multiply by division of the cell into two equal and identical parts. This is binary fission. In the rod-shaped bacteria, division always occurs across the shortest dimension of the cell, at right angles to the long axis. This is sometimes called transverse fission. Some yeasts also produce asexually by binary fission, but the common method is one in which a small portion of the parent cell is pinched off, as it were, and becomes a new actively growing individual. This process is termed budding. Viruses and rickettsia, whose development occurs only in the presence of living tissue, multiply by a means not yet scientifically explored. Protozoa may frequently reproduce by fission but sexual reproduction is very common.

Sexual. Rudimentary sexual methods of reproduction are often encountered among the microorganisms. These involve copulation of two or more cells with interchange of protoplasms, usually resulting in the formation of spores. These may be termed ascospores, zygospores, etc., depending upon their exact characteristics and mode of formation. Sexual methods of reproduction are known to occur among the yeasts, fungi and protozoa even though no visible differentiation between male and female cells exists.

Section III

INHIBITION AND DESTRUCTION

PHYSICAL. Temperature. The most widely used method of preventing microbial growth and of killing microorganisms is by control of temperature. High temperature and refrigeration are extremely effective in preventing multiplication. Sterilization, for the complete destruction of all living forms, requires more drastic measures. When spores are present, temperatures of 240-250 degrees Fahrenheit must be applied for 15 to 30 minutes in the presence of moisture to ensure sterilization. If dry heat is used, even higher temperatures and longer periods of exposure are necessary. The presence of protective materials such as soil or animal wastes interferes with heat penetration and prolongs the period of exposure necessary for sterilization. Incineration by direct exposure to flame is perhaps the most reliable means of sterilizing materials which will stand this treatment.

Dessication. The oldest means of preventing spoilage of food by microorganisms is drying or dessication. Examples are jerked beef, prunes, powdered eggs, other dehydrated foods. When moisture is absent, food cannot be taken into the cell and growth ceases. Vegetative cells are particularly susceptible to drying, while spores are practically unharmed. Drying may effectively reduce the concentration of living organisms, but it cannot be relied on for total destruction.

Starvation. Inhibition of growth can be accomplished by removing or making unavailable essential compounds for the life of the cell. All cells require oxygen, carbon, nitrogen and hydrogen in some form. If any of these elements can be eliminated from the microorganismic environment or is converted to an unusable form, the microorganism is prevented from undergoing further development and may eventually starve to death. Since spores are capable of remaining dormant for long periods without food, such measures do not kill them, although they may effectively prevent germination of the spores. Starvation of microorganisms may also be achieved with substances which are valuable food to them in dilute solution, but which in greater concentrations actually prevents growth.

Sunlight. The ultraviolet rays of the sun quickly destroy microorganisms, but the rays have little penetrating power. The slight protection offered by liquid films or rough surfaces may be sufficient to prevent the death of the organism.

Osmotic pressure. The high osmotic pressures of sugar or salt concentrations of syrups or brines result in preservation of foods from the action of microorganisms because the osmotic pressure removes water from the microbial cell. A common application of this principle is the use of high concentrations of sugar to preserve foods such as jams and jellies. While such high concentrations may effectively prevent growth, they are not reliable for killing organisms, especially in the spore stage.

Filtration. Though it will not prevent passage of viruses or toxins, filtration can be employed to remove many microorganisms from fluids. Removal of the organisms may sometimes be effected by use of substances such as asbestos pads, colloidin membranes or unglazed porcelain, the pore sizes of which are too small to permit passage of cells. For gases such as air, passage through heavy layers of cotton batting effectively removes the airborne organisms. Even closely woven cloth will materially reduce the number of organisms which can penetrate in the form of dust. The efficiency of filtration processes, however, is subject to a variety of factors, including the particle size, number of organisms present and electrostatic charge.

CHEMICAL. Disinfectants and Antiseptics. The use of disinfectants and antiseptics, if used in appreciable concentrations, interferes with the life processes of microorganisms. Growth of the cell is prevented through antiseptic or bacteriostatic action. Injury and death of the cell is caused by disinfectant, germicidal or bactericidal action. Among the common disinfectants and antiseptics are mercuric chloride, silver nitrate, sodium hydroxide, tincture of iodine, chlorine, phenol (carbolic acid), cresol, alcohol, hydrogen peroxide and calcium hypochlorite. Glycol has also been demonstrated as an effective aerosol spray for purposes of disinfection and decontamination.

Antibiotic and Chemotherapeutic Agents. A number of exceptional chemical compounds have been found which are relatively harmless to higher forms of life but are capable of destroying certain disease-producing organisms, even after the organisms have become firmly established in the host body. The more effective of these exceptional agents include arsphenamine (Ehrlich's 606) used to treat syphilis, quinine used to combat malaria and the sulfonamide family — sulfanilamid, sulfathiazole, sulfadiazine, sulfapyridine — which have proved effective against pneumonia, gonorrhea and dysentery. The antibiotic agents, penicillin and streptomycin have proved valuable for treatment of infections not responsive to the sulfa drugs. One other antibiotic, streptothrycin, is still in the development stage. Chemotherapeutic and antibiotic substances are not inhibitory to microorganisms in general, but show a more or less specific action on particular species.

BIOLOGICAL. Bacteriophage. The phenomenon known as bacteriophage is still an unknown substance. It has been described by some as an ultravirus parasite of bacteria, by others as an enzyme produced by the bacteria themselves. It is an autonomous living agent whose immediate origin appears to be in the intestinal contents, blood secretions and organs of animals recovering from bacterial infection. The action of bacteriophage on some cells is to cause them to swell to relatively enormous dimensions and burst. On others, the cell subjected to the action of bacteriophage simply dissolves slowly, leaving an amorphous residue. The action of bacteriophage is very potent. One part in a billion, added to a growing culture of susceptible bacteria will cause the solution of all or nearly all the cells in three to four hours. Bacteriophage, recovered from cholera patients who have succumbed to the disease, has been satisfactorily used in the prevention and treatment of cholera.

Section IV

DETECTION AND IDENTIFICATION

SMEARS AND CULTURES. The first step in the detection and identification of biological organisms is to obtain samples of them for microscopic study. In the case of the human or animal body, smears may be obtained or cultures prepared from the blood or from tissue. A smear may simply be a tiny drop of blood or pus, spread with a needle on a microscopic slide and directly examined. For more specific determination of microorganisms, however, it is necessary to cultivate the organisms in a prepared nutrient media before they are examined.

SAMPLING. In the case of airborne microorganisms, it is necessary to sample the air in order to determine the nature and number of their presence. When such samples are obtained they may then be grown in appropriate culture media. The most common methods of sampling air are by means of solid culture media, bubblers and impingers.

Solid culture media. The exposed agar plate method depends on bacteria particles dropping out of the air and on to the plate. No quantitative determination can be made from this method since only the largest particulates in a concentration would fall to the plate.

The inverted funnel device is a method by which measured volumes of air are pulled by vacuum through a funnel placed over an open agar plate. This is more practical for qualitative determinations than merely exposing the plate, but is impractical in that the developing colonies overgrow one another when two or more types of bacteria are impinged on the agar in the same vicinity.

The sieve sampler is a modification of the funnel device. The metal sieve over the open agar plate prevents colonies from being superimposed upon one another.

The slit sampler pulls air by vacuum through a small slit in a metal disc. Below the disc an open agar plate on a slowly rotating table is exposed to the current of air drawn through the slit.

The Wells centrifuge draws measured volumes of air into a large tube into which molten agar has been placed. By rotating this tube, the molten agar coats the sides of the tube and bacteria in the air which is drawn into the tube are impinged on the agar surfaces.

<u>Bubblers</u>. The single orifice bubbler traps bacteria in air bubbles in liquid. By calibrating the size of the orifice, known volumes of air can be bubbled through the liquid. Its efficiency is limited by the fact that all portions of the air bubble do not

come in contact with the liquid and thus varying percentages of the bacteria in the air fail to become impinged.

The Wheeler bubbler consists of a number of small openings in the end of a piece of glass tubing. By making these openings smaller than is possible on the single orifice bubbler, smaller bubbles are formed, thus ensuring higher percentages of bacteria becoming impinged.

The Moulton collector consists of a capillary atomizer by means of which air being sampled is mixed with a liquid such as water. The mist is then passed through a multiple bubbler system similar to the Wheeler bubbler.

Impingers. The cotton impinger is believed to be a more practical method of sampling air than either solid media or bubblers. It collects over 99 per cent of the organisms sampled from a cloud. When spore suspensions are atomized and set up as clouds, over 98 per cent of the spores may be accounted for with this impinger. Liquid impingers such as the bubblers are 60 to 80 per cent as efficient.

The thermo-precipitator is used to obtain cloud samples in order to determine their particulate size. Samples of a cloud of microorganisms are first taken by means of an evacuated filter flask. The samples are then passed through the thermo-precipitator which, by means of a hot wire, sets up convection currents, impinging the particles on a glass coverslip. These particles may then be stained and observed under the microscope.

STAINING. Staining is the process of artificially coloring microorganisms with dyes or reagents in order to facilitate their study under the microscope. It may be necessary to make certain organisms visible under the microscope, to show the structure of the organism or to inhibit the growth of certain organisms so that others growing simultaneously may be studied. The stains most commonly used for these purposes are aniline dyes such as gentian violet, eosin, methyl violet and safranin. The most useful stain in bacteriology is Gram's Differential Stain, in which gentian violet is used. Those species which retain the violet dye are called gram-positive; those which are decolorized and take the counterstain, red or brown, are called gram-negative.

DIRECT MICROSCOPIC EXAMINATION. When microorganisms are present in large numbers they can be observed and counted by placing a sample under a microscope, but satisfactory differentiation of living from dead cells or of one species from another is not possible by this method alone. Both living and stained preparations must be observed to ascertain the size, shape, arrangement, motility, spore formation and staining reactions of organisms in order to identify them.

GROWTH IN PURE CULTURES. Probably the most satisfactory method of determining the number and variety of living microorganisms in any material is to cultivate the organisms. This may be done by placing a suitable sample in a sterile dish containing prepared nutrient media such as meat macerated in water, together with peptone and common salt. This is a fluid culture media. With the addition of agar, gelatin or starch, which will solidify when cold, a solid culture media is obtained. When the mixture is incubated at a suitable temperature for a few days, the bacteria multiply to such an extent that the offspring from each cell collect in masses or colonies which are plainly visible to the naked eye. Thus the number of living organisms originally placed or deposited in the dish can be determined by counting the colonies. The different appearance of various colonies aids in determining the kinds of organisms present.

BIOLOGICAL TESTS. Detection of disease-producing or pathogenic bacteria is often best accomplished by inoculation of susceptible animals, such as the guinea pig, rat or rabbit, with varying amounts of the sample in order to determine the least amount which will produce definite symptoms of disease, or even death.

BIOCHEMICAL TESTS. Determination of the byproducts of growth and the utilization of specific constituents of the culture medium may be effectively used to identify organisms. Production of acid, gas, alcohol or ammonia by the organism are common determinations that may be made. Tests to determine the utilization by various microorganisms of sugars, proteins and inorganic salts contained in the media may also serve to identify the organisms that are present.

SEROLOGY. All living cells when injected into the animal body produce substances which are antigens; that is, substances which induce the production in the host of specifically reacting substances called antibodies. These antigen-antibody reactions are termed serological reactions because the antibodies usually appear in the blood serum. A variety of tests are used to detect very minute differences between the many antigens produced by microorganisms and the antibodies produced as a result of the invasion of microorganisms.

HABITAT. Microorganisms usually show definite preferences for the type of environment in which they will grow in nature. Thus the source from which the organism is isolated may at times aid in its identification. However, under special circumstances such as the use of microorganisms in warfare, it may be presumed that organisms might be effectively placed in unnatural or at least unusual places, so that reliance on source could easily be misleading.

Section V

INFECTION AND IMMUNITY

INVASIVENESS AND VIRULENCE. That microorganisms have different methods of attack is illustrated by the fact that certain infections tend to remain localized while other spread rapidly through the body. The ability of a parasite to become disseminated and to produce spreading infection is called invasiveness. On the other hand, virulence refers to the ability of an organism to produce infection or death. Some organisms owe all their virulence to high invasive power. Some may be virulent primarily because they produce a potent toxic and owe little of their virulence to invasiveness. In botulism, for example, the toxin alone causes disease; the bacteria have no invasive power. In tuberculosis, virulence depends primarily upon invasive power.

MEANS OF INVASION. Microorganisms produce many substances within themselves which aid in the invasion of host tissues. These include hyaluronidase, leucocidins, hemolysins, fibrolysins and coagulase. Hyaluronidase is an enzyme that hydrolyzes hyaluronic acid, an important constituent of connective tissue. The dissolution of connective tissue allows bacteria to spread more widely. Hyaluronidase is found in bacteria, many animal tissues and snake and bee venom. Leucocidins are produced by some streptococci and staphylococci and cause the death of white blood cells. With the death of the white blood cells, one of the principal defenders against bacteria is removed. Hemolysins act to liberate hemoglobin from the red blood corpuscles, resulting in the dissolution of the red cells. Fibrolysins are capable of dissolving human fibrin, the coagulating factor in the blood. Many strains of hemolytic streptococci and some anaerobic bacteria produce this substance, as a result of which the spread of bacteria is facilitated. Coagulase is a substance produced by certain bacteria which is capable of coagulating citrated or oxylated plasma; that is, it induces coagulation where it is harmful to the body. It is also produced by the body itself and is one of the factors responsible for walling off of pimples, boils and abscesses.

TOXINS. A toxin is a product of the growth of microorganisms, having properties which render it injurious to the host. Some bacteria owe all or a part of their virulence to their ability to produce toxins as metabolic substances. Exotoxins are a secretory product of the bacterium and are given off into the surrounding blood. They generally are potent substances fatal in a very small dose, and are very good antigens. They usually show a characteristic affinity for certain cells of the susceptible host. One of the exotoxins, botulin, attacks the motor nerve endings, producing paralysis of the muscles of respiration. That of tetanus attacks the motor nerve cells, exerting a strychnine-like action on the central nervous system. Diphtheria toxin attacks certain nerve centers, especially those of the vagus and phrenic nerves, as well as the heart muscle and the adrenals. Endotoxins appear to be a part of the internal protoplasmic structure of the organism. They are given up only after dissolution or disintegration of the parent cell. They are often rather weak toxins and usually poor antigens. They have no characteristic affinity for particular cells and a lethal dose is usually quite large. It is an endotoxin which causes typhoid fever.

ROUTES OF INFECTION. Certain organisms require specific routes of infection. Others are effective by a wide variety of routes. Organisms requiring direct contact must penetrate the skin or mucous membrane or enter a wound in order to be effective. Such are the venereal diseases, tularemia and anthrax. Many microorganisms are effective upon inhalation of droplets containing the organisms. These produce most of the respiratory diseases. Most intestinal infections and many others in which the agent is able to penetrate the lining membranes of the mouth or gastro-intestinal tract are inhabitants of food and water. Infection via arthropod vectors such as insects may be of two kinds. The organism may simply survive in or upon the insect and be passed on to a new host by biting, as in the case of microorganisms causing tularemia. The organism may multiply or even pass through one or more changes in form while in the vector and may then be introduced in a new infective form when the arthropod bites a susceptible host, as is the case with malaria.

RESISTANCE. The power which enables the body to dispose of pathogenic agents, even after their entrance into the tissues and fluids, or to prevent them from proliferating and elaborating their poisons, is known as resistance. When this resistance is especially marked, it is called immunity.

The body has three main lines of defense against infection. The skin and mucous membranes act to prevent easy access of the foreign invaders to the deeper tissues. However, if the bacteria do gain a foothold, certain cells of the body act quickly to surround, ingest and destroy the enemy. Finally, a third defense mechanism goes into action should the other defenses be broken through. This is the blood, which contains substances to neutralize the effect of the invasion.

Skin and mucous membrane defense. The skin is not only a mechanical barrier, but also has a mechanism that enables it to free itself of the majority of bacteria that impinge on it. Bacteria which are not part of the normal flora of the skin are rapidly destroyed. No such reduction of bacteria occurs on the dead skin of cadavers. Conversely, no amount of washing will completely remove bacteria whose normal habitat is the skin. It has been observed that dirty skin loses much of its bactericidal activity, indicating absorption or destruction of this mechanism. It is possible this mechanism is a product of the sweat and sebaceous glands of the skin and the normal acidity of skin.

The mucous membranes which cover the gastro-intestinal, respiratory and genito-urinary tracts prevent invasion by bacteria. The mucus which covers the lining epithelium of the mouth is sticky and bacteria adhere to it. The subsequent movement of the bacteria is along uniform lines of flow which converge at the base of the tongue and the particles are removed by swallowing. In the stomach and first part of the intestinal tract, bacteria are subjected to the high acidity of gastric juice. Few survive. In the small intestine, bacteria which have passed through the stomach are subjected to alkaline secretions. In addition, mucus on the lining of the intestines collects the bacteria and movements of the intestines cause the mucus to be rolled into small masses which pass on to the lower bowel.

In the respiratory tract, ciliary action sweeps the mucus and foreign particles which gain entry to the nose backward into the nasopharynx, where the bacteria join those from the mouth and are swallowed. Lysozyme, a substance present in nasal secretion, tears, skin and other tissues, is capable of dissolving some bacteria and may play an important part in freeing the nasal cavities of foreign bacteria. The trachea and bronchi are normally free of microorganisms. Should airborne dust and carbon particles which might be contaminated evade the outer defenses and reach the lung, mucus collects them and they are expectorated, or cilia may drive the bacteria upward, or the cough reflex may act, in the effort to keep the upper lung passages sterile.

The conjunctivae or mucous membranes which line the inner surfaces of the eyelids are protected by the wink reflex, the eye lashes and by the flow of tears which acts as an irrigating mechanism.

The genito-urinary tract is protected by the flushing action of urine which has an acid reaction destructive to most bacteria. In addition, acid-producing bacteria constitute the majority of the normal and protective flora of the genito-urinary tract. The vaginal secretion is bactericidal to most organisms.

Cellular defense. The second line of defense is that of the cells. Should invading microorganisms gain entrance into the deeper tissues, certain white blood cells called phagocytes mobilize within a few minutes at the site of invasion. The phagocytes are special white cells equipped to ingest and destroy foreign bodies in the blood. At the same time the phagocytes mobilize, a network of fibrin from the blood fluids collects around the area, helping to wall off the invaders and forming a scaffold for the phagocytes to progress into the area of conflict. As the invasive organisms penetrate deeper or overcome the phagocytes, they enter the lymph channels and are carried to the lymph nodes where macrophages, giant phagocytes, engulf the bacteria. The swelling and tenderness of lymph nodes indicates the presence of such a struggle. Should the invading organisms overcome both the phagocytes and macrophages, they are carried into the blood stream along with the lymph and are picked up by macrophages lining the liver, spleen and bone marrow. Here the blood flow is slower, allowing more time and greater opportunity to the macrophages to overcome the organisms. For this reason, the liver and spleen are often called the blood filters of the body.

Blood defense. The third line of defense consists of special substances which the body manufactures when threatened with death by invasive organisms. The toxin, enzyme or other secretory product of the microorganism causes the production of an antagonist. Antigen, therefore, is any substance, such as a toxin or enzyme, which when introduced into the body stimulates the production of antibody. Antibody is any substance which makes its appearance in the blood serum or body fluids in response to the stimulus provided by the introduction of an antigen into the tissues. Antibody is specific and acts only to destroy or neutralize the particular antigen which called it into being. Complement is the substance or component of fresh, normal serum which participates in any antigen-antibody reaction and is necessary for the completion of the lytic or bacteria-dissolving reactions that take place.

There are many types of antibodies produced in the fluids and tissues of the body, including bacteriolysins, antitoxins, opsonins, antihemolysins, antifibrinolysins, agglutins and precipitins. Antibodies are the basis for the active immunity which may be produced naturally or artificially in the body as resistance against infecting microorganisms or their poisonous products.

IMMUNITY. Not only must toxic organisms invading the body overcome the mechanical defenses of the skin and tissues and the ingestive defense of the phagocytes, but they may then confront a variety of types of immunity. The types of immunity may be classified as follows:

Genetically inherited. The immunity with which an individual may be born or which he inherits is called genetically inherited immunity. It enables him to resist infection without first producing specific antibodies. It is species immunity when it is characteristic of a particular species. Algerian sheep, for instance, are immune to anthrax, whereas all other species of sheep are susceptible. Racial immunity is that which is characteristic of a race. By nature, the Negro is relatively resistant to yellow fever.

Naturally acquired. Naturally acquired immunity results from recovery from infectious disease or from subclinical infection. Active natural immunity is acquired by contact with the organisms as a non-apparent or non-lethal infection. The minor illnesses of childhood, for example, confer many types of partial or total immunity and the production of many specific antibodies at that time is maintained for years or for life. Passive natural immunity or congenital immunity may be acquired by the newborn following the transfer to the offspring through the placenta of antibodies which were actively produced in the mother. Thus, infants in the first year of life are found to be resistant to diphtheria and scarlet fever. Widespread immunity to the agents of smallpox, measles and diphtheria in well-developed urban populations, as well as the immunity of tropical natives to dengue fever or European cattle to rinderpest may be considered types of congenital immunity or non-susceptibility which have developed over the course of time.

Artifically acquired. Artificially acquired immunity is conferred following the injection into the body of some immunizing substance such as a toxoid or bacterial vaccine or antiserum. Toxoid is produced by treating a toxin so that its toxicity is destroyed, yet its antigencity or antibody-producing power remains unchanged. Vaccines are killed or attenuated bacterial suspensions which cannot cause disease but can stimulate antibody production. Antiserum is serum containing previously manufactured antibodies, obtained from the blood of an animal that has been subjected to repeated sublethal doses of a microorganism or specific toxin.

Active artificial immunity occurs in an individual or animal as the result of injecting an antigen toxoid or vaccine. The individual so treated produces antibody and may be immune for a considerable period of time. Passive artificial immunity results from the injection of immune serum in an individual or animal. This type of immunity is of very short duration.

The kind of immunity which is established in an individual may be either antitoxic or antibacterial. Antitoxic immunity refers to antibodies capable of neutralizing soluble toxic substances produced by bacteria. Antibacterial immunity refers to antibodies which play a role in the destruction of bacterial cells. In this mechanism, other factors usually operate in conjunction with antibody, such as complement or phagocytes.

In an immunized body, the cellular reaction to a virulent organism is much the same as that of a non-immune body to an organism of low virulence. Immunity, therefore, does not necessarily produce complete resistance to an invading toxin or organism. The body sickens, but it has a last ditch weapon with which to fight, and provided the antibodies themselves are strong, the invaders can be overcome.

The problem of employing disease organisms in war is to overcome these natural and artificial defenses of the body, by developing new or greatly improved strains of organisms of exceeding virulence and by assuring dissemination of overwhelming massive doses of the organisms, against which the best defenses of the body, as well as of medical science, may be helpless.

Chapter 2

BIOLOGICAL AGENTS

Section I

DEFINITION

BIOLOGICAL WARFARE. Biological warfare may be defined as the use of bacteria, viruses, rickettsia, fungi and toxic agents derived from living organisms to produce death or disease in men, animals or plants. To this definition of biological warfare there are two specific exceptions: the use of certain synthetic chemicals for crop destruction and defoliation of vegetation, and the use of chemicals as co-agents integrated with a biological agent.

BIOLOGICAL AGENT. A microorganism whose invasiveness, virulence and proliferation or production of a toxic substance may be useful in war to produce disease and death in man, animals or plants.

BIOLOGICAL WARFARE AGENT. A microorganism or chemical substance which, by its ordinary action, produces a delsterious physiological effect when applied to man, animals or plants.

Section II

CLASSIFICATION OF AGENTS

TACTICAL CLASSIFICATION. Persistency. As a result of the ability of some organisms to form spores, which are among the most resistant forms of life existing on earth, certain agents may persist for decades or longer in dry soil. Practically all bacterial spores and many fungal spores are resistant to dessication. Most non-sporing vegetative organisms are nonpersistent. Toxins are nonpersistent because they are subject to inactivation by a number of environmental factors. However, even the more fragile agents may survive for prolonged periods of time if the environment is suitable for continued proliferation or if natural reservoirs are established.

Communicability. Disease agents capable of transmission in series from one susceptible host to another are termed communicable. Nonproliferating biological agents such as toxins and crop destroying chemicals are noncommunicable. Proliferating infectious agents such as bacteria, viruses, rickettsia and fungi are all communicable in some degree, for it is in this manner that they normally survive under natural conditions. Communicability depends upon invasiveness and virulence of the agents and is the basis for the epidemicity of agents.

Severity. The effects of infectious disease agents vary in severity from those resulting in mild and transitory inconvenience through those causing severe incapacitation for varying lengths of time to those which kill the host. Severity is dependent on the strain of the organism used, incubation period, selectivity of infection, dosage and effectiveness of available therapeutic measures.

PHYSIOLOGICAL CLASSIFICATION. Lethal agent. A biological agent whose primary effect is to produce death in the human or animal host. Botulinus toxin and plague are examples of lethal agents.

Debilitating agent. A biological agent whose primary effect is to produce either brief or prolonged incapacitation in the human or animal host. Brucellosis, bacillary dysentery, influenza and typhus fever are examples.

Psychological agent. A biological agent whose primary effect is to produce terror through fear of potential results following localized employment. Cholera or plague are examples.

Animal agent. A biological agent whose primary effect is to produce disease in useful domestic animals for the purpose of reducing or decimating food herds and draught animals. Foot and mouth disease and rinderpest are diseases of food herds; glanders in horses and rinderpest in oxen are diseases of draught animals.

Gallinaceous agent. A biological agent whose primary effect is to produce disease in domestic fowls. Fowl plague and Newcastle disease are examples of fowl diseases.

Plant agent. A biological or chemical agent whose primary effect is to produce reduction in yield or outright death of food and industrial crops, or defoliation of wood plants and trees. Broad-leaf crop destroying agents are brown spot of rice, rice blast, late blight of potato, southern blight, and the chemical agents LN-2, LN-8 and LN-14. Cereal crop destroying agents are southern blight and LN-33. Defoliants are ammonium thiocyanate and zinc chloride.

Section III

REQUISITES OF A BIOLOGICAL AGENT

An organism or substance to be useful as a biological agent should have the following characteristics:

It should be highly infective for the animal or plant species against which it is used for an effective period under local atmospheric and climatic conditions. Infectivity is the capacity of a viable agent to produce disease in a stated proportion of equally exposed subjects as measured in suitable infective units. It includes all effects of infection, including death. It is the term preferred to virulence which is generally one of comparison between strains of an agent, and to pathogenicity, whose impact is qualitative.

It should consistently produce a highly incapacitating or destructive effect on enemy personnel, animals or plants. Such casualty effectiveness may range from rapid lethality to dominantly psychological effects. Regarding crops, casualty effectiveness is the relative effectiveness of a biological agent in reducing the yield of a particular crop, brought about either by the death of the plant, destruction of the reproductive organs of the plant or by partial starvation of the plant. Casualty effectiveness may also be in terms of toxicity, in the case of the so-called true toxins or exotoxins produced by a limited number of bacterial species, such as those causing tetanus and mollusc poisoning, certain plant and animal toxins such as ricin and botulin, and the toxicity of crop destroying chemical agents.

In the case of a microorganism, it should be capable of becoming epidemic, epizootic or epiphytotic for man, animal or plant.

In the case of a microorganism, it should not be excessively retroactive. Retroactivity is the capacity of an agent to attack friend and foe alike or to react against the attacking force. High infectivity, persistence and epidemicity in an agent imply hazard to the attacking forces, especially when they must enter an area after a biological attack. As applied to plant disease, the probable effect of retroactivity on the biological balance of nature must be considered.

It should be capable of being produced in large quantities from available and economical materials. Availability of an agent is its capacity for being produced on a large scale and in suitable high concentrations. Means for initial cultivation of infectious hepatitus or the virus of mumps, for instance, are not available, but similar agents of this class such as measles, dengue and rinderpest have become available. Most plant pathogens can be cultivated in the laboratory, but the rusts and viruses can be cultivated only in the living hosts.

It should be resistant to adverse conditions of temperature, moisture, sunlight and other conditions encountered in storage. Stability of microorganisms during processing, storage and dissemination is not a common characteristic among viable agents, because of course they either grow or they die. The death rate of the bacillus and spore of anthrax is slow and practically negligible. It is therefore an exceptional agent. The agent of tularemia, on the other hand is subject to rapid sterility under ordinary circumstances. Stability in microorganisms is always limited and makes stockpiling of agents and agent-filled munitions almost impossible.

It should be an agent against which protection can be provided by the using force. The ability to apply effective means for the prevention, treatment and control of specific infections is an important factor in the selection of an agent. With plant pathogens, the existence of resistant host varieties, fungicides, bactericides or insecticide sprays and dusts, and sanitation, crop rotation and other cultural practices would be modifying factors in the selection of such an agent.

It should be capable of being easily and stealthily dispersed or transmitted to the enemy by existing munitions or modifications of them or by new techniques.

It should be complemental or supplemental to other methods of warfare.

Section IV

COMPARISON OF BIOLOGICAL AND CHEMICAL WARFARE AGENTS

Biological warfare and chemical warfare are similar in that they employ agents which may be diffused over a large area and thus exert their incapacitating effects on large numbers of individuals simultaneously. They have psychological similarity in that the agents of both may be more or less invisible and intangible so that their action is to a degree insidious, and part of their expected effect is that of demoralization through terror of the mysterious or unknown.

The agents of biological warfare differ in many respects from those of chemical warfare as to physical state, stability, dissemination, detection, persistency, virulence and effects. In addition, biological agents have certain properties not possessed by chemical agents.

PHYSICAL STATE. Biological agents are minute living particulate bodies or large toxic protein droplets derived therefrom. They are thus more complex in chemical structure and greater in molecular weight than chemical agents, yet small enough to remain in suspension over longer periods of time. Most important difference between molecular gases and cellular organisms is that of the effective particle size. Ordinary fog particles have diameters ranging between 10 and 1,000 microns. However, biological particles even as small as 10 microns in diameter will not pass the nasal barriers or enter the lungs of either man or animals. The largest particle capable of entering the lungs is not over 4 microns. Another important difference between the agents is that of volatility. The solids and liquids which are used to produce war gases give off to the air a certain amount of their material in the form of gas or vapor. This property is not possessed by biological organisms. They can be disseminated only as particulates or solid particles, with all the properties of such particles.

STABILITY. As living agents or complex organic compounds, biological agents are less resistant than chemical agents to such influences as heat, pressure, light, drying and chemical action. All biological agents are comparatively unstable and easily subject to attenuation or loss of virulence, with consequent diminution in their ability to produce disease. Most infectious agents require handling under special conditions not required for chemical agents.

PROLIFERATION. Unlike chemicals, living biological agents can grow and multiply. A few bacteria introduced into food, drink or body tissue may increase in manifold numbers. For this reason, the amounts of biological warfare material required to produce disease may be incredibly small in comparison with chemical poisons.

DISSEMINATION. Biological agents may be dispersed as invisible clouds released from bombs or shell, as contaminants in food and water supplies and on ground and vegetation. In addition to the shell, bomb, grenade and spray tank of chemical warfare, biological agents may be dispersed via contaminated missiles, free balloons, insect vectors, guided missiles and similar unorthodox devisements. Sabotage with biological agents is probably far more practicable and effective than with chemical agents.

DETECTION. It is not likely that any biological agent will be detected until suspicious mass outbreak of disease among men, animals or plant crops has occurred. In this respect biological agents are like certain of the newer war gases which are odorless and colorless. Detection in these instances would depend upon effective intelligence and vigilance in public health and military sanitation inspection. It has not been possible to provide means for the rapid detection of pathogenic organisms as is possible in the case of chemical agents with the detector kit.

METEOROLOGICAL CONDITIONS. Dissemination of biological agents is affected very much the same as chemical agents by winds, temperature, humidity, terrain and other meteorological phenomena.

PERSISTENCY. Biological agents may be persistent or nonpersistent, depending upon their form. Even the most fragile organisms are usually somewhat more persistent than the least persistent chemical agents, and the most resistant organisms are far more persistent than the most persistent chemical agents. As vegetative forms, biological agents may quickly die when exposed to air and sunlight. Spore forms tend to be persistent, sometimes for as much as years or decades. Chemical agents like phosgene and hydrocyanic acid are nonpersistent, dissipating shortly after the moment of crash concentration. Mustard, however, may persist in effective form on the ground for as much as two months.

LAG OR INCUBATION PERIOD. Some chemical agents, like phosgene, have a delayed action. Though the individual may be at once apprised of contamination, the full onset of symptoms may be delayed for hours. Other agents, like mustard, have a lag period of several hours during which there may be no indication of contamination. Most chemical agents are more or less immediate in their physiological effects. On the other hand, all biological agents have a lag or incubation period from the time of contact or entrance of the agent into the body and the first appearance of symptoms. This period is never less than a few hours and commonly is several days. Some infectious agents have incubation periods that may extend for weeks, months, or a year or more.

