Technical Aspects of Biological Weapon Proliferation

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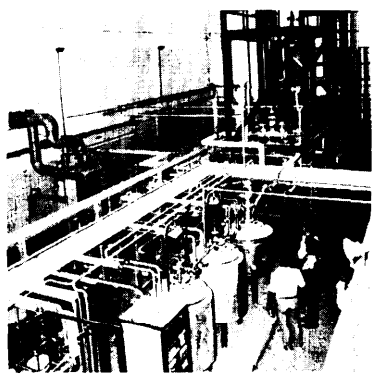
Biological and toxin warfare (BTW) has been termed "public health in reverse" because it involves the deliberate use of disease and natural poisons to incapacitate or kill people. Potential BTW agents include Living microorganisms such as bacteria, rickettsiae, fungi, and viruses that cause infection resulting in incapacitation or death; and toxins, nonliving chemicals manufactured by bacteria, fungi, plants, and animals. Microbial pathogens require an incubation period of 24 hours to 6 weeks between infection and the appearance of symptoms. Toxins, in contrast, do not reproduce within the host; they act relatively quickly, causing incapacitation or death within several minutes or hours.

The devastation that could be brought about by the military use of biological agents is suggested by the fact that throughout history, the inadvertent spread of infectious disease during wartime has caused far more casualties than actual combat.[1] Such agents might also be targeted against domestic animals and staple or cash crops to deprive an enemy of food or to cause economic hardship. Even though biological warfare arouses general repugnance, has never been conducted on a large scale, and is banned by an international treaty, BTW agents were stockpiled during both world wars and continue to be developed as strategic weapons—"the poor man's atomic bomb''—by a small but growing number of countries.[2]



[1] John P. Heggers, "Microbial Invasion—The Major Ally of War (Natural Biological Warfare)," *Military Medicine,* vol. 143, No. 6, June 1978, pp. 390-394.

[2] This study does not address the potential use of BTW agents by terrorist groups. For a discussion of this topic, see U.S. Congress, Office of Technology Assessment, Technology *Against Terrorism: The Federal Effort,* OTA-ISC-481 (Washington, DC: U.S. Government Printing Office, July 1991), pp. 21-22. See also Jessica Eve Stern, "Will Terrorists Turn to Poison?" *Orbis, vol. 37, No. 3,* summer 1993.

The Biological and Toxin Weapons Convention of 1972, signed and ratified by some 130 countries, bans the development, production, stockpiling, and transfer of BTW agents for warfare purposes. This treaty was weakened from the start, however, by the impossibility of banning research on BTW (agents, the fact that the development, production, and storage of BTW agents are permitted for defensive or peaceful purposes, and the absence of formal mechanisms for verification or enforcement.[3] It has also been alleged that key signatory states such as the former Soviet Union have systematically violated the treaty. According to a recent White House report, ''the Russian offensive biological warfare program, inherited from the Soviet Union, violated the Biological Weapons Convention through at least March 1992. The Soviet offensive BW program was massive, and included production, weaponization, and stockpiling. '

The biological disarmament regime has also come under growing pressure from the global spread of biotechnologies suitable for both civil and military applications, and from the revolution in genetic engineering, which has made it possible to manipulate the genetic characteristics encoded in the chemical structure of the DNA molecule. Soon after the publication in 1973 of techniques for cutting and splicing DNA molecules across species lines, a few concerned scientists worried that these powerful methods might be applied to develop new and more dangerous biological-warfare agents. Today, some defense planners believe that genetic engineering and other biotechnologies may eventually remove some of the military liabilities of BTW agents, increasing the attractiveness of these weapons to states of proliferation concern. It is not clear, however, that such techniques would significantly alter the military utility of BW agents compared with the numerous already known agents.

Given the perceived need to strengthen the Biological Weapons Convention (BWC), and the fact that the Chemical Weapons Convention (CWC) includes formal verification measures such as onsite inspections, a number of countries have proposed establishing a similar verification regime for the BWC. (See box 3-A, pp. 74-75. See also ch. 2 for discussion of procedures and technologies that might be used to detect the production of chemical weapons.) Nevertheless, verifying the nonproduction of biological weapons is inherently more difficult than for chemical weapons, for three reasons.

First, since BW agents are living microorganisms that reproduce inside the host, they are much more potent per unit weight. Thus, whereas CW agents must be stockpiled in the hundreds or thousands of tons to be militarily significant, a few kilograms of a BW agent such as anthrax bacteria could cause comparable levels of casualties. Such a small quantity of agent would be relatively easy to hide.

Second, whereas the production of CW agents requires certain distinctive precursor materials, reactions, and process equipment and leaves behind telltale chemical signatures, the production of BW agents involves materials and equipment that are almost entirely dual-use. As a result, it can be extremely difficult to distinguish illicit BW agent production from legitimate activities permitted under the BWC, such as the production of vaccines.

Third, because of the potency of BW agents and the exponential rate of microbial growth, a militarily significant quantity of BW agent could be produced in a matter of days in a small, easily

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[3] Some analysts worry that the Chemical Weapons Convention which was recently opened for signature and includes stringent verification measures, may create incentives for some proliferant countries to acquire biological rather than chemical arms-both because BTW agents can be produced in smaller, more concealable facilities, and because the Biological Weapons Convention currently lacks effective verification mechanisms.

[4] George Bush, "The President's Report to Congress on Soviet Noncompliance With Arms Control Agreements, " Jan. 14, 1993, p. 14.

concealed clandestine facility. All of these factors make the verification of compliance with the BWC a particularly challenging task.

This chapter provides technical background on the difficulty and detectability of BTW production and weaponization. The discussion covers the major technical hurdles involved in the acquisition of biological weapons and the associated ''signatures' that might be monitored to track their spread.

SUMMARY

Although biological and toxin weapons are often grouped together with chemical weapons, they differ in important ways. The most obvious difference is that whereas CW agents are man-made, nonliving poisons, biological agents are infectious microorganisms that reproduce within the host to cause an incapacitating or fatal illness. Toxins, being poisonous chemicals manufactured by living organisms, have characteristics of both chemical and biological agents.

Because of the ability of pathogenic microorganisms to multiply rapidly within the host, small quantities of a biological agent—if widely disseminated through the air-could inflict casualties over a very large area. Weight-for-weight, BTW agents are hundreds to thousands of times more potent than the most lethal chemical-warfare agents, making them true weapons of mass destruction with a potential for lethal mayhem that can exceed that of nuclear weapons. The lengthy incubation period of microbial pathogens places a major limitation on their battlefield utility, except in situations of attrition warfare, sabotage attacks against command and communications facilities deep behind enemy lines, or strikes against massed troops prior to their commitment to battle. Moreover, the delayed effects of biological weapons would not prevent their covert use against crops, livestock, or people as a means of crippling the economy and psychological morale of a targeted country.

Biological and toxin weapons potentially pose greater dangers than either chemical or nuclear weapons because BTW agents are so lethal on a pound-for-pound basis, their production requires a much smaller and cheaper industrial infrastructure, and the necessary technology and know-how are almost entirely dual-use and thus widely available. Despite the drawbacks of biological agents for tactical military use (e.g., delayed action, the dependence on meteorological conditions for their effectiveness, and the difficulty of precise targeting), they might be attractive as a strategic weapon-particularly for small, non-nuclear nations embroiled in regional conflicts or threatened by a nuclear-weapon state.

One technical hurdle to acquiring a militarily significant BTW capability is to ensure adequate containment and worker safety during production and weapon handling. It is also technically difficult to deliver biological agents to a target area so as to cause infection in a reliable and predictable manner. Although a supply of standard BTW agents for strategic attacks against wide-area civilian targets (e.g., cities) would be relatively easy to disseminate using crude delivery systems such as an agricultural sprayer, this means of delivery would be largely uncontrollable and subject to shifting atmospheric conditions. A more predictable-and hence more tactically useful-means of delivery against point targets on the battlefield would require extensive research, development, and testing. In particular, the integration of BTW agents into long-range delivery systems such as cluster bombs poses complex engineering hurdles-although these problems appear to have been solved for a few agents by the United States and the Soviet Union during the 1950s and 1960s.

There are no specific indicators, or "signatures," that can differentiate unambiguously between the development of offensive BTW agents and work on defensive measures such as vaccines, since both activities require the same basic know-how and laboratory techniques at the R&D stage. Moreover, certain types of civilian facili-

Box 3-A—The Debate Over BWC Verification

The Biological and Toxin Weapons Convention (BWC) of 1972 bans agents and delivery systems of types and in quantities that have no justification for prophylactic, protective or other peaceful purposes," yet the treaty does not define permitted activities more precisely and lacks any formal mechanisms for verifying compliance. At the time the BWC was negotiated it was considered politically impossible to obtain international support for onsite inspections and other intrusive verification measures. Since 1972, however, the emergence of genetic engineering and other novel biotechnologies has led to renewed concern over the seriousness of the biological and toxin warfare threat. Given the dual-use nature of the agents and equipment the feasibility of effective verification has been widely debated.

At the Second Review Conference of the BWC in 1986, the participating countries sought to build confidence in the treaty regime through an annual exchange of information on permitted activities and facilities that could be potentially associated with biological and toxin warfare. Additional confidence-building measures were adopted at the Third Review Conference in 1991. None of these measures are legally binding, however, and less than half of the parties to the treaty have participated to any extent in the data exchanges. At the Third Review Conference, several countries supported the drafting of a legally binding verification protocol to supplement the BWC that, inter alia, would require each Party to declare all treaty-relevant biological research and production facilities. The declarations would be confirmed by routine onsite inspections, supplemented by challenge inspections of undeclared facilities.

Proponents of a verification protocol argued that while a BWC verification regime could not provide absolute confidence in a country's compliance, it would serve to deter the proliferation of biological and toxin weapons by:

- imposing a risk of discovery and increasing the cost and difficulty of a clandestine program;
- providing opportunities for parties to demonstrate compliance, and enhancing confidence in the compliance of others;
- decreasing the number of sites of proliferation concern;
- providing an opportunity to act on national intelligence information without public disclosure of sensitive sources and methods;
- creating a legal framework for the conduct of challenge inspections; and
- reinforcing the international legal norm against the acquisition and use of BTW agents.[1]

The Bush administration, however, opposed the negotiation of a formal verification protocol on three grounds:

- the BWC could not be verified effectively because biological production facilities are dud-use and lack distinctive "signatures";
- a negotiated regime could not be sufficiently intrusive to detect clandestine facilities, generating false confidence that a country was in compliance with the treaty when in fact it was not; and
- highly intrusive inspections by multinational teams could expose both government and commercial facilities to foreign espionage. In particular, the loss of valuable trade secrets could weaken the competitive edge of the U.S. biotechnology and pharmaceutical industries?[2]

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1 Federation of American Scientists, Working Group on Biological and Toxin Weapons Verification, "Progress in Identifying Effective and Acceptable Measures for a compliance Protocol tithe Biological Weapons convention," working paper, May 1993.

2 Statement by Ambassador Ronald F. Lehman, II, Head of United States Delegation, Biological and Toxin Weapons Convention Third Review Conference, Sept. 10, 1991. Note that In signing the Chemical Weapons Convention (CWC), the U.S. Government has determined that the highly intrusive inspections mandated in that treaty do not pose an unacceptable risk to proprietary information or national security. However, the Inspections specified in the CWC to verify that chemical weapons are not being produced or stored wwid not necessarily be sufficient for the purposes of verifying the Biological Weapons Convention. Therefore, the fact that CWC Inspections have been judged worthwhile despite their potential for espionage does not automatically mean that proposed BWC inspections wwid be also be seen as acceptable. For a discussion of measures by which industry can protect itself from the ioss of proprietary information due to Chemical Weapons Convention declarations and inspections, see U.S. Congress, Office of Technology Assessment, *The Chemical Weapons Convention: Effects on the U.S. Chemical Industry*, OTA-BP-ISC-1O6 (Washington, DC: U.S. Government Printing Office, August 1993).

While the controversy over BWC verification has focused largely on technical issues, it is fundamentally a political debate over whether the burden of uncertainty associated with BWC verification would hamper more severely the verifier or the violator. Proponents of BWC verification argue that even imperfect monitoring measures would create a finite probability of detection that would have a significant deterrent effect on potential proliferants. Furthermore, a verification regime based on mandatory declarations of treaty-related sites and activities would deter the use of known facilities for BTW production, driving any violations into Clandestine facilities and thus making them more difficult and costly. Verification opponents counter, however, that an ineffective verification regime would create false confidence and hence would be worse than none at all.

There is also a semantic difference over the meaning of the term "verification." The U.S. Government uses this word in a narrow technical sense to mean the ability to detect violations within a specified regime with a high degree of confidence. In contrast, proponents of verification see it as the cumulative result of many sources of information, only some of which would be explicitly contained in a negotiated regime. Indeed, verification proponents admit that no negotiated inspection regime could detect clandestine facilities with a high degree of confidence. Instead, they argue, a formal verification regime would provide a legal instrument to permit inspections of suspicious facilities that have been detected by covert intelligence means. While many countries could be deterred from violating the treaty by a low probability of detection, some determined proliferants would require more intrusive measures.

Despite the Bush administration's opposition to a formal verification protocol for the BWC, it did agree to the establishment of an Ad Hoc Group of Government Experts to identify and evaluate various monitoring approaches from a scientific and technical standpoint. This verification experts (VEREX) group met twice in Geneva during 1992, from March 30 to April 10 and from November 23 to December 4. The focus of its activities was to prepare a list of 21 potential BTW verification technologies, divided into onsite and offsite categories. The onsite measures were exchange visits, inspections, and continuous monitoring; the offsite measures were information monitoring declarations, remote sensing, and inspections. Each of these verification measures was evaluated in terms of 6 criteria:

1. technical strengths and weaknesses, including the amount and quality of information provided;
2. ability to distinguish between prohibited and nonprohibited activities;
3. ability to resolve ambiguities about activities;
4. technology, material, manpower, and equipment requirements;
5. financial, legal, safety, and organizational implications; and
6. impact on scientific research, cooperation, industrial development, and other permitted activities, and implications for the protection of commercial proprietary information.[3]

To determine whether combining some measures would result in synergistic effects, a methodology was developed for assessing measures in combination. The results indicate **that the interaction of two or more measures may yield synergistic capabilities or limitations that are not present** when the measures are evaluated **in isolation.**

Between the first and second VEREX meetings, the U.S. position on BWC verification softened noticeably, and the new Clinton administration initiated a thorough review of its BTW nonproliferation policy. The VEREX group met again on May **24-** June 4, 1993 to evaluate the proposed verification measures. The group met a final time on September 13-24,1993 to prepare and adopt by consensus a final report to be forwarded to the States Parties to the BWC. This final report will provide the basis for a decision by a majority of the participating countries on whether to proceed with the negotiation of a legally binding verification protocol for **the BWC. If such a decision is made in the affirmative, a Preparatory Conference could take place in late 1994 followed by a Special** Conference in early 1995.

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3 Conference on Disarmament, *Final Declaration of the Third Review Conference of the BWC*, Part II, **document no. BWC/CONF.III/23, p. 17.**

ties will inevitably have the capacity to engage in illegal military production activities. Since excessive secrecy might be indicative of offensive intent, however, greater opemmess and transparency would tend to build confidence in a country's defensive intentions.

Advances in biotechnology have made it possible to produce militarily significant quantities of pathogens and toxins rapidly and in small, easily concealable facilities, greatly complicating the task of detecting BTW programs with national technical means of surveillance. To monitor clandestine programs, it is necessary to integrate data from many sources, with a particular emphasis on human intelligence: (agents and defectors).

Even though much of the equipment used to produce BTW agents is dual-use, this is not necessarily true of the agents themselves. Most microbial agents produced for peaceful purposes have no military utility, while those that do are made in very few places and in small quantities. Legitimate applications of dangerous pathogens and toxins (e.g., vaccine production and the use of toxins to treat neurological disorders and for experimental anticancer therapy) are relatively few at present, and are largely confinied to sophisticated biomedical facilities not normally found in developing countries (with the exception of a few vaccine production plants). Moreover, given the fact that the biotechnology industry is still in its infancy around the globe, the background of legitimate activities is still relatively small.

The weaponization of BTW agents entails field testing of biological aerosols, munitions, and delivery systems, as well as troop exercises, which might be detectable by satellite or other technical means of verification. Nevertheless, testing of microbial aerosols might be conceded or carried out at night or under the cover of legitimate dual-use activities, such as the application of biopesticides.

Despite growing concern over the military implications of genetic engineering, this technology is unlikely to result in 'supergerms" significantly more lethal or controllable than existing BW agents or capable of eliminating many of the uncertainties associated with the use of microbial pathogens in warfare. At the same time, however, gene-splicing techniques might facilitate the weaponization of microorganisms and toxins and enhance their operational effectiveness by rendering them more stable during dissemination (e.g., more resistant to heat, ultraviolet radiation, and shear forces) and insusceptible to standard vaccines and antibiotics. Moreover, genetic engineering techniques could be used to develop and produce more effective protective vaccines for the attacking forces.

In the past, most plant and animal toxins had to be extracted from biological materials in a costly and labor-intensive operation, but the ability to ''clone' protein toxin genes in bacteria has made it possible to produce formerly rare toxins in kilogram quantities. For the forseeable future, however, toxin-warfare agents are unlikely to provide dramatic military advantages over existing chemical weapons, although their greater potency makes it easier to transport and deliver militarily significant quantities. While it is possible that bioregulators and other natural body chemicals (or synthetic analogues thereof) might be developed into powerful incapacitants, the nontrivial problem of delivering such agents in a militarily effective manner would first have to be solved.

BIOLOGICAL AND TOXIN AGENTS

Just because a microorganism causes a serious disease does not make it a potential warfare agent. Of the several hundred pathogenic microbes that directly or indirectly afflict humans, only about 30 have been considered as likely warfare agents.[5]

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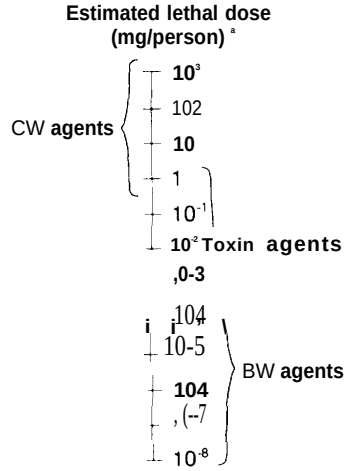
5 Department of the Army, U.S. Army Medical Research and Development Command, *Final Programmatic Environmental Impact Statement: Biological Defense Research Program,* RCS DD-M (AR) 1327 (Fort Detrick, MD: USAMRDC, 1989), p. A7-2.