VIRULENCE AND INFECTIVITY. Virulence is the relative infectiousness of a biological agent, just as it is the relative casualty effectiveness of the chemical agent. Virulence may be enhanced or attenuated by treatment of the organism, just as, to some degree, it may be enhanced by purification of the chemical agent compound. Chemical agents, however, have no power of infectivity.

EPIDEMICITY. Some biological agents have a property not possessed by any chemical agent. This is epidemicity or the capacity to spread disease from one infected individual to another under suitable conditions. A single effective attack with certain biological agents is potentially capable of becoming self-propagating and causing a widespread epidemic or even pandemic. Epidemicity may also be enhanced by endemicity or the natural susceptibility of certain localities to certain agents of disease.

IMMUNITY. On exposure to biological agents the body attempts to build up a specific resistance to the particular agent and by this means may arrest or overcome the infection. Resistance may often be brought about artificially by prior immunization with harmless preparations such as vaccines and toxoids. By contrast, little can be done to increase resistance to chemical agents aside from the employment of chemical and mechanical measures. A certain degree of toleration, though not immunity, can be built up for chloracetaphenone and the irritant smokes.

MECHANICAL PROTECTION. The minute particulate size of biological agents is of considerable importance in devising mechanical means of protection. Only impermeable rubber clothing provides complete protection. Ordinary materials which when impregnated will prevent penetration of liquid or gaseous particles of chemical agents to a large degree, readily admit most microorganisms. The one or two per cent leakage permissible in the standard gas mask when worn in gas atmospheres is almost certainly lethal when the unmodified mask is worn in atmospheres of microorganisms.

MUNITIONS. The chemical efficiency or weight of agent in relation to weight of munition is quite different in biological and chemical munitions. A relatively enormous filling is required in biological warfare to produce an effective amount of agent that will survive the conditions of dispersion in a form suitable for the accomplishment of its purpose. However, the effective dosage of biological agents is many times less than any chemical agent. Another important differentiation is the fact that chemical munitions disperse agents effectively with a fragmentation factor of thousands. Biological munitions must disperse agents with a fragmentation factor of billions.

EFFECTS. While many chemical agents are visible and their effects on the body are relatively rapid, others are invisible and odorless and their effects may or may not be immediate. Most biological agents are both invisible and intangible, and their action is always insidious. Part of their intended and expected effect is that of demoralization through fear of the unknown and the uncertainty of attack.

Section V

AGENTS INJURIOUS TO MAN AND ANIMALS

GENERAL. Bacteria are responsible for many of the serious diseases of man and probably should rank first in the list of agents considered adaptable to use in biological warfare. They offer a wide variety of choice in respect to ease of production, stability, persistence, portals of entry into the body, nature of the associated disease in respect to severity, duration, availability of measures for prevention and treatment, and readiness of transmission from person to person. Among the cocci or spherical forms are the staphylococci which cause boils and food poisoning, the streptococci which cause scarlet fever, the gonococci and meningococci. The bacilli or rod-shaped bacteria include forms which cause diphtheria, tuberculosis, anthrax, tetanus, gas gangrene, botulism, typhoid fever, bubonic plague, tularemia, brucellosis or undulant fever, bacillary dysentery, and glanders. Among these are certain diseases which are due principally to soluble toxins produced by the respective organisms. This is true of diphtheria, tetanus, gas gangrene and botulism. A third form of bacteria are the spirilla, with Vibrio comma as its best representative, the cause of Asiatic cholera. Bacteria show varying degrees of parasitism.

Viruses are strict parasites, existing primarily in living hosts at the expense of the host. They are responsible for many of the well-known diseases of man, ranging from the common cold to such serious conditions as infantile paralysis, rabies, smallpox, influenza, yellow fever, dengue fever, psittacosis and equine encephalitis. The filtrable viruses also cause rinderpest or cattle plague, fowl plague and Newcastle disease. Problems of mass production of viruses, although difficult, are not such as to make the use of these agents in war impracticable.

Rickettsia, like viruses, are all parasitic, infecting man, animals or insects. They are usually transmitted by their original insect or arachnid host. In man they are the causative agents of the important typhus-spotted fever group of diseases. Although production of rickettsia in large quantities would be difficult, their virulence is such that moderate amounts might suffice for purposes of organized biological warfare. They are considered to be potential agents.

Yeasts are associated usually with low grade and superficial infections of man. As a group they are not promising biological agents since they are insufficiently invasive and pathogenic. No. highly poisonous products have been described for yeasts.

Fungi or molds, like yeasts, produce for the most part low grade infections in man. These are mild and often chronic, such as ringworm and athlete's foot. A lesser number of fungi are capable of producing serious disease in man and animals, as for example, actinomycosis which is lumpy jaw of cattle and sometimes infects man. Certain fungi cause pneumonias such as coccidiomycosis and a few invade the deeper tissues. Many of the severe diseases of plants are due to fungi, and should attacks be made on food crops, certain of the agents employed might be in this class. One of the most poisonous substances associated with fungi is ergot, which occasionaly contaminates rye grain. In general, fungi do not produce substances highly toxic for man. On the contrary, their products are widely useful in industry and medicine. Some fungi produce bateriophages. Such is Pencillium notatum, the source of penicillin which checks the growth of streptococci and staphylococci and permits their destruction by the phagocytes.

Protozoa are the cause of amoebic dysentery and sleeping sickness. Showing certain affinities with the protozoa, although generally considered an order of bacteria, are the spirochetes which cause syphilis and relapsing fever. The causal agent of malaria, probably the most devastating disease of mankind, is a protozoon. Although protozoa are occasionally mentioned in connection with biological warfare, the difficulties inherent in production and transmission make it appear unlikely that they would find wide application.

Bacteria, rickettsia and viruses are more likely to be used against man and animals; fungi and chemical agents are more adaptable to use against plants. For these reasons the principal agents which have been studied include the bacteria producing anthrax, botulism, brucellosis, glanders, melioidosis and tularemia; the virus causing psittacosis, and the fungus producing coccidioidal granuloma. In most instances these would be as effective against animals as against man. In addition, a number of other diseases which have special potentialities in biological warfare are included in the text. These include bacillary dysentery, typhoid fever, typhus fever, Rocky Mountain spotted fever, influenza, Japanese type B encephalitis, plague and cholera. Specific animal and fowl diseases which have been studied include rinderpest, foot and mouth disease, Newcastle disease and fowl plague. The crop plant destroyers chosen from among plant fungi include brown spot of rice, rice blast, late blight of potato and Southern blight. Considerable study has been devoted to chemical inhibitors of plant growth and to chemical defoliators of trees and wood plants.

TERMINOLOGY. Two kinds of names are used widely in microbiology: common or colloquial names such as the "malaria parasite" or the "typhoid bacillus," and scientific names such as *Plasmodium vivax* or *Eberthella typhosa,* which refer to the two previous organisms.

ANTHRAX. (*Bacillus anthracis*). *Historical.* Especial historical interest attaches to the anthrax bacillus because it was the first microorganism proved definitely to bear a specific etiological relationship to an infectious disease. The discovery of the bacillus in the blood of infected animals by Pollender in 1849 marks the beginning of modern bacteriology. Not until 1877 was the bacillus first isolated upon artificial media, by Koch who reproduced the disease experimentally by inoculation with pure cultures.

Of all infections attacking the domestic animal, particularly sheep and cattle, no other has claimed so many victims as anthrax. In farm animals anthrax death, due to malignant septicemia, may occur in two or three hours, though more commonly it occurs in one to five days. Anthrax is one of the diseases transmitted from animal to man. In man anthrax occurs usually in those coming in contact with infected animals, such as stablemen, sheep herders, butchers and processors of wool or hair. Although widely prevalent in European animals, it is much less common in North America, and human infection is relatively rare in the United States.

Ordinarily cattle and sheep are affected by an intestinal form of the disease, usually as a result of the ingestion of spores in contaminated soil. The course of the disease is rapid and mortality is between 70 and 80 percent. Investigations of the nature of the pulmonary form of infection has lead to the belief that anthrax causes infection by being carried to the alveoli or air cells of the lungs. There the organism germinates and elaborates a permeable substance which enables it to pass through the alveolar wall into the lymph stream and thence by way of the bronchial lymph nodes into the blood stream. At organs where the blood stream is slowed, as the liver and spleen, the organisms multiply enormously until, at the point of death, they are found throughout the blood stream and lung tissue interstitially. It is probable that in natural infections death is due to purely mechanical means, such as capillary obstruction. When inoculated subcutaneously, the spores are believed to vegetate and enter the blood stream, causing death by septicemia and possibly toxemia.

Characteristics. The anthrax bacillus is a straight rod, occurring singly and in pairs in the blood of infected animals. On artificial media, the bacilli form tangles of long threads. The organism is 1 to 2 microns by 5 to 10 microns in size, and is aerobic and spore-forming. It is gram-positive. The outstanding characteristic of the anthrax bacillus from the standpoint of biological warfare is the resistance of its spores. The spores resist heat, dryness, sunlight and germicidal agents; they will survive and remain virulent for several years in the dark and in contact with air.

Portals of Entry. Man may be infected by the cutaneous, respiratory or intestinal routes. In the cutaneous form, infections occur by implantation in small scratches or abrasions of the skin or by contamination of existing skin infections. The pulmonary form of the infection follows inhalation of infective doses of the organism in its spore or vegetative form. The intestinal form is rare and would probably be contracted only by ingestion of uncooked meat of infected animals.

Epidemicity. In nature, man to man transmission is rare; animal to man is the rule, through direct and indirect contact. Means of artificial transmission include food and water, air-borne, insects and direct and indirect contact with either contaminated fomites or with infected animals. The epidemicity is probably limited.

Symptoms and Prognosis. Following an incubation period which varies from one to five days, the first sign of cutaneous infection is a small, sometimes painful, red nodule, resembling a flea bite. Within 24 hours of its appearance a crop of small pink vesicles appears on the surface of the reddened area; a black hemorrhagic fleck appears in the center and the central vesicles break down, leaving a shallow ulcer which discharges a sero-sanguinous fluid. Concurrently with the initial appearance of the vesicles, edema appears in the adjacent soft tissues and spreads rapidly, sometimes causing considerable distortion in a few hours. The local lesion is seldom painful, although regional lymph nodes may become enlarged and tender and remain so for many weeks. General symptoms such as malaise, joint pains and moderate fever commonly accompany the early stages of the lesion. The lesion may be excised and the patient will usually recover, or local gangrene may set in followed by a systemic infection with a fatal outcome in five to six days. Mortality is between 10 and 20 percent.

The onset of pulmonary infection is characterized by malaise, pain in the chest and slight cough. Progress is usually rapid. Dyspnea, marked cyanosis of face and mucous surfaces and paroxysms of coughing with production of moderate quantities of thin frothy red sputum are common. Physical examination may reveal nothing but usually fine moist rales are detected throughout the chest. Occasionally dullness to percussion is observed in both lungs. Temperature is seldom extremely high and may even be subnormal. Collapse and death occur within three to five days of onset in almost 100 percent of untreated cases.

The onset of the intestinal form of anthrax is characterized by insidious development of malaise. The first definite sympton is that of generalized abdominal pain. In the course of 24 to 72 hours the pain becomes severe and there is frequently vomiting of blood-stained material; less commonly there is diarrhea with blood-stained stools. Abdominal distension is marked and there may be muscular rigidity. Collapse occurs suddenly in from one to five days after onset and leads to death in nearly 100 percent of untreated cases.

Diagnosis and Detection. The cutaneous form is the most readily identifiable by reason of the destructive lesions covered or surrounded by vesicles which become hemorrhagic and are attended by marked regional edema and fever. Direct smears of the lesion usually reveal quantities of the large gram-positive, square-ended rods. Cultures on blood agar within 24 hours show typical colonies one to four millimeters in diameter with a surface suggesting intertwined threads or hairs.

Diagnosis of the intestinal and pulmonary forms of anthrax are more difficult. The abdominal form may be confused with ruptured appendix, perforated peptic ulcer or acute pancreatitis. The symptoms of the pulmonary form make clinical differentiation often impossible for they are suggestive of bronchopneumonia or even lobar pneumonia. Laboratory diagnosis is the only certain identification. Culture and subsequent animal inoculation are necessary even if microscopic examination reveals a significant number of typical organisms. Blood cultures in plain broth medium are made from the blood-flecked vomitus in the intestinal form and from throat swabs in the pulmonary form. Positive blood cultures, however, usually occur late in the disease and indicate a grave prognosis.

Treatment. Large doses of immune serum given in the early stages of the disease may be of value in the human malignant pustule form of anthrax. Animal experiments have indicated that streptomycin is highly effective in the treatment of anthrax, penicillin somewhat less so and sulfadiazine of a much lower order of efficiency. All three substances are considered satisfactory as treatment for the infection.

Prevention and Control. The vaccination of animals has been achieved by the use of living spore suspensions after the method originated by Pasteur in 1881. Such vaccines, however, have not been applicable to man because of the danger that the living spore may produce an active and perhaps fatal infection. While attenuated vaccines have been used with some success, their danger to man precludes their use at this time. Reliance on mechanical protection is still most practicable although immunization against anthrax is almost certainly within reach. This belief is based on recent experiments which seem to indicate that anthrax proliferates a diffusable, soluble substance in living tissue which has been called the tissue destroying factor (TDF). Susceptible animals develop an immunity when injected with this factor, for it seemingly contains the antigen which may lead to the successful development of immunity. Were anthrax employed in biological warfare, the tissue damaging factor containing the anthrax antigen might possibly be employed to protect troops against anthrax infection. Large-scale production of TDF appears practical, approximately 160 immunizing doses being obtainable from each laboratory animal used to produce the vaccine.

In the event that the use of anthrax was anticipated or that its presence was suspected, all personnel would at once observe the following measures:

Regard environment as though contaminated with a chemical agent.

Avoid exposed food and water.

Clear vegetation by flame thrower.

Isolate exposed area.

Expose person and clothing to sunlight and soap baths.

Quarantine affected persons.

Decontaminate water, clothing, equipment and supplies.

Incinerate dead animals.

Disinfect water by boiling 30 minutes. Use of the Engineers' mobile purification unit would provide potable water.

Disinfect clothing by steaming 30-60 minutes in Serbian barrel, treating by boiling in water, or by use of portable or mobile autoclaves.

Disinfect other materials and surroundings by any of the following means:

Direct sunlight which kills spores in 24-28 hours.

10 per cent formalin at 40 degrees C, for 3-4 hours.

0.5-1.0 per cent iodine.

Equal parts of bleach and water for slurries and sprays.

Earth-bleach mixture, three parts in one.

Mercuric chloride.

High test chlorine compounds, such as high test hypochlorite.

Incineration.

Acidified bleach bath.

Hydrocyanic acid.

<u>Usefulness of the Agent in War</u>. <u>Advantages</u>. Anthrax spores can be produced readily in great quantities.

The spores have extraordinary resistance. They are stable in storage and in a suitable munition are capable of efficient dispersion without destruction or loss of virulence.

Anthrax bacillus is surpassed by few microorganisms in its infectivity for animals and surpassed by none in its host range. No mammal appears to be entirely immune. Experiment has shown that all animals having a body temperature approximately 37 degrees Centigrade can be fatally infected.

In the absence of anticipation, detection of anthrax would be difficult and slow and in any case would require equipment and personnel not normally found in the forward areas of the combat zone. Its world-wide prevalence would also make detection difficult.

Clothing for adequate protection against anthrax would certainly render fighting difficult if not prohibitive.

Biological protection by means of vaccination appears to be possible and alone would render adequate protection, but the use of vaccine on large bodies of troops would not be routine. Only expectation of attack by this particular agent would justify the effort and expense.

Disadvantages. Anthrax is non-epidemic.

With all the epithelial cells in perfect condition, infection appears to be possible only through the respiratory tract. The slightest abrasion however will permit ready establishment of the cutaneous infection. This appears to be its most likely portal of entry when employed in the field.

Because of the almost permanent contamination possible with this agent its use in war would probably be prohibited by the War Department and by public opinion.

Once introduced into an area, the agent is likely to persist indefinitely. The area where anthrax was used would thus be permanently contaminated.

BOTULISM. (<u>Clostridium</u> <u>botulinum</u>). <u>Historical</u>. Botulin, the cause of botulism, is the chemical toxin produced as a result of the metabolic processes of the microorganism, <u>Clostridium botulinum</u>. As the most toxic material known in nature, botulin is many hundreds of times more poisonous than the chemical agents phosgene or mustard. The organism causing botulism was first demonstrated by Van Ermengem in 1896 who found the bacilli in large numbers lying between the muscle fibers in a pickled ham whose partial consumption had caused serious illness of all 34 people who had been served the infected meat. The organism has been demonstrated to possess invasive power, capable of elaborating its toxin in the animal body, but it is improbable that man is ever infected in this manner. He is made ill by the preformed toxin.

<u>Characteristics</u>. The organism which produces botulin is an anaerobic spore-former. It is a large bacillus with rounded ends, 0.5 to 0.8 micron in breadth and 3.0 to 8.0 microns in length. It occurs singly, in pairs and in short chains. It is gram-positive. The chemical toxin, botulin, is a protein and therefore incapable of synthetic reproduction. Five different types of the toxin have

been identified, the types marked by serologic differences in specificity. Type A is responsible for most cases reported in the United States, while Type B is more common in Europe. Type E is found in humans in Russia and the United States, but Types C and D are found principally in animals in Australia, South Africa and the United States.

Portals of Entry. The toxin is found principally in infected meats, sausage and canned goods, and the ingestion of such contaminated foodstuffs results in the food poisoning disease in humans and animals. However, poisoning may also follow inhalation of contaminated dust or result from implantation of the toxin on mucous surfaces, cuts, scratches, abrasions, burns or wounds.

Epidemicity. Botulism cannot be transmitted from man to man. It is a matter of individual susceptibility. Massive dissemination, establishing an artificial epidemic, could be controlled by mass inoculation with the specific toxoid provided it were detected quickly enough.

Symptoms and Prognosis. The period of incubation as a result of food poisoning is generally from one to four days. The first symptom is likely to be a burning sensation in the abdomen, followed by nausea and vomiting. The effect of the poison is a paralysis of the muscles of swallowing and of respiration. When introduced by other routes, the toxin makes no digestive disturbance. In botulin poisoning through wounds or by inhalation, the first symptoms are malaise, headache or dizziness, followed shortly by double vision, drooping of the upper eyelids from paralysis and sometimes abnormal intolerance of light. Difficulty of speech and swallowing and general weakness is usually followed by paralysis of the neck muscles and the extremities. Introduction of the toxin through wounds may cause paralysis of muscle groups in the region of the wound before general or remote paralysis becomes apparent. The point of selective action of the toxin seems to be the medulla oblongata at the base of the brain, and these vital nerves are irritated causing dilation of the pupils, difficulty in swallowing, interference with cardiac function and the respiratory center in fatal cases. Fatal cases terminate in untreated persons from three to six days after onset as a result of respiratory paralysis.

Diagnosis and Detection. Clinical diagnosis of botulism would be made in the case of one who showed paralysis, usually of the extraocular muscles and the muscles of speech and swallowing, following ingestion of suspected food or water or implantation on skin injuries or burns. Laboratory studies are of little value except after recovery, when neutralizing bodies may be present in the blood. Examination must be made of suspected materials, injecting such material subcutaneously into mice. Presence of the toxin is indicated by flaccidity of the animal's abdominal muscles, respiratory paralysis and death, all within one to three days. The type of toxin

(A, B, C, D, E) is determined by injecting the sample into several mice, each of which has been protected by the specific antitoxin for each of the five types.

Treatment. Following ingestion, prompt removal of the stomach contents and purgation are indicated. Later, these measures are considered not only useless but harmful. The patient is kept at rest and large fluid intake must be maintained. With the onset of respiratory paralysis, it is necessary to begin and maintain artificial respiration, either manual or mechanical. The type specific antiserum may be effectively administered before paralysis occurs, but it is useless thereafter.

Prevention and Control. Botulism may be prevented through sterilization, processing or cooking of all canned, smoke or dried foods. Water and food supplies may be protected from contamination with the spores by boiling for 6 hours or heating at 120 degrees Centigrade for 4 minutes. They may be protected from contamination with the toxin by boiling and stirring at 80 degrees Centigrade for 10 to 30 minutes. Thirty minutes is required for Type C toxin, which is very resistant. Treatment of drinking water with alum may remove active toxin. Protection of personnel may be provided through the use of masks and protective clothing, cleansing of skin and wounds with soap and water and decontamination of terrain with hypochlorite compounds.

Biological protection is possible by active immunization with the type specific toxoid and by passive immunization with immune serum. The recent development of a means for purification and crystalization of the Type A toxin has prepared the way for preparation of immunizing toxoids of increased efficiency. Toxoids for Types A and B botulins have been shown to protect animals against inhalation of lethal doses of the toxins. There is no gradual production and absorption of the botulinus toxin by the body, as in the case of diphtheria and tetanus. In biological warfare, large amounts of toxin may be ingested at one time or there may be exposure to high concentration. Therefore the degree of immunity must be much greater than ordinary to afford adequate protection.

Usefulness of the Agent in War. Advantages. Large-scale production of botulinus toxin is practicable.

The toxin is considered to be stable and capable of sustaining the shock of detonation.

It is infectious by more than one portal of entry. Infection of animals by respiratory, cutaneous and alimentary routes have been achieved and it is presumed that man can be similarly infected.

The only certain method of detection is serological examination in the laboratory. This would hardly be indicated except in the presence of symptoms, and any delay in identifying symptoms would be irreparable. Botulism is therefore difficult to detect until too late. This would be particularly so in the event that respiratory infections were achieved.

It is the ideal "non-persistent" biological agent, for it is the least retroactive of all the biological agents under consideration.

Disadvantage. Immunity can be provided by means of effective toxoids developed for each of the specific types of the poison.

BRUCELLOSIS. (the Brucella group). Historical. The disease described as brucellosis is also know as Malta fever, Mediterranean fever, goat fever, infectious abortion, Gibraltar fever and undulant fever. Although Malta fever in man and infectious abortion in cattle are diseases with long histories, it was not until 1918 that their organisms were compared and found to be closely similar. Bruce isolated the causative agent of Malta fever in 1887, calling it melitensis. Bang found the small bacterium causing cattle abortion in 1897 and called it abortus. The infectious abortion of swine is caused by a variety of abortus now known as suis, which has been found more pathogenic for man than the bovine variety. Brucella melitensis occurs in goats and man in many of the subtropical countries including those of the Mediterranean area, South Africa, China, the Philippines, Peru, Arizona, Texas and New Mexico.

Brucella abortus is widespread but is particularly heavy in temperate climates where the dairy industry is concentrated. The disease in man follows the distribution of the disease in cattle. *Brucella suis* is widespread wherever the raising of swine is concentrated, as in the Middle West and West Coast of the United States. Brucellosis is said to be the most serious disease in the animal industry next to tuberculosis. Even mass slaughter does not seem to control outbreaks successfully. Between 10 and 20 percent of all United States cattle are believed infected.

The lingering milk-borne disease called undulant fever, produced by the *abortus* organism, is estimated to be the cause of infection in approximately twelve million people in the United States at present. Undulant fever is a systemic or focal infection characterized by weakness, fever with morning remissions, occipital or frontal headache, muscular pain, profuse sweats, drills, constipation, secondary anemia, nervous disturbance, involvement of the joints, eyes and reproductive organs. Although it maims, it seldom kills. Nevertheless it is believed by some, because of the increasing prevalance of brucellosis, that a purely human form may evolve, which will be much more deadly than the present varieties derived from cattle. It has been called a "disease of the future."

Characteristics. The organisms of the *Brucella* group are all parasites which invade animal tissue, producing infection of the genital tract, the mammary gland or lymphatic tissues and the intestinal tract. Brucella melitensis is a strict parasite of goats and the cause of contagious abortion in goats and Malta fever in man; Brucella abortus is a parasite of the milch cow and is the cause of contagious abortion in cattle and undulant fever in man. It also invades mares, sheep, rabbits and guinea pigs, producing contagious abortion. Brucella suis is a parasite of hogs and causes abortion in them and undulant fever in man. In form, the Brucella group are gram-negative rods or bacilli, 0.6 to 1.2 micron long, occurring singly, in pairs or in small chains. They are non-sporing. The organisms are highly resistant, surviving in tap and sea water for as long as 31 days. They withstand dessication for 60 to 80 days outside the body, remain in soil for 70 days and survive in clothing 60 to 90 days.

Portal of Entry. There are four possible routes of invasion by the Brucella organisms: (1) Through the alimentary tract via contaminated food and water. Raw milk, cheese, butter and water are contaminated by infected animals that discharge the organisms in urine and feces. (2) Through the respiratory tract via air-borne organisms. So-called dust fever is brucellosis acquired by the inhalation of dry dust from contaminated goat pens. (3) Skin infection results from direct contact with infected animals. The disease is acquired both through abraded and unabraded skin. (4) Conjunctival infection is rare but possible. As little as one drop of a live culture of Brucella abortus placed in the conjunctival sac of a cow will result in active infection.

Epidemicity. The disease is readily transmitted from animal to animal and from animal to man, but the carrier transmission has not been demonstrated in man. Its epidemicity is probably of a low order.

Symptoms and Prognosis. The incubation period in man varies from 10 to 60 days, although usually it is about 2 weeks. The disease is of variable severity and ranges from a mild, almost unrecognizable form characterized only by neurasthenia to a virulent, rapidly fatal infection. It may be sudden or insidious, accompanied by varying degrees of lassitude, weakness, headache, chills, anorexia, constipation and vague bodily pains. Symptoms range from mild backaches to bone and nerve infections, heart disease and insanity. The attack may be brief, relapsing and chronic, or fulminating and rapidly fatal. Hardly an organ in the body is safe from invasion. The duration of the disease is variable too; it may last for years, but usually it lasts about three months. Mortality is from 2 to 6 percent.

Diagnosis and Detection. Brucellosis resembles influenza, tuberculosis, malaria, typhoid fever, tularemia and appendicitis, among other diseases. Clinical diagnosis is therefore very difficult and requires confirmation by blood culture. Inoculation of blood into guinea pigs and egg-yolk sacs frequently reveals the organisms. The organisms cannot be detected in the air or in clouds, but only in soil, water and milk. Even in water or milk they would escape detection if ordinary methods of examination are employed. Detection is also difficult because isolation of organisms is not a routine matter; they are present in body discharges only intermittently. It is never possible to be certain that the Brucella have been identified because skin, phagocytic and agglutination tests vary in a given patient from day to day. These tests also do not make it possible to determine whether the infection is past or present or whether the patient is merely susceptible to infection. The only positive evidence is isolation of the organism, a difficult laboratory problem.

Treatment. There is no efficacious treatment available to combat brucellosis, nor has any form of immunity been devised. Antimalarial drugs, antibiotics and the sulfa drugs are of no use. Partial success has been achieved with sulfadiazine only. Injections of vaccine made from killed Brucellae and sulfanilamide have been reported as specific remedies but are not yet indicated. Present treatment consists of general supportive measures including administration of tissue stimulants such as liver extracts.

Prevention and Control. The best treatment for brucellosis is prevention. In the event brucellosis were suspected of being employed it would be necessary to regard environment as though contaminated with a chemical agent, avoid exposed water and food, isolate exposed areas, expose person and clothing to sunlight.

Food, water, clothing, equipment and supplies must be decontaminated and all standard sanitary procedures such as disinfection and isolation must be instituted. If infected animals were suspected of being vectors, they must be tracked down and killed. Pasteurization of milk ensures protection against this natural or sabotage vehicle of infection. Sunlight destroys the organisms relatively quickly. Four percent formalin kills them in 5 to 15 minutes. Chlorine compounds in the form of slurries or sprays are also effective in destroying the agent.

Usefulness of the Agent in War. Advantages. Constituents of the culture medium for producing *Brucella* strains are cheap and available in quantity. Methods are available for preparing the organism on a large scale.

The organism is among the most stable of biological agents. It requires only a minimum of refrigeration in shipment and storage. Its stability in cloud form has been demonstrated.

It has four portals of entry in man, the cutaneous, conjunctival, alimentary and respiratory routes.

Although it is a non-spore vegetative organism and therefore delicate, nevertheless it is capable of being effectively disseminated from the airplane spray tank.

The organism cannot be detected in the air or in clouds. It would escape detection if ordinary methods of examination of water and milk were employed, and for this reason would be useful for sabotage.

The disease in man is extremely difficult to diagnose and easily confused with many other diseases.

The symptoms endure so long that a diagnosis of malingering might readily be made. The agent might therefore be used to lower the morale of troops.

There is no known protection against the agent nor any developed vaccine of any certain effectiveness.

The persistence of the organism on contaminated surfaces is not excessive.

The organism is virtually world wide in distribution, it would therefore not represent the introduction of an exotic agent in war.

Disadvantage. The disease probably does not spread from man to man. It therefore rates low in retroactivity, due to its lack of epidemicity.

GLANDERS (*Pfeifferella mallei*). Historical. The microorganism of glanders was first obtained in pure culture and accurately studied by Löffler and Schütz in 1882. This infectious disease of equines which is occasionally transmitted to man was prevalent in this country until 1905 when suppressive measures almost eradicated it. It is still prevalent in the Balkans and in Russia, though uncommon elsewhere.

Glanders, sometimes called equinia, does not occur at all in cattle, and swine appear to have little or no susceptibility. In addition to equines, it affects sheep, goats, cats, dogs and menagerie animals. The course of the infection in equines is frequently latent and is recognized only when a mucoid nasal discharge with enlargement of the lymph nodes of the lower jaw and ulcers on the skin make their appearance. It is spread among equines by water supplies, especially drinking troughs contaminated with nasal discharge. Since the bacilli are also discharged from the skin and mouth, feed bags and harness and grooming equipment, bedding and fodder are also liable to contamination.

Characteristics. The microorganism causing glanders is a slender, straight or slightly curved rod with rounded ends, between 2.0 and 5.0 microns long. It may be arranged singly or in irregular clusters. It is gram-negative, non-sporulating and does not possess a demonstrable capsule.

Portals of Entry. Three definite portals of entry are known, via the respiratory tract, open wounds and ingestion.

Epidemicity. Although a rare disease in man, it is transmissible from horse to man. Laboratory infection have occurred, therefore direct infection is feasible, though epidemic spread would not be probable. Primary respiratory glanders induced by massive air-borne attack might possibly result in severe epidemicity.

Symptoms and Prognosis. The incubation period is usually from 1 to 4 days. Just as in equines, glanders in man manifests itself both in an acute and a chronic form, but the acute form is more common in man. In the acute form it is an extremely painful and incapacitating disease. It behaves like a primary septicemia, pyemia or a pulmonary pneumonia. There is usually a local nodular swelling with spreading pustular skin eruption and foci in the lungs and other internal organs. These manifestations are accompanied by high fever, purulent nasal discharge and extreme prostration following chills, nausea, vomiting and headache. A pneumonia may develop as a result of respiratory infection. Death occurs in 7 to 10 days. In the chronic form, subcutaneous, intramuscular and osseous nodules form and become purulent lesions, draining through the skin. They are tuberculoid in character. Enlargement of the regional glands and foci in the respiratory tract andother organs are usually present. The course of the disease is prolonged and complete recovery is rare. Such recovery confers no permanent immunity. On the contrary, recurrence of the disease almost invariably takes place, though latent periods may be as much as 1 to 5 years.

Diagnosis and Detection. The chief biological methods employed in the diagnosis of glanders are microscopy, isolation of the organism, pathogenicity as demonstrated by the Straus reaction, and application of allergy tests. Allergy develops regularly in chronic cases.

The Straus reaction, used to diagnose glanders, is the tumefaction and purulent inflammation of the testicles of male guinea pigs which results after 2 or 3 days following the intraperitoneal injection with suspected material. A positive reaction confirms the presence of glanders. An allergy test for glanders is based upon the fact that an allergy develops regularly in chronic cases due to the endotoxin, mallein, which the organism produces. The test is similar to the tuberculin test in tuberculosis, but because mallein may cause reactions in the presence of other diseases than glanders, it is not so specifically valuable for diagnosis as tuberculin. The substance mallein is injected subcutaneously into laboratory animals and if glanders is present, local, focal and general reactions will be observed in 10 to 24 hours.

Treatment. Mallein has been employed in therapy for both animal and human cases of disease with some small success. Chronic glanders is recognized as curable and 30 to 50 percent survive independently of treatment. However, prompt and radical surgical measures are the most reliable means of treating the chronic disease. Chemotherapy has been found efficacious, sulfadiazine being completely effective in overcoming the symptoms of chronicity. Streptomycin and penicillin, on the other hand, appear to be useless.

Prevention and Control. The principal prophylactic measures for glanders have been directed to the elimination of the disease in horses. Were an attack made against our animal population, attenuated cultures of mallein or surgical removal of the sites of infection must be employed to eradicate the disease. In an attack upon troops with the microorganisms, procedures similar to those against chemical agents would be instituted, including the use of protective clothing and the mask and decontamination of the affected area. A hypochlorite solution containing 500 parts per million of available chlorine is completely effective in decontaminating glanders-infected ground. In addition, roccal, iodine, mercuric chloride and potassium permanganate are all highly effective decontamination agents. The organism is killed by sunlight in a few hours, by ordinary antiseptics in 10 or 15 minutes.

Usefulness of the Agent in War. Advantages. The organism producing glanders is easy to cultivate, growing slowly but profusely on simple media.

It is fairly persistent, surviving in ordinary tap water for 4 weeks and for as much as 4 to 6 months in slurries, enriched peat moss, synthetic media, nutrient broth or gelatin diluent.

The organisms can be dispersed as an aerosol and 8 to 10 percent of them are recoverable after spraying.

The organisms are fairly stable, virulence not declining in the stored munition or vehicle until after 20 weeks.

As an agent of war, glanders would be a formidable weapon. It is seriously incapacitating and highly fatal. The acute form is usually fatal in 8 to 10 days. The illness may be subacute in type, developing later into a chronic disease which is usually fatal within a few months, but may drag on for several years.

The disease itself does not confer immunity against a second attack.

The organism has more than one portal of entry.

Glanders would be difficult to detect for it resembles and is often classified with the infectious granulomas, tuberculosis and syphilis. Positive cultures from biologic materials are usually rare except at post mortem.

No successful immunity has been reported and none achieved by recent research. The only protection afforded an enemy would be early diagnosis and treatment with sodium sulfadiazine.

Disadvantage. Because the disease is too distressing and protracted, use of the organism in war would probably not be acceptable to the War Department and the general public.

MELIOIDOSIS (Pfeifferella whitmori). Historical. This exotic, glanders-like disease of rats and men has been suggested as a formidable potential agent of biological warfare chiefly because it induces a highly fatal disease in man and is foreign to the experience of most of the world. The microorganism of this rare tropical disease has been recognized only in Burma, Indo-China, Ceylon and British Malaya. It was first described by Whitmore and Krishnaswami in Rangoon in 1912, and was named melioidosis because the ancient Greeks applied the term melis to a variety of conditions resembling glanders. Although primarily a disease of rodents, it is occasionally transmitted to man. It is chronic in wild rats and the organisms are discharged in the feces. It is spread to other animals and to man by the ingestion of food contaminated with rat feces. In general, cattle, horses and pigs are resistant. Guinea pigs and rabbits are most susceptible. Man may not be readily susceptible, but an acute and highly fatal infection can be induced.

Characteristics. The only outstanding differences in characteristics between the agents is that Pfeifferella mallei (glanders) is non-motile where Pfeifferella whitmori is actively motile, glanders has smooth colonial growth and requires 48 hours for development, whereas melioidosis has rough colonial growth and develops in 24 hours. The natural host of glanders is the equine; the natural host of melioidosis is the rodent. Melioidosis organisms will liquefy gelatin and glanders will not.

The diseases produced by the two organisms are also very similar, but melioidosis is much more acute and is more fulminating. The disease has been divided into four clinical forms: (1) The hyperacute septicemia form results in stupor, collapse, delirium and death in 3 to 7 days. (2) The acute septicemia or pyemia form is similar to typhoid clinically and results in death within 2 weeks. (3) The subacute septicemia form with variable localization in the lungs, liver, spleen or other organs is fatal within 3 to 4 weeks. (4) The chronic form results in multiple large abscesses in almost any organ of the body. It persists for from months to years and usually ends fatally. This form is quite rare.

<u>Portals of Entry</u>. Portals of entry, as with glanders, include the respiratory tract, open wounds and ingestion.

<u>Epidemicity</u>. Due to its rarity in man, an attack with these microorganisms would probably produce many casualties. Aside from direct assault with the organisms, spread of the disease from man to man would be unlikely.

Symptoms and Prognosis. The symptoms of melioidosis are varied and obscure. The disease may develop into any of the four clinical types, but most cases are of the acute or sub-acute type and are fatal. Its chief features are septicemia, pyemia and the formation of characteristic nodules in nearly all parts of the body. The lesions produced by both glanders and melioidosis are indistinguishable grossly and microscopically, and the lesions themselves are the same in all affected organs of the various species. The typical lesion is a small white necrotic area of semi-solid consistency, composed of necrotic tissue cells with dense infiltration of polymorphonuclear and large mononuclear leucocytes, many of which contain phagocytized organisms. The lesions occur nearly always in the lungs, but at times appear in almost every part of the body except the brain. The disease organisms spread from the initial focus via the lymphatic and vascular systems, producing miliary lesions comparable in distribution to those seen in miliary tuberculosis.

Diagnosis and Detection. Diagnosis for melioidosis is difficult due to the possibility of wide variations. Symptoms may resemble cholera, plague, severe typhoid, malaria or even lobar pneumonia or miliary tuberculosis. In subacute or chronic cases it resembles glanders, tertiary syphilis, pyemic infections of the lungs, liver or kidney. Early diagnosis can be made only by culture of the organisms in the laboratory, depending almost entirely on demonstration of organisms in the sputum, lesion exudate, blood, urine or viscera. If the patient lives 3 to 4 weeks or more, diagnosis can be made by serological tests.

Identification is made by preparing a suitable medium for growth, incubating and observing the typical colonies by morphology, appearance and slide agglutination. Confirmation of identification is made by inoculation into adult male hamsters or guinea pigs. Fatal infections with specific lesions or a positive Straus reaction confirms the presence of melioidosis as it does glanders. Distinctive features of the organism for purposes of identification are that it is motile, that it liquefies gelatin, digests egg white and that it coagulates milk in 4 days.

Treatment. Treatment for the disease, aside from prompt and radical surgical measures, was not successful until the advent of the sulfa drugs. Sulfadiazine has proved completely effective where diagnosis has been made in time. Previous therapeutic methods used in chronic human cases have included the use of autogenous vaccines, mallein, and chemotherapy with salvarsan or other arsenicals, mercury, silver, iodine or potassium iodide, iron and quinine.

Prevention and Control. Protection against infection would depend largely on detection in the event use of the agent was anticipated or suspected. The necessary prompt diagnosis of suspected casualties is dependent upon the several clinical and bacterial tests that are known. Melioidosis is treated like any highly communicable air-borne disease, with strict isolation of cases.

The agents of both glanders and melioidosis are easily destroyed by disinfectants. Chlorine is effective for the decontamination of water. Moderate heating destroys the organism in food. Control of the disease would have to take into account the persistence of melioidosis in local rodents.

Usefulness of the Agent in War. Advantages. The organisms grow rapidly and profusely in simple media.

The organisms appear to retain their virulence indefinitely when cultivated under proper conditions. They survive at least 8 weeks in tap water, between 4 and 6 months in wet muds or slurries, enriched peat moss, synthetic media, nutrient broth and gelatin diluent.

The organisms can be dispersed in aerosol clouds, 10 to 30 percent of them recoverable after spraying.

The virulence of the organisms is maintained beyond 20 weeks.

The disease has three effective portals of entry. It is believed to have a marked predilection for the respiratory route.

As an agent of war, melioidosis would be an effective weapon of terror, being more acute and fulminating than glanders. It may be said to epitomize the terror which biological warfare has for the popular mind.

It is extremely difficult to diagnose because of its varied and obscure symptoms. Also, it is rare in man and is therefore unlikely to be recognized. Death usually ensues before diagnosis can be made.

The only protection against the disease is early diagnosis and prophylaxis with sodium sulfadiazine.

It is a painful, incapacitating and highly fatal disease.

Cultures of the organism are more virulent for man than pus, exudates or infected material.

Vectors would be difficult to control and the effectiveness of control measures would always be uncertain.

It is possible that melioidosis, which resembles pneumonic plague in its low epidemicity in tropic regions, may also be a cold weather agent, effective in a respiratory infective form.

Disadvantages. Since the organism does not produce spores, it is easily destroyed. However, this permits it to be safely handled by attacking force.

Effectiveness of ingesting the organisms is doubtful; contact infection is likely to produce only sporadic casualties.

Melioidosis has been shown to be carried by fleas of infected rats and this would provide a hazard in its employment.

Observations of the resistance and epidemicity of the agent may indicate a potential high retroactivity.

Because the disease produced is even more distressing and protracted than glanders, it probably would not be acceptable to the War Department and the general public as an agent of war.

TULAREMIA (Bacterium tularense). Historical. Tularemia was first recognized by McCoy in 1911 as a plague-like disease of ground squirrels in Tulare County, California. Also known as rabbit fever, deer-fly fever and tick fever, tularemia is primarily a fatal disease of wild rodents, especially rabbits and hares. Only secondarily is it an infection of man. The disease is transmitted in nature by the bite of insects which carry it in their faces or mouth parts, the particular insects being the wood and dog ticks and the deer fly. In animals the organism causes a fatal plague-like disease with enlargement of the regional lymph glands which become filled with a dry yellowish material. The spleen becomes dark red and greatly enlarged and contains white cheese-like granules. These granules are also seen in the liver. Animals are exceedingly infective. The introduction of but one cell under the skin of a susceptible animal is invariably fatal. Though man is highly susceptible, it is not a common disease because he does not come in contact with infected rodents. The disease in man is milder and rarely fatal, though of long duration. He is infected ordinarily by eating insufficiently cooked rabbit meat, the bite of the deer fly and wood tick, and drinking infected water.

Characteristics. The organism is a rod form, though it also occurs during its life cycle as a coccus. It occurs singly, in pairs or in short chains, the individual organisms ranging from 0.3 to 0.7 micron in length. It is enclosed in a capsule, is gram-negative and non-sporing. Four clinical types of tularemia are known to develop in man, characterized as follows: (1) Ulceroglandular, in which an ulcerating local lesion and enlargement of regional lymph glands occur. (2) Oculo-glandular, in which the local lesion is one the conjunctiva of the eye. (3) Glandular, in which there is enlargement of the regional lymph glands but no local lesion. (4) Typhoidal, in which there are severe general symptoms but neither local lesion nor glandular enlargement.

Portals of Entry. In man infection is possible by all routes, via biting insects, contact, ingestion, and air-borne organism. Laboratory workers have been infected as a result of repeated exposure, while preparing virulent cultures, by direct inoculation from infected animals, through unabraded skin, eye and respiratory routes and by ingestion.

Epidemicity. While large numbers of casualties might be obtained by direct infection, a self-propagating epidemic would not be probable with tularemia organisms.

Symptoms and Prognosis. The incubation period is ordinarily from 3 to 5 days, but a massive does may cause symptoms to appear the first day. The onset is generally sudden. The outstanding features of the clinical picture presented by tularemia are fever, prostration and glandular enlargement. It is most easily recognized when there is inflammation at the point of entry through the skin or conjunctiva and where there is enlargement of the lymph glands. When the agent makes its entrance into the body by inhalation, the picture is that of extremely severe infection, with delirium and frequently death within a week or ten days. Appendicitis, diarrhea, intestinal hemorrhages and pneumonia may be present during the illness. The mortality rate is about 5 percent.

Diagnosis and Detection. Tularemia might first be detected by its plague-like effect on the local rodent population. Until the lesion or glandular enlargement appears, diagnosis is difficult. In the typhoidal form where the clinical picture is not clear, laboratory diagnosis is made by the appearance of agglutins, by isolation of the organism from the lungs or blood stream, and in fatal cases, by specific pathologic findings. Blood specimens injected into guinea pigs result in death of the animal in 4 to 8 days and characteristic organisms may then be recovered from the heart blood, spleen and liver.

Treatment. There is no specific treatment. Immune serum has been tried but without notable success; serum sickness frequently occurs and must be treated with histominase. Penicillin, penatin and the sulfanilamides are of doubtful value. Of recently discovered antibiotics, only streptomycin holds promise of being successful. The disease is usually treated symptomatically. To relieve pain, the buboes may be treated with saturated solutions of magnesium sulfate. Eye lesions may be treated with hot applications of half-saturated magnesium sulfate and lavage with a warm boric-saline solution. Primary ulcers may be treated with a half-saturated solution of commercial urea.

It is characteristic of tularemia that most patients develop a high degree of natural resistance after the initial infection.

Recovery confers life-long immunity which protects against subsequent massive exposures with an effectiveness seldom encountered in bacterial diseases. Second attacks of the disease are unknown.

Prevention and Control. Where the organisms of tularemia are known or suspected, it is necessary to boil or chlorinate contaminated water, decontaminate foods as for chemical agents, discard fatty foods, and ensure thorough cooking of all foods. Protective clothing and masks offer effective protection. Chlorine solutions may be used as a decontaminant. Isolation and decontamination of infected area offer the best means for prevention of further spread of the disease. The control measures employed against the disease as it occurs in nature are equally effective against any deliberate employment of the agent in combat.

Usefulness of the Agent in War. Advantages. Mass production of the organism is feasible.

It is almost unique among disease organisms in its ability to infect both man and animals via multiple portals of entry.

Virulence is maintained without difficulty and the organism has marked abilities for surviving the conditions of dispersion.

The percentage recovery from nebulized clouds is equal to or better than that with any other vegetative agent or simulant.

The organism is highly infectious for it penetrates normal unabraded skin. Large numbers of casualties could probably therefore be obtained by direct infection.

Its short incubation period makes it a relatively quick-acting organism.

It is a severe prostrating disease with prolonged convalescence.

The organism is persistent, surviving for days in soil water. At the same time it is free from the danger of potential retroactivity since it does not spread easily from man to man and is not difficult to destroy in the environment.

Active immunization is as yet only moderately successful.

Disadvantages. No way has yet been found to keep the organism stable for prolonged periods of storage.

Protection for friendly troops with vaccines is not absolute though the incidence and severity of infection in vaccinated personnel is markedly reduced.

It is not difficult to detect, for rapid methods of identifying are available.

It is quickly killed by sunlight and disinfectants. Treatment of infection with streptomycin is fairly effective.

A self-propagating epidemic would not be probable.

Solid, lasting immunity is developed following recovery from an initial attack.

PSITTACOSIS (filtrable virus). Historical. Psittacosis is a disease which was first identified in parrots. It is a communicable disease of parrots transmissible to man and caused by a virus with an affinity in parrots for the liver and spleen. The disease is also transmissible to mice, guinea pigs, rabbits and rhesus monkeys by intracerebral, intranasal, intraperitoneal or intratracheal injections of emulsions prepared from the liver and spleen of infected animals. In parrots the virus causes diarrhea and debility but is seldom fatal. The lungs, as well as the liver and spleen, may also be affected causing respiratory complications.

Characteristics. By means of ultrafiltration and centrifugation, it has been estimated that the virus of psittacosis is about 275 millimicrons or .00275 micron in size. Many different strains of the virus have been isolated from parrots, love birds, pigeons and human cases. The virulence of the organism for man varies from strain to strain. In addition to the original virus isolated from pittacine birds, there is a pigeon ornithosis virus, a mouse neumonitis virus, a feline pneumonitis virus and a meningo-pneumonitis virus of obscure origin. Furthermore, there are two strains which have been isolated from human cases only, which are of particular interest because of their exceptional virulence for man. The virus of lymphogranuloma venereum has been found to be related to these. While all strains are morphologically similar and antigenically related, they differ as to origin, pathogenicity for man and animals and susceptibility to chemotherapeutic agents.

Portal of Entry. The respiratory tract appears to be the only portal of entry for this agent in man.

Epidemicity. Psittacosis is highly infectious. In the pneumonia caused in man, the virus may be recovered from the sputum. The virus has been demonstrated in human sputum from the fifth to the thirty-seventh day after onset of the disease. Recovered cases may become carriers of the disease.

Symptoms and Prognosis. In man the disease resembles typhoid fever and often develops into a pneumonia. The incubation period is from 6 to 15 days. Symptoms of chills, fever and headache, with a focal pneumonia, are seen during the first few days following onset. The course of the disease is debilitating, with frequent relapses. The mortality rate in the absence of treatment is 20 percent or more.

Diagnosis and Detection. Diagnosis is made from clinical symptoms, X-ray and by injection of the patient's sputum into white mice. The development by animals of a characteristic illness which is fatal usually within 5 to 14 days is diagnostic for psittacosis. Post mortem examination of the spleen and liver of the animals is made for elementary bodies. Also, it is possible to detect the virus readily in contaminated animal secretions and excretions following intracerebral injection of the virus in mice.

Treatment. Until recently the only treatment was symptomatic. However, a newly developed convalescent serum has been used in a few cases with some success. All strains of the organism seem susceptible to penicillin and certain of the strains are also susceptible to sulfadiazine. Penicillin appears to offer most promise as both preventive and treatment.

Prevention and Control. Prevention measures against the disease are unsatisfactory, as might be expected with a virus disease. One attack of the disease usually produces an active immunity lasting for some time. Vaccines prepared from both live virus and killed virus seem to produce specific solid immunities, but they do not protect against challenge with other psittacosis strains. All strains, however, may be susceptible to penicillin.

Usefulness of the Agent in War. Advantages. The psittacosis group of viruses are readily produced in quantity and with great potency.

The organisms are comparatively stable. They are easily preserved and are relatively resistant to physical and chemical changes.

They can infect at a considerable distance when prepared and disseminated as an aerosol.

Many species of birds can be used to disseminate the disease.

Most strains of psittacosis produces a severe illness in man which is highly infectious and has a case fatality rate of 20 per-cent or more.

One of the strains of psittacosis which has been isolated in man only has especially good potentialities as a biological agent, causing extremely severe illness with a high fatality rate. It has high degree of stability and seems effective in very low concentrations.

Prevention and treatment of the disease are unsatisfactory.

The disease spreads mainly in winter and would therefore be a good cold weather agent.

Minimal infecting dose is probably small due to the astronomical multiplication of the viruses.

Once established, the disease is probably self-propa gating, both by avian vectors and human carriers. Pigeons are important foci, as are canaries, finches and all the psittacines.

Disadvantages. There is only one route of infection in man, via the respiratory tract.

Persons immunized with intramuscular injections of the active virus develop as high a degree of neutralizing antibodies as accompanies recovery from the disease.

Psittacosis is dangerously retroactive due to human carriers and to the formation of reservoirs of virus in local birds.

COCCIDIODAL GRANULOMA (Coccidioides immitis). Historical. Coccidioidomycosis or coccidioidal granuloma is a fungus disease which is most frequently encountered in the Southwest United States. Numerous outbreaks of the fungus disease have occurred in training camps located in that area, and these have been the scene of recent investigations of the disease. The disease is highly incapacitating but rarely fatal.

Characteristics. The causative agent of the disease is a microscopic fungus. In tissues and pus the organisms appear as a mass of minute round cells each containing a granular protoplasm and possessing a contoured capsule. The cells vary greatly in size, ranging from 5 to 50 microns in diameter. Reproduction is by endosporulation. Colonies are small, elevated, round, paraffin-like plaques covered with fluffy, snow-like flakes.

Portals of Entry. The agent may be introduced into man through a skin abrasion or by inhalation.

Epidemicity. No man to man transmission has been recorded. Its epidemicity would therefore depend on massive dissemination of the organisms.

Symptoms and Prognosis. The organism produces an atypical type of bronchopneumonia when introduced through a break in the skin or by inhalation. The disease usually requires from 1 to 3 weeks to incubate. The infection may be localized and relatively benign in character or it may be systemic, malignant and rapidly fatal. The onset simulates influenza with pulmonary involvement, sometimes without much fever. Symptoms of acute attack include malaise, anorexia, chills, fever, headache, backache, night sweats and coughing. Recovery from acute infection usually occurs in 3 to 6 weeks. The mortality rate is less than 1 percent. The chronic form may follow a recognized acute attack; the initial attack, however, may not have been recognized. Where an acute systemic infection may cause death in a few weeks, a chronic infection may remain localized for years, since it is self-limiting. The chronic infection resembles disseminated tuberculosis, with the primary lesion appearing in the skin or lungs, followed by progressive lesions of the skin, joints, lymph nodes, larynx, adraneals or meninges. Despite this seeming severity in the clinical picture, the chronic form of the disease seldom results in death.

Diagnosis and Detection. Diagnosis may be made by characteristic X-ray shadows or by microscopic identification of the organism in sputum or pus. A skin test with coccidioidin has recently been developed in order to identify the infection. A method has been devised for obtaining quick parasitic growths of the organisms in appropriate tissue culture, making this new skin test possible.

Treatment. No specific therapy is known nor has any biologic immunity been found. The most promising fungicides for use against the organism appear to be triphenylmethane and its water-soluble dye derivatives.

Prevention and Control. Prevention and control of this endemic fungi organism is predicated on the development of an effective fungicide.

Usefulness of the Agent in War. Advantages. Laboratory demonstrations have been made of 90 percent yields of sporulated material on both solid and liquid media, which suggest that quantity production of the organism may be feasible.

Preliminary data on respiratory virulence indicates that very small concentrations are sufficient to produce almost complete infectivity.

Detection under normal circumstances is difficult because the organism can only be identified morphologically by an expert.

It would be an exotic infection.

It would not be excessively persistent since it requires a rather unusual combination of terrain and climate for proliferation.

Disadvantages. The value of *Coccidioides immitis* as an agent, with regard to its production, dissemination, stability, infectiousness and protection has not yet been firmly established.

A relatively quick method for detecting the organism has been developed which would be effective if the presence of the agent were suspected.

SHELLFISH POISONING (*Gonyaulax catenella*). Historical. Shellfish poisoning, which occasionally occurs after eating mussels, oysters or clams, is the result of ingestion of an alkaloid poison and therefore is of the same order among biological warfare agents as botulinus, which is a bacterial poison, or even ricin, which is a protein poison derived from a plant.

Characteristics. Shellfish poisoning is due to the poison content of the plankton, *Gonyaulax catenella*. This microscopic plankton dinoflagellate, characterized by its two whirling flagella at about the middle of its body and generally considered to be a plant rather than animal, forms part of the food of mussels, oysters and clams. Shellfish consuming the plankton become poisonous, the highest concentration of poison being contained in the livers of mussels. When concentrated and purified, the chloride poison is a clear colorless glass-like material which is readily powdered. As a chloride, the poison is soluble in water and alcohols.

Portal of Entry. The powdered poison could probably be administered only in food and water supplies as a sabotage agent.

Epidemicity. The poison is non-proliferative and its epidemicity would therefore be determined solely by the degree of dissemination effected.

Symptoms and Prognosis. The alkaloid poison has a powerful action on the respiratory and cardio-vascular centers of the body. Respiratory paralysis is the primary cause of death. The peripheral effects consist of a strong curare action, paralysing the heart and the motor nerve endings. In addition, the poison affects sensory nerve endings. Because of these concomitant effects, it appears to give a different type of physiological reaction than any other known poison. It is possible to control the extent and intensity of these effects by graded doses of the poisons.

Diagnosis and Detection. The characteristic symptoms of shellfish poisoning, as well as chemical analysis of stomach contents, may serve to identify the causative agent of the illness.

Treatment. There is no treatment available for shellfish poisoning.

Prevention and Control. Since it is a sabotage agent, prevention of shellfish poisoning would depend upon the establishment of effective security measures.

Usefulness of the Agent in War. Advantages. Shellfish poison would be a unique agent in war. Graded doses of the poison give graded effects. It may be possible to immobilize an individual up to any degree including death.

It is an ideal sabotage agent for the poisoning of food and water supplies since it is completely undetectable until its effects on the individual are seen.

It may be possible to synthesize the substance for mass production.

It would produce quick death when administered as a lethal dose. In less than a lethal dose, recovery would be complete, without the stigma by any residual injury.

Disadvantage. Production, stability, dosage and other related aspects of shellfish poison are still in the development stage.

BACILLARY DYSENTERY (*Shigella dysenteriae*). Historical. The bacterial nature of the disease was first discovered and described by Shiga in 1898. The mild or severe inflammation of the lower intestinal tract caused by bacillary dysentery is distinct from amoebic dysentery which is caused by protozoan and is usually ulcerative. Bacillary dysentery is prevalent throughout the world and its debilitating effects become known to almost everyone in his life sooner or later. The disease may be acute or chronic, sporadic or epidemic. In epidemic form, its mortality may be very high. The disease in Japan is frequently over 20 percent fatal. The resistance of the organisms to the ordinary conditions in nature is the most important feature of the epidemiology of the disease. They live for weeks in garden soil, damp sand, on folded linen, in ice, despite the fact that they are not spore-bearers and ought, therefore, to be highly fragile.

All the known varieties of intestinal invaders, typhoid, paratyphoid and the various dysentery bacilli, play a role in natural outbreaks of dysenteric maladies. It is not impossible that many of the mild cases observed in military camps may be true dysenteries, true typhoids or other pathogenic bacterial agencies which have been modified by increased natural resistance and by specific vaccination in the men.

Characteristics. The causative agent of bacillary dysentery in man is a gram-negative, non-motile rod, 1.0 to 3.0 microns long. It is non-sporing and produces a powerful exotoxin which in small amounts produces a typical paralysis and severe nerve lesions in rabbits after an incubation period of a few hours to 4 days. When injected into other animals, the endotoxin produces loss of weight and diarrhea but no paralysis. It is believed that most of the lesions observed in the gastro-intestinal tract of patients with dysentery are due to the toxin rather than to the direct local action of the bacilli. Investigations carried out with the Shiga bacillus have tended to show that the disease itself is probably a true toxema, its symptoms due to the absorption of the poisonous products of the bacillus from the intestine.

Portals of Entry. Ingestion is the principal portal of entry into the human intestine.

Epidemicity. Wherever unsanitary conditions arise, dysentery epidemic is possible. It is likely that massive dissemination of the organisms, particularly by sabotage methods, would result in limited epidemics.

Symptoms and Prognosis. The infection in man is a gastro-intestinal disease which is accompanied by griping pains and constant desire to evacuate. As the disease progresses, the pains and diarrhea increase and the stools become small in quantity and filled with flakes of blood. Marked nervous symptoms, attributable to the absorption of toxic products, may be observed. The infection is profoundly debilitating but seldom fatal. When it is fatal, pathology reveals extensive ulcerations in the mucous membranes of the large intestine.

Diagnosis and Detection. The infection is a common one and may be readily attributed to sanitary conditions in the environment. Even under rigid public health or military sanitary conditions, an epidemic following an organized attack with the organism would probably be difficult to diagnose as such. Identification of the bacterial organism may be made by injection of contaminated material in rabbits.

Treatment. Both immune serum and bacteriophage have been used in the treatment of dysentery in a number of instances with good results. The sulfonamides seem to be most effective in chemotherapeutic treatment. However, in general, there is doubt cast on the complete effectiveness of any present day remedy. Prevention is considered to be the best treatment.

Prevention and Control. A number of vaccines have been employed as prophylactic measures against bacillary dysentery. These have consisted of heat-killed preparations and serum sensitized cultures. Oral vaccination has also been advocated. Vaccines, however, are rarely used due to the toxicity of the vaccine which must be excessively high in order to be effective against all five of the important types of dysentery.

The most practical control measures in the event of an epidemic consist in interrupting the various routes of transmission. These routes are as follows: Person to person infection. Contact with cases during the incubation period of bacillary dysentery is dangerous since organisms are present in stools as much as 24 hours before the appearance of symptoms. Contact with sub-clinical cases may result in infection since patients with dysentery may have no more than a headache, with or without loose stools, and not even require bed rest. Contact with convalescents or chronic cases is hazardous. Dysentery convalescents may harbor organisms for weeks, and between 2 or 3 percent of bacillary dysentery cases may be carriers for weeks or months.

Fomites and soiled articles. Bacillary dysentery in institutional epidemics may be a problem of direct contamination. Infection is very likely to occur in latrines.

Flies. As all military personnel know, or learn, the feces-flies-fingers-food cycle is the most common basis of epidemic infection when lack of sanitary discipline or unavoidable field conditions enable the bacillus of dysentery to flourish. The role played by the cycle is indicated by the correlation of outbreaks of bacillary dysentery with climatic conditions favorable to the development of a large fly population. Flies may carry the bacilli for 24 hours after contamination.

Animal carriers. Rats, monkeys and dogs may contract the disease and become carriers. Rats have been found to remain carriers for as much as 32 days.

Water. Dysentery organisms may survive in shaded water for more than 3 weeks. The presence of organic matter in the water is conducive to rapid growth and proliferation of the microorganisms.

Food. Food may be contaminated by infected food handlers, flies or water and is the most likely means of enabling the bacteria to lodge in the intestines.

Usefulness of the Agent in War. Advantages. Of all biological agents, *Shigella dysenteriae* would probably be one of the most difficult to detect as a deliberate weapon of war.

Dysentery is a disabling infection. It would serve to disrupt military operations and to lower the morale of troops.

The organism is not fastidious in its growth requirements and could probably be produced in great quantities without difficulty. It is moderately stable.

Once established, the disease would be largely self-propagating. The organism itself is fairly persistent on exposed surfaces.

The modes of transmission are numerous.

It would probably be more effective as a ground contaminant than an air contaminant.

Dysentery is prevalent everywhere and endemic in the unsanitary conditions of warfare.

There is no standard inoculation against bacillary dysentery as there is in the case of typhoid. It would therefore be as effective against military personnel as against civilian populations.

The agent might be used to contaminate reservoirs, as part of a scorched earth policy by a retreating force, and small outbreaks could be started by saboteurs.

There are several strains of dysentery, some of which are more virulent and more likely to produce fatalities than the familiar, debilitating types. The disease may be very severe as in the toxic Shiga type or it may be transient as in Salmonella food poisoning.

A major attack would probably result in prompt saturation of medical and hospital resources so that adequate countermeasures would be impossible.

Disadvantages. The sulfa drugs appear to effect quick control over the disease.

Although it is one of the world's greatest epidemic diseases, bacillary dysentery is limited in its military application because normal sanitary precautions may defeat its efforts to get established.

TYPHOID FEVER (Eberthela typhosa). Historical. Eberth discovered the bacillus of the disease in the spleen of typhoid fever patients in 1880. Although once a scourge of nations, typhoid fever has now been apparently controlled through effective sanitation and immunization. In the Franco-Prussian War, typhoid fever caused 60 percent of the total mortality in the German Army. The disease was formerly widespread throughout the temperate zones; it is more common now in tropical areas because of lower standards of sanitation.

Typhoid fever is quite different from typhus fever in that it is transmitted by typhoid bacilli which lodge in human excrement, food and water, and is established in the human host upon ingestion of the bacilli. Typhus fever is transmitted by a rickettsial agent, carried in the alimentary tract of the body louse. The agent is introduced into susceptible persons when the infected feces of the louse are scratched into the skin.

Typhoid fever is one of the outstanding examples of diseases which may be transmitted by individuals who to all appearances are normal and healthy, but are dangerous because they harbor infectious agents. Many epidemics of typhoid fever have been traced to persons who excrete typhoid bacilli in their feces, although they show no outward manifestations of the disease. Vaccine treatment and removal of the gall bladder which is believed to be the reservoir of the typhoid bacillus in some individuals has been suggested as means of curbing those discovered to be typhoid carriers.

Characteristics. The typhoid bacillus is a short plump rod, 2 to 3 microns in length, occurring singly, in pairs and occasionally in short chains. It is gram-negative, aerobic, actively motile and non-sporing.

Portals of Entry. The site of the infection is the intestine, therefore ingestion is the one portal of entry. The organism enters by mouth with food, water or contact with fingers, direct or indirect.

Epidemicity. Once initiated, typhoid fever is a self-perpetuating disease until brought under control.

Symptoms and Prognosis. The bacilli of typhoid fever, after passing through the stomach uninjured, lodge and multiply in the intestines, but they cause no symptoms for from 7 to 14 days. They have a predilection for the alimentary tract, mucous membranes, Peyer's patches (lymph follicles in the walls of the small intestine) and lymphoid tissues. The symptoms begin insidiously by gradual malaise, headache, loss of appetite and sleeplessness. The multiplying bacilli cause irritation of the walls of the gastro-intestinal tract with the formation of ulcers and the production of diarrhea. There is usually a peculiar eruption on the abdomen and chest, appearing the seventh to ninth day, consisting of small, slightly elevated rose-colored spots. These come out in successive crops. The terminal picture is that of continued fever and marked prostration and stupor. In those recovering from an attack of typhoid fever there is usually an immunity which lasts for a period of years.

Diagnosis and Detection. Diagnosis of typhoid fever is relatively simple because it is a well-known and prevalent disease. Laboratory diagnosis is based on the fact that agglutinins are produced against both the antigens of Eberthela typhosa. The Widal test is a specific agglutination test for the diagnosis of typhoid fever, based on the principle that when the serum of persons with typhoid fever is added to a suspension of typhoid bacilli, a specific agglutination reaction is observed.

The organism may be isolated from the feces. It is also demonstrable in large numbers in the spleen and liver of those dying of the fever. During the first week of the disease a blood culture is usually positive and cultures made from the rose spots characteristic of the disease will reveal numerous typhoid bacilli. When inoculated intraperitoneally or intravenously into laboratory animals, the bacilli may produce a fatal bacteremia which is diagnostic.

Treatment. Typhoid serum and the sulfonamide drugs have been used in a few instances with some success, although they remain unproved. Preventive vaccination against typhoid fever is widely practiced with favorable results. Prevention and control are the only certain remedies for the disease.

Prevention and Control. Vaccination is the best preventive measure against typhoid. Control measures, once the disease has broken out and been recognized, consist of interrupting the various routes by which the organisms pass from the intestines of one individual to the mouth of another. Measures consist of quarantine, individual personal hygiene, patient isolation, group sanitation, careful supervision of food and water, control of flies and other insects. Routes of infection and contamination include: Person to person infection. Contact with cases is always a source of infection where large numbers of cases are confined in small areas, as in quarantine. Direct contact in caring for patients is hazardous since patients are often helpless and heavily soiled. Contact with carriers of typhoid is the most dangerous, for the carrier may not be recognized and the condition may persist for years. Whether or not the patient has become a carrier must be determined before any patient is released from isolation, and quarantine must extend until three successive negative stools have been obtained.