Desirable characteristics of a biological agent developed for military use include:

- *the* ability to *infect reliably in* small doses;
- *high virulence,* or capacity to cause acute illness resulting in incapacitation or death, without experiencing an undue loss of potency during production, storage, and transport;
- *a short incubation period* between infection and the onset of symptoms;
- *minimal contagiousness* of the disease from one individual to another, to avoid triggering an uncontrolled epidemic that could boomerang against the attacker's population;[6]
- *no widespread immunity-either* natural or acquired-to the disease in the population to be attacked;
- *insusceptibility to common medical treatments,* such as generally available antibiotics;
- *suitability for economic production in militarily* significant quantities from available raw materials;
- *ease of transport,* and *stability* under wartime field conditions of storage and delivery;
- *ease of dissemination (e.g.,* as an aerosol cloud transmitted through the air);
- *ability to survive environmental stresses* during dissemination (e.g., heat, light, desiccation, and shear forces) long enough to infect; and
- *availability of protection* against the agent for the attacking troops, such as a vaccine, antibiotics, and/or protective clothing and respirators.[7]

Figure 3-1—Toxicity of CBW Agents



a paper clip weighs about 500 mg

SOURCE: Office of Technology Assessment, 1993,

BTW agents differ widely in infectiousness, length of incubation period, and lethality (see figure 3-l). A variety of them, including bacteria, rickettsiae, viruses, and toxins, were weaponized during the U.S. offensive BTW program, which was terminated in 1969. Brief descriptions of some typical BTW agents follow.

Bacteria

Bacteria are single-cell organisms that are the causative agents of anthrax, brucellosis, tularemia, plague, and numerous other diseases. They vary considerably in infectivity and lethality. The bacterium that causes tularemia, for example, is highly infectious. Inhalation of as few as 10 organisms causes disease after an incubation period of 3 to 5 days; if not treated, tularemia results in deep-seated pneumonia from which 30

[6] Some analysts have suggested that a country might deliberately develop contagious BW agents, which might be rendered insusceptible to any drugs that could be used to combat them. Japan, for example, developed plague-a highly contagious disease—as a BW agent during World War II. While contagious agents are commonly dismissed as too dangerous to use, they might convey a decisive military advantage on the attacker if (1) he could give an antidote or vaccine to his own population, (2) the agent was designed to attack crops or livestock specific to the target country, or (3) the agent could be delivered to a distant target by a long-range delivery system such as a ballistic missile. Although mass vaccination of the attacker's own population appears unlikely for logistical reasons, a ruthless aggressor-state might be willing to put its own population at risk.

[7] The effectiveness of defenses cannot be guaranteed, however. No vaccine is 100 percent effective, since even a strong immunity can be overwhelmed by inhaling a heavy dose of agent.

Box 3-B-Anthrax as a Biological-Warfare Agent

Anthrax, a severe illness caused by the bacterium *Bacillus anthracis,* is considered the prototypioal biological-warfare agent. In nature, anthrax is primarily a disease of cattle and sheep but can also infect humans. it can survive for long periods in soil in a dormant (spore) phase; after infection, it reverts to an active phase in which it mulitipiies rapidly in the body and secretes fatal toxins. Natural human infection can result either from skin contact with infected animals, ingestion of contaminated meat or inhalation of anthrax spores, usually from contaminated hides. Cases of pulmonary-and in some outbreaks gastrointestinal--anthrax are almost invariably fatal if not **treated immediately with antibiotics. Inhalation of aerosolized spores would be the primary route of infection if the bacteria were used deliberately as a biological-warfare agent. As extrapolated from animal studies, inhalation of about 1,000 spores or less can produce fatal pulmonary anthrax** in some members of an exposed **population,** while inhalation of about 8,000 spores-weighing about 0.08 microgram-is fatal within less than a week to a large proportion of those exposed.[1]

After inhalation into the lungs, anthrax spores travel to the lymph nodes of the chest, where they become active, multiplying and releasing three proteins-edema factor, lethal factor, and protective antigen. in specific combinations, these proteins function as potent toxins, enabling the bacteria to resist host defenses and to invade and damage host tissues via the bloodstream, resulting in uncontrollable hemorrhaging. in this manner, anthrax bacteria travel to the intestines and other areas, where they cause severe tissue damage. Initial signs of pulmonary anthrax infection include a high fever, labored breathing, choking cough, and vomiting; it is usually fatal within 4 days.[2] Although infections may respond to immediate antibiotic therapy, it is relatively easy to develop antibiotic-resistant anthrax strains.

in addition to its lethality, anthrax has other characteristics that make it an effective BW agent. First the disease is not contagious from one individual to another. As a result, anthrax would not spread far beyond the intended target or boomerang against the attacker's troops or civilian Population, assuming they do not enter a contaminated area Second, anthrax is easy to produce. The organism and its spores can be readily produced

1 Testimony by Barry J. Erlick, Biological Weapons Analyst, Department of the Army, in U.S. Senate, Committee on Governmental Affairs, *Global Spread of Chemical and Biological Weapons: Assessing Challenges and Responses,* 101st Congress, First Session, Feb. 9, 1989 (Washington, DC: U.S. Government Printing Office, 1990), p. 32.

2 Philip J. Hilts, "U.S. and Russian Researchers Tie Anthrax Deaths to Soviets," *New York Times,* Mar. 15, 1993, p. A6.

to 60 percent of victims die within 30 days.[8] Brucellosis, another bacterial disease, has a low mortality rate-about 2 percent-but an enormous capacity to inflict casualties. Infection gives rise to fever and chills, headache, loss of appetite, mental depression, extreme fatigue, aching joints, and sweating.[9] The bacterial agent that has received the most attention is anthrax, whose pulmonary form is highly lethal. (See box 3-B,) Under certain environmental conditions, anthrax bacteria will transform themselves into rugged spores that are stable under a wide range of conditions of temperature, pressure, and moisture. One gram of dried anthrax spores contains more than 10^{11} particles; since the lethal dose by inhalation in monkeys is between l@ and l@ spores, a gram of anthrax theoretically contains some 10 million lethal doses.

8 Testimony by Barry J. Erlick, Biological Weapons Analyst, Department of the Army, in U.S. Senate, Committee on Governmental Affairs, *Global Spread of Chemical and Biological Weapons: Assessing Challenges and Responses,* 101st Cong., 1st sess., Feb. 9, 1989 (Washington DC: U.S. Government Printing Office, 1990), p. 32,

9 J. H. Rothschild, *Tomorrows Weapons: Chemical and Biological (New* York, NY: McGraw-Hill, 1964), P. 212.

in the laboratory in almost unlimited quantities, and antibiotic-resistant strains have been developed with standard selection techniques. [3]

Third, when anthrax bacteria are incubated under particular conditions, they transform themselves into the rugged spore form, which has along shelf-life. Although most spores can be killed by boiling for 10 minutes, they can survive for up to 20 years or longer in soil and animal hides.[4] This spore-forming ability makes anthrax particularity well suited for delivery by missiles or bombs. The spores are stable when suspended in air, can survive explosive dissemination from a bomb or shell, and-unlike most pathogens-will live for several days if direct sunlight is avoided. Indeed, fieldtest data have shown that anthrax spores decay at a rate of less than 0.1 percent per minute, which is very slow for a microorganism.[5]

Nevertheless, anthrax has certain liabilities as a tactical weapon. First, at lower doses there is a wide spread in incubation times, ranging from a few days to several weeks, suggesting that the spore germinations that result in infection can be delayed for considerable periods.[6] This variability greatly reduces the predictability and hence the military utility of the agent. Second, anthrax spores are so persistent that they can contaminate an area for long periods, denying it both to defender and attacker. During World War II, for example, Britain detonated experimental anthrax bombs on Gruinard Island off the coast of Scotland, releasing spores that remained in the top 6 to 8 inches of soil for more than 40 years.[7] By infecting livestock, anthrax bacteria might also create new reservoirs of disease that could result in occasional outbreaks, making it impossible to use the affected area productively for long periods.[8] That might be the desired intent, however, were anthrax to be used as a strategic weapon.

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3 World Health Organization, *Health Aspects of Chemical* and *Biological Weapons* (Geneva: WHO, 1970), p. 74.

4 Donald Kaye and Robert G. Petersdorf, "Anthrax," Eugene Braunwald et al., eds., *Harrison's Principles of Internal Medicine,* 1 Ith ed. (New York, NY: McGraw Hill, 1987), p. 557.

5 World Health Organization, op. cit., footnote 3, p. 94.

6 presentation by Matthew Meselson, Harvard University, at Seminar on Biological Weapons in the 1990s, sponsored by the Center for Strategic and International Studies, Washington, DC, Nov. 4, 1992.

7 These explosive anthrax bombs were crude and inefficient in creating an aerosol cloud composed of small particles. Instead, the bombs compacted the spores into the ground. Effective BW munitions would not do this. William C. Patrick III, former program analysis officer, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, personal communication, 1992.

8 World Health Organization, op. cit., footnote 3, p. 75.

| Rickettsiae

Rickettsiae are microorganisms that resemble bacteria in form and structure but differ in that they are intracellular parasites that can only reproduce inside animal cells. Examples of rickettsial diseases that might be used for biological warfare include typhus, Rocky Mountain spotted fever, Tsutsugamuchi disease, and Q fever. Rickettsiae have a wide variety of natural hosts, including mammals and arthropods such as ticks, fleas, and lice. If used as BW agents, however, they would probably be disseminated directly through the air.

| Viruses

Viruses are intracellular parasites that are about 100 times smaller than bacteria. They can infect humans, crops, or domestic animals. Viruses consist of a strand of genetic material (DNA or RNA) surrounded by a protective coat that facilitates transmission from one cell to another. The Venezuelan equine encephalitis (VEE) virus causes a highly infectious disease that incapacitates but rarely kills. In contrast, some hemorrhagic fever viruses, such as Lassa or Ebola fever, are exceedingly virulent, killing 70 out of every 100 victims. The AIDS virus, despite its lethality, would not be an effective warfare agent because

its mean incubation period of 10 years is too slow to give it any tactical or strategic value in warfare, and because it cannot be transmitted through the air.

Fungi

Fungi do not generally cause disease in healthy humans, although the fungus *Aspergillus,* which infects by inhalation, can cause serious opportunistic infections in people with a weakened immune system. A few other fungi, such as *Coccidioides immitis* and *Histoplasma capsulatum, also* infect naturally by inhalation and can cause severe pulmonary infections in susceptible individuals, but they have never been considered as potential BW agents. Fungal diseases are, however, devastating to plants and might be used to destroy staple crops and cause widespread hunger and economic hardship. Examples of plant fungal pathogens include rice blast, cereal rust, and potato blight, which can cause crop losses of 70 to 80 percent.

Toxins

A toxin is a poisonous substance made by a living system, or a synthetic analogue of a naturally occurring poison. An enormous variety of toxins are manufactured by bacteria, fungi, marine organisms, plants, insects, spiders, and animals, and more than 400 have been characterized to date. Such toxins can exert their effects by three different routes of exposure-injection, ingestion, and inhalation-and their potency derives from their high specificity for cellular targets. For example, many toxins bind to specific sites in nerve membranes, disrupting the transmission of nerve impulses and causing fatal respiratory paralysis. Other toxins selectively block cellular protein synthesis or other vital physiological functions.

From a chemical standpoint, there are two categories of toxins: protein toxins, which consist of long folded chains of amino acids; and nonprotein toxins, which tend to be small but complex molecules.

PROTEIN TOXINS

Most bacterial toxins, including those associated with cholera, tetanus, diphtheria, and botulism, are large proteins. For example, various strains of *Staphylococcus aureus,* a major bacterial pathogen, secrete protein toxins that cause severe nausea, vomiting, and diarrhea lasting from 1 to 2 days. The United States developed one of these toxins, *Staphylococcus* enterotoxin B (SEB), as a warfare agent in the 1960s. Spray-dried SEB, when disseminated through the air in aerosol form, causes a chemical pneumonia that is more debilitating than the toxin's gastrointestinal effects when ingested; it can incapacitate exposed troops within hours, with recovery in 4 to 6 days.[10] Botulinal toxin, secreted by the soil bacterium *Clostridium botulinum,* is the most poisonous substance known. The fatal dose of botulinal toxin by injection or inhalation is about 1 nanogram (billionth of a gram) per kilogram, or about 70 nanograms for an adult male.11 The toxin is also relatively fast-acting, producing death between 1 and 3 days in 80 percent of victims. The U.N. inspections of Iraq after the Gulf War confirmed that the microbiological research facility at Salman Pak had done development work on botulinum toxin as a potential warfare agent. Nevertheless, attempts to weaponize botulinal toxin have in the past failed to prevent extensive loss of toxicity that accompanies dispersion.

Ricin, a plant toxin derived from castor beans, irreversibly blocks cellular protein synthesis and is lethal when inhaled in a dose of about 10 micrograms (millionths of a gram) .12 Castor

10 William C. Patrick III, former program analysis officer, U.S. Army Medical Research Institute for Infectious Diseases, Fort Detrick, MD, personal communication, 1993.

11 D. M. Gill, "Bacterial Toxins: A Table of Lethal Amounts," *Microbiological Reviews,* March 1982, pp. 86-94.

12 G. A. Balint, "Ricin: The Toxic Protein of Castor Oil Seeds," *Toxicology,* vol. *2, 1974, p.* 80.

beans are widely cultivated as a source of castor oil, which has numerous legitimate industrial applications. The paste remaining after the oil has been pressed out contains about 5 percent ricin, which can be purified by biochemical means. During World War II, several countries studied ricin as a potential chemical-warfare agent, and the British developed and tested an experimental ricin weapon known as the "W bomb," although it was not ultimately deployed.[13] In September 1978, the Bulgarian secret police (with technical assistance from the Soviet KGB) assassinated Georgi Markov, an exiled Bulgarian dissident living in London, by firing a tiny metal ball filled with ricin into his thigh from a pellet-gun concealed inside an umbrella; Markov died two days later.[14] According to published reports, Iran has acquired 120 tons of castor beans and is allegedly purifying ricin in pharmaceutical plants.[15]

NONPROTEIN TOXINS

Nonprotein toxins are small organic molecules that often have a complex chemical structure. They include tetrodotoxin @educed by a puffer fish), saxitoxin (made by marine algae known as dinoflagellates, which are taken up and concentrated by clams and mussels), ciguatoxin and microcystin (synthesized by microscopic algae), palytoxin (made by a soft red Hawaiian coral), and batrachotoxin (secreted by poisonous frogs indigenous to western Colombia). Typical characteristics of nonprotein toxins are high toxicity, the absence of antidotes, heat stability (unlike most protein toxins), resistance to other environmental factors, and speed of action. Saxitoxin, for example, produces initial symptoms within 30 seconds after ingestion and can cause labored breathing and paralysis in as little as 12 minutes. There is no known prophylaxis or therapy, and the lethal dose in 50 percent of those exposed maybe as low as 50 micrograms, a potency 1,000 times greater than the chemical nerve agent VX.[16]

Trichothecene mycotoxins are a family of about 100 poisonous compounds manufactured by certain strains of the mold *Fusarium* that grow on wheat, millet, and barley. When ingested by people or livestock, these toxins kill rapidly dividing cells such as those of the bone marrow, skin, and the lining of the gastrointestinal tract; they also block certain clotting factors in the blood, causing severe bleeding after injury. In aerosol form, about 35 milligrams of the trichothecene mycotoxin T-2 can kill a 75-kilogram man; unlike most other toxins, it is also absorbed through the skin. Although mycotoxins are significantly less potent than chemical-warfare agents such as VX, they are relatively easy to produce and are highly stable.

In 1982, the Reagan administration alleged that the Soviet Union and its allies were using a toxin-warfare agent in Southeast Asia known as "yellow rain" whose active ingredients were trichothecene mycotoxins.[17] The Soviets denied the allegations, and the United States was unable to provide convincing public evidence to back up its charges in the face of criticism on the part of

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13 Stockholm International Peace Research Institute, *The Problem of Chemical and Biological Weapons, Vol. I: The Rise of CB Weapons* (Stockholm: Almqvist & Wiksell, 1971), p. 123.

14 Robert Harris and Jeremy Paxman, *A Higher Form of Killing: The Secret Story of Gas and Germ Warfare* (London: Chatto & Windus, 1982), pp. 197-198; David Wise, "Was Oswald a Spy, and Other Cold War Mysteries," New *York Times Magazine,* Dec. 6, 1992, p. 44.

15 Douglas Wailer, "Sneaking in the Scuds," *Newsweek,* June 22, 1992, p. 42.

16 Erlick, op. cit., footnote *8,* p. 32, See also B.J. Benton and F.C.T. Chang, "Reversal of Saxitoxin-Induced Cardio-Respiratory Failure by Burro IgG Antibody and Oxygen Therapy," *Proceedings of the 1991 Medical Defense Bioscience Review (Fort* Detrick, *MD:* U.S. Army Medical Research Institute of Chemical Defense, Aug. 7-8, 1991), p. 176.

17 U.S. Department of State, *Chemical Warfare in Southeast Asia and Afghanistan, Special* Report No. *98,* Mar. 22, 1982, P. 30.

many in the U.S. scientific community.[18] Following early reports of the presence of trichothecenes in samples from alleged attacks, the U.S. Army and the U.K. Ministry of Defense initiated large analytical studies but were unable to confirm the early findings.[19] Nonetheless, U.S. intelligence officials, based on all the: information available to the U.S. intelligence community, remain confident in the yellow rain allegations and have not retracted them.