Fomites and soiled articles. Feces are one of the original sources of contamination. In typhoid fever the organisms are also excreted in the urine. Soiled clothing is always a potential source of infection.

Flies. The cycle, feces-flies-fingers-food, is perhaps the most common route of infection. It is the same that exists in the epidemic transmission of bacillary dysentery and of cholera.

Animal carriers. Rodents are occasionally carriers of typhoid and for that reason must be guarded against.

Water. Water is responsible for many of the great outbreaks of typhoid fever. Organisms of typhoid fever have been known to survive for as long as 7 days in well water. The presence of organic matter in water may allow multiplication of the organisms.

Food. Food may be contaminated by food handlers, flies or contaminated water. It is a particularly good vehicle as it is usually damp and gives good protective covering to the organisms. Milk and ice cream are especially dangerous. Outbreaks of typhoid fever have also been traced to infected shellfish.

Usefulness of the Agent in War. Advantages. Typhoid is prevalent the world over, easily established and is nourished particularly by unsanitary conditions that are commonly established in the wake of war. It would be especially effective against war-torn civilian populations where public health agencies were disrupted or nonexistent.

The modes of transmission of the disease are numerous and difficult to interrupt.

There is no one effective remedy for treatment of the disease in the individual.

The incidence of organisms causing infection is multiplied many times as they are excreted. This would make the typhoid bacillus of special value where it is desired to initiate a self-perpetuating outbreak of disease.

Disadvantages. Typhoid would be difficult to establish in a modern army due to mass inoculation against it.

Public health agencies are familiar with the disease and with epidemic control measures.

There is only one portal of entry and ensuring the inital infection would present problems of dissemination.

TYPHUS FEVER (Rickettsia prowazeki). Historical. In the Thirty Years' War an estimated 8,000,000 Germans were wiped out by louse-borne typhus fever and flea-bourne bubonic plague. On Napoleon's retreat from Moscow, typhus, dysentery and pneumonia killed 450,000 of the Grand Army of 500,000. World War I was the first war in history in which guns were more deadly than germs. In that war battlefield deaths totaled 8,000,000; deaths from disease were 3,000,000. Yet during the same war typhus swept through Serbia, spread to Russia, were during the four years of the war it killed 3,000,000 peasants.

The causative agent of typhus was named by da Rocha-Lima in 1916 for Ricketts and Von Prowazek, both of whom had died from typhus in the course of their investigations of the disease. In 1909 Ricketts first described the bacillary bodies (<u>Dermacentroxenus rickettsii</u>) seen in the blood of patients with Rocky Mountain spotted fever and soon after found similar organisms in typhus blood smears. In 1913 Von Prowazek made similar observations of the agent of typhus fever in Serbia.

The rickettsia of typhus fever is a strict parasite, growing only within the cells of living tissue. The disease, normally transmitted from man to man by lice, has two forms: European or classical typhus whose natural reservoir is man and is louse-borne, and murine typhus whose natural reservoir is the rat and is flea or louse-borne. The louse does not transmit infection to man directly through its bite, as does the mosquito infected with malaria or yellow fever, but through its feces which contain the organisms in enormous numbers. Infection in man results upon entrance of the organisms through abrasions caused by scratching. The blood of a typhus patient contains an average of 100,000 rickettsia per cubic centimeter, and lice are readily infected by feeding on the patient. Lice thus infected die within 8 to 10 days after their infection because the multiplication of the organisms in the cells lining their alimentary tract interferes with digestion and adsorption. Before that time, however, they have been actively instrumental in spreading the infection.

Characteristics. The exact nature of rickettsial bodies is unknown but they are generally considered as intermediate between the bacteria and filtrable viruses to which they are related. Specific characteristics of Rickettsia prowazeki cannot be given, and its identity is established only through the character of the disease it produces. It, like other rickettsia, resembles bacteria in shape, is approximately 0.5 micron in length, and may be cocci, diplococci or short bacilli. It is gram-negative, non-motile, and in common with all rickettsia, is transmitted to man by an intermediate host, in this case, the flea and louse.

Portals of Entry. As a biological agent the organism would probably be air-borne and cause infection through the respiratory tract. However, the use of infected fleas and lice for dissemination of the disease is not impossible since the use of anthropod vectors in biological warfare appears to be practicable.

Epidemicity. Dispersed as a aerosol, infection would be limited to the individuals exposed. There would probably be no person to person transmission in the absence of lice. Only conditions of filth and famine present proper conditions for wide-spread epidemicity.

Symptoms and Prognosis. The incubation of typhus fever is from 5 to 21 days. The onset is like a severe case of influenza. The disease is characterized by alternate chills and fever, with a typical rash forming on the fourth day, which may cover the entire body. Extracellular and intracellular hemorrhages may occur and fatal cases develop a pneumonia from toxemia. The mortality rate is approximately 50 percent.

Diagnosis and Detection. It is difficult to cultivate the rickettsial bodies and therefore diagnosis and identification based on demonstrating the bodies is uncertain. The Weil-Felix reaction, an agglutination reaction of the serum of persons with typhus fever, is the standard method of diagnosis. The appearance of the typical rash is indicative of the disease.

Treatment. There is no treatment for typhus fever. Preventive measures appear to be most practical and promising. Several vaccines have been developed which give varying degrees of immunity. Also, following an attack of typhus fever there is usually an immunity conferred against subsequent infection.

Prevention and Control. Whether typhus fever is disseminated as an air-borne agent or by infected lice or fleas, the spread of the disease depends upon control of the factors promoting epidemicity. These factors include vermin, which constitute the only means whereby typhus is normally transmitted from man to man; filth, which is conducive to lousiness; and famine, leading to lowered resistance against infection. Control of the disease once it has been established requires: (1) Removal of famine conditions.

This is even more important than the elimination of overcrowding, which materially assists in the spread of the disease. (2) Education of the general public concerning transmission and control of typhus epidemic. (3) Early diagnosis and isolation of patients with typhus fever. (4) Establishment of delousing and disinfecting stations. (5) Establishment of isolation hospitals. (6) Establishment of effective sanitary cordons around unaffected and infected areas. Delousing of all persons going into unaffected areas. (7) Vaccination.

Control through biological protection is based upon the use of vaccines. Living vaccines have been prepared from the peritoneal washings of guinea pigs or by grinding up the intestines of lice. Killed vaccines have been prepared from the lungs of rats intranasally infected with the rickettsial bodies. Living vaccines produce an early and high degree of immunity with a single injection and the immune state is prolonged, but they also make it possible to induce an infection capable of transmission by lice, thereby constituting a new potential focus for epidemic spread. Living vaccines, however, may be used in the event of widespread epidemics for prompt protection. The risks involved are less than those of the disease. Killed vaccines are more safe, but repeated injections are necessary. The immunity develops slowly and the protection is transient. They may be used against limited outbreaks of typhus fever in a stable, non-infected population.

The use of vaccines against the deliberate dissemination of the agent may be expected to give at least partial protection and possibly complete protection to younger individuals. Their use is well established in reducing the incidence of laboratory infections. Vaccination, however, is only a subsidiary method of control and the immunity may be only partial at best. Delousing remains the standard control measure for the insect-borne infection.

Usefulness of the Agent in War. Advantages. Only influenza is capable of greater epidemic proportions than typhus, and of the two, typhus is the greater killer.

Man is highly susceptible when his resistance is lowered.

Disadvantages. Maintenance of modern methods and of sanitation would make widespread epidemics almost impossible, although local epidemics might be induced.

The continuing development of vaccines may offer a completely effective means of prevention.

ROCKY MOUNTAIN SPOTTED FEVER (*Dermacentroxenus rickettsii*). Historical. The bacillus-like microorganism causing Rocky Mountain spotted fever was found in the blood of patients with the disease and described by Ricketts in 1909. The whole class of organisms which seem to be an intermediary form between the bacillus and the virus now bear their discoverer's name. Rocky Mountain spotted fever is a tick-borne disease with high infectivity for man.

Characteristics. All the spotted fever rickettsia (the agents of Rocky Mountain, scrub typhus and tsutsugamushi fevers) are transmitted hereditarily in the tick. The infected tick shows no effects of the parasite it harbors. The nymphs feed and develop normally into adults despite rickettsial invasion of the cells of all their tissues.

Portals of Entry. Although Rocky Mountain spotted fever is produced by an obligate vector-borne organism and is transmitted in no other way in nature except by the biteof the tick, it is possible the causative agent may be developed as an airborne organism, infective upon inhalation or even ingestion.

Epidemicity. No epidemic spread is likely with this agent whether it is transmitted by the tick or as an air-borne disease. Epidemicity would therefore depend upon degree of dissemination.

Symptoms and Prognosis. The period of incubation may be from 2 to 14 days but is usually less than a week. The symptoms that develop are serious and incapacitating, although variantly severe. The characteristic rash of the disease usuallyappears on the second to fourth day of fever, spreading centripetally from the wrists and ankles. The rash is preceded by headache, fever, chill, loss of appetite, irritability, photophobia and bone and muscle pains. Severe fever symptoms persist for 2 or 3 weeks. Even in relatively mild cases the period of convalescence may last from a month to a year or even more. The average mortality for the disease is approximately 20 percent, but it varies from 4 to 90 percent.

Diagnosis and Detection. Identification of Rocky Mountain spotted fever is not especially difficult due to the rash which appears shortly after onset of the disease. Agglutination and complement-fixation tests make it possible to detect the presence of the agent. Inoculation of guinea pigs also will identify the agent for the rickettsial bodies may be demonstrated in smears taken from the scrotal sac of the animal.

Treatment. There is no satisfactory therapy available for the disease. It is treated symptomatically. Protection against the disease may possibly be conferred by vaccination, although this has not yet been established.

Prevention and Control. All races of man are susceptible to the spotted fever rickettsia and no natural immunity to them is known. Where the incidence of the disease can be attributed to the prevalence of infected ticks, insect control measures would eradicate the source of disease. As an airborne agent, primary control would be difficult. Secondary control measures would be directed against insects which might become infected and serve to propagate the disease.

Usefulness of the Agent in War. Advantages. The agent has high infectivity for man and all races of man are susceptible.

The disease is serious and incapacitating, with prolonged convalescence.

Although at present it is only a tick-borne disease, it may be capable of development as an air borne agent.

The rickettsia can be maintained in tissue and egg cultures and in ticks or guinea pigs without loss of virulence. It can be grown in egg yolk for large-scale production.

It is not likely to be highly retroactive as an air-borne disease, for epidemic spread is not possible except by the tick-borne route.

Disadvantages. No satisfactory vaccine has yet been developed for the disease.

An area might be more or less permanently contaminated through the persistence of infected ticks, and even airborne dissemination of the agent might be capable of infecting large tick populations.

INFLUENZA (filtrable virus). Historical. The virus of epidemic influenza was not isolated until 1933. That a filtrable virus might be the agent of influenza was suggested by the characteristics of the uncomplicated mild cases with which the epidemic begins, the extreme infectiousness of the disease, and the lack of uniformity of bacterial findings in early cases. Influenza is a pandemic as well as epidemic disease. The morbidity rate of pandemic influenza is extremely high. In the 1889 pandemic it was 345 per 1,000 persons afflicted; in the 1918 pandemic the rate was 280 per 1,000.

Characteristics. Until recently the disease was attributed to Hemophilus influenzae, which is an extremely small rod bacillus and is the cause of primary and secondary infections of the respiratory tract, septicemia, endocarditis and meningitis. However, this bacillus has been shown to represent an incidental invader rather than the primary etiological factor in epidemic influenza, and has served to conceal the real cause of influenza, the filtrable virus.

Portals of Entry. Infection is the result of airborne inhalation of coarse and fine droplets containing the virus agent.

Epidemicity. Diseases in the influenza group, all of which are considered to be of viral etiology, vary in epidemicity from sporadic cases in which contagion is limited, to world-wide pandemics that rank with the Black Plague of the 14th century.

Symptoms and Prognosis. The onset of infection is abrupt, typical cases becoming ill without premonition. The first symptoms are headache, feverishness, loss of appetite, pains in the back and calves of legs and often mild sore throat. This condition continues for 2 or 3 days, when the patient returns to normal but is left quite exhausted. The morbidity is high, but the mortality is practically nil. This three-day fever, however, prepares the body for the secondary respiratory infection which is highly fatal. The agent of the secondary infection may be Hemophilus influenzae, which is frequently found in the upper respiratory passages and in sinus infections. This bacterium is ordinarily an isolated organism of low virulence which is harbored in a quiescent state in the body until the influenza virus has lowered the resistance of the host body. The bacterium then become highly virulent. The other secondary invader following the fever caused by the influenza virus is the highly fatal bacterium, Hemophilus influenzae meningitis. The infection caused by this agent is almost 100 percent fatal.

Diagnosis and Detection. Although the clinical features are wellknown, diagnosis of influenza may be difficult. The primary infection, usually of 3 days duration, is misleading, and only when the epidemic has been established is the causative agent fully recognized. Detection is possible in uncomplicated cases within a relatively short period by means of neutralization and complement-fixation tests.

Treatment. There is no specific therapy available for the treatment of influenza.

Prevention and Control. A vaccine has been produced and has recently been widely used by the Army which appears to confer a satisfactory degree of immunity of several months duration. A serum has also been devised which offers a means of treating the most dangerous of the secondary infections, that caused by *Hemophilus influenzae meningitis*. The serum is obtained by prolonged immunization of horses with strains of *Hemophilus influenzae*. Glycol mists and other disinfectants for dispersion as aerosols in enclosed spaces offer a means of combating the infectious diseases produced by airborne viruses.

Usefulness of the Agent in War. Advantages. Among the airborne virus diseases, influenza alone or in conjunction with bacterial or other viral agents would probably be one of the most effective agents because of its destruction of the primary defenses of the body against biological invasion.

It is both epidemic and pandemic.

The virus is highly resistant to natural destruction. Dried on cotton or serge cloth or as impregnated house dust, it retains considerable activity for long periods of time. The virus has been lyophilized and found to be infective after 15 months.

Diagnosis is difficult and can easily be made more difficult by the addition of secondary pathogenics or camouflage agents.

Effective dissemination can probably be effected by spreading large quantities of the virus in dust, together with Hemophilus influenzae, pneumococci, streptococci, and possibly meningococci.

Disadvantages. Influenza ranks with plague as the agent of greatest potential retroactivity.

Immunization of populations against the disease appears practicable and effective.

JAPANESE TYPE B ENCEPHALITIS (filtrable virus). Historical. A number of infections of the central nervous system have been recognized as of viral origin. Such diseases have been termed epidemic encephalitides, one of which is Japanese Type B encephalitis.

Characteristics. The etiologic agent of this disease has been isolated by intracerebral inoculation of mice. The agent has not been described, but it is believed to be a herpes or crawling virus. Japanese Type B encephalitis has been distinguished from a closely similar chronic infection called Type A by the prevalence of the Type B infection in the summer months. The type A enceph litis is more common in the winter months.

Portals of Entry. Infection may occur by inhalation of contaminated sprays or dust or by the bite of an infected mosquito which has had access to infected animals.

Epidemicity. The epidemicity of the infection is high as a result of the favorable routes of infection.

Symptoms and Prognosis. The incubation period is from 4 to 21 days. Clinical manifestations include sudden onset of headache, severe malaise, fever, backache, abdominal pains, nausea and vomiting. Encephalitic signs such as very severe headache, mild rigidity of the neck, mental confusion and stupor may occur early, but more commonly appear just before the peak of fever, on the second or third day of illness. These symptoms may become worse and the patient may die, or there may be recovery with some disability, or the patient may make a complete recovery. Ordinarily the disease has a short course and few sequelae.

Diagnosis and Detection. The most certain diagnosis is through laboratory tests. Spinal fluid upon examination shows slight to moderate increase in pressure, and cell count of the blood varies from 25 to 500 per cubic millimeter. An increase in antibody titer in an infected mouse is diagnostic. There is no known method of detecting the presence of the organism, and unless it was suspected, prompt diagnosis would not be likely.

Treatment. An attack of the disease results in the production of antibodies which confer a lasting immunity in the individual. There is no known treatment.

Prevention and Control. Prevention and control of the infection would be almost impossible beyond isolating the individual cases. Insect control measures would be helpful in preventing vector transmission of the infection.

Usefulness of the Agent in War. Advantages. As an agent producing an encephalitis, this would be a powerful psychological weapon of terror.

Mass production may be feasible due to the development of new techniques in growing viral agents.

Detection of the agent would be unlikely until too late. Because of the rarity of the disease, it might not be recognized as an agent of war if used discriminately.

There is no treatment as yet available.

Disadvantages. Because the agent may be carried by the mosquito, the disease might become dangerously retroactive.

Mass production of the virus may lead also to mass production of a vaccine for the disease.

As an encephalitis, this biological agent might not be acceptable to higher echelons for use in war.

PLAGUE (<u>Pasteurella pestis</u>). <u>Historical</u>. The plague bacillus was discovered independently by both Kitasato and Yersin during the plague that appeared in Hongkong in 1893. Both found the organism in pus from the buboes of infected victims.

Historically, the primary plague focus is in Yunnan Province, China, from whence it periodically spreads to India and Europe. In the Middle Ages it was known as the Black Death, from the hemorrhagic, blackening spots developing on the skin in the course of the disease. At least one-fourth of central Europe's population died in the Black Plague which appeared in 1347. In 1661 the plague again swept into Europe from the Levant and tens of thousands died. The plague reached the United States, probably by way of ship-borne rats from Honolulu, and broke out in San Francisco in 1900, killing more than a hundred in the 2 years it lasted. By 1935 the plague had infiltrated as far east as New Mexico and Wyoming. Plague in the United States is still sporadic and cases are rare, due to the fact that it is not the rat-borne but the wild rodent form of disease, known in the west as sylvatic plague.

There are three forms of plague in man, based on different means of acquisition. Bubonic plague goes through a rat-flea-man-man cycle. Large-scale epidemicity requires large rat or other rodent populations, human crowding, poor sanitation and temperatures between 60 and 80 degrees Fahrenheit with high humidity to ensure flea maintenance and rat proliferation. Bubonic plague parasites cannot leave the rat except via the flea. The exception to this is the pneumonic form of plague. Pneumonic plague, originating from the bubonic form, requires no intermediate host for its maintenance as it passes from person to person. It is far more deadly than bubonic plague. Outbreaks of pneumonic plague are always small in warm climates, are devastating in colder areas. The one or two pneumonic plague outbreaks in the United States have originated from bubonic cases of squirrel origin which developed secondary pneumonia. The third form is sylvatic plague, to which wild rodents of the forest such as the chipmunk, ground squirrels and jackrabbit are subject. The disease is usually only sporadic in man, upon infection by flea or animal bite or by contact. While the incidence of rat plague rises, falls and disappears within a century, for reasons unknown to epidemiologists, sylvatic plague is permanent once it has been allowed to establish itself in the wild rodent population.

Characteristics. The Pasteurella pestis of the three types of plague is a short, plump rod, .05 to .07 micron in breadth and 1.5 to 1.75 microns in length. It occurs singly. It is a gram-negative, non-motile, non-sporing organism and possesses a gelatinous capsule.

Portals of Entry. The pneumonic type of plague could probably be induced by airborne attack with virulent culture bacilli.

Epidemicity. Pneumonic plague is highly transmissible and would probably be devastatingly effective against troops in cold climates who were not vaccinated against it. Biting vectors are essential intermediates in nature, but in the deliberate establishment of pneumonic plague enormous numbers of uniformly highly virulent microorganisms are coughed up in the sputum and discharged into the environment, forming a self-perpetuating source of infection.

Symptoms and Prognosis. In bubonic plague the organisms entering the skin may cause a localized lesion at the point of infection. They then pass into the lymphatics and produce the so-called bubo characteristic of plague, an inflammatory swelling of lymph glands, especially in the groin or armpits. Secondary buboes may arise in other parts of the body, along the distribution of the lymphatics, and the organisms rapidly enter the bloodstream causing a septicemia. Bubonic plague may be relatively mild, or it may take an acute septicemic form with the formation of skin carbuncles which is rapidly fatal. The pneumonic form is more severe and the mortality is almost 100 percent. The organisms attack the lungs, causing high fever and multiple pulmonary hemorrhages. There is often a chill, headache, and fever which reaches 103 or 104 degrees within a day of the onset, accompanied by a very rapid pulse. A cough appears within 24 hours. The expectoration soon becomes abundant and consists of blood-tinged mucus. Later it becomes thick and bright red. There are marked signs of cardiac involvement, and delirium and coma frequently appear just before death. Pathology shows a general engorgement and edema of the lungs, with hemorrhages under the pleura, pleurisy and even pneumonic infiltration. The distribution of the pneumonic lesions may be either lobar or lobular. The sylvatic type of plague affects the circulatory similar to bubonic plague and results in septicemia, with the formation of skin carbuncles. All three forms of plague when they are fatal usually produce death within a week of onset.

<u>Diagnosis and Detection</u>. The oval plague bacilli can be found in blood cultures in about 30 per cent of cases. Since smears from buboes and other plague lesions show typical bacilli in very small numbers and are usually badly contaminated with other microbes, it is often necessary to inoculate guinea-pigs by rubbing the material into the unbroken skin. The organisms can then be isolated from the ensuing culture produced in the animal. In the pneumonic form, enormous quantities of plague bacilli are found in the blood-tinged sputum, or they may be present in the blood in such large numbers that a simple microscopic

examination suffices for their detection. Post mortem diagnosis of pneumonic plague is made from the enormous numbers of the oval organisms found in the peribronchial lymph spaces and in the adjoining alveoli. They may also be present in large numbers in the inter-lobula septa and under the pleura.

Treatment. There is little or no data to suggest that serum therapy, sulfonamides or penicillin can be used to treat or control plague, although sulfadiazine has been reported successfully used in China both as a cure for and preventive of pneumonic plague. Vaccination for pneumonic plague alone seems hopeful. A curative plague serum prepared by Yersin by the immunization of horses with plague cultures has been extensively used but its positive values are not confirmed.

Prevention and Control. Active immunity against plague with favorable results has been demonstrated with living virulent cultures, heat-killed cultures, sensitized cultures, filtrates from old cultures and nucleoproteins. The serum of immunized animals has slight protective properties and has been widely employed in the control of the disease. It appears to contain specific bacteriolytic or bactericidal substances, but no definite antitoxins.

Control of the transmission of plague is exceedingly difficult. It depends upon prompt detection. Stringent isolation of cases and 10-day quarantine of all contact personnel, as well as prompt evacuation of all dispensable non-contacts, is necessary. Every means for control of airborne infections known must be instituted, including masking, barrier or cubicle nursing, oiling of surfaces and use of aerosol sprays.

Plague bacilli are easily destroyed by sunlight, heat and disinfectants. Plague is likely to persist in a warm climate environment only if the local rodents become infected. In cold climates, the bacilli may remain viable and virulent for several weeks when frozen or dessicated or both, as these are the special conditions for the maintenance of the pneumonic form of plague.

Usefulness of the Agent in War. Advantages. Plague bacilli grow well in artificial media.

Pneumonic plague might be very effective against troops in cold climates.

Where plague is endemic, the filth and famine that follow war make it an easily propagated agent. It might readily be established in non-endemic regions in the wake of war.

Plague is a terror disease and therefore has great psychological value.

The pneumonic form requires no animal intermediates for epidemic spread.

Pneumonic plague is difficult to diagnose and is easily confused with other respiratory diseases.

Great numbers of casualties might be obtained before measures could be taken to prevent them for plague kills quickly.

Disadvantages. There is no certain treatment yet developed for pneumonic plague.

Airborne organisms would be difficult to control by the using forces.

Ground contamination in cold regions with the pneumonic form of plague would render areas dangerous for long periods of time. It would thus be highly retroactive.

Sylvatic plague is a permanent disease when established in a wild rodent population.

CHOLERA (Vibrio comma). Historical. This organism was unknown until 1883 when Koch discovered the "comma bacillus" in the defecations of cholera patients. Cholera is essentially a disease of man; it never appears as a spontaneous disease in animals. Practically all pandemics of cholera have originated in the delta of the Ganges, from whence at times it has spread to Persia, Russia, Sweden, England and the United States. The most recent cholera epidemics occurred in Manchuria in 1932 and in Japan in 1932, 1937 and 1938.

Cholera, typhoid and bacillary dysentery are sometimes considered as a group because in all three the site of infection is the intestine, the problems of transmission control is the same and the three diseases have similar antigenic make-up; that is, their essential immunizing antigens are closely related chemically. Special problems of the three infections are based on the fact that contamination from the environment is an important source of infection with them, geographical distribution becomes a significant factor in the problem of transmission control, and in all three infections the diseased patients excrete large amounts of fecal material which may serve as highly infective sources for secondary cases.

The climatic influences which appear to favor the spread of the cholera are low-lying areas and soils easily permeable to water, especially if the water is polluted with decomposing matter. If there is a lack of cleanliness in drinking water and food, together with bad sanitation, the disease may spread even where the climatic influence is ordinarily opposed to it.

Characteristics. The cause of Asiatic cholera, *Vibrio comma*, is a short, slightly bent rod, 0.4 to 0.6 micron broad and 1.5 to 3.0 microns in length, occurring singly and in spiral chains. It is gram-negative and non-sporing and may have from one to three polar flagella, which make the organism actively motile. It has not as yet been ascertained with certainty whether a true toxin is produced by Vibrio comma or whether the symptoms of the disease are referable only to the endotoxins it produces.

Portals of Entry. Cholera is introduced into the body by ingestion following transmission of the organisms by personal contact, by fomites or by contaminated food, milk and water.

Epidemicity. Cholera may be epidemic or even pandemic, widely prevalent over an entire country or even a continent. It is an enteric disease, easily spread by means of human excreta containing the comma organisms. Important is the epidemiological fact that certain epidemic foci exist, especially in Lower Burma and India, where cholera is always going on and from which all epidemics and pandemics originate.

Symptoms and Prognosis. After an incubation period of 2 to 5 days, the disease is first characterized by a preliminary diarrhea which become progressively more severe. The organisms proliferate rapidly in the intestines, often completely outgrowing the normal intestinal flora. They attack the walls of the intestines, particularly the lower half. Intense vomiting begins and as the purging and vomiting persist, body water is depleted. The urine diminishes and may stop, the fluids depart from the subcutaneous tissues, which therefore contract so that the face alters, the nose becoming sharp, the cheekbones prominent and the eyes sunken.

There is also a systemic toxemia, indicated by subnormal temperature together with prostration and collapse. The disease runs a short course, resulting in death in some cases within 12 hours after the onset of the disease. It is almost 100 percent fatal.

Diagnosis and Detection. The vibrio is detected in large numbers in the stools and other dejecta of the infected patient. Laboratory diagnosis may be made from smears prepared from a flake of mucus in the stools for evidence of the characteristic comma-like rods. The cholera-red reaction is obtained in cultures when 3 to 5 drops of concentrated sulphuric acid are added to a peptone water culture. The development of a pink color indicates the presence of *Vibrio comma*. In addition, intraperitoneal injection of the culture into a guinea pig will result in death of the animal from a peritonitis within 24 hours. Pfeiffer's reaction, which depends upon microscopic observation of granular degeneration, swelling and loss of motility of the vibrios, also is used to confirm the diagnosis of cholera.

Treatment. There is no satisfactory treatment for cholera although bacteriophage has been used with some success both in the prevention and treatment of the disease. Prevention and control of the disease remain the best treatment. Cholera vaccination is of distinct value.

Prevention and Control. Various vaccines, including heat-killed, sensitized and mixed vaccines, have been used in the prevention of cholera with good results. Injections of mixtures of *Eberthella typhosa Salmonella paratyphi, Salmonella schottmuelleri* and *Vibrio comma* have been found to confer a reasonable immunity of short duration, persisting for a period varying from 6 months to a year. Prophylactic and therapeutic administration of bacteriophage has been attempted with some evidence that early administration may be of value in reducing the severity and mortality of the disease.

Cholera breeds in filth. Control measures consist of quarantine, group sanitation, careful supervision of food and water and war against the fly population, in order to interrupt the common routes of infection. These routes are as follows:

Person to person infection. Contact with cases during the incubation period is dangerous since cholera organisms are present in stools as much as 24 hours before the appearance of symptoms. Contact with subclinical cases may result in infection since healthy persons may become infected and excrete vibrios without sign of disease. Cholera carriers, following recovery, may become an important factor in distribution of the disease in epidemic centers.

Fomites and soiled articles. Soiled clothing is always a potential source of infection. Even the act of washing contaminated clothing has resulted in cases of cholera; the vibrios remain viable for as long as 5 weeks on moist linen.

Flies. Cholera vibrios have been isolated from the feet of flies 17 hours after contamination. Cholera has no known animal carriers.

Water. Cholera vibrios may survive for 16 days in water. All drinking water must be sterilized.

Food. Food is commonly involved in outbreaks of cholera. It is a particularly good vehicle as it is usually damp and gives good protective covering to the organism. All food, therefore, must be cooked.

Usefulness of the Agent in War. Advantages. Healthy carriers who excrete cholera vibrios without exhibiting signs of the disease play an important role in the transmission of the disease.

The filth of war is propitious for cholera epidemics and pandemics.

Cholera is a highly and rapidly fatal disease and once established would be difficult to bring under control.

Its principal vector, the fly, is not eradicable and may even be deliberately used to transmit the disease.

There is no satisfactory treatment for cholera. The immunity conferred by vaccination is not absolute.

Disadvantages. The incidence of cholera offers no problems of detection.

There is but one portal of entry.

The rapid spread of the disease would be apt to endanger friend and foe alike. Once the health and sanitation agencies broke down, cholera might become a scourge.

Vaccination against cholera is now standard procedure for troops going into areas where cholera is endemic.

RINDERPEST (filtrable virus). Historical. Rinderpest or cattle plague is an acute, highly fatal disease of cattle and less often of sheep and goats. It is especially prevalent in parts of the Orient, in India and in Africa. The disease has never occurred in Canada or the United States. Wartime interest in the disease centered on this latter fact, and research efforts were almost solely directed to producing an adequate stock of virulent rinderpest virus and to converting this virus into a safe vaccine for the effective protection of cattle against rinderpest.

Characteristics. The virus cannot be described; it can only be demonstrated. By inoculating a calf with the virus strain, the disease is reproduced. It may be possible to demonstrate further the incidence of the disease by examining lesions produced in the calf which may be considered pathognomonic of this particular virus infection.

Portals of Entry. The lesions that occur in the mouth and intestines of infected animals suggest that ingestion is the usual route of infection.

Epidemicity. The disease is highly contagious, being transmitted by contact from animal to animal.

Symptoms and Prognosis. The period of incubation is from 3 to 9 days. The fever which marks the onset of the disease lasts through the course of infection, from 4 to 7 days. The disease is characterized by catarrhal conditions of the nasal mucous membrane, with fever, conjunctival infection, profuse diarrhea and rapid emaciation. Animals die usually on the tenth or eleventh day. Mortality of the disease ranges between 15 and 75 percent.

Diagnosis and Detection. The virus may be detected in the blood, secretions and intestinal contents of infected animals. A neutralization test has been devised for certain diagnosis of the disease. The test is based on the neutralization of the virus when a solution prepared from the spleen of a suspected infected animal is mixed with an equal amount of prepared immune serum.

__Treatment.__ There is no treatment for rinderpest. The immunity of cattle which have recovered from either experimentally produced or naturally occurring disease, however, is solid and lasting, as is that conferred by the new attenuated vaccine.

__Prevention and Control.__ Until recently a chloroform-inactivated virus vaccine was used in the immunization of cattle or carabaos in the Philippines where it had given good results. However, the disease could be spread by such vaccinated animals, and the vaccine itself could only be made on a small scale. Now a method of mass production of vaccine is possible, by producing cultures of the virus in embryonating eggs. The new avian-attenuated rinderpest vaccine that has been developed has been shown to confer a solid and lasting immunity in cattle and does not cause spread of the disease even by close and intimate contact. The immune response is prompt, most animals demonstrating neutralizing antibodies within 10 days. The dried vaccine, packed in vacuum, will maintain its potency for as long as 15 months when stored at a temperature of 2 to 5 Centigrade.

In the absence of a vaccine, destruction of all infected animals is the only certain means of controlling the disease.

__Usefulness of the Agent in War.__ __Advantages.__ Rinderpest is not generally prevalent. Where it does not presently exist, the introduction of the disease would probably result in a serious epizootic among dairy and food herds.

The disease is highly infectious and considerable initial destruction might be effected before vaccination could be instituted.

Disadvantages. A tissue vaccine is available to provide rapidly the means of surrounding an epizootic, should it occur, with a ring of immunized animals.

An avian vaccine which, because it does not require infected animals as a source of live virus, is economical and capable of being produced in great quantities.

FOOT AND MOUTH DISEASE (filtrable virus). Historical. The infectivity of this disease for man appears to be very low, but it is highly infective for cattle, sheep, pigs and other domestic animals. On infected premises the virus has been known to remain active for as long as 345 days. Control by slaughter of infected animals has freed the United States of this disease since 1933.

Characteristics. The causative agent of foot and mouth disease is unknown, but is believed to be a filtrable virus which as yet has not been demonstrated.

Portals of Entry. Transmission of the disease appears to occur through contaminated pasturage and may be water-borne as well. The presence of the virus in the vesicle fluid in blisters appearing in the mouth and on the feet of infected animals seems to provide a ready source of animal to animal infection.

Epidemicity. Foot and mouth disease is considered the most contagious infection known to veterinary science.

Symptoms and Prognosis. The disease is characterized by the appearance of vesicular eruptions localized upon the mucosa of the mouth and upon the delicate skin between the hoofs. With the onset of the eruptions there may be increased temperature, refusal of food and general depression. Usually the disease is mild. The vesicles become small ulcers and pustules and go away. Occasionally the disease is complicated by gastro-enteritis or inflammation of the respiratory tract, in which case death follows. The mild form of the disease has a 2 to 3 percent mortality; the malignant form frequently has as high as 50 to 70 percent mortality.

Diagnosis and Detection. The characteristic vesicles on the mouth and hoofs of animals indicate the disease.

Treatment. There is no satisfactory treatment for the disease. One attack of the disease protects against reinfection for years. It may therefore be presumed that a vaccine is possible against the disease.

Prevention and Control. Control of the disease in animals is effected by destruction of all infected with it.

Usefulness of the Agent in War. Advantages. The virus is stable and resistant and will remain active for 6 months or more if kept moist and cool.

The disease is epizootic and at present can only be eradicated by animal destruction.

It is highly infectious for cattle, sheep and goats and might therefore be used to deplete the dairy and food herds of a nation.

It is a mild disease, prevalent throughout the world and would probably not be conspicuous as an agent of warfare.

Infectivity for man is low, and therefore the agent is safe for him to handle.

Disadvantages. Because of the resistance of the virus and its high infectivity, retroactivity of the disease would probably be great.

Infected premises remain sources of disease for a year or more following contamination.

NEWCASTLE DISEASE (filtrable virus). <u>Historical</u>. This disease was first described in England in 1926 and obtained its name from the locality where it was first found. Since that time its occurrence has been reported in the Philippines, India, Ceylon, Korea, Japan and Australia. Identification with Newcastle disease of a virus disease of fowl in California in 1944 was the first recognition of the malady in the Western Hemisphere. The disease that appeared on the West Coast has been called avian pneumoencephalitis. Its virus is immunologically related to the Newcastle disease found on the East Coast, but where the West Coast disease is difficult of transmission and has small mortality, the East Coast fowl disease is readily transmissible and causes severe death losses. It is an acute infectious disease resembling fowl plague in many respects. The species affected are chickens, guinea fowls and turkeys, but many other species can be infected artificially.

<u>Characteristics</u>. The filtrable virus causing Newcastle disease is estimated to be from 80 to 120 millimicrons in size. The virus infection subsides during the summer months. It is most active in cold weather.

<u>Portals of Entry</u>. Natural transfer of the disease is by means of infected exudates, excreta and offal of infected birds. The digestive and respiratory routes constitute the usual channels of natural infection. Traffic in live birds accounts for the normal

dissemination of the infection. Free-flying birds such as sparrows and pigeons have been suggested as possible vectors but there is no actual proof as yet.

Epidemicity. The virus can be readily introduced into a poultry area and once established it spreads rapidly. Since it is essentially 100 percent fatal, entire flocks are usually wiped out wherever the disease appears.

Symptoms and Prognosis. Newcastle disease has a comparatively long incubation period, from 4 to 11 days. It may even be as long as 25 days. In the acute experimental disease, an initial dullness in the fowl develops rapidly into marked depression accompanied by progressive weakness. Diarrhea often appears early. Cyanosis of the comb, advanced debility or complete prostration usually precedes death which usually follows within 24 to 48 hours following onset of the infection. Most striking are the early signs of incoordination or slight tremor or the suddenly developing rapid twitching of one or more extremities or of the head and neck. Even after almost complete paresis and prostration, the muscle spasms may still be quite prominent, indicating the severe nervous system involvement caused by the disease. Pathologic phenomena are chiefly hemorrhagic and inflammatory in nature. Severe hemorrhagic leasions are found in the proventriculus or true stomach of the fowl and throughout the intestines. Proliferative necrosis takes place in the lung and central nervous systems.

In disease strains isolated in this country, subacute or chronic cases of experimental Newcastle disease are not uncommon, with partial or complete recovery occurring in a small minority of subjects. European strains of the disease are almost always acute and fatal.

<u>Diagnosis and Detection</u>. Severe respiratory as well as nervous symptoms are manifestations of Newcastle disease. It may be confused with other fowl diseases such as laryngotracheitis or infectious bronchitis. It is distinguished, however, by the obvious marks of nerve involvement.

<u>Treatment</u>. There is no treatment possible once the disease has become established. A specific immunizing serum has been developed for the prevention of the disease and where this is not available, control measures alone must be effected.

<u>Prevention and Control</u>. A formalin-inactivated or ultraviolet light-irradiated vaccine has been devised recently which stimulates within a period of ten days a specific active immunity of high degree. It is possible to enhance and prolong this immunity by the addition of lanolin and fixed oils or aluminum hydroxide. By repeated injections of this vaccine, hyperimmune sera have been secured. A substantial immunity may persist for periods of several months to as much as 4 years with living modified virus vaccine or with virulent Newcastle virus. Lyophilized sera in quantity can be made available for Newcastle disease in the case of suspected outbreaks

of the disease. The serum is specific for this disease. A passive immunity is conferred upon the chick via the egg yolk of an immune hen. This appears to be a factor which may modify the epizootic character of the disease in the course of time.

Control of Newcastle disease consists in rigid quarantine and in destruction of affected flocks.

Usefulness of the Agent in War. Advantages. The relatively high tenacity of Newcastle virus obviously favors indirect as well as direct dissemination of the disease.

Whole flocks are wiped out upon introduction of the disease.

Newcastle disease would be an effective sabotage agent and could be used to make serious inroads on a basic food staple.

No treatment has been found for the disease.

Many routes of transmission are suggested as sources of infection.

Disadvantages. The availability of an effective vaccine against the disease would nullify the operations of sabotage agents once deliberate dissemination of the disease was suspected.

FOWL PLAGUE (filtrable virus). Historical. The character of fowl plague is very much like that of Newcastle disease and the two viruses behave similarly. It is only in their pathology that they are now recognized as separate entities. Fowl plague has long been known in the Mediterranean region of Europe when it has repeatedly spread northward into Switzerland, Germany, Holland, Russia and England.

Characteristics. The virus of fowl plague is believed to be somewhat smaller than that of Newcastle disease and is probably 60 to 90 millimicrons in size.

Portals of Entry. Like Newcastle disease, natural transmission of fowl plague is by means of infected exudates, excreta and offal of infected birds. The digestive and respiratory routes are the usual channels of natural infection and both may be used for artificial infection.

Epidemicity. The virus is easily introduced and spreads rapidly in unprotected fowl.

Symptoms and Prognosis. The incubation period is from 3 to 7 days, with death within the following 12 to 24 hours. Clinical manifestions are characterized by depression, cyanosis and polydipsia or excessive thirst. General malaise becomes pronounced, together with conspicuous congestion of the comb and wattles, their color gradually changing from a bright red to a deep bluish hue. The voice gradually changes from a shrill quality to a weak squawk, which often is the forerunner of terminal convulsive seizures.

Chickens that die of the acute form of fowl plague reveal a variety of congestive, hemorrhagic and transudative changes including cyanosis of the comb and wattles and larynx and trachea, lesions of the heart and edema of the lungs and blood stained mucus in the trachea. The virus appears to concentrate in the nervous system when it disappears from the blood. Basic microscopic changes include multiple focal necrosis in the visceral organs and the central nervous system, accompanied by inflammatory reactions leading, in the brain, to meningoencephalitis.

Diagnosis and Detection. Detection and diagnosis of fowl plague depend upon the appearance of its characteristic symptoms. Laboratory demonstration of the virus is difficult.

Treatment. There is no specific treatment for fowl plague.

Prevention and Control. Effective control measures for fowl plague were unknown until recently. The development of a specific vaccine for the protection of fowl from this disease now makes it possible to establish an active immunity of high degree. The vaccine is prepared from virulent plague virus inactivated by formalin, or virus that is irradiated by means of ultraviolet light.

Usefulness of the Agent in War. Advantages. Fowl plague is a highly infectious and rapidly fatal disease among domestic fowl.

All flocks in a poultry region might be quickly wiped out by sabotage methods.

There is no treatment for the disease.

Disadvantage. The development of a specific vaccine for fowl plague makes it possible to establish a barrier against either the deliberate or accidental introduction of the disease.

Section V

BIOLOGICAL AGENTS INJURIOUS TO CROPS

GENERAL. The use of biological agents for the destruction of crops is intended to serve as a weapon of the same order as the classical blockade. Selection of agents for plant destruction in biological warfare is based on their effectiveness against such crops as potatoes, sugar beets, rice, cereals and truck garden vegetables. In general, the effects of crop destroying agents are slowly achieved. Few pathogens kill crops outright. Their chief mode of action is reduction of yield by local injury to a portion of a particular plant. The symptoms and effects in crop destruction that may be reasonably attained are:

Local lesions on leaves and stems.

Damping off, which is an attack on the root or stem near the soil surface.

Wilting, by blocking moisture and nutrient-conducting tissue.

Formation of galls or cankers on woody tissues.

Rotting of flesh parts of plant such as tubers or fruits.

Dwarfing or distortion of the growing point or top of the plant.

Plant diseases are either of parasitic or nonparasitic origin. Parasitic agents may be animal, plant or virus. The use of animals (insects such as the Japanese beetle, Colorado potato beetle and

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Plant diseases are either of parasitic or nonparasitic origin. Parasitic agents may be animal, plant or virus. The use of animals (insects such as the Japanese beetle, Colorado potato beetle and

Mexican bean beetle, leaf hoppers and seed corn maggots) has been considered in spite of the difficulties inherent in production and dissemination, as well as their restricted usefulness against the particular targets under consideration. Bacterial agents are less selective in the crops they attack. Those which may be considered for biological warfare purposes usually attack the conducting tissue of plants and interfere with water movement in the plant so that the plant wilts. Bacteria may also attack soft tissues of leaves or roots causing rotting or producing galls. The great majority of parasitic plant diseases which may be useful in war are caused by pathogenic fungi. These, by virtue of their filamentous growth process, resulting in the production of masses of filaments termed mycelia, are the cause of epiphytotic disease in plants. Growth of fungi on plants can be initiated by small segments of mycelia (hyphae), by spores or by sclerotia (compact masses of mycelia). Fungi spores are ordinarily produced in great numbers, are stable in drying and are easily windborne. Fungi have the additional advantage, aside from their epiphytic character, of being able to infect their host through natural openings such as the stomata of leaves, and they invade the intact epidermis of aerial parts and roots. They are also believed able to effect penetration by secreting an enzyme that partly dissolves the cell wall of the host. Virus diseases are considered less satisfactory than either bacteria or fungi as agents because they are for the most part dependent upon insect vectors for dissemination. Nevertheless, the virus diseases of plants are very numerous and effective. For the most part they produce a systemic infection in the plant. Juices or crushed tissues of plants are highly infective. Plants are seldom filled outright by viral agents, but their growth habits may be seriously affected.

Nonparasitic agents seem most practicable for use in biological warfare. These are organic chemical compounds which are known as plant growth regulators. Regulating chemical substances may cause distortion, malformation or dwarfing even in high dilution. The destruction which they cause is usually induced by establishing unfavorable growth conditions. Many diseases of plants result from adverse environmental conditions and by controlling these conditions, the desired results are obtained. They are to be distinguished from chemical agents which have been developed as weed-killers.

BROWN SPOT OF RICE (<u>Helminthosporium oryzae</u>). <u>Historical.</u> This fungal disease is prevalent almost everywhere rice is grown. Under normal conditions, infested rice straw from rice refuse is probably the most common source of infection. Certain weeds also serve as hosts and provide a reservoir of infection. The only way the disease is transmitted in nature is via wind-borne spores.

Effects on Plants. The organism of brown spot produces a variety of symptoms on rice plants, which vary with the age of the plant. On very young plants, damping off is observed; on older plants, leaf lesions appear. On plants in the heading stage, infections of the kernel, known as pecky rice or rotten nick, are produced. These symptoms appear as elongated leaf spots, brown with a greyish center. The initial leaf lesion, barely visible in 24 hours, reaches maturity in about 3 days.

Control of Disease. General field sanitation such as burning all rice straw, and seed treatment with hot water or red copper oxide, afford some protection against the disease. Although a few rice varieties show resistence to brown spot, none are known to be entirely immune.

Usefulness of the Agent in War. Despite the demonstrated effectiveness of brown spot, its usefulness as an agent is limited by its special environmental needs. Its requirements of high humidity, free water on leaves and 69 to 86 degrees Fahrenheit severely restrict opportunities for its employment.

The organism has been prepared for warfare purposes in a form which will permit it to be stored, transported and disseminated effectively. Rapid production in large quantities with uniformity of product and maintenance of virulence under conditions of storage and use have also been achieved. Virulence of the organism has been maintained for a period of more than 900 days. The organism will survive through winter and can withstand 60 hours of freezing at -40 degrees Centigrade. Infection is initiated by two forms of the organism, by spores and by fragments of mycelium. The spores, however, are five to ten times more infective than the mycelia. Both spores and mycelium are more effective when applied to plants in dust form than in an aqueous suspension. Despite the feasibility of large scale production of brown spot, the chemical agents of the LN group, later discussed, are considered more efficient agents against rice.

RICE BLAST (Piricularia oryzae). Historical. Rice blast is one of the most important and destructive enemies of rice. The primary source of infection in nature is caused by mycelia which live through the winter in dead rice stubble or other hosts such as weeds. The mode of transmission is by air-borne spores.

Effects on Plants. The name of the fungus disease is derived from its effect on the plant during the heading stage which partially or completely prevents the formation of rice kernels. A single leaf or sheath lesion or the coalescing of two or more lesions results in killing the leaf. The period of incubation, from infection to appearance of symptoms, is from 4 to 6 days. Secondary infection from infected plant to uninfected plant, requires an additional period of 6 to 12 days. High humidity and the presence of free moisture on the leaves are necessary to the spread of the disease. Rice is susceptible to blast infection during two periods of its growth: from seedling stage to the appearance of extra shoots from the original blade, and from the emergence of the grain out of its protecting sheath to the flowering stage.

Control of Disease. Field sanitation is partially successful in preventing and controlling rice blast. Some work has been done on the development of a fungicide which may prove effective against this particular organism.

Usefulness of the Agent in War. Susceptibility of rice crops depends on the variety of rice plant and extends from the very susceptible to the completely immune. Air temperatures above 21 degrees Centigrade are necessary for infection of rice with rice blast. A heavy rain within 8 to 10 hours after initial dissemination of the agent would materially reduce the amount of infection if a dust inoculum for secondary infection were used. This would be only slightly bettered if the infection were in the form of spore suspension. Better infection is attained with spores in the dust form if applied in the early morning or late evening when plants are wet from dew or rain.

Large scale production of rice blast is possible and the spores so produced may be processed and prepared in dust form for use directly as a dust or for resuspension in water. They are capable of retaining their viability and infectivity in storage for a considerable period. Spore cultures survive satisfactorily in transit and storage if kept dry and cool. Rice blast is the most promising of all the rice pathogens, causing extensive damage in the early stages of plant development and later in the heading stage.

LATE BLIGHT OF POTATO (Phytophthora infestans). Historical. This was the disease that produced the famine of 1845 in Ireland, following total loss of the potato crop. The fungus causing late blight is intensely pathogenic on susceptible varieties of potatoes. It is the most devastating pathogen of that crop and is found in all potato-growing areas of the world. Discarded potatoes left in the field under refuse piles and infected seed potatoes are the common source of the plant infection.

Effects on Plants. The incubation period on stems and leaves varies from 3 to 5 days. Symptoms consist of small dark water-soaked areas which spread under ideal conditions to the total leaf surface and stem. Roots are not affected. On the underside of blighted leaves a white growth appears consisting of fruiting sporangia-spores. On infected tubers the symptoms appear at the stem end or eyes. Infected potatoes in storage rot until they are reduced to a putrid mass.

If the potato is not destroyed by freezing, the spores themselves can survive the winter. The sporangia or spore cases, however, are of very short life and can survive but a few days under favorable weather conditions. Sporangia are wind and rain-borne, but high humidity and low dew point with the temperature not higher than 70 degrees Fahrenheit are necessary to facilitate transmission of the spores. Transmission from plant to plant is possible only if they are grown close enough to permit aerial transmission.

Control of Disease. Control of late blight is affected by elimination of infected seed potatoes and refuse piles. Spraying with fungicides from blossom time until the vines die in the fall is affective.

Usefulness of the Agent in War. Because of the short life of the sporangia, production of the agent of late blight is made from the mycelia rather than sporangia. Seed substrata infested with the blight mycelium may be maintained in a viable condition for a period of at least 3 months, and under suitable conditions sporangiospores emerge from such seeds and produce sporangia from which infection is initiated. Preformed pellets inoculated with late blight are also effective initiators. The pellets withstand storage for 12 to 14 weeks without loss of viability or pathogenicity. In addition to infested seed and preformed pellets, inocula in the form of sporangia, mycelium and infected tubers are also capable of initiating infection under suitable environmental conditions such as free moisture on the plants, high humidity and cool temperature. While late blight is extremely destructive, its use as a weapon is limited by its instability after prolonged storage and its requirement for special climatic conditions for initiation.

SOUTHERN BLIGHT (Sclerotium rolfsii). Southern blight has a wide host range, affecting rice, cereal grains, soybeans, sweet potatoes, Irish potatoes, sugar beets and garden vegetable crops. It is found in all of the Southern states, in Europe, Asia, Africa and Australia, and in the islands of Japan, Ceylon, Cuba, Hawaii and the Philippines. Southern blight is typically a disease of tropic or subtropic areas, its distribution limited by the fact that its mycelium are killed by exposure to soil temperatures below freezing. The period of greatest activity is generally confined to summer, late spring and early fall.

Effects on Plants. The host plant dies when the fungus destroys the base of the stem to such an extent that it will no longer support the plant. When attacked by Southern blight, the host stem of the plant is rotted at ground level with attendant interruption of food supply from the leaves of the plant. General wilting occurs, increasing in severity with extension of the rot until the plant dies. Death is accomplished in from 4 to 20 days, depending upon the severity of the attack. The results of the attack appear similar to those following the girdling of a tree in that the first apparent injury is the starvation and death of the roots. The top of the plant wilts only when the water supply is cut off by the death of the roots.

Control of Disease. Where soil is contaminated with this agent, a naturally resistant crop such as sorghum may be sown in place of susceptible crops. While crop rotation will not save susceptible crops, it is an indirect means of decreasing the loss in total food crops. Sanitation measures such as burning or plowing under the contaminated crop debris, disinfection of tools and cultivation machinery and isolation of the infected field may tend to lessen damage to successive crops. The spraying of fungicides may be effective in controlling the disease.

Usefulness of the Agent in War. Sugar beets, white potatoes, carrots and lettuce are most susceptible to Southern blight; soybeans and field beans are intermediates; and rice, cereals and sweet potatoes are highly resistant. Root crops such as sugar beets and carrots are very susceptible, for their roots serve as an excellent source of food for the fungus. In moist weather when the agent grows over the surface of the soil, it often attacks the bases of the leaf petioles or stalks of root crops. The fallen petioles serve as a medium for growth of the fungus in the soil and in many instances act as a bridge for growth from plant to plant. While almost all field and garden crops are susceptible to Southern blight at some stage of growth, cereal grains, rice and sweet potatoes are so highly resistant that the agent is of no value even in the seedling stage of their growth.

The organism seldom produces spores of any type in artificial culture or in nature. Utilization is therefore made of the sclerotia and mycelia. The sclerotia can be used directly; mycelia require culture on a carrier such as seeds or bean pellets which have been thoroughly infected with the hyphae. These infested particles after being dried then serve as carriers of the mycelial inoculum. Although ideally adapted to the infestation of soil, inoculum can not be adapted easily for use with an organism which produces abundant wind-borne spores. The strains of agent selected for their military value are quite stable, no change in cultural characteristics having been observed in over a years period of time. The virulence of the organism is attested by the fact that it is capable of growing and attacking seeds in soil too dry to support the growth of a plant or to permit germination of seeds.

The agent has been prepared in three forms, as sclerotia, infested wheat, seeds and pellets of infested bran. Infested seeds appear to be the most effective carriers of the agent. Dissemination by aircraft over wide areas given over to production of susceptible crops is the most practical means of employing large quantities of the agent.

SECTION VI

CHEMICAL PLANT GROWTH REGULATORS AND DEFOLIANTS

GENERAL. In order to overcome many inherent difficulties in the use of biological agents for the destruction of food and industrial crops, a number of synthetic chemical compounds have been developed which in many respects are superior substances for obtaining the same results. The difficulties of producing, storing and disseminating fragile viable agents or insect-vectors are not met with in the use of chemical agents. Almost the only advantage lost in employing chemical plant regulators is that of persistence. The infective biological agent may be harbored in soil for months or years after an attack, fully capable of reviving and renewing the attack when conditions became favorable again. A saturation attack with a chemical compound may render soil sterile and achieve a similar effect but it has not the insidious character of a viable agent.

PLANT GROWTH REGULATORS. Characteristics. The plant growth regulating or inhibiting agents are organic compounds which are capable of producing various types of growth response at very low concentrations and may inhibit growth or even cause death of the plant if applied in higher concentrations. The injury caused by these agents results from changes within the plant produced by the stimulus of the agent and not to cumulative effects or localized damage to the tissues, such as is produced by the more common inorganic herbicides or weed-killers. The discovery that certain chemical compounds could be used to cause death or produce abnormalities of growth causing reduced fruitfulness arose out of work on the auxins, the so-called plant hormones and growth-promoting substances.

Compounds belonging to the chlorinated phenoxy acetic acid series have been found to be very destructive to broadleaf crops in small amounts, resulting in bending of the plant, chlorosis or blanching, formation of galls on the stems and finally death. The carbamates have been found to be most effective on cereal crops, producing a withering effect, bulbous crowns and eventual death to the plant.

The plant regulators may be produced as either solid or liquid agents. Solid agents may be applied as dusts of 100 percent agent or the agent may be impregnated in clay, talc or Fuller's earth. It may be applied as (1) granular material of 100 percent agent or (2) agent, Fuller's earth and carbowax or (3) agent, Fuller's earth, carbowax and starch. Dusts may be used in low level operations for direct contamination of crops. Granular material may be used in high level operations to produce ground and irrigation water contamination. Liquid agents in the form of aerial sprays may be made up as water sprays or oil sprays. In most instances five times as much water as oil is necessary to achieve comparable results. The use of water acts to reduce the payload, but the effectiveness of certain crop agents is reduced when applied in oil.

The LN compounds. The compounds which have been designated as LN-8, LN-14 and LN-33 are the principal chemicals covering the widest range of effects desired. Other compounds have been devised with special properties not possessed by these three.

LN-8 (2,4-dichlorophenoxyacetic acid) in oil severely injures or destroys all broadleaf crops except Irish potatoes. It produces significant reduction in yields of rice when applied at the flowering stage, but does not produce wholesale destruction of the plant. None of the phenoxyacetic acid compounds are effective against field crops of cereals. LN-8 has two forms. As Vegetable Killer Acid (VKA), it is a granular powder. As Vegetable Killer Liquid (VKL), it is a solution either as an ammonium salt in water or acid dissolved in oil and tributyl phosphate. VKA may be packaged in paraffined fiber cartons containing 200 pounds of agent. The contents of a carton are sufficient to produce 500 gallons of 5 percent solution of active agent, enough to treat approximately 120 acres. VKL may be transported in 55-gallon drums containing 30 percent active agent. The VKL is diluted with diesel fuel oil to produce a 5 percent solution of active agent. The solution may be liberated from the M10 tank at the rate of 30 gallons per 7 acres. A drum of VKL will cover 77 acres of land.

LN-14 (2,3,5-trichlorophenoxyacetic acid), either in water or oil, is the agent of choice for the destruction of Irish potatoes. It is also effective against all other broadleaf crops. LN-32 (2-methyl-4-chlorophenoxyacetic acid) is in the same class with LN-14.

LN-33 (isopropyl phenyl carbamate) is the agent of choice for the destruction of cereal crops. It destroys rye when applied in the late boot stage and other young cereals which are less than 10 inches in height. A 10-pound per acre concentration is the most effective dosage.

LN-2 (para-chlorophenoxyacetic acid) is particularly effective against sweet potatoes. Soybeans are more susceptible than sugar beets and Irish potatoes. The esters of the compound, as well as those of LN-8, LN-14 and LN-32, are all good inhibitory agents in small concentrations.

LN-974 (butyl 2,4,5-trichlorophenoxyacetate) is as effective a crop destroying compound as LN-14 and in addition is directly oil miscible.

Effects on Broadleaf Plants. Plant inhibitors sprayed on the top growth of young dicotyledonous plants such as beans, sugar beets, tomatoes and sweet potatoes, or applied in one or more droplets, may cause either marked curvature of the stem and shoot and dropping of the leaves within a few hours, or partial or complete inhibition of subsequent shoot growth. If growth continues, the form of the plant may be abnormal, with modification of leaf shape, thickening and splitting of the plant stem and occurrence of gall-like swellings at the nodes of the plant. The cumulative effect is to produce a plant which may be dwarfed or distorted, with proportionately reduced yield.

Effects on Cereal Crops. The first visible response of plant inhibitors on cereals such as rye, wheat and other grains may be a contact injury, browning or death of the leaves or of small areas of the leaves. This may appear within a day, or as much as 3 or 4 days, after spraying. If sprayed on young cereals, there may be a stunting of growth. If applied later it may prolong the period of vegetative growth and delay the heading.

Detection. Chemical means of detection may aid in determining the exact nature of the crop destroying agent or the concentrations of agent as in irrigation water sources, but in general it is inferior to biological means of detection such as plant response. Soil substances may interfere with chemical tests, the tests require large concentrations of the agent, and they are time consuming. Plant response will be observed in a matter of a few hours.

Prevention and Control. No method of arresting the effects of the LN compounds has been found. Defensive measures are almost impossible. The effects produced by soil or irrigation contamination are to a degree insidious and the damage would be done when the symptoms appeared. In the event of a sprayed chemical, the only course would be to remove the crop and replant, probably with a forage crop if the spray came late in the season. If the soil is contaminated by a plant inhibitive, a new crop may possibly be established after plowing, unless the level of contamination is excessively deep. In the case of an irrigated crop such as rice, draining and reflooding might possibly reduce the loss due to the plant inhibitor, were it carried out promptly enough.

CHEMICAL DEFOLIANTS. Characteristics. Defoliants are chemical compounds which cause trees and shrubs to drop their leaves.

In peacetime employment, defoliating chemicals have been used extensively for the removing of leaves from cotton plants prior to the harvest of the bolls. Agricultural experiment stations and chemical companies have also worked to find substances for removing the leaves of soybeans and other such crops prior to harvesting. Calcium cyanamide is used to defoliate cotton plants. Thirty pounds of agent per acre when applied as a fine dust to the moist plants is effective sometime between 3 and 10 days following application. Ethylene and other unsaturated hydrocarbons are known to cause defoliation of many plants at very low concentrations and commercial experiments are continuing with the hydrocarbons. Until recently, however, little work has been done on chemical substances for the defoliation of trees or other woody plants. In the process of defoliation, the leaves of trees go through a period of discoloration. The military applicability of this fact has also been considered in the selection of defoliating agents.

Ammonium thiocyanate and zinc chloride. The most effective chemical compounds causing rapid leaf discoloration and defoliation are ammonium thiocyanate and zinc chloride. Leaf discoloration often occurs within 4 hours of treatment and defoliation begins 3 to 4 days after treatment with either agent. The ultimate degree of defoliation often reaches 75 to 100 percent. Leaf discoloration is as rapid and defoliation as complete for tropical plant species as for temperate zone plants. One-eighth saturated solution will produce as much plant necrosis as a saturated solution and cause as much defoliation. Heavy rainfalls occurring 5 or more hours after spraying cause no decrease in the effectiveness of the spray. Considerable damage is done even with rain 1 hour after treatment. Leaves in the shade are discolored and defoliated as rapidly as those in sunlight.

Ammonium thiocyanate turns leaves a bright maple-red color within 48 hours. The color lasts almost 10 days. Zinc chloride turns leaves a yellowish-brown color in 2 to 3 days following application. The maximum in color is obtained on the fifth day.

Defoliation. For defoliation, ammonium thiocyanate and zinc chloride are equally effective in the degree of defoliation ultimately produced. Defoliation with ammonium thiocyanate is initiated more rapidly, for a one-third saturated solution produces results beginning on the third day. An 0.37 saturated solution of zinc chloride produces results beginning on the fourth day. Leaf fall in both cases is most rapid on the fourth and fifth days, and maximum effects are achieved by the tenth day. Eight to twelve M10 tanks of agent in two or three planes, simultaneously released 50 feet over the forest canopy produce the most complete effects and the best penetration to low level vegetation. Eight tanks produce a visible swath approximately 110 to 130 yards wide. Following 75 percent or greater defoliation, prominent objects on the forest floor, such as a panel, may be clearly seen from directly above.

Target marking. For target marking, ammonium thiocyanate is the agent of choice, due to the rapidity and intensity of the color changes produced in vegetation. One-third saturated solution produces satisfactory color intensity. The optimum altitude of release for clarity marking is less than 50 feet above the forest canopy. Swaths produced by four tanks of agent, 25 gallons per tank, is visible at 9,700 feet at 5 miles distance.

Detection. Chemical analysis for the determination of the exact nature of the defoliant may be made, but the biological or plant response of the agent reveals the nature of the attack within a short period of time.

Prevention and Control. No effective means of protection against the defoliation of vegetation is likely other than preventing the aircraft from bombing, dusting or spraying forested areas.

SECTION VII

SIMULATED AGENTS

A simulated biological agent may be defined as a non-pathogenic microorganism or microorganism of restricted virulence which is useful in demonstrating the principles of microbiology and principles of dissemination and tactics. The simulated agents are usually chosen from among the larger forms of microorganisms or from the chromogens. Chromogens are bacteria capable of producing a pigment and are therefore readily identified in colonial growths. Among the simulated agents which are commonly used in the laboratory and in the field are:

Serratia marcescens (or Bacillus prodigiosus). This is a small rod, non-spore forming, which grows easily and rapidly into large colonies. It produces a bright red pigment. It is found in water, soil, milk and foods and is non-pathogenic.

Bacillus globigii. The spores formed by this bacterium are the same size as anthrax spores and because the organism produces a characteristic pigment on agar and is readily cultivated, it is frequently used as simulated Bacillus anthracis.

Bacillus subtilis. This spore-forming organism, sometimes called the hay bacillus, is related to anthrax. However, it is nonpathogenic. It is a straight rod, 2 to 8 microns long, is gram-positive and grows in long chains. Microscopically, the colonies are made up of interlacing threads, irregularly round with fringed edges. It is widely distributed in hay, soil, dust, milk and water. Bacillus subtilis is a common saprophytic organism on the normal skin. For that reason, it is often a source of annoyance to the medical bacteriologist because it contaminates cultures taken from lesions of the skin or from materials obtained by puncturing the skin.

Escherichia coli. This is a short, plump, gram-negative, nonsporing, mobile rod form which is 1 to 3 microns long, usually appearing singly. Although it is no chromogen, its yield is abundant, being plainly visible when grown on potato. Cultures of the colon bacillus are characterized by a fetid odor not unlike that of diluted feces. In nature it ferments dextrose and lactose and is present normally in the intestines of man and other vertebrates. It usually grows as a harmless parasite on the human and animal body and is capable of leading a purely saprophytic existence, though under certain circumstances it may become pathogenic for its host. In man, the coli bacillus appears in the intestinal tract normally soon after birth, at about the time of taking the first nourishment. From that time on the bacillus is a constant intestinal inhabitant without dependence upon the diet. Its function in the intestine is thought either to aid in the fermentation of carbohydrates or, as well, to act as antagonist to certain putrefactive bacteria.

Staphylococcus aureus. This is a pathogenic organism which produces not only an exotoxin and endotoxin, but also a hemolysin and leucocidin. It is found in boils, abscesses, furuncles and suppuration in wounds. It is a gram-positive, spherical cell, with an average diameter of 0.8 micron, found singly and in pairs, but usually in grape-like clusters. Its pigmentation is golden, though it appears as white, orange or lemon-yellow. Staphylococcus aureus is used by the United States Public Health Service to determine the germicidal coefficient or bactercidal value of ointments, tooth-pastes and other pharmaceutical preparations.