Compared to microbial pathogens, toxins offer the following tactical advantages:

- The most toxic toxins (e.g., botulinal toxin) are exceedingly potent, so that small, easily transportable quantities would be militarily significant for certain missions.
- Toxins tend to deteriorate rapidly once released into the environment, whereas anthrax spores and persistent chemical agents can centaminate soil for months or years. For this reason, territory attacked with toxin agents could be occupied more rapidly by attacking forces.
- Toxins are well suited to covert warfare. Whereas chemical agents leave telltale degradation byproducts that persist for long periods in the environment, some toxin agents break down completely over a period of weeks or months, leaving no traces. Moreover, even fresh samples of toxin might not provide conclusive evidence of military use if the agent occurred naturally in the region where it was employed.

Despite these operational advantages, however, toxins have drawbacks for battlefield use:

- Protein toxins such as botulinal toxin decompose rapidly on exposure to sunlight, air, and heat, and thus would have to be used at night.
- Protein toxins may be inactivated by the mechanical shear forces caused by passage through an aerosol sprayer.[20] While low-molecular-weight toxins such as saxitoxin and trichothecene mycotoxins are more stable than protein toxins, they are less stable than chemical-warfare agents.
- Most toxins (with the exception of trichothecene mycotoxin T-2) do not penetrate the skin, nor would toxin lying on the ground create a vapor hazard.[21] Weaponization therefore requires the creation of a small-particle aerosol cloud, in which the toxin must remain airborne to be effective.
- The inhalation threat posed by protein toxins such as botulinal toxin can be countered effectively with modern gas masks (although a surprise or covert attack might expose personnel to lethal concentrations before they could don their masks).

For conventional battlefield use, toxins offer few military advantages over chemical nerve agents. Toxins would, however, probably be superior for small-scale clandestine operations.

ACQUIRING A BTW CAPABILITY

The acquisition of a militarily significant BTW capability would probably involve the following steps (see figure 3-2):

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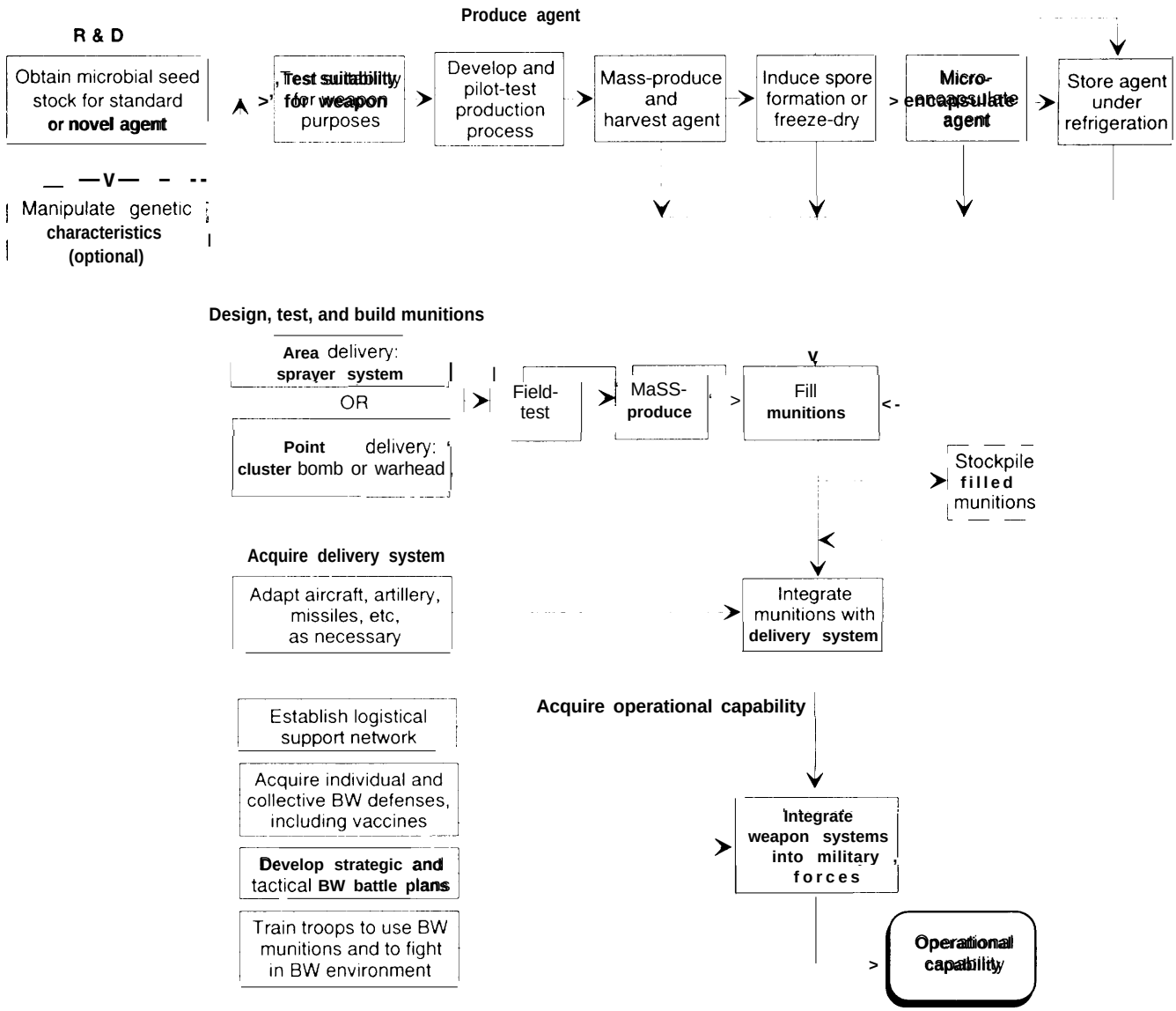
18 Julian Robinson, Jeanne Guillemin, and Matthew Meselson, "Yellow Rain in Southeast Asia: The Story Collapses," Susan Wright, cd., Preventing a *Biological Arms Race* (Cambridge, MA: MIT Press, 1990), pp. 220-238. See also U.S. Senate, Committee on Foreign Relations, Subcommittee on Arms Control, Oceans, International Operations, and Environment, 98th Cong., 1st sess., *Yellow Rain: The Arms Control Implications,* Feb. 24, 1983 (Washington DC: U.S. Government Printing Office, 1983).

19 Robinson et al., op. cit., footnote 18, pp. 228-229.

20A few protein toxins are quite stable: SEB in milk, for example, is not destroyed after boiling for 30 minutes. In addition, the stability of protein toxins can be increased through a technique known as microencapsulation (see production section below), David S. Huxsoll, former director, U.S. Army Medical Research Institute for Infectious Diseases, personal communication, 1992.

21 Shirley Freeman, "Disease as a Weapon of War," *Pacific Research,* vol. 3, February 1990, p. 5.

Figure 3-2—Biological Weapon Acquisition



SOURCE: Office of Technology Assessment, 1993.

1. Establishment of one or more facilities and associated personnel with organizational and physical provisions for the conduct of work in secret;
2. Research on microbial pathogens and toxins, including the isolation or procurement of virulent or drug-resistant strains;
3. **Pilot** production of small quantities of agent in flasks or small fermenter systems;
4. Characterization and military assessment of the agent, including its stability, infectivity, course of infection, dosage, and the feasibility of aerosol dissemination;

5. Research, design, development, and testing of munitions and/or other dissemination equipment;
6. Scaled-up production of agent (possibly in several stages) and freeze-drying;
7. Stabilization of agent (e.g., through microencapsulation) and loading into spray tanks, munitions, or other delivery systems; and
8. Stockpiling of filled or unfilled munitions and delivery vehicles, possibly accompanied by troop training, exercises, and doctrinal development. (In some but not all cases, a country planning the offensive use of BTW agents would take measures to protect its own troops, such as immunization, the acquisition of respirators, and training in self-protective measures.)

The key steps in this acquisition sequence are examined below in terms of their technical difficulty.

| Research, Development, and Weaponization

Countries seeking a BTW capability are likely to start with the development of standard agents that have been weaponized in the past, such as anthrax, tularemia, and botulinum toxin. Nearly all proliferant states lack the sophisticated scientific and technological infrastructure needed to develop novel agents such as exotic viruses, whose military characteristics are poorly understood.

BTW agents are widely accessible. Pathogenic microorganisms are indigenous to many countries and can be cultured from infected wild animals (e.g., plague in rodents), living domestic animals or infected remains (e.g., Q fever in sheep, anthrax in cattle), soil in endemic areas (which may contain trace amounts of anthrax bacteria and other pathogens), and spoiled food. Certain biological supply houses also ship strains of microbial pathogens to scientists throughout the world. For example, American Type Culture Collection (ATCC), a nonprofit company in Rockville, MD, acts as a clearinghouse for research institutions around the world, shipping each year approximately 130,000 cultures of weakened (''attenuated") pathogens to 60 nations.[22] While such attenuated strains are not virulent and hence could not be converted directly into biological weapons, they would be useful for BTW research and development and for preparing self-protective vaccines. Methods for culturing organisms and for inducing spore formation are also described in the open scientific literature, and standard microbiological procedures can be used to produce more virulent or antibiotic-resistant strains of microbial pathogens.

Once a proliferant had acquired BTW agents, they might be modified genetically through simple selection techniques to increase their virulence or effectiveness. For example, incubating microbial pathogens in the presence of standard antibiotics can induce the emergence of drug-resistant strains, which can then be subcultured and mass-produced. Agent development would also involve "weaponization," or a thorough assessment of the agent military potential, including its stability, infectivity, course of infection, and effective dosage. This step would include the testing of candidate agents to determine their effectiveness, including the feasibility and reliability of aerosol dissemination. Such tests might be carried out either in a sealed aerosol chamber or in field studies of simulant microorganisms at a remote testing range.

THE DUAL-USE DILEMMA

A fundamental problem in countering the proliferation of biological and toxin weapons is the fact that much of the necessary know-how and technology is dual-use, with legitimate applications in the commercial fermentation and biotechnology industries. Many developing countries

22 Eric Nadler and Robert Windrew, ''Deadly Contagion,'' *The New Republic,* vol. *204, No. 5,* Feb. 4, 1991, p. 18.

have acquired industrial microbiology plants for the production of fermented beverages, vaccines, antibiotics, ethanol (from corn or sugar cane), enzymes, yeast, vitamins, food colors and flavorings, amino acids, and single-cell protein as a supplement for animal feeds.[23] This global expansion of the civilian biotechnology industry, combined with the growing number of molecular biotechnologists trained in the West, has created much broader access to the expertise and equipment needed for the development of BTW agents. Sophisticated laboratories that might be used for the design of novel BW agents are inexpensive compared with nuclear weapon plants. Moreover, biotechnology is information-intensive rather than capital-intensive, and much of the relevant data **are** available in the published scientific literature. For these reasons, it is virtually impossible for industrialized states to prevent the diffusion of weapon-relevant information to states of proliferation concern.

It has been estimated that more than 100 countries have the capability-if not necessarily the intent-to develop at least crude biological weapons based on standard microbial and toxin agents.[24] In addition to the United States, Russia, Western Europe, and Japan, countries with an advanced commercial biotechnology infrastructure include Argentina, Brazil, Chile, Cuba, India, Israel, the People's Republic of China, Taiwan, and Thailand. Cuba, in particular, has an advanced biotechnology industry that exports vaccines and reagents to other Latin American countries .[25] While Iraq lags somewhat behind this group of countries, Baghdad established a national Center for Genetic Engineering and Biotechnology in the late 1980s, initially staffed with only four scientists.[26] As an increasing number of developing countries become involved in commercial biotechnology, they may be tempted to explore its military potential.

In addition, the legitimate use of toxins for medical therapy and biomedical research is increasingly widespread. Botulinal toxin, for example, is used to treat abnormal muscle spasms known as dystonias by selectively paralyzing the spastic muscles; it has also been applied cosmetically to smooth wrinkles.[27] Toxins such as ricin, when linked to antibodies that selectively target cancer cells, have shown promise in clinical trials as an anticancer therapy.[28] Furthermore, saxitoxin and other exotic toxins that bind specifically to channels or receptors in nerve-cell membranes are valuable research tools in neuroscience. The inherently dual-use nature of many pathogens and toxins makes the prevention of BTW-relevant research extremely difficult. Consumption of toxins for medical therapy and research has already expanded to the current level of hundreds of grams per year, and the anticipated further growth of such therapies will eventually blur the

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23 For an overview, see U.S. Congress, Office of Technology Assessment, Biotechnology *in a Global Economy,* OTA-BA-494 (Washington, DC: U.S. Government Printing Office, October 1991).

24 Testimony of Thomas Welch, Deputy Assistant Secretary of Defense (Chemical Matters), reported in *Defense Week,* May 9, 1988.

25 Raymond A. Zilinskas, "Biological Warfare and the Third World," *Politics and the Life Sciences,* vol. 9, No. 1, August 1990, p. 61.

26 Presentation by Raymond Zilinskas, Washington Strategy Seminar, Washington, DC, July 14, 1992.

27 *Tom Waters,* 'The Fine Art of Making Poison,'' *Discover,* vol. 13, No. 8, August 1992, p. 32; and Anna Evangeli, ' 'Botulism Gives Faces New Lease of Life, " New *Scientist,* vol. 137, No. 1859, Feb. 6, 1993, p. 18. See also Fritz P. Gluckstein and Mark Hallet, *Clinical Use of Botulinum Toxin: January 1987 through September 1990, 318 Citations* (Washington DC: National Library of Medicine/U.S. Government Printing Office, 1990).

28 David Fitzgerald and Ira Pastan, ''Targeted Toxin Therapy for the Treatment Of Cancer, " *Journal of the National Cancer Institute,* vol. 81, No. 19, October 1989, pp. 1455-1463; Andrew A. Hertler and Arthur E. Frankel, "Imrnunotoxins: A Clinical Review of Their Use in the Treatment of Malignancies," *Journal of Clinical Oncology,* vol. *7, No. 12,* December 1989, pp. 1932-1942; Lee H. Pai and Ira Pastan, ''Immunotoxin Therapy for Cancer,' *Journal of the Amen'can Medical Association,* vol. 269, No. 1, Jan. 6, 1993, pp. 78-81.

distinction between medically useful and militarily significant quantities of toxins. 29 The legitimate applications of toxins will therefore have to remain relatively open to preclude their use for illicit purposes.

Finally, the development of defenses against BTW attack-an activity explicitly permitted by the Biological Weapons Convention-draws on much of the same knowledge base needed to develop offensive BTW agents. Indeed, "threat assessment, an important aspect of some biological-defense programs, includes the evaluation of defenses under simulated warfare conditions and may be indistinguishable from the development of offensive BTW agents .30 Furthermore, certain defensive activities may have offensive applications. According to one assessment, ''the virulence of micro-organisms is studied both for its relevance to the field of natural infections and in order to produce living, attenuated vaccines. Such knowledge can obviously be used more or less directly to make a BW agent more virulent."[31] For these reasons, biological-defense activities such as the development of vaccines may arouse concerns about offensive intentions unless they are conducted openly and in an unclassified environment..

In sum, research on potential BTW agents does not necessarily imply an offensive weapon program because much of the relevant knowledge is multiuse. This inherent ambiguity means that at the R&D stage, the only difference between offensive and defensive activities is one of *intent. The* policy dilemma is that progress in controlling infectious diseases requires the free and open flow of information, so that researchers can build on and validate the work of others; imposing controls on the publication of results with potential military implications would seriously impede legitimate scientific research worldwide. Nevertheless, openness may impose some limits on the misuse of biomedical research for malicious purposes.

| Large-Scale Production

BTW agents would be relatively easy and inexpensive to produce for any nation that has a modestly sophisticated pharmaceutical or fermentation industry. Indeed, mass-production methods for growing pure cultures are widely used in the commercial production of yogurt, yeast, beer, antibiotics, and vaccines. Nearly all the equipment needed for the production of pathogens and toxins is dual-use and widely available on the international market, increasing the potential for concealing illicit activities under the cover of legitimate production. Whereas a typical vaccine production facility costs a minimum of $50 million, a much less elaborate industrial fermentation plant suitable for conversion to BTW agent production could be built for about $10 million.[32] In such a' 'no-frills' facility, bacteria could be grown in standard dairy tanks, brewery fermenters, or even in the fiberglass tanks used by gas stations.

In contrast to chemical-warfare (CW) agents, no specialized starting materials are required for the production of biological and toxin agents except for a small seed stock of a disease-producing organism. Nutrients such as fermentation medium, glucose, phosphates, peptone, and a protein source (e.g., casein, electrodialyzed whey, or beef bouillon) are widely available and are routinely imported by developing countries that have commercial fermentation industries. A state seeking a CW capability, in contrast, re-

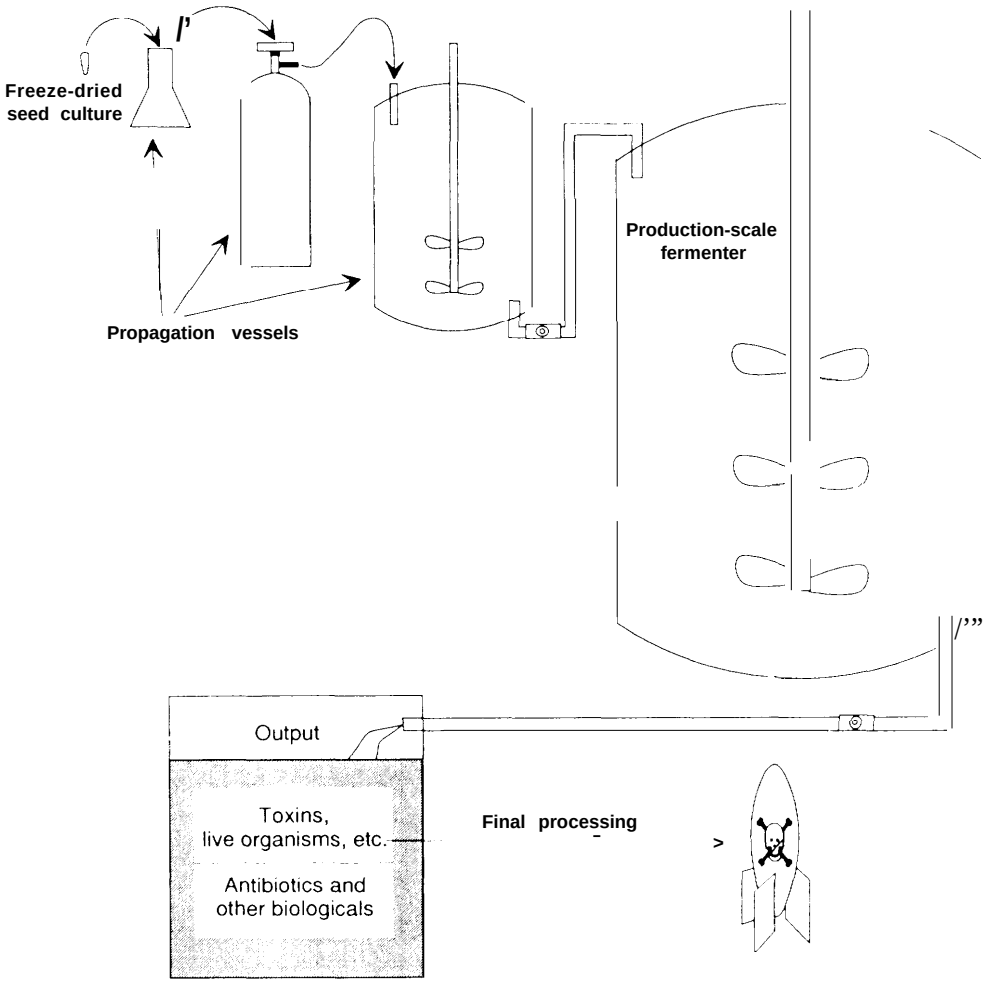
29& P. Zelicoff, Senior Member, Technical Staff, Sandia National Laboratories, personal communication, 1992.