Staphylococcus albus. A gram-positive cell similar to Staphylococcus aureus, it too is found in wounds, boils and abscesses. Its normal habitat is the skin and mucous membranes. It is pathogenic but less so than Staphylococcus aureus, and produces a characteristic white pigment. Albus and aureus staphylococci can almost always be isolated as normal habitants of the mouth, as well as the skin, where they lead a more or less commensal existence in the crevices, pores of sweat glands and hair follicles of the skin. Unless the skin is injured they are harmless. Unexplainable is the surprising fact that despite their presence and that of other virulent micro-organisms, frequent accidental injury of the gums and oral and pharyngeal mucous membranes rarely leads to serious infection and the injury heals rapidly.

Chapter 3

MUNITIONS

Section I

PRINCIPLES OF DISPERSION OF BIOLOGICAL AGENTS

The success of biological warfare as a weapon against men and animals is ultimately related to the ability to disperse the agents in aerosol form. Cutaneous infection is rarely grave enough to justify its use in war. Ingestion of organisms can only be accomplished with present techniques by sabotage, and therefore has very limited usefulness.

With these limitations in mind, certain principles of dispersion with biological warfare agents may be established:

AGENTS. The agents to be dispersed may be living organisms in dry or slurry form, toxins from previously living organisms in dry or slurry form, or chemical compounds, such as the plant growth regulators and defoliants.

AEROSOLS. In the employment of agents as aerosols, all primary particulates are governed by two functions: aggregation and settling. It is convenient to classify aerosol particles relative to the degree to which they tend to adhere or aggregate and to the degree of rapidity with which they settle.

Class A (non-settling) - Particulates having diameters less than 5 microns.

(non-aggregating) - Concentrations estimated to be between 10,000 and 50,000 particles per cubic centimeter.

Class B (slow settling) - Particulates between 5 and 75 microns in diameter.

Class C (rapid settling) - Particulates, sprays and granular solids having diameters above 75 microns.

METHODS. Biological warfare agents may be effectively applied in clouds of finely divided particles, ground contamination, formation of dust clouds, contamination of the skin or personnel through abrasions or previously induced vesication and contamination of food and water supplied.

MEANS. Instruments which may be used to disperse biological agents include explosive munitions, the airplane spray tank, canister base-ejection bombs, impregnated small bore ammunition, darts, land mines, arthropod vectors, the free balloon principle, the gas expulsion principle, sabotage and carriers.

METEOROLOGY. The principles of meteorology applicable to chemical warfare are in general applicable to biological warfare. The ideal cloud laid down by a biological warfare munition must be low-lying and finely divided with regard to particle size. As the cloud approaches the consistency of a dust, it becomes a ground rather than air contaminant. Of all meteorological factors that of wind direction is most important for the efficient tactical use of the munition. The higher the ground temperature, the greater the tendency for the cloud to rise. Wind speed determines the rate of cloud travel and liability of the cloud to pillar or to dissipate.

RECOGNITION. Most munitions of biological warfare may be difficult to disguise due not only to radical departures in design but also to the unusual appearance of biological agent clouds and differences in sound and functioning of these munitions by comparison with the accepted munitions of war.

LIMITATIONS. Among the principal difficulties to be encountered in the munition dispersion of biological agents are contamination of handling personnel, storage difficulties due to corrosion and leakage, the difficulty of obtaining high dispersion efficiency due to the deleterious effects of explosives, and difficulty of obtaining a completely airborne cloud.

Section II

FACTORS IN CHOICE OF MUNITIONS

TYPE OF MUNITION. Close-range weapons probably cannot be used for the dissemination of biological warfare agents because of the possibility of infecting friendly troops. The bomb and the airplane spray tank are therefore the logical weapons of biological warfare. The possibility of contamination of the plane and subsequent infection of the crew and ground force indicate that spray tanks may be of doubtful value except for the dispersal of crop destroying agents.

Air contamination is the most effective means of employing biological agents on a large scale. Most agents that are pathogenic to men and animals are effective or can be made effective via the respiratory route. The majority of plant pathogens, as well as the plant chemical compounds, are also effectively applied by airborne techniques. Contamination of the air may be effected by the use of bombs, rockets, shell or gas expulsion missiles containing liquid or solid fillings which form finely divided particles on burst. Spray apparatus on aircraft or adapted for guided missiles may also be used to achieve air contamination. Air contamination eventually becomes surface or ground contamination due to the settling of the agent cloud. Some of the plant pathogens that infect through the soil, as well as direct soil contamination with chemical agents for crop destruction fall within this method.

SIZE OF MUNITION. The efficiency of a biological warfare bomb depends upon the size of the cloud produced at burst. Increasing the size of the bomb enormously does not increase the relative size of the cloud. On the contrary, the smaller the bomb the larger the burst-cloud, relative to the amount of agent filling. An efficient biological munition is therefore a small bomb that can be used in great numbers. High velocity propulsion of the agent is also necessary to the production of an aerosol with satisfactory particle size and concentration, and for that reason bombs made from shotgun shell and bombs that function on impact in much the same manner as a mortar shell are most successful.

PARTICLE SIZE. For contamination of the air when munitions are directed against men or animals, an aerosol or air suspension of liquid or solid particles must be created in which all particles should range from one to five microns and be of such concentration that rapid aggregation will not occur. This range of diameters is necessary in order to ensure extremely slow settling of the aerosol and afford particles which will traverse the nasal passages and will be retained in the lungs. For ground contamination, when employing either biological agents against man or plant chemicals, the particle size may be somewhat larger. Little agent may be required in the case of a sporulating organism, since after a week or ten days the spores have multiplied and are spread by the wind over the entire area.

EFFECTIVE CT. Ct represents the ideal dosage and is the product of the concentration of aerosol cloud and the time a given point is exposed to the cloud. It is expressed as milligram minutes per cubic meter. Ct requirements for biological agents range from as high as 0.5 to as low as 0.0001 milligram minutes per cubic meter of agent. Airburst bombs, sprays or dusts provide the most effective high concentrations. Sprays of crop agents require more or less uniform distribution at low concentrations.

PLACEMENT. When air contamination bombs are involved, a near miss is nearly as effective as a target hit. The anticipated travel of a cloud is at least several hundred yards and may be much greater. If self-propagating agents such as sporulators are used, uniform distribution is not necessary. A polka dot pattern with each dot representing an area of several square yards and each containing a high concentration of agent is completely effective. In achieving such pattern with bomb clusters, a cluster of 110 Mark I, Type F, bombs dropped from 11,000 feet and opened at 2,400 feet give an elliptical pattern on the ground with major and minor axes of 340 and 225 feet respectively. Dropped at 18,000 feet and opened at 3,000 feet, the pattern is 500 by 350 feet.

EFFECTS OF MUNITIONS. The greater amount of explosive used in a munition, the finer the dispersion. However, the greater the explosion produced, the greater is the destruction of agent by heat, pressure and toxic gases produced by the explosion.

Detonation. The effects of explosion on biological warfare agents is a critical factor in the development of munitions. It has been demonstrated that spore forms of organisms are less subject to the deleterious effect of munition explosion than yeast cells, which are the vegetative form of the organism.

Gases. The gases produced by an exploding munition are toxic to most microorganisms and the efficiency of bomb munitions is thus limited significantly by the use of nitro explosives. For example, explosion-produced gases have been found to destroy as much as 39 percent of a concentration of anthrax. EC powder, on the other hand, is much less toxic to anthrax organisms, the effect of its gases on the viability of the spores being almost negligible.

Heat. The heat generated by an exploding munition destroys some of the agent. The simulant agent Bacillus globigii will tolerate 100 degrees Centigrade for one second, but suffers 85 percent destruction at 200 degrees and is almost completely destroyed at 300 degrees Centigrade.

Brisance. The brisance of the detonating munition also contributes to the destruction of agent. The pressure shock effect of an exploding munition on vegetative cells is much greater than that on sporulated cells. A bomb exploded near containers of the simulant agent Serratis marcescens, a non-spore former, and Bacillus globigii, a spore former, has resulted in a loss of 47 percent of the vegetative cell material as against only 7 percent loss in the sporulated material.

Section III

PREPARATION OF CHARGES OR FILLINGS

CHOICE OF FILLING. Because of the hazards in filling and handling munitions, a wet, solid or slurry form of agent is preferred, rather than a dry powder or dust form. The plant, shellfish and bacterial toxins as well as viable agents present special problems in handling. Only the plant growth regulators are completely acceptable in powder or dust form. However, no tactical advantage is lost as a result of this limitation since a slurry used for ground contamination becomes a powder or dust upon drying.

PRESERVATION OF THE AGENT. Preservation of the viability and virulence of the organisms is the most important consideration in the preparation of munitions. A heavy aqueous suspension or slurry is ordinarily used in bursting bombs. A slurry is difficult to preserve over any long period of time and tends to become susceptible to heat and brisance as a result of its agency in the munition. A concentrate of the organisms as "mud" can be preserved as a rule for longer periods. It is made by mixing the organisms in a vehicle such as peat or charcoal.

A powder or dust form of agent is required in the case of microorganisms which are best preserved when subjected to lyophilization. The viable agents are first frozen and then reduced to a dry or dessicated form resulting in full retention of their virulence despite the unnatural state.

PARTICULATE SIZE. In order to prepare biological warfare agents as fillers for munitions, they must first be dried and then pulverized to a high degree of fineness. The mass mean diameter of the individual organism should be no greater than five microns, and in most instances must be considerably less than that. It is also necessary to pulverize peat moss to a similar degree of fineness, since it must act as the vehicle or carrier for the organisms when dispersed as an aerosol.

Ball-milling is the customary method of grinding material into exceeding fine particles, but this process when applied to dried organisms tends to destroy them. The heat generated in the process has been found to destroy between 30 and 50 percent of Bacillus globigii spores. Ball-milling also does not always assure production of the required particle size and is further unsatisfactory because of a high loss in viability. The use of a whirlwind mixer, however, which produces fine particles by grinding them against one another, has been found to pulverize simulated agent organisms into particles as small as 2.5 microns and produce peat moss particles smaller than 10 microns, all without loss of viability or virulence to the dried spores of Bacillus globigii.

LOADING. In the filling of biological munitions, loss of agent material greater than a few micrograms per day is considered highly hazardous. While handling a wet or moist filling requires great care, dry fillings are especially dangerous. As a result, a method has been developed for introducing the agent into a munition which has been tightly sealed prior to the injection of the agent. The agent compartment of the munition is sealed with a heavy rubber diaphragm. The agent is then introduced under vacuum from its supply container into the munition through a hollow needle which punctures the diaphragm. By agitating and mixing the dry material under vacuum with air, ready flow of the material into the munition is achieved. Possible exposure to contamination from the open end of a filled munition is entirely obviated by vacuum loadings.

INSPECTION. Inspection to detect leakage around the cap and burster adapter of the Mark I bomb is made possible by the addition of fluorescein to the liquid filling. Any leakage that occurs may be absorbed on filter paper. Even exceedingly small amounts of leakage may then be detected when examined by ultraviolet light. Since the addition of fluorescein might aid an enemy in the detection of biological munitions, fluorescein concentrations of 1;20,000 or less are used. These low concentrations cannot be detected in bomb craters or surrounding ground by ultraviolet lamp examination, yet are adequate for inspection and surveillance tests.

Section IV

EXPERIMENTAL MUNITIONS

Except in the case of the airplane spray tank, the standard devices used in chemical warfare which might be used to carry and disseminate biological warfare agents require extensive modifications. In addition to the adaptation of existing munitions for biological agents, it has also been necessary to develop several entirely new munitions, incorporating special features necessary to the preservation of the viable agent and to its efficient dissemination in the field. The munitions developed up to the present time remain in the experimental stage and indicate no more than the direction of design required by the instruments of this form of warfare.

BOMBS. SPD Mark I Type F Bomb. This munition is an adaptation of the British light case 4-pound chemical bomb, as an air contaminating bomb producing a Class A aerosol. It is designed to enable a string of individually dropped bombs to be exploded in rapid succession in the air, each bomb being set off by the blast of its predecessor. The total capacity of the bomb is 400 cubic centimeters, but the recommended filling is 320 cubic centimeters (0.725 pounds), or 17.6 percent of the total weight. The weight ratio of the pentolite explosive charge to filling is 1 to 3. A rubber diaphram is sealed in the tail end of the bomb to permit filling by the needle-injection process. The Mark I type F bomb permits clustering in a modified M10A1 adapter holding 110 of the bombs.

SPD Mark 2 Bomb. The SPD Mark 2 bomb has been specifically designed for the dissemination of the LN series of crop chemical agents in solid form. The bomb weighs 91 pounds empty and has a capacity of 192 pounds of agent. It is an air burst bomb, detonated by primacord and a nose fuze. It has a high pay load, functions satisfactorily and provides effective dispersion of granular agents such as VKA.

M74 Bomb. The 10-pound M74 chemical or incendiary bomb has been modified as a biological warfare munition for surface contamination with solid agents. To avoid the deleterious effect of explosion on living agents, this bomb ejects the agent-container by means of gas pressure. A compartment of 300 cubic centimeter capacity in the nose end of the bomb casing contains the agent. The seal of the compartment is punctured by a spring-operated striker pin. The released gas pushes the dry agent out past crossed vanes which produce the dispersed cloud. The agent compartment holds approximately 250 grams of agent. Two types of the modified M74 bomb have been developed and designated "GXS" for Gas Expulsion, Solid, and "GXL" for Gas Expulsion, Liquid.

M77 Bomb. A munition to disperse a chemical and biological warfare agent simultaneously has not been satisfactorily developed as yet. The problem is to secure a cloud of co-agent in close proximity to the agent cloud. Mixing the two agents is not feasible and for that reason separate bombs in the cluster are indicated. The 10-pound M77 HC smoke bomb with cadmium substituted for the zinc offers the most promise as a co-agent device, to be used in conjunction with the SPD Type F Mark I in a ratio of one M77 per cluster for each two Mk I bombs filled with agent slurry.

SS Bomb. The SS bomb is an adaptation of the ordinary shotgun shell. It is a primed 10-gage cement-coated shell casing into which is inserted a drawn aluminum vial 1-3/4 inches long containing the agent. The explosive consists of two grams of Bullseye powder. The entire bomb is about 6 inches in length and 1-1/2 inches in diameter. The SS bomb is adaptable to either liquid or dry filling and produces either a Class A or Class B aerosol. The bomb may therefore be used for dispersing both antipersonnel and antivegetative agents. It is estimated that one cubic centimeter of liquid filling in the SS bomb will furnish 250 billion (2.5×10^{10}) particles each 2 microns in diameter. There is no crater loss as is associated to most other biological warfare munitions. It is possible to achieve production of dilute clouds over a wide area with this bomb, as well as a concentrated cloud in a small area. It may be possible to cluster the bomb in lots of from 500 to 1,000 per cluster.

AIRPLANE SPRAY TANKS. The airplane spray tank suggests itself as the most practical device for the proper dispersal of liquid chemical agents for crop destruction and defoliation. The spray tank ordinarily is best adapted to lightweight short range bombers or fighters, although heavy bombers, capable of extended flight and high altitude appear most practicable for attack on crops. The M10 tank is well suited for the distribution of crop chemical agents. At 75 to 100 feet altitude and at 220 miles per hour, a strip 40 to 200 yards wide and 600 yards long can be sprayed with a single tankload. On the other hand, the M33 tank has too great a discharge rate without extensive modifications and is therefore limited in usefulness. An experimental 250-gallon tank, the E2, for the bomb bay of the B25 plane is well suited for crop destruction. At 100 feet altitude and 220 miles per hour, a strip 6,500 yards long and 50 to 80 yards wide can be covered. A second experimental tank, with a total of 550 gallons capacity has also proved an effective dispersing device, spraying an area 16,000 yards in length.

MISCELLANEOUS MUNITIONS. Among the miscellaneous munitions which have been investigated for the dissemination of biological agents has been an effervescent gas bomb which has been tested for the production of biological agent aerosol. Suggestions have been made to impregnate small bore ammunition, leaflets and paper money with microorganisms. Darts coated with botulin toxin, and anthrax-inoculated linseed meal biscuits may be the type of unorthodox weapons expected in biological warfare. It is conjectured that agent-impregnated shrapnel which caused slight wounds would prove fatal within a few hours. The development of a low melting wax film impregnated with sporulating agents might prove valuable for sabotage purposes. Perhaps most practicable and feasible for sabotage purposes would be small glass vials containing micronized agents.

Guided missiles such as rockets and robot planes may be valuable for placing high dosages of agent in difficult and inaccessible places. Among non-explosive munitions which may be considered for biological warfare adaptation are smoke pots and smoke generators. Land mines would be effective for the erection of barriers. Mortar and artillery shell might be used where high accuracy was required, such as placement on a road or bridge.

Section V

POSSIBLE MUNITIONS FOR BIOLOGICAL WARFARE AGENTS

Based on preliminary tests in this country and abroad, it is possible to give some indication of the probable effective munitions which may be used to disperse certain biological warfare agents.

ANTHRAX. Actual dissemination of anthrax has not been accomplished in the United States, although the British have repeatedly tested it in England and Canada. Anthrax simulants have been tested in this country in various munitions, but anthrax itself has only been used for the purpose of testing the longevity of the spores as ground contaminants. To disperse the simulant, a mud form is processed into a dry powder and the material then used as a filling in the dry state or suspended in distilled water in the desired concentration. The simulant has performed satisfactorily in the M47, M70 and M74 bombs, in the British Mark I 4-pound chemical bomb, in shotgun shell bombs and gas expulsion bombs. The resulting clouds formed upon static explosion of the bombs indicate these munitions are efficient, the best being the M74, the British Mark I and shotgun shell. A lacquer coating for the bomb interiors has been found least injurious to the organisms. The most satisfactory explosive charges have been pentalite, tetryl or Bulls eye powder, at a ratio to the fill of 1:3.

BOTULISM. Dissemination of the botulinus toxin has been considered from the point of view of its use as a liquid for ground contamination and as a dry powder for infection by the respiratory route. It has been learned that an acid mud slurry makes an effective munition filling. It may be dispersed as an aerosol both by spray tank and explosive munitions, or may be effectively employed on small arms missiles as a contaminating coating.

BRUCELLOSIS. Field trials of brucellosis in Canada indicate that two types of bombs, the SPD Type F Mark I and the SS bomb are most effective. Bomb fillings consist of the agent in slurry, stabilized with dextrin. The clouds obtained are remarkably stable and effective doses can be obtained. Ineffective concentration have been maintained throughout the entire width of the cloud for 100 yards downwind. It has been definitely established that brucellosis can produce infection under the conditions of dispersion in the field. Brucellosis is also effective as an airplane dust spray, in spite of the fact that it is a non-sporulating vegetative organism, which ordinarily will not survive dispersion into the air.

TULAREMIA. The organisms of tularemia must be dispersed as a wet material. Drying of the agent is prevented by the use of glycerol. It is extremely effective when dispersed as nebulized clouds via bombs and shotgun shell.

GLANDERS AND MELIOIDOSIS. The ability of the organism to survive with full virulence in a number of media including ordinary tap water makes this one of the simplest agents to disperse in present munitions. Good results have been obtained when the agent is dispersed as an aerosol either by bomb or shotgun shell.

PLANT DISEASES. Fungus disease organisms of food and industrial crops can be disseminated for the establishment of primary infection either as sprays, dusts, infested substrate, in explosive munitions and by arthropod vectors.

Spray. For agents which are to be used in the form of spores, use of the airplane spray tank is particularly adaptable. Sticking and spreading substances may be incorporated in spray suspensions to augment the amount and uniformity of the infection.

Dust. Certain agents which retain their infectivity in the drying process, as those of brown spot of rice and rice blast, are adaptable to dust application via airplane dusting devices. The advantage of dust is its ease of transportation to the area of operations and the high efficiency of agent as a pay load. However, dry particles do not adhere to dry plant surfaces as well as spray droplets and they are more likely to be removed by rain.

Infested substrate. The agents of late blight and Southern blight are best distributed from the air as a substrate infested with the viable agent. The substrates may be bean seed or wheat seed, which supply a reservoir of nutrient to the agent to aid in initiation of growth in the new environment. The plants being attacked may be indirectly inoculated or the soil may be effectively contaminated by this method. The disadvantage is the added bulk of the inoculum in the munition.

Bomb. The SS bomb is an effective munition for the dissemination of brown spot of rice. The low-hanging cloud formed on explosion of the bomb results in a high concentration of agent, without damage to the spores or mycelial particles.

Vectors. Arthropod vectors such as the insect borers may be useful in establishing a primary infection as well as in developing a secondary infection. It may even be possible to disseminate specific crop agents indirectly by means of contaminated vectors.

PLANT REGULATORS AND DEFOLIATORS. Effective dissemination of the LN series of plant regulating agents is possible when the agents are made up either as liquid or solid. Both standard and experimental airplane spray tanks are available for dispersion of the liquid agent. Oil sprays involving oil-miscible solvents are the most effective forms for dispersion. The solid or granular agent may be used effectively in the SPD Mark 2 bomb. The chemical defoliants, like the liquid LN compounds are dispersed via spray tanks.

Chapter 4

TACTICS AND TECHNIQUES

Section I

GENERAL

OBJECTIVES OF BIOLOGICAL WARFARE. Offensive biological warfare. The primary object of offensive biological warfare is to employ viable agents or their products or chemical plant regulators and defoliants in such a way as to induce disease in large numbers of men, animals or plants, in order to lower by disease the vitality of the enemy's potential manpower or food reserves, to divert and immobilize his medical and industrial forces, and to break his morale so as to result in the deficiency of the supply forces of his combat troops.

Defensive biological warfare. The primary object of defensive biological warfare is to prevent the occurrence of artificially induced disease, or if such prevention fails, to limit the direct effects and the spread of such disease.

SIGNIFICANCE OF BIOLOGICAL WARFARE. The employment of microorganisms in warfare is a potentially important weapon because certain of the organisms produce disease in man, domestic animals and crop plants. They may multiply astronomically in a relatively short time in favorable environments. Unless subjected to drastic treatment, many of the organisms remain alive for long periods of time and may be difficult to eliminate once an area is heavily contaminated.

Biological warfare comprises not one weapon but a large and varied array of weapons of diverse overall effect both in kind and degree. It may be used to provoke effects ranging from a mere local nuisance to devastating epidemics equal to, if not surpassing, those natural outbreaks which in the past have many times decimated armies and nations. Biological agents may be directed against food and industrial crops and against animals of economic importance, principally for long range strategic effect. When directed against man, the objective may be to induce casualties in large numbers, either through rapid killing or through incapacitation. In some instances there may be the promise of eventual recovery; in others convalescence may be indefinitely prolonged. It may be intended to provoke an outbreak of disease that will remain limited to the initial casualties or, at the opposite extreme, to start a major epidemic. The tactical objective may require the development of only a few cases of a disease with a high demoralizing factor. In this connection, it is possible to launch a biological attack the nature of which may be apparent or disguised, well marked in onset and clear in its characteristics, or insidious and obscure, with calculated psychological effects in view.

Where atomic warfare is utterly destructive, biological warfare is selective for living things, and in most instances for a very narrow class of living things. Like gas warfare, it is concerned with the man destruction of personnel, not materiel. It does not destroy structures or property, but it may be used to destroy the effectiveness of structures through the destruction of their operating personnel. It is adaptable to strategic operations which often precede formal declarations of war, as well as those involved in declared war. It has a potentiality of surprise not shared by other forms of warfare except chemical warfare. Its agents are highly concentrated in munitions, yet they are effective in extraordinarily low concentrations. Biological warfare munitions may therefore be very compact.

CRITERIA FOR TACTICAL AGENTS. The qualities which will determine whether a biological or chemical agent possesses tactical value in biological warfare are:

Pathogenicity or toxicity

Specificity of action or reaction

Rapidity of action

Detectability

Extent of hazard to using personnel

Persistency

Degree of protection possible for using troops

Communicability of the agent or its products

Stability of the organism in manufacture, storage and in its munition

Efficiency of the method of dissemination

Section II

FACTORS IN THE TACTICAL EMPLOYMENT OF BIOLOGICAL WARFARE

FEASIBILITY. The general principle that biological agents can be disseminated with significant military results can no longer be held seriously in question. Virulent strains of many pathogenic organisms can be grown in large quantities and effectively dispersed. Although the use of biological weapons against economically important animals and the whole area of artificially induced human disease has of necessity been largely evaluated by inference, evidence is available of the effectiveness of certain agents directed against plants, and limited experience with promising results has been gained from trials against domestic and laboratory animals.

Recent developments which have tended to make offensive and defensive biological warfare feasible are: —

The possibility of developing new diseases by the "faith passage" technique; that is, by the adaptation to man of virulent organisms at present highly pathogenic only to some other species than man. A reverse example has already been demonstrated: yellow fever, after a sufficient number of passages, has been adapted as a new and virulent disease of mice.

The possibility of developing drug-resistant strains of known bacteria which are generally assumed to be controllable by that drug.

The development of the chick embryo technique for the large-scale production of effective virus agents which have hitherto been unobtainable by artificial means. This technique also makes it possible to mass produce antivirus vaccines.

The development of lyophilized bacteria, permitting the easy and probably effective distribution of new airborne diseases. Lyophilization of bacteria is a process by which virulent organisms are first frozen and then dried, in order to retain the virulence otherwise lost when they are simply dried.

Advances in the preparation of synthetic media for the mass culturing of infectious materials.

The development of a rationale which may permit the systematic development of a number of new chemotherapeutic agents.

The development of new masks capable of stopping particulate materials.

The development of techniques for air disinfection by the use of ultra-violet radiation and germicidal sprays or aerosols.

SELECTIVITY. The selection of proper agents and provision of protection to our own troops permits the use of biological agents to weaken enemy forces and make them susceptible to attack by conventional means. Biological agents thus employed would succeed in diverting the strength of opposing forces to the medical care of their casualties. Biological warfare may also be used simply to harass an opponent, forcing his troops into masks and clothing, or to remain in circumscribed areas which have been rendered proof against biological agents.

SURPRISE. As with gas warfare, the element of surprise is the most important consideration in both the tactic and strategic use of biological warfare. The effectiveness of a new organism will depend upon the state of preparation by the enemy. If he is unprepared, the agent can be used decisively. It should be used in massive doses at an unexpected time. It is improbable that an enemy would be likely to run the risk of trying a series of new agents in small doses, for an enemy daring biological warfare could not afford to permit retaliation.

INFECTIVITY. The period of disability to an individual following exposure to a pathogenic agent may last from six months to many years, and possibly a lifetime. Mortality would depend on the degree of exposure, the agent used, the speed of diagnosis and availability of therapy. The psychological effects of biological warfare as a weapon of terror would probably be enormous. Employed against animals, biological warfare would destroy or incapacitate animals of burden. It would affect the food supplies of cattle. It would spread disease to man by way of the animals. Against plants, biological warfare would ensure the destruction of all vital food crops, contamination of soil for short or long periods of time, and in certain instances might act to upset seriously the biological balance of nature.

RETROACTIVITY. Biological warfare, like atomic warfare and some forms of chemical warfare, is characterized by persistency of effect. The persistent effects of biological warfare may vary from those which are very brief and easily controlled to effects which are extreme and possibly unmanageable. While biological warfare will generally have no effect on inanimate materials and structures, persistent contamination may result from the use of certain bacterial and fungus spores and tick-borne agents which may make property more or less permanently unapproachable and therefore, unless it can be decontaminated, require its destruction.

The use of infectious biological agents in tactical situations of fluid movement is probably impractical. Infections once established among the enemy would spread from man to man and finally even to those using the agent or agents. There might well be lasting postwar effects as a result of ground contamination and the establishment of rodent and insect reservoirs of disease.

DETECTION. There is no simple way to detect the presence of microorganisms. Their minute size makes possible their presence without detection, and facilitates their spread by wind, dust or contact.

PROTECTION. Protection against biological agents would probably be impossible under conditions where large numbers of individuals are present in a relatively small area. A few agents put down in high explosives can thus create havoc unless or until troops are rendered immune. The lag incubation period of pathogenic organisms will ensure massive infections before the first symptoms appear, revealing the nature of the hazard.

Section III

ARTIFICIALITY IN BIOLOGICAL WARFARE

ARTIFICIAL ROUTES OF INFECTION. As a means of confusing an enemy, unnatural portals of entry may be used to introduce an agent into a host. Airborne infection with brucellosis, tularemia, glanders or yellow fever, for example, would not be expected since in nature these diseases are caused by ingestion or through an insect vector. In crop destruction, artificiality would mean the introduction of greater amounts of inoculum than is met with in nature or the introduction of foreign plant disease strains.

NEW AGENTS AND ARTIFICIAL VARIANTS. Problems of detection and treatment in biological warfare may be complicated by the use of agents of newly discovered or little known diseases, such as melioidosis, agents developed for extravagant infection or distinct pathogenicity, agents developed to be immunologically specific so that existing vaccines would be of little or no use against them, or agents developed to be drug-fast so that specific chemotherapeutics against the normal strain of the disease would be ineffective.

In addition to the development of new strains of disease organisms, newly identified diseases in nature may be employed. An example is the recent identification of a disease which causes a high fever for several days, followed by a convalescence period of two weeks. The disease has been called rickettsialpox because its microorganisms are carried by mice and transmitted, like any rickettsial disease, to mites which feed upon them. The mites carry the organisms from mice to human beings. In biological warfare such a new disease of low virulence and pathogenicity in its original manifestation may be developed to new heights of virulence and given the status of an exotic infection.

MIXTURE OF AGENTS. Mixing biological agents is usually more successful than similar attempts to mix chemical agents. The effects of one disease can be superimposed on another, and the use of two disease agents can produce results more violent than those of either agent alone. The common respiratory pathogens such as the staphylococci, hemolytic streptococci and pneumococci, when acting with one another or with influenza, makes them much more effective. Similar results may be obtained in plant pathology.

The appearance of two or more infectious diseases, both having approximately the same incubation period, in a large group of individuals would be expected to alter the clinical picture, prognosis, laboratory diagnosis, treatment and other aspects of the diseases involved. The use of several agents with different incubation periods timed so as to act consecutively might increase the morbidity and mortality. Thus brucellosis coming on during the convalescent stages of tularemia might carry a higher mortality than at present. Non-pathogenic agents might be mixed with pathogens to further complicate the laboratory diagnosis.

CO-AGENTS. The combination of a bacterial agent and a toxin may produce effects greater than single agents or produce new and unusual symptoms which would result in confused diagnoses, so that rapid recognition and proper counteraction would be difficult.

For example, the co-existence of typhoid fever and botulism might well lead to delay in diagnosis and treatment, by its mimickry of an encephalitic syndrome. Biological agents might well be used in association with chemical agents because of the synergistic effects of chemicals for increasing the virulence of biological organisms, injuring the body tissues whose normal function is protection against bacterial invasion or lowering the host resistance to bacterial infection. For example, the metallic agent cadmium, either as an oxide, chloride or chromate, is effective in producing the membranous abrasion or irritation required to permit introduction of certain pathogenic organisms into the body. Co-agents may also be found among non-toxic chemical agents, either of biological or non-biological origin, such as spreading factors like hyaluronidase, or allergens, or active compounds such as vasomotor depressents and stimulants or astringents.

ARTHROPODS AS AGENTS. In addition to the use of bacteria, viruses, fungi and bacterial and other toxins as biological agents, it is possible to employ certain of the arthropods which include the insects, arachnids and myriapods, as vectors of disease organisms. The arthropods may be considered as primary or intrinsic agents, as in the case of the corn borer which itself is the cause of crop destruction, or as secondary or vector agents, as in the case of the infected tick, flea or louse.

There are certain disadvantages to the use of arthropods against man and animals. The arthropod agent is large enough to be seen and can therefore be easily combatted. It does not possess the insidious nature and quality of surprise inherent in most biological agents. It is susceptible to control measures which are effective and easily applied. The production of house flies and fruit flies is relatively easy and the use of these arthropod vectors as transmitters of disease to man appears to be practical. The use of borers and locusts against food crops and natural vegetation is entirely feasible. There are, however, fungal, viral and chemical agents which are equally effective and more easily produced. These are also more easily disseminated and less susceptible to control measures.

Section IV

STRATEGIC AND TACTICAL EMPLOYMENT OF BIOLOGICAL WARFARE

STRATEGIC APPLICATIONS. Pre-War Sabotage. Successive crop failures may be established by systemic sabotage. This may be possible even by a nonresident saboteur by taking advantage of the fact that nature transmits rusts, smuts and fungi in high-level winds and natural clouds.

Wartime Attack. Biological attack may be used to interrupt production of war plants by bombing urban populations with long-range rockets, guided missiles and bombs. With biological agents, machinery and equipment would not be destroyed but would be available to attacking forces after decontamination.

Disorganization or incapacitation of industrial areas deep in enemy territory may be readily accomplished. Such areas would be almost as suitable biological warfare targets as isolated strongholds. The concentration of individuals indoors would lend itself to the use of epidemic agents. Botulinus toxin in reservoirs feeding these centers would also probably be effective.

Ground contamination of war plant areas would prevent repair to any plant or factory initially damaged by explosives or incendiaries. Ground contamination might also make it difficult for the enemy to destroy an intact plant that had to be abandoned following biological attack.

Transportation such as subway systems could easily be sabotaged, as would any construction with great centralized ventilation systems.

Demoralization as a strategic end would be realized as hysteria and panic resulted in chaos in great urban centers.