30 See Susan Wright and Stuart Kc@@ ''The Problem of Interpreting the U.S. Biological Defense Research *program,'* Susan Wright, cd., *Preventing a Biological Arms Race* (Cambridge, MA: MIT Press, 1990), pp. 167-196.

31 Stockholm International Peace Research Institute (SIPRI), *The Problem of Chemical and Biological Warfare, Vol. VI: Technical Aspects of Early Warning and Verification* (Stockholm: Almqvist & Wiksell, 1975), p. 24.

32 Interview with Dr. Ron Thibeautot, Wyeth-Ayerst vaccine production plant, Marietta, pA, Oct. 22, 1992.

Figure 3-3-Production of Biological Agents by Fermentation



SOURCE: U.S. Senate, Committee on Governmental Affairs, Global *Spread of Chemical and Biological Weapons*, 101st Cong., 1st sess., Feb. 9, 1989 (S. Hrg. 101-794), p. 241.

quires hundreds or thousands of tons of unusual precursor chemicals that may be difficult to obtain.

PRODUCTION OF BACTERIAL AGENTS

A biological-warfare plant would contain fermenters and the means to sterilize and dispose of hazardous biological wastes on a large scale. A small vial of freeze-dried seed culture, grown in a fermenter in a nutrient medium kept at constant temperature, can result in kilograms of product (e.g., anthrax bacteria) in as little as 96 hours.[33] (See figure 3-3.) Microbial pathogens such as plague bacteria can also be cultivated in living animals, ranging from rats to horses.

Fermentation can be carried out on a batch basis or in a continuous culture from which organisms are constantly removed and an equal volume of new culture medium is added. An advantage of continuous culture is shorter turna-

[33] Erlick, op. cit., foomote 8, p. 32.

round time, increasing the productivity of each fermenter. Indeed, if nutrients are supplied continuously and natural growth-inhibitors are removed as soon as they are formed, the bacterial culture can be maintained indefinitely in a phase in which it multiples exponentially. A continuous culture can therefore yield nearly 10 times as much product per volume of culture medium as the batch approach.[34] Nevertheless, batch culture has generally been used to cultivate BW agents in the past because continuous culture is technically more complex and sometimes results in a loss of potency. High levels of purity are not required for BW agents; 60 to 70 percent purity will suffice and is easy to obtain. The main technical hurdles in bacterial production are:

- the danger of infecting production workers;
- genetic mutations that may lead to a loss of agent potency; and
- the contamination of bacterial cultures with other microbes (e.g., bacterial viruses) that may kill them or interfere with their effects.

Although biological agents can be grown in ordinary laboratory flasks, an efficient production capability would require the use of specialized fermenters. Until fairly recently, large-scale production of bacteria for commercial or military purposes required tank-type bioreactors containing thousands of liters of culture, with mechanical stirring or a flow of air to oxygenate the culture medium. During World War II, for example, the Japanese Army ran a top-secret BTW facility in occupied Manchuria at which more than 3,000 workers grew kilogram quantities of pathogenic bacteria (including the agents of anthrax, brucellosis, plague, and typhus) in giant vats.[35]

Also during World War II, the United States and Britain planned to produce anthrax bacteria in large quantities for use in a strategic bombing campaign against Germany. In 1943, a pilot anthrax production plant became operational at Camp Detrick, MD, staffed with about 500 bacteriologists, lab assistants, chemical engineers, and skilled technicians.[36] Based on this experience, the decision was made to build a fill-scale plant at Vigo, Indiana, at a cost of $8 million, where 1,000 workers would manufacture more than 500,000 anthrax bombs a month (or, alternatively, 250,000 bombs filled with botulinal toxin). Since both agents store well, they could be stockpiled in large quantities. The Vigo plant was completed in early 1945 but never actually went into production.[37] *Although it is* far *from* certain the anthrax bombs would have worked as designed, it is possible that large areas of Germany could have been rendered uninhabitable for decades.

In 1950, the U.S. Congress voted $90 million to build another BTW plant called X-201 at a renovated arsenal near Pine Bluff, Arkansas. The new production facility had 10 stories, 3 of them underground, and was equipped with 10 fermenters for the mass-production of bacterial pathogens on short notice.[38] To give some idea of the scale involved, the Pine Bluff facility and its associated munitions-falling plant required a water supply of 2 million gallons per day, an electrical power supply of 5 megawatts, and an initial workforce of 858 people.[39] Production of BW

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34 SIPRI, vol. VI, op. cit., footnote 31, p. 43.

35 The Japanese BTW facility was code-named Unit 731. John W. Powell, "A Hidden Chapter in History," *Bulletin of the Atomic Scientists*, vol. 37, No. 8, October 1981, pp. 44-52. For a more detailed description, see Peter Williams and David Wallace, *Unit 731: Japan's Secret Biological Warfare in World War II* (New York, NY: Free Press, 1989).

36 and Paxman, op. cit., footnote 14, pp. 1(X L101.

37 Ibid., p. 103.

38 Ibid., p. 160.

39 Matthew Meselson, Martin M. Kaplan, and Mark A. Mokulsky, "Verification of Biological and Toxin Weapons Disarmament," *Science & Global Security*, vol. 2, Nos. 2-3, 1991, p. 237.

agents on such a vast scale is far in excess of what a country would need to wreak enormous destruction on an adversary.

Over the past decade, technological advances associated with the commercial biotechnology industry have made it possible to produce large quantities of microorganisms in much smaller facilities. The introduction of computer-controlled, continuous-flow fermenters and compact ultrafiltration methods has vastly increased productivity, making it possible to reduce the size of a fermenter to about 1,000-fold less than conventional batch fermenters that give equivalent production.[40] Real-time sensors and feedback loops under microprocessor control have also optimized culture conditions, resulting in much higher yields and better quality products than in the past. The resulting increase in productivity has made it possible to reduce the amount of trained manpower needed to operate large-scale fermenters and to use smaller, more concealable production equipment. Of course, a developing country could produce many small-scale batches of BTW agents in laboratory glassware without the need for high-technology fermenters.

PRODUCTION OF VIRAL AND RICKETTSIAL AGENTS

Pathogenic viruses and rickettsiae are intracellular parasites that can only reproduce inside living cells. There are two approaches to cultivating these agents: in intact living tissue (e.g., chick embryos or mouse brains) or in isolated cells growing in tissue culture. The latter approach is technically simpler because it requires only flasks and nutrient medium, but certain viruses (e.g., influenza) do not grow well in tissue culture and must be cultivated in fertilized eggs. In 1962, Fort Detrick used more than 800,000 eggs for the cultivation of pathogenic viruses.[41]

Growing viruses and rickettsiae in cultured mammalian cells offers greater control but involves certain technical hurdles. The cells must adhere to a surface to grow and also require a complex culture medium based on blood serum obtained from horses and cows. Until recently, cultured mammalian cells were grown on the inner surface of rotating glass bottles, which limited the volume of production. Over the past decade, however, new methods for cultivating mammalian cells have been developed that permit higher concentrations of cells and greater recovery of product. For example, allowing the cells to grow on surface of beads suspended in culture medium has permitted the scaling-up of production. Yield has been improved further by replacing the beads with microcarriers, which have a porous internal structure into which animal cells can grow.[42]

Hollow-fiber technology offers an even more efficient method of growing anchorage-dependent mammalian cells in high concentrations for the cultivation of viruses or rickettsiae. The cells are grown on the outer surface of thin fibers that are immersed in the growth medium; air is pumped through the fibers and diffuses through the fiber wall to reach the cells.[43] Since a single hollow-fiber bioreactor is equivalent to several thousand one-liter roller bottles, it occu-

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~ Government of Australia, ' 'Impact of Recent Advances in Science and Technology on the Biological Weapons Convention' Background *Document on New Scientific and Technological Developments Relevant to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction,* Third Review Conference of the BWC (Geneva, Switzerland), Document No. BWC/CONF.III/4, Aug. 26, 1991, p. 3.

41 Seymour M. Hersh, *Chemical and* Biological *Warfare: America's Hidden Arsenal* (Indianapolis, IN: Bobbs-Merrill, 1%8), p. 78.

42 S.B. Primrose, *Molecular Biotechnology,* 2d ed. (Oxford, England: Blackwell Scientific Publications, 1991), p. 116.

43 Government of the U.S.S.R, "Selected Scientific and Technological Developments of Relevance to the BW Convention,'' *Background Document on New Scientific and Technological Developments Relevant to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction, Third* Review Conference of the BWC, Geneva, Switzerland, Document No. BWC/CONF.III/4/Add.1, Sept. 10, 1991, p. Al 1.

pies less than one-twentieth the volume of the previous technology.[44] Advantages include economy and the high concentration and purity of the end-product, which reaches 98 percent on leaving the reactor. In sum, the new cell-culture techniques greatly simplify the production of viruses and rickettsiae and allow large-scale yields from very small facilities.

PRODUCTION OF TOXINS

The most efficient way to produce bacterial toxins is through fermentation. Botulinal toxin, for example, is derived from a culture of Clostridium *botulinum* bacteria, which multiply rapidly under the right conditions of temperature, acidity, and the absence of oxygen. It takes only about 3 days to grow up a dense culture of the bacterial cells, which extrude botulinal toxin into the surrounding culture medium. (Purification of the toxin is neither necessary nor desirable, since it tends to reduce stability.) During World War II, Japan's Unit 731 produced kilogram quantities of botulinal toxin in a fermenter approximately 10 feet high and 5 feet wide.[45] A crude preparation of toxin can be freeze-dried down to a solid cake, which is then milled into a fine powder suitable for dissemination through the air. The milling operation is exceedingly hazardous, however, and must be carried out under high-containment conditions. Plant toxins such as ricin, whose raw material is widely available, could easily be produced in the hundreds of kilograms.

With recombinant-DNA techniques, rare animal toxins—formerly available only in milligram amounts-can be prepared in significant quantities in microorganisms. Although these techniques are still largely restricted to the advanced industrial countries, they are spreading rapidly around the world. One method, known as the "cloning" of toxin genes, involves identifying DNA sequences in plants and animals that govern the production of protein toxins, transferring these genes to a suitable microbial host, and mass-producing the toxin in a fermenter. In this way, ordinary bacterial cells can be transformed into miniature toxin factories.[46] Production of animal toxins in bacteria involves certain technical hurdles, however. Bacteria typically produce and secrete toxins only under special conditions, which may not be met in an artificial environment; and bacteria may be unable to perform certain biochemical "processing" steps needed to convert a protein toxin to its active form.[47] For these reasons, it maybe necessary to clone plant and animal toxins in yeast or mammalian cells, a technically more challenging task.

Nonprotein toxins are considerably harder than protein toxins to produce in militarily significant quantities. Until recently, even small amounts of nonprotein toxins such as saxitoxin had to be extracted from large quantities of biological material with costly and labor-intensive purification methods. For example, 270 kilograms of toxin-containing clam siphons yielded less than 5 grams of saxitoxin.[48] Although some nonprotein toxins such as saxitoxin and tetrodotoxin can be synthesized in the test tube with multistep procedures, the overall yield is only

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44 Government of the United States, "Technological Developments of Relevance to the Biological and Toxin Weapons Convention" *BackgroundDocument on New Scientific and Technological Developments Relevant to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction,* Third Review Conference of the BWC (Geneva, Switzerland), Document No, BWC/CONF.III/4, Aug. 26, 1991, p. 30.

45 Williams and Wallace, op. cit., footnote 35, p. 124.

46 Alan Wiseman, "The Organization of Production of Genetically-Engineered Proteins in Yeast," *Endeavor* (new series), vol. 16, No. 4, 1992, pp. 190-193,

47 Richard Novick and Seth Shulman, "New Forms of Biological Warfare?' Susan Wright, cd., *Preventing A Biological Arms Race* (Cambridge, MA: MIT Press, 1990), p. 115.

48 Edward J. Schantz et al., "Paralytic Shellfish Poison. IV. A Procedure for the Isolation and Purification of Poison from Toxic Clam and Mussel Tissues," *Journal of the American Chemical Society,* vol. 79, Oct. 5, 1957, pp. 5230-5235,

Table 3-I—Key Production Techniques for BTW Agents

Type of agent	Low-tech production	High-tech production
Bacteria	Batch fermentation, production in animals	Genetically engineered strains, continuous-flow fermentation
Rickettsiae and viruses	Cultivation in eggs, mouse brains, or tissue culture (roller bottles)	Culture in mammalian cells grown on beads, microcarriers, or hollow fibers
Protein toxins	Batch fermentation and purification of a bacterial toxin, or extraction of toxin from a plant or animal source	Cloning of toxin gene in microbial host, extraction
Nonprotein toxins	Extraction from plant or animal source	Cloning of a series of genes, each governing production of one of the enzymes needed to complete a step In the biosynthetic pathway

SOURCE: Office of Technology Assessment, 1993.

about 0.1 percent, making it unlikely that militarily significant quantities of toxin could be produced by chemical synthesis.[49] Biotechnological approaches are possible but technically challenging, involving the synthesis not of a single protein but of an entire series of enzymes, each necessary to catalyze one step in a complex series of reactions .50

Production techniques for the various types of BTW agents are summarized in table 3-1. With advanced fermentation techniques available today, a militarily significant supply of BTW agents could be produced over a period of several days, obviating the need for the long-term stockpiling of agents. As a result, a BTW production facility might remain largely quiescent in peacetime. After completing R&D, weaponization, and pilot-production tests on BTW agents, a proliferant could build production and storage facilities and either keep them mothballed or in use for legitimate commercial purposes. Clandestine production facilities might be kept in reserve, ready to be diverted to the rapid manufacture of BTW agents in the event a major conflict breaks out. Alternatively, a commercial production facility could be kept in operation and converted to BTW production in wartime. The advantage of the latter option is that it would be easier to retain the necessary trained staff and up-to-date equipment, albeit at some cost in secrecy.

CONTAINMENT MEASURES

Since working with pathogenic microorganisms is extremely hazardous, specialized physical-containment or ''barrier' measures are needed to protect plant workers and the surrounding population from infection. Great care must be taken to prevent BTW agents from escaping from a production facility and causing a devastating plague in the country producing the weapons.

In advanced industrial countries, work on highly infectious microbial agents is carried out in high-centainment (Biosafety Level 3 or 4) facilities. In a BL3 facility, all personnel have been immunized against the infectious agents they work with. Since no vaccine is 100 percent effective, however, they also wear protective

49 Manuel L. Sanches et al., Chemical *Weapons Convention (CWC) Signatures Analysis (Ml* ington, VA: System Plannin g Corp, Final Technical Report No. 1396, August 1991), p. 89.

50 Primrose, op. cit., footnote 42, p.84.

clothing, goggles, and face masks. Microorganisms are manipulated in special biohazard safety cabinets maintained under "negative' pressure (lower than the outside atmosphere), so that air flows into the work area. In addition to these primary barriers, secondary barriers to the spread of infectious materials include the use of high-efficiency particulate air filters and the incineration of exhaust.[51] Because viral particles are about a hundredth the size of bacteria, they are more difficult to contain with filters and other means. Moreover, spore-forming bacteria (e.g., the agents of anthrax and tetanus) foul the air-handling system with long-lived spores, which can easily contaminate other products. As a result, these bacteria are normally produced in separate facilities.[52]

A BL4 facility, the highest level of containment, is designed to isolate the human operator from the infectious agents. Since research on dangerous microbial pathogens requires handling much lesser quantities of" hazardous material than does production, it is generally performed in small BL4 enclosures inside a less stringent BL3 facility .53 Such enclosures generally consist of sealed boxes with rubber-glove ports that provide absolute containment, and 'hoodlines' that make it possible to move hazardous cultures directly from a glove-box to an autoclave, which destroys the infectious microorganisms with superheated steam. In a larger BL4 research or production facility, the human workers are isolated from the microbes, rather than vice-versa. Each operator wears a self-contained "space suit" and is completely isolated from the surrounding room, which is contaminated with infectious agents. He or she enters the laboratory through an air-lock and hooks up to a supply of compressed air. The **suit is** kept under positive pressure so that if there is any loss of physical integrity, the leaking air will blow outwards, reducing the risk of infection.

A developing country seeking to develop biological weapons would probably use much less elaborate containment measures. During World War II, for example, the Japanese Army's Unit 731 produced vast quantities of highly infectious agents, yet the workers were protected only by wearing rubberized suits, masks, surgical gloves, and rubber boots, and by receiving vaccinations against the agents they were working with. The United Nations inspections of Iraq after the 1991 Gulf War revealed that BTW researchers in that country's BTW program used surprisingly rudimentary containment measures, at the level of a BL2 facility. Laboratory technicians were vaccinated against the infectious agents they worked with and used simple laboratory hoods, but they did not wear masks or protective clothing.

Commercially available containment systems used for vaccine production might be suitable for cultivating highly pathogenic organisms. To comply with environmental and occupational-health standards and to ensure the purity of products for human use, many pharmaceutical plants carry out the microbial production of antibiotics and other drugs in a "clean room" that is comparable to a formally designated BL4 facility. Clean rooms for drug production are normally kept Under positive pressure to keep contaminants out, whereas areas of a vaccine plant used for the culture of in fectious microorganisms are kept under *negative* pressure to prevent the dangerous microbes from escaping. In principle, the direction of air flow could be reversed, albeit with some difficulty.

Methods for sterilizing equipment after use with hazardous microorganisms include physical measures such as dry heat or pressurized, superheated steam; ionizing radiation such as x-rays

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51 Department of the Army, U.S. Army Medical **Research** and Development Coremand, op. cit., footnote 5, p. 7-15.

52 **Gary Ebert, vice president for** operations, **Connaught** Laboratories **(Swiftwater, PA), personal communication, 1992.**

53 **Herbert Marcovich, "Verification** of High-Containment **Facilities,"** S.J. **Lundin,** cd., *Views on Possible Verification Measures for the Biological Weapons Convention* (Oxford, England: **SIPRI/Oxford** University **Press,** 1991), p. 55.

and high-energy ultraviolet radiation; and chemical treatment with formaldehyde or bleach.[54] Sophisticated biotechnology plants often have self-sterilizing fermenters and process equipment, whether or not they are handling hazardous microorganisms. Such plants would therefore have an inherent capability to work with BTW agents.

Stabilization of BTW Agents

Once BTW agents have been produced, it is necessary to process them into a form that enhances their stability in storage and after dissemination, so that they remain viable long enough to infect. Since microbial pathogens are living organisms, they will eventually deteriorate and die unless their metabolism is slowed down or stopped. Such a process of suspended animation occurs naturally in the case of spore-forming microorganisms such as anthrax, which can survive for decades in the dormant spore form. Nonspore-forming microbes and most toxins, however, tend to break down rapidly in the environment if not protected. For this reason, BTW agents are generally most effective if disseminated within a few days after production. If rapid use is not feasible, the live agents must be converted into a more stable form so that they can survive the stresses of storage, transport, and dissemination.

FREEZE-DRYING

One method for enhancing the stability of BTW agents is rapid freezing and subsequent dehydration under a high vacuum, a process known as freeze-drying or *lyophilization. In* a few hours, a lyophilizer, a device mainly used in the pharmaceutical industry, reduces a solution of bacteria and a sugar stabilizer to a small cake of dried material that can then be milled into any desired state of freeness. Lyophilization avoids the need to maintain microorganisms in inconvenient and dangerous liquid suspensions during storage and transportation. It also makes possible a significant increase in agent potency by direct inhalation of particles of dried agent into the lungs.[55] This technique is also applicable to toxins; a fine dust of dried toxin, if inhaled, can be deadly in extremely small quantities.

If kept in cold storage, the desiccated organisms will remain viable for long periods, although they still deteriorate. For example, freeze-dried brucellosis bacteria can be stored for several months, and Q-fever rickettsiae for up to 8 years.[56] Lyophilization also extends the shelf-life of protein toxins: freeze-dried *Staphylococcus* enterotoxin B (SEB) can be stored for up to a year. Even so, the virulence and viability of lyophilized BTW agents decays over time: there is a loss of potency of a factor of 10 to 100 over a period of 1 to 5 years, so that much larger quantities of older agent are required to produce the same military effect.[57]

CHEMICAL ADDITIVES

The stability of a microbial aerosol can be increased by adding a variety of compounds to the spray material.[58] Moreover, antiagglomerants such as colloidal silica help prevent the clumping of freeze-dried microbial agents and toxins that have been milled into a fine powder. Agricultural research on biological pesticides, such as the insect-killing bacterium *Bacillus thuringiensis,* has provided much information on methods for stabilizing bacterial agents in the field. For example, new formulations of *B. thuringiensis* have been developed that extend the life of the

54 Department of Army, U.S. Army Medical Research and Development Command, op. cit., footnote 5, pp. A-132, A-13-3.

55 Williams and Wallace, op. cit., footnote 35, p. 72.

56 Rothschild, op. cit., footnote 9, pp. 206-219.

57 SIPRI, vol. VI, op. cit., footnote 31, p. 50.

58 Robert J. Goodlow and Federic A. Leonard, "Viability and Infectivity of Microorganisms in Experimental Airborne Infection," *Bacteriological Reviews,* vol. 25, 1%1, p. 185.

disseminated bacteria by means of ultraviolet protestants and other additives that ensure compatibility with existing agricultural sprayers.[59]

MICROENCAPSULATION

Another approach to stabilization, known as microencapsulation, emulates natural spore formation by coating droplets of pathogens or particles of toxin with a thin coat of gelatin, sodium alginate, cellulose, or some other protective material. (An industrial example of microencapsulation is the production of carbonless carbon paper, in which ink droplets are coated in this manner.) Microencapsulation can be performed with physical or chemical methods.[60]

Micromcapsulation production methods can be set up to generate particles of a selected size range (e.g., 5 to 10 microns).[61] The polymer coating protects the infectious agent against environmental stresses such as desiccation, sunlight, freezing, and the mechanical stresses of dissemination, and permits cold-storage of microbial pathogens for several months. Microcapsules can be charged electrostatically to reduce particle clumping during dissemination, or ultraviolet-light blocking pigments can be added to the microcapsule to protect microorganisms against degradation by sunlight. Once in the target environment, such as the interior of the lung, the polymer coating dissolves, releasing the agent. Microencapsulation can also be applied to toxins, making them more stable, predictable, and safer to handle.

Integration With Delivery Systems

A biological or toxin agent is of little military utility if it does not produce consistent and reliable effects and cannot be delivered to a target. BTW agents are all nonvolatile solids that would be disseminated either as a liquid slurry or a dry powder of freeze-dried organisms or toxin.[62] Possible delivery systems range in complexity and effectiveness from an agricultural sprayer mounted on a truck to a specialized cluster warhead carried on a ballistic missile. The difficulty of delivery-system development depends on the proliferant's military objectives. It is not hard to spread BTW agents in an indiscriminate way for the purpose of producing large numbers of casualties over a wide area. It is much more difficult, however, to develop BTW munitions that have predictable or controllable military effects against point targets, such as troop concentrations on the battlefield.

Many pathogens infect man naturally by means of an intermediary organism ("vector' '), such as a mosquito or tick.[63] Military microbiologists discovered during World War II, however, that BTW agents can be disseminated through the air, making it possible to infect large numbers of people simultaneously. Many microbial pathogens and toxins-even those normally transmitted by vectors or in food-can invade the body through the lungs, giving rise to foci of infection or traveling through the bloodstream to other parts of the body. The key to producing large-

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59 Government of the United Kingdom, ''General Developments Relevant to the BWC,'' in *Background Document on New Scientific and Technological Developments Relevant to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction,* Third Review Conference of the BWC (Geneva, Switzerland), Document No. BWC/CONF.III/4, Aug. 26, 1991, p. 25.

60 Arthur Osol et al., eds., Remington's *Pharmaceutical Sciences, 15th* ed. (Easton, PA: Mack Publishing Co., 1975), P. 1604.

61 A micron is a thousandth of a millimeter.

62 C.V. Chester and G. P. Zimmerman, ''Civil Defense Implications Of Biological Weapons,'' *Journal of Civil Defense, vol. 17,* No. 6, December 1984, p. 6.

63 The disease vector is usually some type of arthropod: mosquitoes transmit yellow fever and dengue fever; fleas transmit plague; and ticks transmit tularemia and Q fever, During 1932-45, the Japanese BW facility known as Unit 731 set up flea "nurseries" for the production of 135 million plague-infested fleas every 4 months. As a delivery system, porcelain bombs were developed that could contain about 30,000 infected fleas. See Williams and Wallace, op. cit., footnote 35, p. 27.

scale respiratory infections is to generate a biological "aerosol": a stable cloud of suspended microscopic droplets, each containing from one to thousands of bacterial or virus particles. (Fogs and smokes are examples of visible aerosols.) Biological aerosols can be produced with a relatively simple piece of machinery, analogous to a home vaporizer, that sprays a suspension of microorganisms through fine nozzles, converting about 85 percent of their starting material into droplets in the desired size range.[64] The concentration of organisms in the starting solution influences the distribution of organisms among the aerosol particles.[65]

Aerosol dissemination of many vector-borne diseases, such as yellow fever, Rocky Mountain spotted fever, tularemia, and tick-borne encephalitis, can produce atypical infections of the respiratory tract. Respiratory infection with such agents bypasses normal protective mechanisms such as local inflammatory processes and increases the virulence of pathogens that normally have a low lethality, such as Venezuelan equine encephalitis (VEE).[66] In the case of microbial pathogens that can be transmitted by different routes, such as anthrax bacteria, respiratory infection results in by far the most virulent form of the disease. For example, whereas untreated skin anthrax is fatal in only about 5 percent of cases, pulmonary anthrax is fatal in more than 90 percent of cases.[67]

Freeze-dried toxins can also be disseminated in the form of an aerosol. Recent studies have shown that saxitoxin and T-2 trichothecene mycotoxin are at least 10 times more toxic when administered by aerosol than by intravenous injection.[68] Because protein toxins are large organic molecules, however, they are susceptible to environmental stresses such as heat, oxidation, and shear forces. As a result, attempts to aerosolize toxins have encountered problems in maintaining the stability of the agent before and after dissemination. It is also difficult to formulate protein toxins capable of penetrating the skin.

The primary challenge in weaponizing BTW agents for long-range delivery is to keep them alive long enough to infect enemy troops. The agent must be capable of withstanding the physical stresses involved in the dissemination process without losing activity. Technical hurdles involved in the design of self-dispensing biological weapons are as follows:

- the munition or delivery system must generate a cloud of aerosol particles with dimensions that allow them to be inhaled deep into the lungs of the target personnel;
- the agent must be physically stabilized so that it can survive the process of dissemination long enough to infect the target personnel;
- the agent must disseminated slowly to permit aerosolization while avoiding loss of viability or toxicity; and
- the overall size and shape of the aerosol cloud and the concentration of agent within it must be reasonably predictable, so that the dispersion pattern can be matched to the target.[69]

These technical hurdles are discussed below.

64 Milton Leitenberg, "Biological Weapons, ' Scientist *and Citizen,* vol. **9, No. 7,** August-September 1%7, p. 157.

65 Goodlow and Leonard, op. cit., footnote 58, p.184.

66 World Health Organization, *Health Aspects of Chemical and Biological Weapons* (Geneva: WHO, 1970), *p.* **61.**

67 Zelicoff, op. cit., footnote 29.

68 Government of the U. S. S. R., "Selected Scientific and Technological Developments of Relevance to the BW Convention" op. cit., footnote 43, p. A lO.

69 Stockholm International Peace Research Institute (SIPRI), *The Problem of Chemical and Biological Warfare, Vol. II: CB Weapons Today* **(Stockholm:** Almqvist & Wikell, 1973), pp. 72-73.

EFFECT OF PARTICLE SIZE

The particle size of an aerosol is critical to both its atmospheric stability and its military effectiveness. Whereas larger particles tend to settle out of the air, microscopic particles between one and five microns in diameter form a stable aerosol in which the particles remain airborne for a long time. The very low settling velocity of the particles will by itself keep a biological aerosol cloud suspended in the air for long periods. Such a cloud may therefore be transported by the wind over long distances. Moreover, losses resulting from fallout and washout are negligible and do not significantly reduce the concentration of an aerosol cloud. Particles less than 5 microns in diameter generally do not collide with smooth surfaces in their path but are carried over them by air currents. In contrast, transport over rough surfaces for distances of more than a kilometer can result in significant deposition.

Aerosolized BTW agents generally do not penetrate the skin and thus do not represent a significant contact hazard; instead, they infect individuals only if inhaled into the lungs .70 Particle size is also critical for respiratory infection. Almost all particles larger than 5 microns in diameter are trapped in the phlegm and passages of the upper respiratory tract, while particles smaller than 1 micron diameter are exhaled without being retained in the deep lung tissue. Only particles between 1 and 5 microns in diameter are small enough to reach the tiny terminal air sacs (alveoli) of the lung, bypassing the body's natural filtering and defense mechanisms. In one set of experiments on the effect of particle size on respiratory infection, tularemia bacteria were administered to guinea pigs as an aerosol. When the aerosol. particles were 1 micron in diameter, only 3 bacterial cells per animal were needed to kill 50 percent of the guinea pigs, but when the particle size was increased to 7 microns, the number of bacteria per animal required to kill half of the guinea pigs rose to 6,500.[71]

PHYSICAL STABILIZATION

The use of mechanical devices to generate aerosols from a bulk storage tank places a variety of mechanical stresses on microorganisms, reducing the number of viable, infectious cells. Relatively few microbial pathogens can meet the stability requirements of bulk dissemination.[72] Those agents best suited for long-range attack can infect with a small number of microorganisms and are hardy enough to survive for a fairly long period floating in the air. Once released, however, the aerosol cloud "decays" over time as the microorganisms die as a result of exposure to oxygen, atmospheric pollutants, sunlight, and desiccation, resulting in a loss of viability (ability to survive and multiply) and virulence (ability to cause disease and injury). A BW agent disseminated into a given environment may also retain its viability while losing its virulence.[73]

Decay of an aerosol cloud occurs in two stages. Initial dissemination is followed by a period of very rapid cell death during the first several seconds after the cloud has been released. Indeed, producing a liquid aerosol by explosive dispersion or passage through a spray device may kill as many as 95 percent of the microorganisms. This initial stage is followed by a much slower rate of decay, so that the aerosol cloud may persist for long periods of time. A relative humidity of over 70 percent promotes microbial survival.[74] Large-particle clouds are more resistant to the lethal effects of solar radiation than small-particle

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70 Ibid., p. 29.

71 Maj. William D. Sawyer, "Airborne Infection," *Military Medicine,* February 1%3, pp. 90-92.

72 SIPRI, vol. II, op. cit., footnote 69, p. 30.

73 Group of Consultant Experts on Chemical and Bacteriological (Biological) Weapons, *Chemical and Bacteriological (Biological) Weapons and the Effects of Their Possible Use,* United Nations Report No. E.69.I.24 (New York NY: Ballantine Books, 1970), p. 13.

74 Department of the Army, *U.S.* Army Medical Research and Development Command, op. cit., footnote 5, p. A7-16.

clouds, and dry disseminated aerosols are more resistant than wet aerosols.[75] As the plume disperses, long-lived particles (e.g., anthrax spores) may be deposited on the ground, where they may then adhere to large particles of surface soil and dust. If the surface is disturbed, either by the wind or by human activities, the spores can again be resuspended, potentially causing additional infections.[76] The inhalation hazard is much reduced, however, owing to the large particle size.

TYPES OF AEROSOL ATTACKS

There are two types of aerosol dissemination of BTW agents. "Area" attack involves releasing an aerosol cloud upwind and allowing it to drift over the target area. In contrast, "point" attack involves projecting the agent in a cannister that releases the agent immediately over the target.[77]

Area attack

A BTW weapon designed for area attack would disseminate its payload as an aerosol cloud containing a sufficient concentration of viable microorganisms to infect the targeted personnel with particles in the 1 to 5 micron size range. The simplest means of area delivery is with spray tanks mounted on manned aircraft, unmanned remotely piloted vehicles (RPVs), or cruise missiles, which can release a large quantity of agent over a controlled line of flight. A slow-flying aircraft such as a crop duster could discharge a line of agent that, as it travelled downwind, would reach the ground as a vast, elongated infective cloud. Such a linear cloud of agent, known as a ''line source, can cover a larger area than a cloud released from a single spot, or ''point source. '

Air rushing past the spray tank can be used to force out its contents; alternatively, compressed air or carbon dioxide may be used to disseminate the agent. Aerosol generators might also be operated from offshore ships or submarines parallel to a coastline, producing an invisible cloud of BTW agents that could be carried by the prevailing winds over key coastal cities or military bases.[78] The discharge rate must be slow enough to generate a stable aerosol, yet slow-flying aircraft and RPVs are extremely vulnerable to air defenses.

Area attack with a biological aerosol depends heavily on atmospheric diffusion and wind currents to dilute and spread the agent over the area being attacked. The most stable atmospheric conditions occur on cold, clear nights or early in the morning, when the ground and the layer of air above it are cooler than the next higher 1ayer of air. This phenomenon, called an *inversion,* is ideal for the delivery of BTW agents because the stable interface of warm and cold air prevents the vertical mixing of the cloud and causes it to hug the ground, keeping the organisms at a low altitude where they can be inhaled. In contrast, bright sunlight causes atmospheric turbulence that breaks up the aerosol cloud, and also contains ultraviolet rays that kill many microorganisms. For these reasons, a BTW attack would be most likely to come at dusk or at night.[79]

The effectiveness of an area attack also requires detailed knowledge of the prevailing wind direction and speed. Under favorable wind conditions, an aerosol cloud could contaminate the air over large areas, but if the wind is erratic or excessively strong, the agent might fail to reach the target or might be dissipated too rapidly to be

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75 Goodlow and Leonard, op. cit., footnote 58, p. 184.

76 Ammon Birenzvige, *Inhalation Hazard from Reaerosolized Biological Agents: A Review,* Report No. CRDEC-TR-413 (Aberdeen Proving Ground, MD: Chemical Research, Development and Engineering Center, September 1992).

77 SIPRI, vol. II, op. cit., footnote 69, p. 28.

78 W. Seth Carus, *''The Poor Man's Atomic Bomb?'': Biological Weapons in the Middle East,* Washington Institute Policy Papers No. 23 (Washington, DC: Washington Institute for Near East Policy, 1991), p. 11.

79 Chester and Zimmerman, op. cit., footnote 62, p. 7.

effective. Assuming the target is nearby, the attackers will know the wind direction and can plan the attack at a favorable time. If the target is deep inside enemy territory, local meteorological conditions would be harder to assess without access to current weather data. Nevertheless, hundreds of airports worldwide broadcast wind direction, speed, cloud cover, and temperature every 3 hours according to World Meteorological Organization guidelines.