Camps and training centers are good strategic targets of biological warfare. The density of population would likely be lower, indoor environment less prominent and climatic conditions more varied than in an industrial area. However, airborne agents of high infectivity might be used against the barracks at night. Dengue fever would probably be an effective agent.

Crop destruction and destruction of domestic animal and fowl populations would render an extended war improbable. Such attacks would produce results similar to blockade. Crops would be selected for destruction on the basis of information on normal production and consumption. Interchangeability of basic food items and stockpiling of food items would have to be considered, as would the delay between production and consumption which, except for truck crops and perishable fruits, would be several months. Since food rationing and distribution plans in wartime are based on conservative yield estimates, destruction would have to reduce the total yield well below the level of normal yield. Nations not normally self-sufficient would be most vulnerable as food reserves became depleted.

Secondary results of biological attack on food crops and animals would be the wastage of labor in intensifying defensive measures where these were possible, and in clearing and replanting crops after attack or in restocking depleted herds. Enormous burdens would be placed on transportation to equalize the distribution of food. Starvation of cities and impairment of civilian morale through fear of famine would be concomitant results of such attack.

Biological attack on crops with industrial uses, with wastage of labor as well as impairment to production activities, would tend to make a prolonged war difficult or impossible.

The use of defoliating agents to destroy vegetation cover used for large-area camouflage would serve to mark out bomb targets. Fires would be relatively easy to start in areas where defoliating agents had been used.

TACTICAL APPLICATIONS. Military Tactics. Like chemical warfare, biological warfare is not suitable probably in close contact fighting or in situations of rapidly changing positions. A possible exception might be the use of a virus organism where attacking troops were picked men who had recovered from the infection, with conferred immunity, or had been actively immunized against the infection.

Biological agents projected by rocket, bomb or spray may be used to set up a barrier. Beachheads may be covered by both air and ground contaminations. Roads, wooded areas and open country would probably be effectively blocked by ground contamination. Barriers thus established may be used to isolate troops for flank maneuvers or to prevent large scale invasions. Biological agents are not armor piercing, but they are armor penetrating, and though a combat force might move against a biological barrier, the contamination picked up by vehicles or stirred up as dust would soon cause the death of all operating personnel. Such a barrier could be quickly set up in front of a moving army by rocket barrage. Land mines could be used if more time were available. Such contamination would be substantially invisible, exceedingly difficult to remove under combat conditions, and deadly to any size unit that moved into the field.

For the reduction of isolated strongholds such as islands, naval bases or air bases, biological warfare possesses the most notable advantages and is most free from tactical disadvantages. Any agent suited to the climatic and terrain circumstances could be used, including the agents of greatest potential retroactivity such as plague, influenza and malaria.

The seige of cities or other static warfare offers effective employment of biological agents. Where the object of operations is to bypass rather than seize the city, any of a number of biological agents would be useful. Depending upon favorable climatic conditions such agents would include psittcosis, yellow fever, botulism, brucellosis, tularemia and the insect-borne diseases.

A strategic retreat or scorched earth policy lends itself to biological warfare. Persistent agents with little tendency to spread and not likely to be retroactive should enemy after passing the infective area make contact with opposing forces would be most effective as part of a scorched earth policy. Agents such as brucellosis, tularemia, tick-borne infections and water contaminants would probably be most effective.

Biological attack may be made on subsistence crops of isolated garrisons. Since such crops are irregularly distributed in small patches, no broad destructive attack could be made. Repeated application at intervals or heavy ground contamination might completely deny the use of land to the enemy.

Clearing terrain by extensive defoliation would not only aid visibility but also remove undergrowth so that ground fortifications become readily detectable. Natural camouflage and concealment afforded the enemy may thus be removed if foliage is reduced or destroyed by chemical sprays or spray and burning. Present defoliants are effective only against deciduous or broadleaved trees. They do not cause the shedding of palms or conifers.

Terrain may be marked by the use of defoliants. Changes in color or appearance of jungle or heavily wooded areas would aid aircraft missions, providing a bomb line, safety line or rendezvous point for aircraft. The change of color which precedes defoliation may also be used for the disclosure of camouflaged positions.

Naval Tactics. A curtain of biological aerosols set up in front of a warship or merchant convoy would probably be intercepted by the fleet before the ventilating systems could be closed. A single bomb with half an ounce of agent, were it to land on deck, would probably be sufficient to subject 50 percent risk of death for all personnel. Once the ventilating system of a ship was contaminated, the hazard would remain until elaborate decontamination measures were taken.

Biological agents are a greater threat to navies and to the merchant marine than all other instruments of destruction. The crowded conditions aboard ships favor the spread of disease and casualties would soon tax isolation facilities possible on a ship. The immunization of personnel against all agents would be impossible.

Section V

DEFENSIVE AND OFFENSIVE EMPLOYMENT OF BIOLOGICAL WARFARE

DEFENSIVE EMPLOYMENT OF BIOLOGICAL AGENTS. Against invasion. Biological agents might be used against rear area installations of enemy forces mobilized for invasion. The confusion and the production of casualties would be almost certain following spray and bomb attacks on concentrations of troops. The disadvantages of such employment of biological agents would be the difficulty of ascertaining the degree of effectiveness of the agents, of timing such an attack properly, and the hazard in revealing that biological agents were being employed.

Across bodies of water. Agents could be disseminated by the use of spray, bombs, submarines or floats against enemy forces on another shore. Such defensive employment would serve to pocket the enemy force and would not be likely to reveal the source of the attack. In addition, the separation of forces by a body of water would ensure protection of the defending forces. Disadvantages of such tactics would be the difficulty of ensuring effective dispersion, the impossibility of determining favorable meteorological factors, and the possibility that enemy forces, apprised of the attack, would be free to move rapidly and evade it.

Against landing operations. Bombs, mines, infected small arms, sprays or insect vectors might be used against landing operations of an enemy. The advantages would be the full control over the agents permitted the defending forces and the ample preparations that such use of biological warfare affords. Disadvantages would be the likelihood of wind shifts and the necessity of determining possible sites of landing operations.

Against forces already landed. Bombs and sprays would probably be most effective and they could only be used against enemy rear areas. The disadvantage of such defensive tactics would lie in the possible hazards of using terrain thus contaminated when it was recaptured.

OFFENSIVE EMPLOYMENT OF BIOLOGICAL AGENTS. Preparatory attack. It is possible that experimental attacks of a deceptive nature might be made with biological agents, to test the enemy's defenses against such attack as well as to effect a tactical or strategic advantage. An experimental attack of this nature might be made by the release of one biological charge by plane during the course of release of great numbers of conventional types of explosives or incendiary bombs. The single biological charge might well pass undetected for considerable periods of time and in this manner the desired increase in density of distribution of a disease agent in the environs of the target might be built up.

Against inland troops. Bombs, sprays, darts, rockets might be used against concentrations of reserve troops. Defoliators and plant pathogens would be used to destroy food crops of the enemy.

Against beachheads. Biological agents would probably not be used against beachheads whose occupation was planned or anticipated.

Against cities. The use of biologic agents against cities would be the signal for a war of extermination. The psychological toll of terror obtained by the use of biological warfare against great civilian populations would undoubtedly be great. Epidemic diseases would create great havoc and in a short time all industry would be retarded or even stopped entirely.

Against islands. Where it was planned to occupy an island, nonpersistent biological agents would be employed, with careful timing of the bombs, sprays or projectiles employed against the island. Where the island was to be neutralized, with no plan of occupation intended, either persistent or nonpersistent agents would be used in saturation concentrations.

Section VI

TACTICAL ASPECTS OF SPECIFIC AGENTS OF DISEASE

ANTHRAX. The world-wide prevalence of anthrax would make its detection difficult. If the use of anthrax was threatened or anticipated, the clothing necessary for adequate protection against it would render combat operations difficult or impossible. The area where anthrax was used would be permanently contaminated. Animal vectors in which the bacillus is parasitic include cattle and sheep.

BOTULISM. As an aerosol produced by spray or munitions, botulinus toxin may be absorbed through wounds, abrasions or blisters, through the respiratory tract by inhalation and through the conjunctivae of the eyes. Botulin toxin would be effective and deadly as a sabotage agent of food and water supplies, and may be used to contaminate dust. Infection is not dependent upon gradual production and absorption of the toxic. Large amounts of the toxin might be ingested at one time or there may be exposure to high concentrations of the aerosol, and even immune defenses would probably be overwhelmed. The botulin toxin is one of the few biological agents that can be used to contaminate missiles.

BRUCELLOSIS. Because the period of incubation, which varies from ten to sixty days, depends upon the resistance of the individual at the time of exposure, use of this agent is indicated for an area intended as a theater of future operations. Brucellosis would probably be effective against bodies of troops holding ground in a static situation, where plans were made to take that ground a month or two months after the biological attack. More immediate disease reactions could be produced by the use of a co-agent to lower individual resistance. The agent is very effective in the field when put up as an air-borne cloud and may be expected to produce in man a disease that, while not fatal, would be extremely incapacitating. It is impossible to detect the organism in the air or in clouds. The agent would be effective for sabotage. Ordinary methods of examination of water or milk would fail entirely to detect its presence. Animal vectors of brucellosis include milk cows, mares, sheep, rabbits, guinea pigs, goats and swine which may be infected with the disease to reduce food productivity or be used to infect human population.

TULAREMIA. In almost all respects this agent meets the requirements of an ideal biological warfare agent. Although tularemia is of minor importance as a cause of death, it has considerable importance in areas of high endemicity as a cause of prolonged morbidity and disability. Its capacity to disrupt the normal activities of a military encampment would probably be high. The infection is acquired with extreme ease. However, recovery from the attack confers life-long immunity which would protect against subsequent massive exposures with great effectiveness. It could only be used once against a local population. The habitat of tularemia is in ground squirrels, rabbits, hares and other rodents, and these may become vectors of the disease in man.

GLANDERS AND MELIOIDOSIS. Introduction of either glanders or melioidosis into enemy territory would probably produce disastrous results. Melioidosis, which is more acute and more rapidly fatal than glanders, would be an effective weapon of terror. Since the organisms do not produce spores, they are easily destroyed and can be handled with a certain degree of safety by forces using them. Infection and recovery does not confer immunity against a second attack. Rat populations would serve to propagate the disease, for the fleas of infected rats transmit the disease. It is obvious from a biological warfare standpoint that if either the organisms of glanders or melioidosis were introduced into a water supply, devastating results would be likely to occur. If that water supply contained a small amount of nutrient matter such as could be expected from vegetative decay, the agents would not only survive for a long period of time but might actually increase in numbers. Glanders is normally found in horses, sheep and goats; melioidosis inhabits wild rats, guinea pigs and rabbits.

COCCIDIOIDAL GRANULOMA. As one of the few fungi producing serious disease in man, this would probably be an unsuspected agent of war. It is seldom fatal but frequently becomes chronic and as such may remain localized for years.

PSITTACOSIS. Certain of the strains of the hardy virus producing this disease cause extremely severe illness with high fatality rates. Minimal infecting dosages are small due to the astronomical multiplication of the virus, and once established the disease is likely to be self-propagating due to its vectors. Pigeons are an important carrier of psittacosis, as are canaries, finches and all the parrots.

JAPANESE TYPE B ENCEPHALITIS. This virus infection of the central nervous system is severely disabling. There is no known method of detecting the disease and no treatment. Aerosol dissemination is feasible and the rate of epidemicity is exceedingly high. Infected mosquitoes can transmit the infection.

TYPHOID FEVER. Since all troops are vaccinated against typhoid, it would probably be of limited use against military personnel. It would be devastating against unprotected civilian populations. Water and food are the principal sources of the disease and for that reason it is an effective sabotage agent against disrupted civic centers. The fly is the most common vector. Once established, it is a self-perpetuating disease.

BACILLARY DYSENTERY. It is a common disease, profoundly debilitating but seldom fatal. Because of its prevalence it would not be likely to be recognized as deliberately established. Rats, monkeys and dogs can contract the disease and become carriers, but flies, food and water are the usual foci of infection. Since there is no inoculation for it, as in the case of typhoid, it would be equally effective against military and civilian populations. It would be effective in disrupting military operations and lowering the morale of troops.

TYPHUS FEVER. Conditions favoring the spread of a flea-borne or louse-borne typhus fever are in direct proportion to the disorganization of civilian life and consequent economic distress, the breakdown of sanitary administration, influx of refugees and famine. In armies, the movement of masses of troops under conditions of active service, involving fatigue, exposure, anxiety, all of which lower resistance, favor spread of typhus fever when epidemic or endemic regions of the disease are entered. Used as an aerosol agent, typhus fever would break out initially only in proportion to the degree of dissemination of the agent. Thereafter spread of the disease would occur only as favorable conditions prevailed.

PLAGUE. Pneumonic plague might be one of the few effectiv microorganisms against troops in cold climates. It requir animal intermediates for epidemic spread and once estab control would be most difficult. Because it is the of the plagues, great numbers of casualties might tained before such measures as vaccination could be taken to prevent decimation of forces.

CHOLERA. Essentially a disease dependent upon conditions of filth for widespread propagation, cholera would be difficult to establish. As a sabotage agent in contaminated food, milk or water, it would be a weapon of terror, incubating in the human body in as little as two days and resulting in death in as few as twelve hours after onset.

RINDERPEST. In a country where the disease was non-existent, cattle plague might be introduced with certain destruction to all animal herds exposed to it. Even though vaccinated against the virus, animals can still spread the disease to uninfected or non-vaccinated animals. The disease would be used to destroy live-stock; it is not found in man.

NEWCASTLE DISEASE AND FOWL PLAGUE. The susceptibility of chickens, guinea fowl and turkeys to these highly infectious fowl diseases would serve to wipe out entire flocks wherever the agents were introduced. Wide-spread destruction might be achieved by sabotage methods before vaccines could be distributed to stop the plagues.

PLANT PATHOGENS. In the tactical employment of plant pathogens, favorable meteorological conditions are the chief factor for the establishment of infection. If the weather is propitious, defensive measures against crop plant attack, however extreme, are likely to be ineffectual. Some agents by infecting the soil can maintain themselves over long periods of time. This would be localized contamination. Agents which attach the aerial parts of plants, however, have enhanced potentialities for spread, for these usually sporulate more or less profusely and the spores are windborne. Aircraft dissemination is the simplest means of contaminating crops with biological agents. The wastage involved is within tolerable limits. Soil-infesting agents require very low concentrations of agent per acre. Spore infestations on the other hand would require greater concentrations, but on fewer targets since the potential spreading characteristics of such agents ensure successive foci of infection.

CHEMICAL PLANT GROWTH REGULATORS. The choice of agent depends on whether cereal or broadleaf crops are to be destroyed. If broadleaf crops, it is necessary to determine whether Irish potatoes constitute a high percentage of the acreage or not, for most of the LN compounds have varying degrees of specificity. Aerial observation and general knowledge of agricultural practice in the area of attack must be known in order to apply agents at the most susceptible stage of growth. In order to produce the desired inhibitory effect, an agent must be disseminated so that all plants receive at least a drop of agent. Crosswind release of agent is most satisfactory; for lateral dispersion, steady winds from 5 to 10 miles per hour are optimum. Early morning or evening are the best hours for dissemination. Bright sunlight tends to speed up the action of most agents. By varying the ratio of chemical to solution, crops may be severely injured, killed outright, or the soil may be thoroughly contaminated for several seasons or more.

CHEMICAL DEFOLIANTS. The meteorological principles applicable to the dissemination of chemical warfare agents by spray methods are equally effective in defoliation operations. The degree of rapidity and intensity of color change and defoliation desired will determine the selection of agent for the particular operation.

Chapter 5

PROTECTION

Section I

PRINCIPLES OF PROTECTION

The methods for physical protection against the agents of biological warfare are similar in many respects to those against chemical warfare agents, and are based on the development of protective masks and clothing. The difference in protection is quantitative rathern than qualitative, for the agents of chemical warfare are almost elephantine by comparison with biological agents. A minimum lethal dose of phosgene or mustard if os considerable magnitude in comparison with an organism one micron or less in diameter, which may be equally lethal. The peripheral leakage tolerate in the gas mask, therefore, cannot be permitted in a biological mask. For this reason, the gas mask designed to keep out toxic gases or smokes must be adapted for one million or more times greater efficiency in order to be of use in biological warfare. Similarly, protective clothing which is almost completely effective against mustard gas is, due to its porous weave, highly vulnerable to penetration by most bacterial organisms.

In addition to physical means of portection, there are biological means of preventing casualties due to biological agents. These include the use of vaccines, toxoids and immune sera and correspond in general to the few prophylactic measures available in chemical warfare.

Absolute protection from biological warfare agents for a large number of personnel for a period of time is not considered feasible. The group protection devised for chemical warfare, such as the posting of sentries and operation of gasproof shelters and collective protectors, is believed impractical at the present time.

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...re in conjunction with the simultaneous operation in

...of a decontaminating spray. For the present, however, sani... asures, the application of public health principles and immunization are the known means of group protection.

The protection of the individual against any infectious disease is based on three factors concerning microorganisms: the virulence of the agent, the number of organisms and the resistance of the individual or host. In biological warfare, no control would be possible over the factor of virulence. The factor of resistance of the host is largely based on immunization and the maintenance of discipline concerning environmental sanitation. Physical protection is based upon reducing the number of organisms to which an individual may be exposed.

Section II

INDIVIDUAL PROTECTION

Complete protection against the organisms of biological warfare might possibly be given to an individual by placing him in an impermeable suit and an oxygen rebreather. But he could tolerate this only for a maximum of a few hours, and in such an outfit could be expected to carry out only operations involving the most restricted of movements.

BIOLOGICAL MASK. A modified gas mask is the principal device to prevent contact of man with air which is heavily contaminated with bacteria. The standard gas mask has been modified as a biological warfare mask because it is already designed to filter solid smoke particles from the air and because it is believed desirable to have a single mask to provide protection against both biological and chemical agents. The mask that has been modified is the M5 assault type.

The degree of leakage which is tolerated in the standard gas mask is of relatively small consequence in gas atmospheres because each respiration by the individual tends to dilute the gas that entered the mask from the previous respiration, resulting in mechanical control of the rate of increase of intrapulmonary concentration of the gas. However, since biological agents are not volatile particles but are suspensions of solids in the air, respiration tends to concentrate the agent leaking into the mask. The organisms that are inspired become impinged on the increased moisture present in the mucous membranes in the lung and air passages. Even an immeasurably small amount of leakage would therefore be quickly fatal in the case of a respiratory biological agent. British scientists working in biological warfare have calculated that if a man inhales 500 organisms of certain pathogens over a five-day period, the man will become ill. This postulate is the basis for tests of the biological mask and is the measure of the small margin of error that can be permitted. In biological warfare it would be entirely possible for a man to be exposed to an aerosol containing 100,000 organisms per liter of air over a period of ten minutes. Walking at the rate of three miles per hour, such a man would breathe at the rate of 30 liters per minute. Thus he would be exposed to 30,000,000 organisms. The standard gas mask whose approximate leakage is 50 milliliters per minute, would therefore admit approximately 50,000 organisms for the ten minute period, or one hundred time the dosage necessary to produce illness when inhaled over a five-day period.

In the standard gas mask there are three principal sources of leaks, namely, in the canister, facepiece and outlet valve. Recent developments and improvements in the gas mask canister, resulting in standardization of the M10A1 and M11 models, now provide as efficient filters against biological agents as against chemical agents. Two types of mechanical filters are effective against biological agents, filtering out particles of 1 micron or more in size. These are the asbestos-bearing paper used in American and German canisters and the electrostatic-resin wool filter used in British and Italian canisters. Wool filters, however, can be broken down by heat, oil smokes, short waves and metallic particles, and for that reason asbestos-bearing paper filters are considered superior for biological warfare canisters.

The outlet valve is a serious source of leakage, varying with the cleanliness of the valve and the closing pressure of the valve. The C15 outlet valve for the biological mask is the result of the addition of a resin wool filter to the present outlet valve which acts to filter any leaking air, gives additional dead air space and protects the valve from exterior dust and debris. In the absence of the filter, a snout of flannel will give the necessary added protection to the valve.

Leakage around the facepiece of the gas mask presents the greatest hazard from biological agents. Proper head harness adjustment is more important in reducing facepiece leakage of biological than of chemical agents. In addition, the use of a special hood over the facepiece of the assault mask serves to magnify the marginal seal and to act as an additional impinger to catch organisms. The hood is made of a tightly-woven material called permeable Oxford type IV wind-resistant cotton cloth and is designed not only to reduce the peripheral leakage of the mask facepiece but to provide a dead airspace around the outlet valve of the mask, thus affording a high degree of protection at that point. The hood covers the entire head and facepiece except for the eyepieces and canister. It is attached to the combat facepiece by means of aluminum eyerings and tied tightly at the juncture of the canister and canister mounting piece by means of a drawstring. A drawstring at the neck provides a snug fit there. The complete mask is designated as the leakproof service mask E2R4-M11-M6 and consists of the leakproof headpiece E2R4 in combination with the combat canister M11 and the M6 carrier. Positive pressure exerted within the headpiece makes entry of air impossible except through the canister. In test demonstrations only several spores have been found admitted by leakage in the E2R4 mask, at the same time that standard masks permit leakage of 1,000,000 or more spores.

Decontamination of the biological mask canister and the hood is accomplished by autoclaving at 15 pounds pressure for 20 minutes. The facepiece and hose may be decontaminated by washing with a 40 percent solution of alkaline chlorox.

PROTECTIVE CLOTHING. Clothing must be devised to exclude biological warfare agents which are effective against intact skin and others which gain entrance to the body through minute skin abrasions. It is doubtful whether any but impermeable clothing affords 100 percent protection against biological agents. Penetration of standard herringbone twill (HBT) by biological organisms is directly proportional to its air permeability. Materials having a nap, such as outing flannel, are penetrated less readily even though they have greater air permeability than closely woven cloth. Washing or autoclaving of nap cloth such as flannel or G.I. winter underwear decreases air permeability, but washing HBT materially increases its permeability as a result of removal of the sizing.

The standard two-piece permeable HBT suit, when treated by the CWS aqueous method is probably the best protective clothing which can be worn in comfort and which at the same time permits the fewest number of organisms to pass through the cloth. The protection provided by this uniform against aerosols of biological agents is limited, excluding approximately 50 percent of the organisms to which the body would be exposed without the clothing. The addition of ankle length G.I. underwear under the HBT suit increases protection since with it the exclusion of organisms is raised to almost 90 percent. Sleeves of the uniform must be tied tightly at the wrist, pants legs tied at the ankles or stuffed inside the collar of combat shoes, and all other openings carefully secured in order to assure this degree of protection (Figure 1).

The CC-2 impregnite of protective clothing has been found to possess considerable sporocidal properties. The standard aqueous impregnation of clothing appears to give the most powerful bactericidal and sporocidal properties to the clothing. Such clothing requires relatively high atmospheric humidity for the most efficient sporocidal action. On the other hand, M5 ointment has been found to have negligible bactericidal and sporocidal properties when applied as skin protection.

When they must be worn for long periods of time, gloves made of water-repellent Oxford type IV cloth are more comfortable than impermeable rubber gloves. The use of cloth gloves, however, is limited to work in relatively dry aerosol agents.

Biological protective clothing can be completely decontaminated by autoclaving at 15 pounds pressure for 20 minutes. Laundering impregnated clothing reduces the number of organisms impinging on contaminated clothing by almost 97 percent.

For plant workers in biological warfare, almost complete protection is provided by a uniform made of wind-resistant, water-repellent Oxford type IV cotton cloth. Its filtration efficiency is high but due to its high degree of impermeability, it is not practical for combat troops.

A special ventilated suit for biological workers provides total protection (Figures 2 and 3). It is constructed of two layers of Nylon cloth bonded together with Neoprene. It is a one piece suit with gloves and shoes of rubber cemented to the fabric. A transparent curved glass plate, clamped and cemented to the headpiece, provides for vision. A wire frame which rests on the shoulders holds the headpiece in position. A zipper across the back of the suit, covered by a flag, provides the opening into the suit. Air is introduced into the back of the suit by hose and a system of multiple rubber tubing carries the air to the extremities. The amount of air required for the suit and the necessity of refilling precludes general field use of the ventilated suit.

PROCEDURE IN EVENT OF BIOLOGICAL ATTACK. Immediate measure for individual protection. In the event that the actual presence of biological agents has been detected, the individual will:

Put on mask. See to it that the facepiece is properly adjusted to avoid leaks. In the event the special filter for the outlet valve is not available, tie a patch of heavy outing flannel over the outlet valve. A strip of herringbone twill or toe of cotton or wool sock may be used instead of flannel.

Button clothing. Tie clothing at wrists and ankles with extra shoe laces. Put on coveralls and tie wrists and ankles with shoe laces.

Tie cloth over head as hood and button collar around the hood.

Put on gloves if available.

Cover all skin cuts and have wounds attended to immediately.

Wash exposed body surfaces.

General instructions for individual protection. The steps which must be taken by the individual in the presence or suspected presence of biological agents are as follows:

Regard surroundings, materials and equipment as though contaminated with toxic agents.

Expose persons and clothing directly to sunlight. Air clothing.

When hood, mask and clothing are removed, use the same care as in the case of vesicants.

Decontaminate clothing by boiling or disinfecting with bleach or high test hypochlorite (HTH).

Avoid all exposed food and water. Eat from boiled mess gear. Handle food as in chemical warfare.

Clear underbrush and vegetation by burning (use flame throwers if available).

Isolate exposed areas and exclude personnel from unexposed areas.

Avoid crowding of exposed personnel.

Destroy rodents. Delouse clothing if indicated and prevent in any way the further spread of agents by insects.

Section III

GROUP PROTECTION

RESPONSIBILITY. Group protection in the event of biological warfare is primarily a responsibility of the Medical Department of the Army, with the active cooperation of the Chemical Corps and all other military personnel as well. The principal measures available for the maintenance of group protection are sanitation, protection of food and water supplies and employment of vaccine, toxoids and immune sera.

SANITATION. Military sanitation comprises the application in the military service of practical measures for the preservation of health and the prevention and control of disease. It is the first line of defense against the ordinary and unnatural occurrence of disease organisms in the field. In the event of threatened or actual employment of biological agents in war, sanitation discipline becomes of paramount importance, for it may assist materially in the reduction of primary casualties and also restrict secondary spread of the agent. Army Regulations constitute the general sanitary code of the Army. The performance of environmental sanitation, as it related to the source and methods of purification of water supplies, the methods and efficiency of waste disposal, the food supply and the sanitation of messes, personal hygiene and sanitation, the elimination of insect and rodent pests, and all other measures for the prevention and control of disease is based on FM 8-40; Field Sanitation and FM 21-10; Military Sanitation and First Aid.

PROTECTION OF FOOD AND WATER SUPPLIES IN FORWARD AREAS. Source of water. Provision of adequate and safe water supply is the responsibility of the Corps of Engineers. The Medical Department approves the quality of the water supply.

Artesian well water is most likely to be pure, but, with the exception of flowing wells, is liable to deliberate contamination. Dug wells are especially susceptible to accidental or deliberate contamination. Surface supplies, from rivers, ponds or lakes, are obviously liable to contamination. Water should be free from war gases as well as pathogenic agents and when war gas contamination is suspected, water may be tested with the CWS water-testing kit. At present there is no quick comprehensive test for detecting biological warfare agents in water. Only laboratory analysis is practical and efficacious.

Purification of water. All water supplies in the field must be considered impure and must be sterilized. Chlorination is the almost universal method for field sterilization. The mobile purification unit of the Engineers filters and chlorinates water at the rate of 100 gallons per minute. The portable purification unit of the Engineers prepares potable water at the rate of 15 gallons per minute. The storage tanks of the Engineers can be used for sterilization, if necessary, as well as for storage. The Lyster bag is used for field sterilization of water to be used by small bodies of men. In emergencies, Halazone tablets or calcium hypochlorite solution may be mixed with water in the canteen in order to assure its safety. In special cases, where chlorination is not practicable, boiling ensures proper sterilization of water.

Superchlorination and dechlorination may be used for the destruction of the most persistent organisms when they have been detected in water supplies. Tests for residual chlorine and the chlorine demand test assure potability of water supplies.

Food protection. Many of the same rules established for the protection of food from chemical attack hold true for biological attack, but in general prevention of contamination is simpler in the event of biological agents. The packaging of types C, D and K rations is completely effective against all such agents. With the exception of self-protected fruits such as oranges, coconuts and bananas, fresh fruits and vegetables must not be eaten uncooked where biological attack is possible.

[illegible]

An overseas theater of operations is in most instances a particularly good potential target for sabotage. Special defenses against sabotage must be provided for the protection of local water supplies, fresh milk, ice cream, bottled soft drinks, beer, ice and cold storage plants, bread, meat and butter and biological supplies. The principal reliance for defensive control is placed on measures relating to physical security and personnel security by isolating vulnerable areas where sabotage would be most effective and making these areas accessible only to authorized and selected personnel.

It is possible to introduce contaminants into storage tanks holding water supplies or directly into the water distributing system. The most effective measure is maintenance of adequate chlorine residual in all active parts of the system, with frequent checks to see that the residual content is maintained. Vulnerable areas in ice cream plants are usually the rooms in which mixing, blending, pasteurizing, cooling and holding are performed. For bottled soft drinks, the most vulnerable area is the syrup room. Bottled beer is not a good vehicle for the spread of bacterial contamination. Chemical poisoning, however, may be attempted in the brewing, cooling and fermentation rooms. Ice is not a likely vehicle of biological agents, but it is a possible source and cannot be overlooked. Bread is a poor vehicle of biological organisms, but is very vulnerable to chemical poisoning, chiefly in the mixing room and the room in which ingredients are stored. Deliberate large-scale contamination of meat in storage is difficult. Butter is an excellent vehicle and presents greater opportunities for wide disseminations of disease organisms.

<u>Food Poisoning</u>. The use of chemical as well as biological agents may be anticipated for the poisoning of foodstuffs. It must also be remembered that either type of agent may be present as a result of natural causes as well as by deliberate implantation.

Antimony poisoning may result from foods cooked in cheap gray-enameled cooking utensils. Cadmium poisoning occurs from acid foods or drinks prepared in cadmium-plated utensils. Arsenic and mercury compounds may be maliciously added to foods.

Shellfish poisoning is caused by the plankton eaten by mussels and causes respiratory paralysis. Snakeroot poisoning is usually caused by milk from cows pastured on snakeroot. Mushroom poisoning is a common occurrence. Ergotism is caused by a parasitic fungus of rye and causes a gangrenous involvement of the fingers and toes. Botulism is caused by the ingestion of food in which the organism has grown and produced toxin. The most common types of food poisoning in the United States, however, are caused by staphylococcus and streptococcus organisms.

Dried, evaporated or whole milk will all harbor disease organisms. Dried or fresh eggs may contain poisoning organisms or biological agents may be prepared and cultivated in the eggs. Both fresh and dehydrated meats are susceptible to disease organisms. Fresh vegetables and fruit may be contaminated with enteric bacteria or with helminth larvae. Insects frequently contaminate food.

Medical Supplies. Such medical supplies as blood plasma, [illegible] and [illegible] may be contaminated by seeding with hemolytic streptococci, staphylococci, spores of anthrax or tetanus bacilli. Other biological products such as drugs, poisons and specific antisera must be protected against contamination or tampering.

VACCINES, TOXOIDS AND IMMUNE SERA. In general, the best means of attaining increased resistance to specific disease organisms is by the administration of vaccines or active immunization. The protection vaccination affords beginning usually two weeks after the initial course of treatment will persist for relatively long periods of time and can be maintained in an effective state by means of periodical booster doses.

It must be remembered that immunity against specific diseases is not an absolute but a relative factor in the protection of individuals. Practical considerations which limit the value of immunization are as follows:

The vaccines and techniques customarily used are intended to protect against ordinary exposures to disease organisms. While the immunized individual will be more resistant than the unimmunized, he still cannot risk repeated and heavy exposure without some danger. In biological warfare, the virulence and dosage of agent, as well as the use of unorthodox portals of entry, may result in the breakdown of an otherwise satisfactory degree of resistance. Infections that do occur in vaccinated personnel, however, are likely to be milder than those in the unprotected.

To accomplish good immunization, a period of time is required. It takes from a few weeks to a few months to establish a sufficient degree of antibody substances for successful destruction of invading microorganisms.

Immunity reaches its highest level soon after completion of the immunization procedure and thereafter falls off over a period of months and years, more or less rapidly depending upon the individual and the product used.

Immunization is specific in nature. The protection afforded an individual against a particular agent by the use of a particular vaccine will not be effective against any other agent.

Individuals vary in their response to inoculation. While most individuals build up a satisfactory store of protective substances in the tissues and blood stream, a few, for reasons largely unknown, react poorly.

Even in the vaccinated person a state of poor general health, exhaustion, malnutrition, exposure and the presence of other disease conditions may reduce resistance below the protective level.

General experience with vaccines indicates that very few of those available at present can be expected to confer sufficient protection to overcome high concentrations of agent that may be expected in biological warfare.