A remotely piloted vehicle or subsonic cruise missile flying at low altitude might reduce such problems by disseminating the toxic cloud close to the ground just upwind of the target—assuming, of course, that the attacker had the means of knowing which way the wind was blowing over the target area. In 1960, the U.S. Army began developing a drone aircraft that could be used to deliver chemical and biological weapons. The pilotless plane was designed to carry 200 pounds of germ agents as far as 115 miles.[80] Even relatively unsophisticated cruise missiles might be capable of generating line-source aerosols for off-target attacks.[81] For this reason, the simultaneous proliferation of biological weapons and cruise-missile capabilities may become a major security threat in the future.

Point attack

A point BTW attack would be performed with munitions delivered by artillery, rockets, missiles, or aircraft. Although the targeted personnel would be warned of the attack by the arrival of the munition, the rapid formation of a concentrated aerosol (within 15 to 30 seconds) means that many soldiers would inhale an incapacitating or lethal dose of agent before they had time to put on their gas masks properly, assuming they were available.[82] A point attack that dropped the agent directly over the targeted personnel would also be much less dependent on meteorological conditions, although it would require a much higher payload of munitions per area covered.

MUNITIONS FOR POINT ATTACK

Munitions developed for chemical-warfare agents are generally unsuited for biological warfare because of the lower stability of BTW agents and their susceptibility to environmental factors such as ultraviolet radiation and air pollution. There are two basic methods for disseminating BTW agents from a munition: *explosive* and *pressurized.* Whereas explosive dissemination produces an almost instantaneous build-up of aerosol concentration over the target, it destroys a large portion of the infectious agents and tends to produce drops that are considerably larger than the optimal droplet size for inhalation.[83] In contrast, pressurized munitions do not disperse agent as rapidly as explosive munitions but provide better control of particle size, are gentler on the microorganisms, and produce an aerosol cloud that is visible for a shorter period of time. One method of pressurized delivery is to force a liquid suspension of agent through a fine nozzle, which breaks up the material into droplets of the appropriate size.

A BTW bomb or warhead may either be filled with bulk agent or with numerous self-dispensing cluster-bomb units (CBUs). A cluster bomb has a casing that breaks open during delivery to scatter a large number of smaller submunitions over a wide area. The submunitions then fall to earth and are triggered to go off at an altitude of about 15 to 20 feet off the ground. Each of these bomblets generates a small aerosol cloud; these multiple point sources are then coalesced by air currents into a single large cloud. During World War II, the United States and Great Britain

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80 Hersh, op. cit., footnote 41, p. 71.

81 Carus, op. cit., footnote 78, pp. 10-11.

82 Rothschild, op. cit., footnote 9, p. 76.

83 Ibid., p. 60.

jointly developed a 500-pound bomb for the delivery of anthrax spores. Each bomb casing contained 106 four-pound bomblets designed to burst in midair, producing dense aerosols of spores. Twenty of these bomblets could cause a high fatality rate among humans and livestock over a one-square-mile area, and British war plans called for using as many as 40,000 anthrax bombs against six German cities.[84]

Nevertheless, the technical difficulty of dispensing submunitions should not be underestimated. Missile delivery would place severe environmental stresses on microbial agents, including freezing temperatures prevailing at high altitudes and friction-heating of the missile nosecone during reentry through the atmosphere, which could be fatal to microbes without sufficient insulation. The timing of agent dissemination would also be critical, since if it occurred at the wrong altitude, the agent would not form a militarily effective aerosol. Releasing the agent too high would cause it to dissipate before it could be inhaled by the targeted troops; releasing it too low would merely produce a puddle of toxic material on the ground. In sum, effective agent dissemination requires a series of mechanical steps to work perfectly, and atmospheric conditions to cooperate.

Other problems associated with weaponization include the hazards of loading munitions with agent, and corrosion and seepage from filled munitions. Despite these technical hurdles, however, effective biological munitions have been developed and deployed in the past. In 1951, the first anticrop cluster bombs were placed in production for the U.S. Air Force; each bomblet contained turkey feathers contaminated with cereal rust spores.[85] Also during the 1950s, the United States developed small, self-dispersing BTW bomblets for the Honest John missile.[86]

In conclusion, strategic BTW attacks against cities might be carried out with relatively simple off-target delivery systems, such as a spray tank carried by an aircraft. After dissemination, the aerosol cloud might behave in unexpected ways in response to changes in the wind and weather, potentially boomeranging against the attacker's own troops or population.[87] More controlled point attacks with BTW agents for military purposes would require the use of missiles or bombs, possibly equipped with cluster munitions, but such attacks would be technically more difficult to carry out.

Some analysts contend that the most probable use of BTW agents would be for covert warfare against crops, livestock, or human populations for purposes of economic destabilization. In this case, relatively small quantities of BTW agents might be introduced by human saboteurs directly into the air or water supply of a city or military installation.

INDICATORS OF BTW AGENT PRODUCTION

Detection and monitoring of BTW agent production is an extremely challenging task for the following reasons:

- All equipment and feedstock materials used to make BTW agents is dual-use and could be used for legitimate purposes in the biotechnology or pharmaceutical industries.
- Utilizing new biotechnologies, production could take place in facilities that are much smaller and less conspicuous than in the past,

84 Barton J. Berstein, "Churchill's Secret Biological Weapons," *Bulletin of the Atomic Scientists*, January/February 1987, pp. 4&50.

85 SIPRI, vol. II, op. cit., footnote 69, p. 160.

86 Rothschild, op. cit., footnote 9, p. 78.

87 In 1942, during World War II, special Japanese troops spread diseases such as cholera, typhoid, plague, and anthrax in China. Subsequently, more than 10,000 Japanese soldiers fell ill after they overran a contaminated area, presumably because regular soldiers had not been informed about the use of biological weapons. Barend ter Haar, *The Future of Biological Weapons, The Washington Papers No. 151 (New* York, NY: Praeger, 1991), p. 5.

with no obvious signs to indicate illicit activity.

- Legitimate facilities might be diverted to the production of BTW agents in a relatively short time.
- Since microbial agents can be grown in an advanced fermenter from a few cells to many kilograms of agent over a period of days or weeks, it would not be necessary to maintain large stockpiles (although filled or empty munitions would need to be stored).

The extreme potency of BTW agents means that as little as a few kilograms can be militarily significant.

- BTW agents would be hard to distinguish from naturally occurring pathogens, particularly if the agents are endemic to the affected area.

There is an enormous variety of potential BTW agents, each requiring a specific detection method.[88]

According to a State Department official, "In many ways, recent progress in biological technology increases the ease of concealment of illicit manufacturing plants, particularly for biologically derived chemicals such as toxins. . . . Not only has the time from basic research to mass production decreased but the ability to create agents and toxins with more optimal weapon potential has increased. Simply put, the potential for undetected breakout from treaty constraints has increased significantly."[89] Thus, while the characteristics of a given facility may be consistent with an offensive BTW program, the odds of finding a "smoking gun'' -such as a munition filled with BTW agent—are quite low.

Despite these difficulties, however, a few factors constrain the detection problem. Microbial pathogens and toxins of military concern have relatively few civilian uses in scientific research and medical therapy, and such applications are generally confined to sophisticated biomedical research centers not often found in developing countries. As a result, there are some production and weaponization signatures that-if integrated effectively with national intelligence collection and declarations of activities and facilities relevant to the Biological and Toxin Weapons Convention-might provide strong circumstantial evidence of a clandestine BTW program.

| Research and Development Signatures

Many research and development activities related to BTW agents are inherently ambiguous in that they can support both defensive and offensive purposes. It is therefore essential to evaluate the evidence in the context of a country overall behavior and the openness and transparency of its nominally defensive program. Telltale indicators of a BTW program *might* include the existence of biological research facilities operated under military control, the large-scale production of vaccines in excess of legitimate domestic needs, or the purchase of dual-use biological materials and equipment. Analysts searching for indicators of foreign BTW activities should avoid "mirror-imaging' '—the temptation to judge other countries by U.S. standards. Only by first understanding a country's commercial standards for biological containment and good manufacturing practice is it possible to identify anomalies that do not fit this basic profile.[90]

SCIENTIFIC PUBLICATIONS

The collection and systematic analysis of scientific and technical information published by a country of proliferation concern can help to

88 Stephen S, Morse, ''Strategies for Biological Weapons Verification" *Proceedings of the Arms Control and Verification Technology Conference*, Williamsburg, VA, June 1992 (Washington, DC: Defense Nuclear Agency, in press.)

89 H. Allen Holmes, ''Biological Weapons Proliferation,'' *Department of State Bulletin*, vol. 89, July 1989, p. 43.

90 Graham S. Pearson, ''Biological Weapons: The British View,' presentation at a Seminar on Biological Weapons in the 1990s, sponsored by the Center for Strategic and International Studies, Washington DC, Nov. 4, 1992.

monitor research trends, identify institutions and scientists associated with such research activities (including cooperation with foreign states), and identify gaps or abrupt halts in open research on particular topics that may be suggestive of military censorship. Although such literature analysis is unlikely to reveal BTW activities that have been deliberately concealed, it can raise questions about the capabilities and activities of various facilities and is useful when combined with other sources of information. For example, useful intelligence on Soviet BW activities was reportedly gleaned from clues picked up in the Soviet scientific literature. By tracking the award of academic honors and by noticing obvious gaps in a series of published papers, Western intelligence analysts could judge which fields of biological research Soviet military scientists had entered.[91] During the 1970s and '80s, for example, the Soviet literature contained a remarkably large number of publications on toxin research.[92]

Nevertheless, publication tracking is not a reliable indicator of a BTW program. First, it requires the existence of a preliminary scientific research effort before a country undertakes production of BTW agents. In the past, countries with offensive BTW programs, such as Japan, Germany, the United States, and the Soviet Union, launched a major scientific research effort in relevant areas of microbiology before they could develop biological weapons. Today, however, much of the basic science is already well understood, and explicit weapon-development programs could be undertaken with relatively little preliminary research.

Second, because many variables affect scientific productivity and publication rates, publication tracking can only provide a broad indication of a state's BTW activities. The scientific culture in many developing countries does not demand large numbers of publications, so there will be fewer to monitor. Finally, the biomedical databases available for tracking (e.g., MEDLINE) do not have representative numbers of scientific abstracts from the countries of greatest proliferation concern, particularly for papers not published in English.[93] For this reason, the number of abstracts in the database dealing with microbial and toxin agents may not correlate either positively or negatively with a country's BTW activities. For all these reasons, publication analysis is an unreliable-although potentially useful—indicator of BTW activity.

HUMAN INTELLIGENCE

Because of the limited value of technical collection systems such as satellites for monitoring BTW activities, human intelligence is vital in this area. For example, Vladimir Pasechnik, a Russian microbiologist with first-hand knowledge of the Soviet BTW program, defected to Britain in 1989 while attending a scientific conference in London and provided extensive information on Soviet BTW activities that was unobtainable by other means.[94] Human agents and defectors can also confirm suspicions about sites and activities based on other sources of intelligence. Nevertheless, the UNSCOM biological inspections of Iraq have shown that human intelligence can be unreliable or misleading if the individual reporting does not have direct knowledge or is unfamiliar with the technical details of what he is describing.[95]

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91 Harris and Paxman, op. cit., footnote 14, p. 114.

92 David B&r, "Chemical and Biological Warfare Agents—A Fresh Approach," *Jane's Intelligence Review*, vol. 5, No. 1, January 1993, p. 44.

93 Zelicoff, op. cit., footnote 29.

94 Bill Gertz, "Defecting Russian Scientist Revealed Biological Arms Efforts," *The Washington Times*, July 4, 1992, p. A4.

95 Telephone interview with David Huxsoll, dean, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, Aug. 17, 1992.

| Weaponization and Testing Signatures

Weaponization involves the determination of whether a candidate BTW agent is militarily effective and how it would be used. These activities have no obvious civilian counterpart, and hence would be indicative of a clandestine BTW development program. Signatures associated with the testing of candidate BTW agents might be easier to detect than agent production signatures, and inspections focusing on weaponization activities would also be more acceptable to the biotechnology industry than production monitoring, since they would not compromise trade secrets.

Examples of weaponization and testing signatures that might be observable with overhead photography include field tests of aerosol dispersal patterns, tests of effectiveness against large animals, and the surreptitious burial of dead animals from weaponization tests .96 Other weaponization signatures would only be visible during an onsite inspection. For example, specialized aerosol test chambers might be used to study the behavior of biological aerosols in the environment or the detonation of BTW munitions. If such a test facility were found in a nominally civilian vaccine plant, it would be extremely suspicious. Indeed, the secret Soviet BW facility at the All-Union Research Institute of Applied Microbiology in Oblensk reportedly contained two such test chambers. The "aerosol-dissemination test chamber' consisted of a steel cube, roughly 50 feet on a side, in which experimental animals were tethered to the floor and exposed to BW aerosols released from ceiling vents. There was also a reinforced ''explosive-test chamber' in which the detonation of BW munitions was simulated.[97]

Although some analysts contend that weaponization could be carried out in enclosed, unobtrusive facilities, others argue that the integration of signatures from a variety of sources would make a militarily significant weaponization program difficult to hide. Nevertheless, there are a number of potential concealment strategies:

- Weaponization studies short of actual field tests could be performed inside production facilities.
- Open-air testing of BTW weapons would be difficult to detect if the test grid were masked, or if there were no distinctive delivery systems or advance indications of where to look. In addition, since many BTW agents are sensitive to sunlight, they would be tested at night.[98]
- Certain legitimate activities, such as the dissemination of biopesticides on crops, or the use of conventional smoke bombs, might be used as a cover for BTW weaponization testing.

A growing number of countries, for example, are replacing chemical pesticides with certain microorganisms that are natural insect-killers. Although over 100 bacteria, fungi, and viruses infect insects, only a very few are in commercial production. The most widely used is *Bacillus thuringiensis,* a bacterium that produces a crystalline protein toxic to insects and is applied to crops as an aerosol from agricultural sprayers.[99] Although B. *thuringiensis* does not have the characteristics of a BW agent and would be a poor simulant for weaponization studies, field tests with bacteria more closely resembling BW agents could be disguised as biopesticide application. Thus, if biopesticides are already in use in the

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96 Test ranges will likely be at remote locations. The former Soviet Union, for example, operated a BTW test site on isolated Vozrozhdeniye Island in the Aral Sea.

97 John Barry, ''Pl arming a Plague?" Newsweek, Feb. 1, 1993, p. 40.

98 James Adams, Engines *of War: Merchants of Death and the New Arms Race (New* York, NY: Atlantic Monthly Press, 1990), P. 221.

99 primrose, op. cit., footnote 42, p. 76.

local agricultural sector, a covert proliferant could use them to test the open-air dissemination of microbial aerosols in preparation for germ warfare, and also to justify the acquisition of hardware needed to disperse the agent. Nevertheless, substantial field tests using grams or kilograms of micron-sized particles could be detected in samples taken at great distances downwind if the organism had distinctive DNA sequences and those doing the detection knew what to look for.

Production Signatures

Production of BTW agents is nearly impossible to detect by visual inspection alone, although this approach may add some useful pieces to the puzzle.

EXTERNAL VISUAL SIGNATURES

BTW production facilities may sometimes be detected or monitored with overhead photography, although the evidence is nearly always ambiguous. For example, the Institute of Microbiology and Virology in the Russian city of Sverdlovsk, 850 miles east of Moscow, reportedly aroused the suspicions of U.S. intelligence analysts in the 1970s because of certain characteristics observed by satellite. Photointerpreters identified tall incinerator stacks, large cold-storage facilities, animal pens, sentries, and double barbed-wire fences. These features, not unlike those at the former U.S. offensive BTW facility at Fort Detrick, suggested that the Sverdlovsk facility might serve military purposes.[100] In Much 1980, the U.S. Government attributed a serious outbreak of pulmonary anthrax in Sverdlovsk the previous year to Soviet BW activities at the microbiology institute. Although Soviet officials denied the allegations at the time, in May 1992 Russian President Boris Yeltsin finally admitted that the anthrax epidemic had been caused by an accident at the military facility. Russian military spokesmen have insisted, however, that the anthrax work at Sverdlovsk was "defensive" in nature, and this question has yet to be resolved.[101]

Recent innovations in biotechnological production technology aimed at increasing productivity, cutting costs, and improving safety have further blurred distinctions important for verification, such as between a laboratory and a production facility. In the past, large batch fermenters and refrigerated storage vaults provided signatures of BTW production; today they are being replaced in advanced industrial countries with small, continuous-flow fermenters that can produce large quantities of highly infectious materials rapidly in a laboratory-scale facility. Such advances in production technology have greatly increased the difficulty of detection by reducing the size of plants needed to produce militarily significant quantities of BTW agents and the amount of time needed to break out of disarmed status. With the new production technologies, a clandestine BTW plant might be more easily camouflaged, buried underground, or hidden within a larger complex that produces legitimate commercial products.

Still, satellite or aerial photography might help to monitor sites judged suspicious on the basis of other sources of intelligence. The following signatures of BTW agent production might be detected or monitored with overhead imaging:

- "Excessive" secrecy and security surrounding a nominally civilian microbiological facility, such as a brewery, sugar refinery, infant-formula plant, or single-cell protein plant. Telltale security measures might include double or triple fencing, watch towers,

[100] Elisa D. Harris, "Sverdlovsk and Yellow Rain: Two Cases of Soviet Noncompliance?" *International Security*, vol. 11, No. 4, *spring 1987*, pp. *41-95.*

[101] See *Milton* Leitenberg, "A Return to Sverdlovsk: Allegations of Soviet Activities Related to Biological Weapons,' *Arms Control, vol.* 12, No. 2, September 1991 (published April 1992), pp. 161-190; and Milton Leitenberg, "Anthrax in Sverdlovsk: New Pieces to the Puzzle, " *Arms Control Today*, vol. *22, No. 3,* April 1992, pp. 10-13.

and air-defense missile batteries-although concealment, camouflage, and deception operations are possible.
- Elaborate microbiological production facilities inconsistent with the level of sophistication of other, clearly civilian plants.
- Facilities for housing large numbers of primates, horses, rats, mice, rabbits, sheep, goats, or chickens (for producing eggs), when such animals are not clearly associated with vaccine production.
- Changes in activity at nominally civilian production facilities.

PLANT DESIGN AND LAYOUT

Other signatures of BTW production would not be visible from outside a suspect facility, and thus could only be detected during an intrusive onsite inspection. The basic equipment in a BTW production facility would be much the same as that in a vaccine plant, including equipment and materials for microbial fermentation, cell culture, or egg incubation, followed by harvesting, purification, and lyophilization. Both a vaccine plant and a BTW production facility would require a source of pharmaceutical-grade distilled water to remove bacterial contaminants in tap water that would interfere with the growth of desired microbial agents. And both types of facilities would require autoclaves to sterilize the growth media and decontaminate the equipment after production.

It is also important to evaluate BTW-related activities in their socioeconomic context. In developed countries, civilian production facilities that utilize microorganisms, such as pharmaceutical plants and even breweries, now incorporate safety and environmental equipment that were once unique to BTW production facilities. This fact has made it easier to use commercial production as a cover for illicit military work, although the presence in a vaccine-production plant of processes that cannot be justified on technical or economic grounds may provide indicators of possible conversion or diversion to BTW production. Nevertheless, a clandestine BTW production facility could be so small that it could be easily hidden.

PHYSICAL CONTAINMENT

In developed countries, an important difference between a vaccine plant and a BTW production facility may be the level and type of physical containment measures that are employed. Three aspects of physical containment might be suggestive of a clandestine BTW program:

First, production of vaccines involves the use of living, attenuated microbial strains that are either further weakened to produce a live vaccine or killed immediately after cultivation. As a result, stringent containment measures are required only during the *initial phase* of vaccine production, which involves the cultivation of agents before they are attenuated or killed.[102] According to one assessment, "Extensive safety precautions during the whole production cycle for a vaccine are hardly defensible economically and would hence be suspect. ' '[103]

A second difference between a vaccine plant and a BTW production facility is the presence in the former of costly measures to protect the purity, sterility, and reproducibility of the products so that they are suitable for human use. Thus, an indicator of illicit BTW production in a pharmaceutical facility might be the *absence* of measures to purify the product and ensure its sterility.

Although BTW agents would probably be cultivated under *negative pressure* to prevent dangerous microbes from escaping from the plant, this would not be a reliable signature because negative pressure would also be needed

102 David L. Huxsoll, ~@ D. Parrott, and William C. Patrick III, "Medicine in Defense Against Biological Warfare," *Journal of the American Medical Association,* vol. *262, No. 5,* Aug. 4, 1989, p. 679.

103 SIPRI, vol. VI, op. cit., footnote 31, p. 45.

in a legitimate pharmaceutical plant that is producing live, attenuated vaccines. It would also be possible to reverse the pressurization of a facility before an inspection by changing the direction of flow of the faltered air, but only if the system were engineered for this purpose in advance. Moreover, governments engaged in the covert production of BTW agents would probably not hesitate to cut comers on containment and worker safety in order to avoid signaling their intentions.

In addition to the type and level of physical containment, several other potential signatures of clandestine BTW production might be detected during an onsite inspection.[104] The utility of such signatures is highly controversial, however, and each one is open to criticism. These signatures might include:

- Bad odors associated with microbial fermentation, since multiplying bacteria produce a variety of volatile and odiferous gases. However, odors do not travel far and are nonspecific.
- Seed stocks and cell lines inappropriate for declared activities such as production of vaccines or single-cell protein, or in amounts exceeding immediate research needs. However, such materials would probably not be declared and could be easily hidden.
- Activities related to microorganisms and toxins that cannot be explained by civilian needs, such as development of vaccines against rare, nonindigenous disease agents. However, such activities could be easily hidden.
- Production capacity greatly in excess of demand for the plant's legitimate products, such as vaccines. However, such excess capacity would not be required for a BTW capability.
- A discrepancy between a small quantity of output product (e.g., packaged vials of vaccine) and a large quantity of input materials (e.g., fermentation media). However, calculation of a precise material balance is probably impossible.
- Air compressors, air tanks, or lines for air-supplied protective suits as a means of enhancing physical containment. However, compressors are easily hidden.
- Facilities for rapid decontamination and cleaning of the production line, or evidence of recent large-scale decontamination operations, fumigation, or removal of materials or equipment. However, decontamination routinely occurs in pharmaceutical facilities.
- Large stockpiles of bleach (sodium hypochlorite or sodium hydroxide) for use as a decontaminating agent. However, such agents are also widely used in legitimate commercial facilities.
- Specialized equipment for the lyophilization, *milling,* or microencapsulation of BTW agents. However, lyophilization machines are ubiquitous in the pharmaceutical industW.
- Refrigerated storage bunkers, freezers, or large quantities of liquid nitrogen for storing stockpiles of live or freeze-dried pathogens. However, since significant quantities of biological agents can be grown relatively quickly, long-term stockpiling of agents in refrigerated bunkers is unnecessary.
- Anomalous transport of microbial products or wastes off-site. However, microbial waste can be steam-sterilized.
- Incomplete or anomalous plant production records. However, a proliferant engaged in illicit production is unlikely to provide such records voluntarily. Records might also be

104 Federation of American Scientists, Working Group on Biological and Toxin Weapons Verification, ''A Legally Binding Compliance Regime for the Biological Weapons Convention: Refinement of Proposed Measures Through Trial Facility Visits," draft manuscript, March 1992, p. 12. See also SIPRI, vol. VI, op. cit., footnote 31, p. 35.

forged, although it is hard to do so convincingly.

Still, although the indicators listed above would not necessarily be associated with illicit production activities, a pattern of them might arouse suspicions.

BIOCHEMICAL SIGNATURES

BTW agents do not possess a single common chemical signature, such as the phosphorus-methyl bond characteristic of most nerve agents, but pathogenic microorganisms can be identified in minute quantities using sensitive immunological, biochemical, and genetic techniques. (See box 3-C, pp. 108-109.) Telltale traces of DNA from virulent strains of bacteria and viruses might be discovered in samples collected during an onsite inspection of a suspect site, even after the facility had undergone decontamination. Such traces might be indicative of previous research or production activities at the facility. Fermenters also generate large quantities of liquid wastes that might contain unusual metabolic byproducts and other telltale chemicals even after decontamination treatment.

Nevertheless, the fact that certain toxins and microbial pathogens have defensive or medical uses means that it can be difficult to distinguish legitimate from illicit BTW activities. Toxins also differ from chemical-warfare agents in that they do not leave persistent traces in the environment and are easily destroyed by autoclaving with superheated steam. The extent to which heat-neutralized protein toxins could be detected by immunological methods is unknown. Finally, the ability of modem analytical techniques to detect trace amounts of biological organisms could make legitimate biotechnology facilities reluctant to submit to such intrusive inspection for fear of losing proprietary information. Analytical instruments could probably be "blinded," however, to detect only the presence or absence of known BTW agents.

BIOMARKERS

Since the workforce in a BTW production facility would likely be immunized against the agents being produced, another approach to verification would be to determine whether the blood of workers in a suspect fermentation or vaccine facility contains antibodies against known BTW agents. Monitoring of immunization programs would involve taking blood samples from plant workers for onsite immunological analysis. Another approach would be to take blood samples from wild animals (e.g., rodents) in the vicinity of a suspect facility to detect possible exposure to unusual infectious agents.

Although most vaccine production plants require all workers to undergo initial and periodic blood collection and analysis, it would be difficult to negotiate a verification regime that requires such intrusive inspections. Furthermore, performing such tests as part of routine onsite inspections might violate U.S. constitutional protections against "unreasonable searches and seizures," since it would be difficult to protect confidential medical information unrelated to the purpose of the inspection. According to one legal scholar, "No treaty could empower inspectors to conduct random intrusive body searches for possible telltale evidence of radiation or biological weapons."[105] Another analyst argues, however, that biomedical sampling might be upheld by the courts on grounds of national security if there is a clear connection between the objectives of the regime and the analysis of biochemical indicators.[106]

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105 David A, Koplow, "Arms Control Inspection: Constitutional Restrictions on Treaty Verification in the United States," *New York University Law Review, vol. 66,* May 1988, p. 355.

106 Jerry R. Stockton, Edward A. Tanzman, and Barry Kellman, *Harmonizing the Chemical Weapons Convention With the United States Constitution* (McLean, VA: BDM International, Report No. DNA-TR-91-216, April 1992), p. 59.

Stockpile and Delivery System Signatures

Stockpiling of agent or loading into sprayers, munitions, or other delivery systems might be associated with a number of signatures. The following might be observable by aerial or satellite photography:

- cold storage of bulk BTW agents in refrigerated bunkers or igloos, although small quantities of stored agent would probably not be detected;
- storage depots for BTW-capable munitions and delivery systems in proximity to possible production facilities; and
- heavy trucks for the transport of empty or filled munitions in the vicinity of a biological production facility.

The remaining signatures could only be detected during an onsite inspection:

- inappropriate metal-working equipment or stock that might be used to fashion munitions;
- specialized equipment for filling BTW agents into munitions and warheads;
- breeding of insect vectors, or acquisition of equipment for disseminating biological agents and toxins as an aerosol cloud;
- munitions or parts thereof for disseminating BTW agents; and
- the training of troops in the tactical use of BTW agents.

Weapon Use Signatures

BTW agents might either be used deliberately or escape accidentally from a secret military research or production facility, as happened in the Soviet city of Sverdlovsk. It is therefore important to determine whether an outbreak of infectious disease in an area where it is not endemic is the result of clandestine biological warfare activities and, if possible, to identify its source. Field epidemiology can help investigate alleged cases of biological and toxin warfare.[107] Indeed, the Centers for Disease Control's Epidemic Intelligence Service was originally founded in 1951 out of concern that terrorists or foreign intelligence agencies might launch a covert BTW attack against the United States.[108]

As a first step, all of the likely natural causes of an epidemic must be investigated and excluded—a difficult task given the enormous variability of infectious diseases. Covert attacks aimed at economic sabotage are most likely to involve animal or plant pathogens. The best known case of a suspicious epidemic took place in 1981 in Cuba, which suffered a severe outbreak of dengue fever, a mosquito-borne viral illness. Of the estimated 350,000 people who developed the disease, approximately 10,000 suffered from severe (hemorrhagic) symptoms and 158 died, a mortality rate of 1.6 percent.[109] Cuban President Fidel Castro blamed the epidemic on covert U.S. biological warfare, which he alleged was being run by the Central Intelligence Agency. Epidemiological analysis indicated, however, that the outbreak was of natural origin. The Cuban epidemic occurred a few months after a major outbreak of hemorrhagic dengue fever in Southeast Asia. Epidemiologists determined that Cuban construction workers building a hotel in Hanoi, Vietnam, had become infected with the disease. After returning home to Cuba, they were bitten by

107 Peter Barss, "Epidemic Field Investigation as Applied to Allegations of Chemical, Biological, or Toxin Warfare," *Politics and the Life Sciences*, vol. 11, No. 1, February 1992, pp. 5-22.

108 Stephen S. Morse, "Epidemiological Surveillance for Investigating Chemical or Biological Warfare and for Improving Human Health," *Politics and the Life Sciences*, vol. 11, No, 1, February 1992, pp. 28-29.

109 Jay P. Sanford, "Arbovirus Infections," Eugene Braunwald et al., *Harrison's Principles of Internal Medicine*, 11th ed. (New York, NY: McGraw-Hill, 1987), p. 727.

Box 3-C-Biochemical Detection of BTW Agents

Inspections of microbioiogical laboratories or production facilities for indications of BTW activities might involve the collection and analysis of samples to detect the presence of undeclared pathogens or toxins. Such samples might include wipes from equipment and air filters or liquid samples from the waste stream or the environment near the plant Alternatively, air filters might be used to screen large volumes of air inside, or even at a considerable distance from, a plant.

Immunological techniques. The fastest method for detecting pathogens is to use specic antibodies that have been labelled with a tag of some type, such as a fluorescent molecule or a radioactive atom. Such antibodies would bind to the pathogen with high specificity, providing nearly unambiguous evidence of its presence. Techniques for producing large quantities of "monocional" antibodies, all specific to a single marker protein on the surface of a microorganism, permit the detection and identification of minute quantities of bacterial and viral agents (and protein toxins) in Mood or environmental samples. Such immunological screening techniques include radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA). A drawback of such assays is that they could not identify BTW agents that had been genetically modified to alter their immunological characteristics.

Bioassays. A pathogen or toxin can be detected by measuring its physiological effects on intact organisms or on isolated cell or enzyme systems. For example, many toxins work by specifically inhibiting the enzyme acetylcholinesterase involved in nerve transmission, thereby reducing its ability to break down the messenger chemical acetylcholine. Devices known as 'biosensors" are under development that use receptor molecules or enzymes immobilized on the surface of a chip to detect the binding of toxins or viral agents. One biosensor capable of detecting toxins, developed by engineers at Arthur D. Little, is moving into a manufacturing prototype.[1]

Genetic analysis. The advent of recombinant-DNA techniques has made it possible to identify minuscule quantities of microorganisms in complex samples.The first step is to prepare standards by isolating single-strand DNA sequences from specific microorganisms or synthesizing them chemically and labeling them with a radioactive isotope or a fluorescent dye. These labeled DNA fragments, known as "DNA probes," can pair up or "hybridize" with DNA in a sample if the sample contains DNA from the same microorganism. The advantage of DNA probes is their unique specificity, which enables them to identify a single type of pathogen even in complex mixtures.

Because of background noise, it can be difficult to detect probe/target hybrids when only a small number of microorganisms are present in the sample. This problem was solved in 19$5 with the development of a powerful new technique known as polymerase chain reaction (PCR), which can amplify a given DNA sequence as much as $1O^{8}$ times. PCR therefore makes it posssible to use DNA probes to identify pathogens present in trace quantities-as few as tensor hundreds of microorganisms-without having to grow them into larger colonies over a period of days or weeks. Since PCR reagents are available in kit form, this technique has greatly speeded the diagnosis of infectious diseases, including potential BW agents such as anthrax bacteria.[2] PCR is also useful for analyzing biological samples in the field or during an onsite inspection of a suspect facility it has even detected killed bacteria in autoclaved samples?

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1 Richard F. Taylor, Arthur D. Little Corp., "Portable, Real-Time Biosensors for Chemical Agent Verification," presentation at the Chemical Weapons Convention Verification Technology Research and Development Conference, Herndon, VA, Mar. 2-3,1993.

2 Department of Defense, *Annual Report to Congress* on *the Research, Development, Test and Evaluation of the Chemical/Biological Defense Program for the Period October 1, 19$0 Through September 30, 7991,* RCS:DD-USDRE(A) 1085, p. 51. See also, M. Carl et al., "Detection of spores of *Bacillus anthracis* using the polymerase chain reaction," *Journal of Infectious Diseases,* vol. 185, 1992, pp. 1145-1148.

3 T. Barry and F. Gannon, "Direct Genomic DNA Amplification From Autoclaved Infectious Microorganisms Using PCR Technology," *PCR Methods and Applications,* VOL 1, 1991, p. 75.

Nevertheless, PCR has some limitations. First, it is only suitable for identifying known organisms, since one must decide in advance which DNA sequences to use as probes. Second, because many pathogenic microbes (e.g., anthrax bacteria) are ubiquitous in the environment in trace amounts, a probe of sufficient sensitivity may find "prohibited" DNA everywhere! Third, the accuracy of PCR depends on both the length of the target DNA sequence and the length of the PCR "primers," which bind to the target DNA to initiate the amplification process. As one tests for shorter sequences (e.g., 100 instead of 1,000 DNA base-pairs), the sensitivity of the technique increases but its specificity declines, since several different microbial species may have identical short DNA sequences. For this reason, two levels of detection have been proposed, depending on the characteristics of the DNA probe. The first level would identify the group of pathogenic bacteria to which a suspect agent belongs by detecting a DNA sequence common to all species in that group; the second level would provide species-specific identification by using longer DNA probes specific to each microorganism targeted for detection.[5]

Genetic *fingerprinting.* Known technically as restriction-fragment length polymorphism (RFLP) analysis, this technique involves the use of special "restriction" enzymes that cut microbial DNA at specific sites. This treatment results in a pattern of DNA fragments of different sizes, which can be analyzed by separating the fragments on a gel. Genetic fingerprinting can also be done with RNA viruses. The result of this technique is a characteristic pattern of spots on the gel. Since different DNA sequences will result in a different pattern of spots, comparing such maps will reveal the extent to which two strains of a bacterium or virus differ genetically.

All microbial pathogens can be "fingerprinted" by analyzing their genetic material (DNA or RNA). Since there are always minor genetic differences among various strains of a pathogenic microorganism, it is very likely that a laboratory-developed strain is genetically distinct from an indigenous strain. Moreover, an indigenous strain that has been produced in large quantities is likely to be more genetically homogeneous than the causative agent of a natural epidemic. For many microbial pathogens, scientists have compiled a library of characterized strains t hat can be compared with any newly discovered strain. Thus, genetic fingerprinting often provides enough information to determine the source of a virus and whether it has been modified genetically in the laboratory. Trace amounts of genetic material can be amplified for further analysis using PCR.

Town analysis. For toxins, analytical techniques for detection and characterization include immunoassay, as well as analytical chemistry techniques such as combined gas chromatography/mass spectrometry (GC/MS), liquid chromatography, and others. Quadruple mass spectrometry is used to analyze protein toxins, as well as samples dissolved in water.[6] Very large biomolecules must be broken down into smaller components for analysis. To this end, a technique known as pyrolysis mass spectrometry involves heating complex materials in a controlled manner to generate characteristic chemical signatures that can then be analyzed by a mass spectrometer.[7] These signatures are compared with a large computer database of known chemical spectra to identify the compounds present Finally, if a pure sample of a protein toxin or peptide bioregulator is available, it may also be possible to identify it from its amino-acid sequence. Off-site detection of toxins is nearly impossible, however, because they lack volatility.

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4 Moreover, a laboratory might detect a contaminant from past rather than current work, such as anthrax spores from earlier samples.

5 Barbara J. Mann, *Detection of Biological Warfare Agents Using the Polymerase Chain Reaction* (Research Triangle Park, NC: Battelle Memorial Institute, September 1992), DTIC No. AD-A259391.