Variably effective vaccines have been prepared for use against smallpox, yellow fever, cholera, typhoid and paratyphoid fevers, diphtheria, tetanus, plague, typhus fever and influenza. Smallpox and yellow fever vaccines appear to be exceptionally effective, but the remaining vaccines are of distinctly limited value against their diseases even as they occur in nature, and probably have considerably less protective value against an artificial attack employing high concentrations of the disease agent. Among the recently developed vaccines which appear to have some degree of effectiveness are those prepared for use against type A and B botulinus toxin and the psittacosis virus. Vaccines have also been utilized to advantage against such diseases as rinderpest, foot and mouth disease, Newcastle disease, fowl plague, bacillary dysentery, anthrax, and Japanese Type B encephalitis. With further research it is possible that satisfactory vaccines may be found for brucellosis, tularemia, glanders, melioidosis, coccidioidal granuloma and other organisms and toxins which may be used in biological warfare.

In addition to the vaccines there are other means of affording biological protection to individuals, including the use of specific antisera, chemotherapeutic agents and the use of antibiotic agents such as penicillin and streptomycin. To act as preventives, these substances must be given before or immediately following contact with the biological agent. Their effectiveness is lost in a relatively short time if their administration is not continued once it has been initiated. Chemotherapeutic and antibiotic agents have an advantage over vaccines in that protection is conferred immediately upon administration. There is evidence that the sulfa drugs and antibiotics are effective against such diseases as plague, glanders, anthrax, psittacosis and tularemia.

Section IV

DECONTAMINATION

GENERAL. Decontamination from the standpoint of biological warfare is the process of rendering an area or materials and equipment safe after exposure to a biological agent in order to destroy pathogenic agents that are present and prevent their being spread.

DECONTAMINATING AGENTS. The destruction of antibiotic organisms may be effected through the use of physical agents and chemical agents.

Physical agents. Physical agents which may be used in decontamination include heat, light, cold, sound and pressure. The most practical of the physical agents is heat which may be applied in the form of incineration, dry heat or moist heat. The use of moist heat includes boiling, flowing steam or steam under pressure. The autoclave employs the principle of steam under pressure and is the most effective device for the decontamination of clothing and instruments, rendering them sterile in 20 minutes at 15 pounds pressure and 120 degrees Centigrade.

Chemical agents. The chemical agents which may be used for the decontamination of materials contaminated by microorganisms include phenol, the cresols, alcohol, formaldehyde, hexylresorcinol, iodine, chlorine, hydrogen peroxide, potassium permanganate, bichloride of mercury, mercurochrome, metaphen, propylene glycol and calcium hypochlorite. The chemical agent which most nearly meets the conditions of an ideal decontaminant for military purposes is calcium hypochlorite or bleach.

Calcium Hypochlorite. Chloride of lime or calcium hypochlorite is effective against a multiplicity of organisms, it decontaminates quickly, can be made available in quantities for immediate use, is not hazardous to use, is easily inactivated, is not prohibitive in cost, and can be used in a variety of apparatus.

Calcium hypochlorite contains 30 to 35 percent available chlorine. High test hypochlorite (HTH), which contains between 60 and 70 percent available chlorine or twice the amount of ordinary calcium hypochlorite, is also twice as effective. A 7 percent solution of calcium hypochlorite will successfully decontaminate materials, equipment and even the air itself within 15 minutes. A 20 percent slurry of calcium hypochlorite will decontaminate top soil within 5 minutes.

Seven-percent bleach solution may be prepared on the basis of

1/2 canteen cupful of bleach per gallon of water
0.6 pound of bleach per gallon of water
6 shovelfuls of bleach per 50 gallons of water
30 pounds of bleach per 50 gallons of water

Twenty-percent bleach slurry may be prepared on the basis of

1½ canteen cupfuls of bleach per gallon of water
1.7 pounds of bleach per gallon of water
17 shovelfuls of bleach per 50 gallons of water
85 pounds of bleach per 50 gallons of water

DECONTAMINATING APPARATUS. There are many items of standard equipment which can be used to decontaminate areas, materials and other equipment. They include:

M2 1½-quart decontaminating apparatus.

M1 3-gallon decontaminating apparatus.

M3A1 or M4 400-gallon power-driven decontaminating apparatus.

Large wooden, earthenware, porcelain, stainless steel or aluminum containers, which may be used for immersing contaminated equipment.

Other large containers such as tubs, tanks, drums and Lyster bags, which may be used for preparing hypochlorite solutions.

The paint spray apparatus of the Ordnance Department.

The field autoclave of the Medical Department.

Delousing equipment and mobile laundries of the Quartermaster Corps.

Carboxide cylinders, used for fumigation purposes by the Navy Department, which are also excellent for the destruction of microorganisms.

Steam generators, including the M1 and M2 mechanical smoke generators.

Clothing impregnating plants.

DECONTAMINATION METHODS. Immersion methods. The use of 7 percent hypochlorite solution is the most efficient immersion method. Cotton clothing, leather, wool, rubber and metal articles are decontaminated in 30 minutes. This may damage cotton clothing to a slight extent but does not affect rubber. Leather and wool will deteriorate and metals will corrode unless they are thoroughly rinsed after immersion.

DANC will decontaminate cotton and wool clothing in 15 minutes without significantly damaging the cloth. There is less corrosion on metal materials than with the hypochlorite solution.

Field washing or rinsing reduces contamination. Warm water, soap or detergents and agitation may increase the degree of decontamination.

Boiling water will render clothing or equipment essentially sterile in 15 minutes.

Autoclaving. The use of steam under pressure in the autoclave, at 23 psi (123 degrees Centigrade) for 45 minutes, renders most materials sterile.

Vapor-phase chemicals. Methyl bromide, which is found in the Quartermaster delousing kit, is an effective sterilizer. Clothing and equipment placed in a container with five 20-milliliter ampules of methyl bromide will sterilize the equipment in 12 hours. Shoes, leather, metal and wool materials may also be safely decontaminated in this manner.

Carboxide, the Navy fumigating agent composed of 10 percent ethylene oxide and 90 percent carbon dioxide, will sterilize all clothing and equipment subjected to a chamber concentration of 300 milligrams per liter of space for 16 hours.

Formaldehyde when dispersed with steam under pressure in enclosed spaces is a good decontaminating agent. Use has been made of the M2 smoke generator, vaporizing a 50 percent formalin-water mixture to approximately one milliliter of formalin per cubic foot of space. Formaldehyde gas for sterilizing air is effective when a concentration of 5 milligrams per liter of air at a relative humidity of 75 percent and 30 degrees Centigrade (86 degrees Fahrenheit) is maintained for ½ hour.

Laundering. Quartermaster laundries or Chemical Corps impregnating plant operations remove all but a small percentage of highly resistant organisms. Modifications in procedures can be made to provide complete sterilization.

DECONTAMINATION PROCEDURES. Clothing. Ordinary cotton or HBT clothing should be immersed in 7 percent calcium hypochlorite solution for 15 minutes. After removal, the clothing is rinsed in water and allowed to dry. Woolen material should be decontaminated only by autoclaving. Shoes may be decontaminated by spraying with the 3-gallon hand apparatus. Biological protective clothing is best decontaminated by autoclaving at 15 pounds pressure, 120 degrees Centigrade for 20 minutes.

Equipment. Helmets, mess kits, rubber equipment may be decontaminated by immersion or spraying with a 7 percent calcium hypochlorite solution. Gas mask canisters may be decontaminated by autoclaving or wiping or spraying with hypochlorite solution. Facepieces and hoses may be washed with hypochlorite solution or 40 percent alkaline chlorox. All equipment should be flushed, rinsed or wiped with water after 15 minutes. Large items such as field pieces or trucks may be decontaminated by spraying with power-driven decontaminating apparatus, with special care taken to flush metal equipment which might corrode.

Buildings. Exteriors and interiors of buildings may be sprayed with solution or slurry and then flushed with water.

Terrain. Top soil, beaches and roadways may be successfully decontaminated with a 20 percent slurry of calcium hypochlorite. Less than a 20 percent suspension is insufficient to kill spores in soil. The hypochlorite is inactivated by the large amount of organic matter present in the ground before any marked bactericidal action can be exerted.

Air. Air in enclosed spaces such as chambers or rooms may be successfully decontaminated with a fine mist of 7 percent calcium hypochlorite for 5 minutes. Hypochlorite solutions should be filtered before nebulization. The mist will not decontaminate surfaces in an enclosed space unless the surfaces are actually wetted with the mist. Propylene and triethylene glycols may also be used as effective air-decontaminating mists. Formaldehyde gas is an effective air decontaminant provided high temperatures and humidities can be maintained. Large volumes of formaldehyde gas with a high humidity can be obtained with the M2 smoke generator, substituting formaldehyde for the fuel oil in the apparatus. It has been shown that FS and HC smokes are effective in decontaminating aerosols of certain biological spores and the use of smokes might prove effective in the field.

Water. Ordinary chlorination of water is generally effective in removing most harmful organisms. In special cases where chlorination is impractical, boiling will ensure proper sterilization of water. To treat water heavily contaminated with biological agents, it may be necessary to resort to superchlorination and dechlorination. The decontamination of sewerage presents a special problem in that the organic material present serves to inactivate chlorine, but once the necessary residual chlorine concentration of 10 parts per million has been reached, decontamination is effected.

Food. Exposed food that has been subjected to biological attack must be destroyed. Packaged food and self-protected fruits and vegetables may be decontaminated by spraying or immersion in a hypochlorite solution. Thorough cooking will ensure effective destruction of microorganisms. Foods contaminated by toxins must be destroyed.

Personnel. Individuals exposed to heavy clouds of biological agents should be subjected to a spray of 7 percent calcium hypochlorite from the 3-gallon decontaminating apparatus before disrobing. Clothing should be carefully removed and a soap and water shower taken as soon as possible. The hands are most satisfactorily decontaminated by washing with disinfectants such as hypochlorite, cresols or hydrogen peroxide. Equally effective is the surgical practice of washing the hands thoroughly with a good soap. In the event of cuts or abrasions, the various mercurial preparations and tincture of iodine are satisfactory cleansing agents. It has been shown that only the proprietary article Mercresin among the mercurials has a truly bactericidal effect. The balance of the mercurials are merely bacteriostats.

Chapter 6

ORGANIZATION FOR BIOLOGICAL WARFARE

Section I

GENERAL

The Army Chemical Corps is concerned with the preparation of all possible defenses against the initiation by an enemy of biological warfare and with retaliation should higher authority so direct. Biological warfare is an insidious form of warfare and may be highly deceptive both to the forces of intelligence seeking to detect preparation for the use of microorganisms and their toxic products in war and to the medical services attempting to ascertain the difference between a natural or sporadic outbreak of disease and an organized effort to establish disease.

Biological warfare through sabotage can be developed in a relatively short time by any progressive nation. Preparatory activities would be difficult to detect since they can be conducted under the guise of legitimate research. Sabotage would be the logical precursor to large-scale biological attack. It would succeed only in proportion to the breakdown of public health measures in the country under attack. Open, large-scale biological warfare can be developed only by a nation with ample resources in scientific personnel and industrial facilities. However, the cost of an effective arsenal of biological agents is less than for any other form of warfare.

Section II

ORGANIZATION FOR DEFENSE AGAINST BIOLOGICAL ATTACK

PERSONNEL. All organization commanders are responsible for the proper training of their respective commands in defense against biological attack, and within the means available to them, are responsible for taking proper measures for the care and maintenance of protective equipment and for the protection of their troops, equipment and supplies against biological warfare. They will have on their staffs specialists in defense to advise them on the proper protective measures. These specialists will actively supervise the execution of all such measures under the authority of the commander.

Specially trained officers with duties corresponding to those of the division chemical officer and the theater chemical officer will carry out the directives of higher headquarters and act in the interests of their immediate organizational commanders.

The division biological warfare officer, who is an officer of the Chemical Corps or of the Sanitary Corps of the Medical Department, will be the adviser to the division commander. He has three specific functions: biological warfare intelligence, training, and supply. Under the direction of the division commander and in cooperation with the G-2 section of the general staff, he will gather information of enemy biological warfare activities, transmit it to higher headquarters and recommend to the division commander the issuance of such instructions to subordinate units as are necessary in each instance. It is part of his duty to prepare a standing order for defense against biological attack for his division. In addition, he will cooperate with the G-3 section in matters of biological warfare training, and through the division G-4 he will requisition supplies from the Army and issue them to regiments and separate units within the division.

The theater biological warfare officer, who is a specially trained officer of the Chemical Corps, is assigned to the staff of the theater commander in order to advise on all biological warfare matters affecting the security of the theater.

BIOLOGICAL LABORATORY COMPANY. The principal agency in the field for biological intelligence and the detection of specific organisms that may be employed by an enemy is the biological laboratory company. It is the duty of this company to investigate all matters of a biological nature that the theater biological warfare officer may request. Its principal missions are to maintain a complete and active biological intelligence section and to perform all the necessary laboratory operations with respect to the work of the intelligence section. The intelligence section is organised into a number of mobile units, each capable of operating independently in the field, but maintaining close liaison with the main laboratory section of the company.

The members of the biological laboratory company would include bacteriologists, physiologists, pathologists and epidemiologists, who would maintain close relations with all agencies of the Medical Department of the Army in the field in order to assist the medical services in the identification of organisms being used and in the difficulties of diagnosis that might be expected.

Section III

METHODS AND OPERATING PROCEDURES FOR DEFENSE

The organizations that are established in the Army for defense against biological attack include the intelligence services, the medical services and the Army Chemical Corps which is charged with all research and development for the Army of the materials and equipment of biological warfare. The methods that are available to these agencies for the defense of the Army in the field are:

Biological warfare intelligence.

Detection.

Mechanical and physical protection and decontamination.

Sanitation

Immunization.

Management and treatment of casualty cases.

Epidemiological control.

INTELLIGENCE. Biological warfare intelligence activities are based on the assumption that an enemy in desperation may resort to biological warfare. It is therefore necessary for the intelligence and medical services to be keenly alert to possible methods of attack and to be able to recognize presumptive evidences of its contemplated or employed or attempted use of disease organisms or their toxic products.

Sources of Intelligence. Peacetime sources of intelligence include the use of information channels of various Government agencies, the diplomatic channels of the State Department, information obtained from travellers and friends in foreign countries, and the newspapers, reports and periodicals of foreign countries. In time of war, intelligence is obtained from such special organizations as the Office of War Information, Office of Strategic Services, Office of Economic Warfare, as well as from the Military Intelligence Service, State Department and Federal Bureau of Investigation. Besides these sources, much information may be obtained in the field from agents behind the lines, underground organizations and refugees and friendly individuals in unoccupied or neutral countries. Information is also obtained from captured documents such as manuals and orders; captured munitions, biologicals and defensive and offensive equipment; censored correspondence; examination of enemy technical literature; examination of the blood of prisoners of war; interrogation of prisoners of war; aerial requiring the use of large numbers of trained personnel and large laboratories. Research and development centers for biological warfare would probably be marked by:

Strict security measures.

Numerous separated laboratory buildings, and perhaps greenhouses and field plots.

Provision for sterilizing and disposing of contaminated air, solids and liquids on a large scale, involving considerable use of fuel and steam.

Delivery of large quantities of agar, casein, sugar products, autoclaves, laboratory glassware, microscopes, centrifuges, refrigerators, and incubators.

Delivery of large quantities of small animals and cages.

Inclusion of many bacteriologists on the staff.

Inclusion of military personnel on the staff, or as frequent visitors.

Presence or delivery of specific protective equipment which may be similar to chemical warfare equipment in appearance.

Presence of obnoxious odor of decay, which usually accompanies large-scale production of bacteria, on or near the installation.

Production plants for biological warfare would be indicated by:

Similarity to chemical plants in external appearances.

Delivery of empty shells or bombs to the plant.

Existence of large vats for the growth of bacteria.

Removal of charged projectiles with unusual markings or with unusual security precautions.

Biological test installations would be marked by:

Closely guarded, isolated large fields.

Presence of vacuum pumps with cotton-collectors for the detection and testing of bacterial clouds.

Unusual decontamination measures effected in the field, such as burning, flooding or use of chemical sprays.

Presence of planes with special equipment for biological bomb and spray tests.

General evidence would include:

Organization and training of military units for biological warfare.

Abnormal immunization of military and civilian personnel.

Psychological preparations such as accusations of use of biological agents by other nations.

<u>Suspicious circumstances</u>. In time of war, the evidence of plans may become more apparent. The nature of the materials of biological warfare is such that they cannot be used without a great deal of preparation and special precautions, and this is difficult to conceal completely. Evidence that the enemy was contemplating the use of disease organisms as an instrument of war might be inferred from:

The appearance of specialized troops in strategic areas, such as certain types of chemical and engineer troops trained in biological methods, for otherwise unexplained purposes.

The appearance of unusual types and amounts of special equipment such as delousing units, field laboratories, field autoclaves and decontaminating equipment.

Unusual types of weapons and offensive techniques, including material dropped or sprayed from aircraft such as smokes or mists, flasks or ampules, gelatinous masses, food particles; parachutes, balloons or other devices for distributing animals or insects; unusual types of bombs and shells, especially those containing compressed air or pistons; or any type of weapon which appears to have little if any immediate effect.

Unusual types of inoculation of enemy personnel that may be learned from paybooks, medical records or blood serum tests.

Unusual types and amounts of biological supplies in medical stockpiles for the prevention and treatment of disease.

Transportation or storage of highly classified material, particularly by refrigeration, and usual security precautions in relation to such.

New issue, reissue or alteration of protective equipment such as gas masks, special decontamination clothing, gloves or hoods.

Evidence of enemy instruction of line troops in protection against biological warfare.

In relation to sabotage, the unusual taste and appearance of food, water or beverages, suspicious actions of individuals handling them, or the sudden reduction or disappearance of the residual chlorine in water supply systems.

Manifestations of Successful Biological Attack. Evidence of a successful biological attack made by an enemy from within or without the country might be made manifest through:

The appearance of a disease in a previously non-infected area, which cannot be accounted for by normal means of transmission.

The occurrence of numerous widespread and unexplained outbreaks of unusual diseases, or numerous unexplained deaths occurring in a given area.

Simultaneous appearance of many cases of disease, all in approximately the same stage of development.

Unusual symptomatology and severity of well-known diseases.

Simultaneous appearance of two or more infectious diseases in the same individuals.

Unusual incidence of sickness and death in the animal population, either domestic or wild.

Abnormal deaths among the rat population in a given area, or a sudden decrease in the number of rats.

DETECTION. If the first indication of biological attack is the actual illness of large numbers of troops, detection, identification and treatment become largely a medical problem. Medical responsibility requires that qualified medical personnel will, in the event of biological warfare:

Isolate and observe exposed domestic animals.

Detect biological agents by direct examination, culture and animal inoculation of specimens from shell fragments, air, water, exposed materials and equipment, and by use of specifically immunized animals with controls.

Detect biological agents by clinical diagnosis of exposed personnel and animals and examination of materials from same.

Detect biological agents by epidemiological study of cases.

Isolate biological cases.

Have available such specific therapeutic agents as are of established value.

In the absence of an outbreak of disease, but where employment of biological agents is suspected, field troops or units of the biological laboratory company will collect samples of material for transmission to the laboratory.

It is imperative that the first use of biological agents on the part of the enemy and the use of particular agents for the first time be confirmed by agencies of the highest authority. If the proper individuals are constantly alert and sensitized to the possibilities of biological attack, it might be possible to complete detection of an agent during its incubation period. Early detection and speedy identification are of the utmost importance for until the specific nature of the agent is known, the most effective measures of defense and individual protection are impossible. With quick detection, it may be possible to institute specific therapy and other positive measures including immunization against spread of the infection.

Whenever physical evidence is found and preliminary examination warrants completion of appraisal, the services of the nearest laboratory equipped to conduct the investigation will be promptly utilized. The laboratory technicians may require from one to seven days to complete identification of specimens submitted. No time, therefore, must be lost in securing and transmitting suspected materials.

Collection of Suspected Materials. The samples should be taken over a wide area and may consist of such materials as air, soil, water, vegetation, clothing, gas mask filters, bomb fragments and especially dud shells or bombs. All samples should be refrigerated if possible while in transit. All such material should naturally be treated as contaminated. Sick animals such as birds, mice or other rodents should be captured without direct handling and sent to the field laboratory for examination.

Samples should be collected as aseptically as possible and sealed in sterile containers. Sterile cotton swabs should be used to collect samples from contaminated surfaces. They should then be placed in sterile test tubes and plugged with cotton. Samples of soil and water should be placed in sterile rubber-stoppered test tubes.

Field Sampling Equipment. A satisfactory field sampling kit should have in it the following articles:

One quart jar container holding cotton swabs in cotton-stoppered test tubes, sterile rubber-stoppered test tubes, and a 5 ml syringe with #18 and #20 needles.

Two half-pint cardboard cartons.

Two large test tubes containing sterile capillary pipettes.

Sterile instruments such as scalpel, scissors and forceps.

Gummed labels.

Sterile gloves.

Six 5 ml pipettes.

Two pint thermos jars containing nut-sized cracked ice.

One cotton impinger or liquid impinger apparatus for air sampling.

The cotton impinger (Figure 4), used to collect biological agents from the air, is designed to filter air through cotton wool. Suspended organisms, being trapped on the filter, may then be recovered and identified in culture plates. The cotton impinger consists of 4 pellets of cotton packed into a glass holder to which an aspirating device is attached. The glass holder is made by cutting off the end of a test tube and sealing a smaller tube to the end, or by pulling down one end of the original tube to a smaller bore. Air may be drawn through the impinger by means of a small hand pump, such as that found in the chemical agent detector kit [illegible], or by use of an atomizer bulb.

The liquid impinger or bubbler (Figure 5) may also be used to collect suspended agents in the air. The liquid impinger consists of a 50-milliliter round bottom flask with side arm and a straight glass tube with a small bulb blown onto one end. About 25 small holes are punched through this small glass bulb with a hot tungsten wire and the tube is then fitted with a one-hole rubber stopper and plugged into the flask. Ten milliliters of chlorine-free water is placed in the flask and the small bulb of the straight tube is brought below the surface of the water. The side arm and tube openings are plugged with cotton. Air is evacuated from the side arm by means of a small hand pump or atomizer bulb. Organisms are trapped in the liquid where they may be recovered and identified in the laboratory.

As a field expedient, in the absence of either the cotton or liquid impinger, the filter of the gas mask may be used as a means of collecting airborne agents.

<u>Identification</u>. In very few instances is it possible to identify precisely the organisms that may be obtained from sampling the air and cultivated on agar plates. The most reliable detection test for almost all the biological agents involves animal inoculation and response. By making immediate inoculations of

the culture growth into several animal species and by several routes of introduction, pathogenic rickettsia and viruses as well as bacteria and toxins, upon exerting their characteristic reactions in the animal host, may be detected. Examination by smear is then possible with exudates from wounds or lesions, with sputum, feces, blood or urine. Corroborative findings may be made by means of blood chemistry, blood cell counts and urine examination. As the infection declines or recovery is effected, detection is possible by the appearance and identification of antibodies. These may be demonstrated by agglutination reactions, complement fixation reactions, toxin neutralization or virus neutralization.

Only the biological laboratory company, general medical laboratories, general hospitals and hospital centers are properly equipped to detect and identify the agents of biological warfare. Proper and adequate facilities are not likely to be found in aid, collecting or clearing stations, evacuation hospitals, convalescent hospitals, small station hospitals or hospital ships.

<u>Probable Complications in Detection.</u> Detection in actual practice in biological warfare may have many complicating or confusing factors involved. By the time an agent sample is inoculated into a laboratory animal, for instance, it may have become nonviable, even though it may already have infected human or animal

hosts. Contaminants in the suspension may need to be suppressed or eliminated by pretreatment with antibiotics or other specific inhibitors, particularly if rickettsia or viruses are to be demonstrated. Confirmatory tests such as cultural or serological tests are slow and they discriminate less easily between pathogens and contaminants. Other complications likely to be confronted under combat conditions may arise from the use of standard procedures of sanitary and diagnostic microbiology which may fail under the artificial conditions of combat. For example, polluted water may not contain the colon bacillus of dysentery, contaminated milk may have an insignificant bacterial count, or the undifferentiated viable count of highly infective air may be low or completely misleading. The agent may be unrecognizable by standard tests because it is unfamiliar or immunologically distinctive. Animal symptomatology and pathology may be confused in the presence of a strange mixture of agents. It is possible that the agent employed may be non-infective for any test animal and undetectable by laboratory means, yet be highly infectious for man or domestic animals. The common signs of an epidemic disease, frequently useful in identification under normal conditions, may be quite different under the artificial methods of biological warfare. This would be particularly so if more than one agent was used concurrently. Thus success in detection requires a high degree of organization with a well-conceived plan of procedure and analysis, a suitable array of equipment and materials, and personnel with adequate skill and training.

PROTECTION AND DECONTAMINATION. The materials, equipment and procedures for individual and group protection and for decontamination in the event of biological attack are given in Chapter 5.

Training in physical protection and decontamination. With the development of the biological mask and special clothing as protection against biological agents and the provision of these and the materials for decontamination, organizational aspects of protection devolve into the training of military personnel in preparation for biological attack. Training and instruction should be such as to alert all personnel from the higher echelons on down to troop level to the possibilities of biological warfare. Proper psychological instruction will allay fears and unnecessary apprehensions. Training in mechanical and physical protection and methods of decontamination and in environmental sanitation is the basis of an adequate defense against biological attack. Good discipline and good personal health habits must be fixed.

ENVIRONMENTAL SANITATION. Commanding officers of all grades are responsible for sanitation and for the enforcement of the provisions of sanitary regulations and orders within their organizations and the boundaries of areas occupied by them. The Medical Department of the Army is responsible for investigating, reporting on, and making recommendations relative to all matters affecting the health of the army, including the location of camps and stations, the source and methods of purification of the water supply, the methods and efficiency of waste disposal, the food supply and the sanitation of messes, the suitability of clothing and housing of troops, efficiency of training in personal hygiene and sanitation, the elimination of insect and rodent pests, and all other measures for the prevention and control of disease. The senior medical officer of a command is responsible for the preparation of orders relating to sanitation.

IMMUNIZATION. Immunization is the method of choice to be employed in the control of any infectious disease. New immune sera, vaccines and toxoids that are developed through continuing research must find their way to the proper medical agencies as quickly as they are readied for field employment.

[redacted] OF CASUALTY CASES. At the first sign that biolog- [redacted] may be employed, biological warfare officers and medical officers must be alerted to the potential dangers. While biological warfare and medical viewpoints are not the same, an alert medical and sanitary program includes many anti-biological warfare measures.

Medical Department facilities. The Medical Department upon alert will be concerned with the following:

Hospitals and dispensaries will be surveyed to ascertain patient capacity and facilities for caring for biological warfare casualties.

Laboratory facilities will be strategically located and have adequate facilities for such tests and examinations as may be required for biological investigations.

The Sanitary Corps will be alerted to various types of possible biological attack, especially with regard to the sabotage of water supplies, food and milk sources. Inspection and testing measures will be rigorously maintained to guard against sabotage.

The Veterinary Corps will make certain that all dairies and slaughtering establishments are adequately protected against sabotage.

Wherever possible, civilian health authorities and facilities in the theaters of operations will be mobilized for biological defense.

Medical handling of casualties: Personnel exposed to biological agents should be hospitalized for what might otherwise be considered slight justification. Even slight fever or malaise, when a deliberately disseminated disease organism is a possible causative agent, should be viewed with suspicion until specific infection is ruled out. All possible means of detection, identification or diagnosis will be employed to establish the significance of suspicious manifestations. Chest X-rays may be helpful when an agent producing pulmonary disease is suspected, such as anthrax, coccidioidal granuloma, glanders, melioidosis, plague, psittacosis, tularemia or brucellosis.

Quarantine will be used only in connection with diseases which are highly transmissible and for individuals whose exposure has been clearly established. Prompt segregation and isolation of individuals with contagious diseases must be carried out.

Specific biological drugs and convalescent sera must be used promptly and continued where they are appropriate and available. The use of the sulfonamides and antibiotics will be considered in all cases of infection.

Extremely long periods of convalescence may be expected in severe cases of many diseases which may be used in biological warfare, particularly tularemia, brucellosis and glanders.

<u>Possible complications of diagnosis</u>. During the stages of onset, recognition may be impossible. Common to most infectious diseases are symptoms of general malaise, headache, fever, nausea, generalized aches and pains. It would not be possible therefore

We do more than isolate men showing these symptoms. Not until most infectious diseases have achieved their climax do differentiating signs appear making diagnosis at all certain. Then the clinical appearance or laboratory findings or both may make it possible to ascertain the identity of the invading organism. The clinical appearance of biological warfare diseases may be influenced by the route of infection. Natural infection, as by vector or contact, may differ from infection induced by artificial means such as respiratory or cutaneous infection. In addition, large doses of pathogenic organisms launched in a deliberate attack may shorten the normal incubation period to be expected and result in overwhelming infection. Fatigue or malnutrition may accelerate the course of disease; good physical condition may reduce its severity. Immunization may prevent or render mild the attack. Clinical appearances may also be confused by mixtures of other agents, either chemical, metallic or biological.

EPIDEMIOLOGICAL CONTROL. Epidemiology is concerned with the [illegible] disease in large numbers of individuals or animals. [illegible] study of the chain or cycle of events by which a disease [illegible], in order to find one or more vulnerable links in the chain which may be broken to interrupt the continuity of the disease. With the typhoid group of fevers, the weakest link is at the point where sewerage pollutes drinking water. With epidemic yellow fever, it is the breeding places of _Aedes aegypti_ mosquitoes. In biological warfare, however, the epidemiologist is not only concerned with the transference of disease as it occurs in nature, but with the means of unnatural transference. The observation that yellow fever virus can be transmitted apart from mosquitoes, as has been demonstrated by the laboratory infection of men, is of special epidemiological significance.

Factors in epidemiology. During the last twenty years prevention of the spread of the enteric group of infections has been controllable by means of water purification, milk control, food handler examination and insect and rodent control. Progress in the control of airborne infections has not been as rapid. The study of airborne infections has been carried out principally in children's hospitals, and during the late war, in air raid shelters and in army barracks. The spread of a number of diseases such as the common cold, influenza, meningitis, measles and a typical pneumonia have been attributed to airborne contagion.

The conditions of epidemicity depend upon the manner of transmission of the particular disease. That is, whether the agent is [illegible] in the respiratory tract and discharged into the air by sneezing or coughing, whether the agent is discharged in excreta, or whether it is found in circulating blood from which it may be released and transmitted by insect vector.

<u>Epidemic investigation</u>. Accurate and prompt reporting is usually the first indication the epidemiologist has of the occurrence of unusual illness. He must then investigate all circumstances concerned with the particular outbreak of disease. He will confirm the clinical diagnosis. Laboratory tests must be made on each case for possible carriers. Tracing the source of infection is the final step of the investigation. All or pertinent samples of cases must be visited to collect information which may lead to the source. Case cards and spot maps are prepared to plot the site and cause of epidemicity.

<u>Epidemic control</u>. Isolation with concurrent disinfection and decontamination is the first step in epidemic control. Isolation means the confinement of an individual case or carrier for the duration of infectiousness. Total isolation would be required in the case of pulmonary anthrax, glanders, melioidosis, pneumonic plague or psittacosis. Medial isolation is required for cases of cutaneous and intestinal anthrax, brucellosis, coccidioidal granuloma, bubonic plague and tularemia. Only minimal isolation would be [illegible] botulism or typhus fever.

[illegible] means the isolation or confinement of exposed normal individuals or animals for the longest possible incubation period of the disease in question. As generally practiced, quarantine is one of the least effective methods of disease control. It is without value in those diseases where there are very high carrier rates.

Carrier control depends upon the biological behavior of the particular disease. An extreme example of control is the removal of the gall bladder in typhoid carriers or the administration of sulfadiazine to a large section of a population to cure undetectable meningococcus carriers.

Intensive environmental sanitation is one of the most important factors in epidemic control. Proper control of food, water and milk supplies and of rodent and insect population are basic. Active immunization is an effective and certain control measure when an effective immunizing agent has been developed or is available.

Section IV

GENERAL DEFENSIVE MEASURES

Tactical dispositions depend upon the local situation and the nature of the attack. In many cases, commanders will not know immediately which particular agent is being used. Immediate defensive measures must therefore be of a general nature.

TERRAIN. During the height of an attack, vegetation affords considerable protection against airborne agents. After an attack, vegetation should be avoided until decontaminated. Dispositions will be made to higher lands and upwind of contaminated areas insofar as possible.

CONTAMINATED AREAS. Areas known to be contaminated will be avoided if the tactical situation permits. Otherwise they will be held as lightly as possible by troops who are properly protected. Such troops should be replaced at short intervals and properly decontaminated on leaving the area. Messing and bivouacking in contaminated areas will be prohibited except when unavoidable. Such areas will be placarded and guards will be posted. Decontamination will be carried out as soon as possible.

COUNTERATTACK. Counterattack by all available means will be directed against the installations and weapons used in the attack. It will be borne in mind that in all cases there will be a period of a few hours to a few days following exposure before troops are incapacitated. During this period, and pending the receipt of specific orders from superior authority, commanders will carry on all activities essential to the established plan of battle.

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By J. A. Newlander, Lt Col CmlC
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TOP SECRET