6 External Affairs and International Trade Can-Verification Research Unit, *Verification: Development of a Portable Trichothecene Sensor Kit for the Detection of T-2 Mycotoxin in Human Blood Samples* (Ottawa: External Affairs, March 1987).

7 Diane M. Kotras, "New Detection Approaches for Chemical and Biological Defense," *Army Research, Development and Acquisition Bulletin*, January-February 1989, p. 2.

mosquitoes, which then transmitted the disease to others. [110]

Another suspicious epidemic that still remains to be explained took place during the civil war in Rhodesia (now Zimbabwe) from 1978 to 1980. An unprecedented outbreak of cattle anthrax was almost entirely confined to the Tribal Trust Lands-the areas then assigned to Rhodesia's blacks and accounting for about 17 percent of the country's land area. [111] Since cattle were the primary source of wealth for black farmers, the epidemic led to the severe impoverishment of the ected rural populations. The outbreak of cattle thrax was accompanied by a secondary human idemic, which resulted in more than 10,000 infections and 182 human deaths. Since anthrax is not contagious from one individual to another, the explosive nature of the human epidemic was striking: the reported incidence of human anthrax cases during the 1979-80 period was more than 400 times the average incidence of the previous 29 years. Some epidemiologists believe that the losing Rhodesian government forces may have resorted to biological warfare with anthrax against cattle in order to impoverish the rural black population, as a desperate tactic in the final months of the civil war, and that humans were infected secondary to contact with infected animals or animal products. [112]

EPIDEMIOLOGICAL ANALYSIS

Distinguishing natural disease outbreaks from those produced deliberately requires careful investigation and knowledge of local diseases and endemic infections. There is at present no generally accepted methodology for investigating the possible use of BTW agents. But Dr. Jack Woodall, an epidemiologist with the World Health Organization in Geneva, has identified a number of characteristics of a disease outbreak that would suggest it was not of natural origin: [113]

- *The appearance of an endemic disease far outside its established range.* A natural disease outbreak might be distinguished from a BTW attack by determiningg whether its source is an agent endemic to the region. Although jet travel has made it easier for infectious agents to spread discontinuously from one continent to another, the progression of an epidemic typically involves gradual spread to contiguous regions or along transportation routes.
- *Appearance of a vector-borne disease in the absence of natural vectors or reservoir hosts.* Plague epidemics, for example, typically begin in rats and are spread to man by infected fleas. The initial form of the disease in humans is the bubonic form affecting the lymph nodes, which later converts into the more lethal and contagious pneumonic form. Thus, the sudden appearance of pneumonic plague in humans (1) in the absence of infected rats and fleas, and (2) without precursor cases of the bubonic form, would be suggestive of a covert BW attack.
- *Pulmonary disease in the absence of natural mechanisms for producing high-concentration biological aerosols. Since* many infectious diseases do not naturally infect the lungs, the anomalous appearance of a respi-

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110 Telephone interview with Dr. Scott Halstead, Associate Director of the Health Sciences Division, Rockefeller Foundation, New York, NY, Aug. 6, 1992.

111 See J.C.A. Davies, "A Major Epidemic of Anthrax in Zimbabwe, Part l," *Central African Journal of Medicine,* vol. 28, 1982, pp. 291-298; and J.C.A. Davies, "A Major Epidemic of Anthrax in Zimbabwe, Part 2," *Central African Journal of Medicine, vol. 29, 1983,* pp. 8-12.

112 Meryl Nass, "Anthrax Epizootic in Zimbabwe, 1978-1980: Due to Deliberate Spread?" The *PSR Quarterly,* vol. 2, No.4, December 1992, pp. 198-209.

113 John P. Woodall, "WHO Health and Epidemic Information as a Basis for Verification Activities Under the Biological Weapons Convention" S.J. Lundin, cd., *Views on Possible Verification Measures for the Biological Weapons Convention,* SIPRI *Chemical* & Biological Warfare Studies No. 12 (Oxford, England: Oxford University Press, 1991), pp. 59-70.

ratory form of such a disease might be indicative of a deliberate aerosol attack. Other human activities than deliberate military attack may generate infectious aerosols, however. The outbreak of Legionnaires' Disease at a hotel in Philadelphia, for example, was traced to a natural microbial contamination of the building's air-conditioning system.

- *Unusual epidemiological patterns that differ from natural disease outbreaks.* A deliberate BW attack by aerosol dissemination would infect a large number of exposed individuals simultaneously, causing a majority of them to develop symptoms at approximately the same time. Thus, instead of a gradual rise from a smaller number of precursor cases, there would be an "explosive' outbreak of disease in many thousands of people.[114]

While these characteristics are all plausible, the recent natural outbreak in New Mexico of ' 'Navajo flu, ' a virulent respiratory illness with greater than 30 percent mortality, meets nearly all of the criteria Woodall proposes. Hanta virus, now known to be the cause of the illness, had never before been known to occur among humans in the Western Hemisphere, Whereas all previous reported cases around the world were hemorrhagic fevers with shock and kidney failure, this recent outbreak took the form of a respiratory illness. Finally, the epidemiology of the disease was extremely unusual and confused investigators for months. This episode points out the difficulty of distinguishing a highly anomalous disease outbreak of natural origin from the deliberate or accidental release of biological-warfare agents.[115]

An important task of epidemiological analysis is to characterize the strains of disease-causing agents indigenous to the affected area, thereby making it possible to distinguish preexisting "background" strains from BW agents introduced from the outside. Even if it could be ascertained that a disease outbreak was of artificial origin, however, it might still not be clear who had initiated the attack. It might also be difficult to collect the necessary data if investigators were denied permission to visit the sites of the alleged attacks.

A current problem with depending on epidemiology to detect the use of BTW agents is that such skills are unlikely to be available in those regions of the world where biological warfare is most likely. In order to detect new infectious diseases such as AIDS before they reach epidemic proportions, the epidemiologist Donald A. Henderson has proposed the establishment of an international network of research centers to monitor the emergence and spread of new infectious diseases, linked to a global rapid-response system.[116] Beyond its obvious public-health benefits, such a global surveillance system would make it easier to distinguish artificially induced epidemics associated with the covert use of BTW agents from ordinary background noise. ''117 It would thereby help to deter biological warfare and also to identify false claims of BW, an important objective.

Table 3-2 summarizes the various potential signatures associated with BTW development,

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114 Nevertheless, the anthrax epidemic in the Soviet town of Sverdlovsk (now Yekaterinburg) in 1979-now recognized to have been the result of an accidental release of anthrax spores from a Soviet military biological facility-was associated with a gradual increase in the number of cases over a period of several weeks, a pattern that appeared consistent with a natural epidemic. It is known, however, that at low levels of exposure, anthrax spores germinate at different rates in exposed primate hosts, resulting in highly variable incubation periods. Matthew Meselson, Harvard University, personal communication 1993.

115 Zelicoff, Op. cit., footnote 29.

116 Donald A. Henderson, "Surveillance Systems and Intergovernmental Cooperation" Stephen S. Morse, cd., *Emerging Viruses (New* York: Oxford University Press, 1992), pp. 283-289. See also Joshua Lederberg et al., *Emerging Infections: Microbial Threats to Health in the United States* (Washington, DC: National Academy Press, 1992), pp. 134-137.

117 Mark L. Wheelis, "Strengthening Biological Weapons Control Through Global Epidemiological Surveillance, " *Politics and the Life Sciences,* vol. 11, No. 2, August 1992, pp. 179-189.

Table 3-2—Biological Weapon Program Signatures and Concealment

Program stage	Signature	Detection methods (examples)	Concealment methods, comment
Research & development	Scientific and technical publications (presence or absence)	Literature survey and analysis	1. Manage publication activities 2. Use widely available technical information rather than design new agents or techniques
	Nondeclaration of work with potential BTW agents or with pathogen aerosols	Human intelligenoe (humint), on-site inspections	Conceal undeclared activities
Clandestine production plant	Security measures	Overhead imaging or humint	Conceal measures, or place plant within other secure facilities
	Large numbers of eggs or laboratory animals for virus production	Humint	Use tissue culture rather than animals for production of viruses
	Storage depots for BTW-capable munitions	Overhead imaging or humint	Conceal depots underground (although faciiity building would be visible)
	imports of dual-use equipment (fermenters, lyophilizers, microencaipsulation systems)	Tracking of exports to suspected proliferants	1. Obtain equipment from multiple suppliers, or through intermediaries 2. Divert equipment from legitimate civil activities 3. Make equipment indigenously
Converted or multipurpose pharmaceutical plant	Security measures	Overhead imaging or humint	Conceal measures
	Residues of virulent microbial strains or genetically modified agents	Sampling of air, water, or soil in or near suspect plants; together with various forms of biochemical analysis (e.g., ELISA, bioassay, DNA probes, PCR)	1. Decontaminate production line wlth bleach or superheated steam and autoclave cultures 2. Remove wastes for off-site disposal 3. Claim that BTW agents are being used for defensive activities
	Special safety and containment measures	Onsite inspection of suspect plants	1. Sacrifice worker safety 2. Modern biotech plants increasingly have these features
	Processes or capacity that cannot be justified on technical or economic grounds	Onsite inspection of suspect plants	Such assessments are highly subjective
	Seed stocks, cell lines, and equipment (e.g., microencapsulation) inappropriate for declared activities	Onsite inspection and sampling	Claim that material and equipment is being used for legitimate medical applications, although possibilities may be limited
	Omission of costly measures to ensure purity and sterility of pharmaceuticals or to inactivate agent to make vaccine	Onsite inspection	Employ measures to simulate pharmaceutical production (costly)
	Facilities for rapid, large-scale decontamination	Onsite inspection	Use legitimate vaccine production activities as a cover

Program stage	Signature	Detection methods (examples)	Concealment methods, comment
	Evidence of immunization to BTW agents in plant workers or evidence of infection in people or animals nearby	Blind tests	Refuse permission to take blood sampils
Weaponization and testing	Uniquely configured arsenals (e.g. distribution of storage bunkers)	Overhead imaging	Pattern facilities after conventional arsenals
	Cold storage of BTW agents	Thermal infrared imagery Excess electrical capacit y	1. Produce large quantities of agent shortly before use to minimize need for storage 2. Mask thermal-infrared emissions from refrigerators
	Specialized equipment for filling agents into munitions	Onsite inspection	Conceal filling operation at some remote location
	BTW testing facilities, such as small aerosol chambers	Onsite inspection, sampling and analysis	Carry out tests inside dosed buildings
	Field testing of aerosol generators and delivery systems	Overhead imaging, onsite inspection, sampling and analysis	1. Mask test grid 2. Use legitimate activity such as biopesticide dissemination as a cover for illicit activities, although high security might be a giveaway
	Large animals for aerosol testing		
	Field training of troops		
	Uniquely configured test facilities	Overhead imaging	1. Make special features temporary 2. Test on overcast days, at night, or in absence of overhead imaging systems
Weapon use	Anomalous characteristics of a disease outbreak (e.g., atypical agent, explosive disease spread, pulmonary disease in absence of natural respiratory	1. Field epidemiology 2. Genetic fingerprinting of disease agent	Use a disease agent indigenous to the area being attacked

SOURCE: Office of Technology Assessment, 1993.

production, weaponization, and use. Many of these indicators are nonspecific, since their presence could be associated with other, legitimate activities. Even so, a pattern of such signatures would be suggestive of a clandestine BTW program that could then be confirmed by other means.

MILITARY IMPLICATIONS OF GENETIC ENGINEERING

The past two decades have seen revolutionary advances in the ability to manipulate the genetic characteristics of living organisms at the molecular level. Genetic engineering involves identifying regions along the DNA molecules that encode desirable genetic characteristics and cutting and splicing these segments of DNA with enzyme tools to create "recombinant" strains. Since all living creatures contain DNA, it is also possible to combine genes across species lines to give an organism novel traits that do not occur in nature.

| Novel Agents?

Techniques for the engineering of genes in bacteria and animal cells, and for the modification

of proteins, have become widely available. Although advanced genetic-engineering capabilities are still rare in the developing world, gene-splicing "kits" containing the necessary equipment and reagents (e.g., restriction enzymes) can be easily obtained by mail order, and much of the necessary know-how is openly published in the scientific literature. Some analysts have speculated that gene-splicing technologies could be used to develop 'second-generation' BW agents with greater military utility by making the behavior of these agents in the environment more predictable.[118] Toxin genes and Virulence factors might also be transferred from one species of microorganism to another. According to John Birkner, a foreign technology analyst for the Defense Intelligence Agency, "recombinant-DNA techniques could open up a large number of possibilities. Normally harmless, nondisease-producing organisms could be modified to become highly toxic and produce effects for which an opponent has no known treatment. Other agents, now considered too unstable for storage or biological warfare applications, could be changed sufficiently to become effective. ' '[119]

Although it could theoretically add a toxin gene to a harmless bacterium to render it virulent, recombinant-DNA technology is unlikely to produce novel pathogens more devastating than the highly infective and lethal agents that already exist in nature. The reason is that any attempt to combine genes from unrelated organisms is likely to interfere with the highly developed and integrated pattern of genetic traits that give rise to pathogenic behavior. Since a whole constellation of genes must work together for a microorganism to cause disease, altering a few genes with recombinant-DNA techniques is unlikely to yield a novel pathogen significantly more deadly than natural disease agents.[120] It is therefore doubtful that genetic engineering could result in novel BW agents with greater potency than naturally occurring agents.

Increased Controllability of Microbial Agents

Nevertheless, the genetic modification of standard BTW agents might, however, overcome specific obstacles that currently limit their military utility. In particular, genetic engineering and modern biotechnologies can facilitate microbial production, improve storage and delivery, create antibiotic resistance, and enhance the controllability of existing pathogens. It is not clear, however, that these modifications would significantly alter the military utility of BW agents compared with the numerous already known agents.

SHORTER INCUBATION TIME

Modifying BW agents to act more rapidly would increase their tactical utility on the battlefield, although this is unlikely to be accomplished anytime soon.

ENVIRONMENTAL STABILITY

Genetic engineering might be able to increase the ability of microorganisms and toxins to withstand some of the stresses associated with storage and dissemination, for example, by inserting complexes of genes for resistance to inactivation by temperature, ultraviolet radiation, drying, and the shear forces associated with aerosol formation. Most of these traits are genetically complex, however, and are not well understood.

INCREASED VIRULENCE

Development of a system for the super-expression of toxin genes has made it possible to develop recombinant bacterial strains that pro-

118 Erhard Geissler, ''Implications of Genetic Engineering for Chemical and Biological Warfare,'' *World Armaments and Disarmament: SIPRI Yearbook 1984* (London: Taylor & Francis, 1984), pp. 421-451.

119 John Birkner, cited in R. Jeffrey Smith, "The Dark Side of Biotechnology," Science, vol. 224, June 15, 1984, p. 1215.

120 Jonathan B. Tucker, ''Gme: Wars,'' *Foreign Policy, No. 57,* winter 1984-85, p. 62.

duce 10 to 100 times more toxin than natural strains.

ANTIBIOTIC RESISTANCE

Inserting antibiotic-resistance genes into naturally infectious agents can make them resistant to one or more prophylactic or therapeutic drugs, rendering such defenses useless. At the same time, an attacker could immunize his troops against the modified agent, protecting them without the need for antibiotics. Reportedly, the Soviet Union launched a secret program in 1984 to develop a genetically engineered form of plague that was resistant to antibiotics.[121]

VACCINE PRODUCTION

Recombinant-DNA techniques make it easier and safer to produce specific vaccines to match novel agents, thus enabling the attacker to protect his own forces while denying a vaccine to the defender. In the past, the difficulty of producing effective protective vaccines was a major obstacle to acquiring an offensive BTW capability. Nevertheless, recombinant vaccines are not always effective because they represent one or a few antigens rather than the full set of antigens present in the actual pathogen.

CONTROLLED PERSISTENCE

Genetic engineering might result in more controllable BW agents through the manipulation of genes to program the survival of a bacterial population released into the environment. For example, it might be possible to program microorganisms genetically to survive only under a narrow set of environmental conditions. Alternatively, one might design regulatory sequences known as ''conditional suicide genes, ' which cause a microorganism to die off after a specified number of cell divisions.[122] By inserting such genes into pathogens, it might be possible to create a BW agent that would cause disease for a limited period of time and then spontaneously die off.

IMMUNOLOGICAL MODIFICATION

By means of gene transfers, it would be possible to modify the antigenic (antibody-inducing) proteins on the outer surface of a pathogenic virus or toxin, thereby rendering the modified agents insensitive to a preexisting host immunity or to standard vaccines and antitoxins. (Since most toxin antigens are located on the scaffolding of the molecule rather than near the site responsible for its toxic effects, it would be possible to alter the immunological characteristics of a toxin without changing its biological activity.) Further, since most diagnostic procedures for BTW agents rely on the detection of certain surface antigens with antibodies, modification of the antigens would make it harder to detect, identify, and counter the modified agents.

HOST SPECIFICITY

Some analysts have raised the grotesque possibility of making microbial pathogens more discriminate by designing ''ethnic weapons' that exploit differences in gene frequency between populations to selectively incapacitate or kill a selected ''enemy' population to a greater extent than a''&iendly' population. Yet human populations are not uniform enough to be uniquely targeted by a given pathogen.[123]

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These possibilities notwithstanding, the practical obstacles to developing more controllable BW agents remain enormous. Even if genetic engineering could produce recombinant pathogens that survived for a predetermined length of

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[121] Barry, op. cit., footnote 97, p. 41"

[122] Preparatory Committee for the Third Review Conference of the Parties to the BWC, *Background Document on New Scientific and Technological Developments Relevant to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction,* Document No. BWC/CONF.III/4, Aug. 26, 1991, p. 11.

[123] Novick and Shulman, op. cit., footnote 47, p. 114.

time in the environment, they would remain incalculable in their effects, since their dissemination would still rely on wind and weather, and mutations might change the behavior of a genetically modified agent after it had been released. Once released,, living pathogens might propagate, evolve, and develop ecological relationships with other living things in ways that cannot be entirely foreseen. Furthermore, genetically engineered pathogens would require extensive trials to verify that they would survive long enough to infect target personnel after being disseminated by a weapon system and exposed to the natural environment, in which most microorganisms are extremely fragile. Thus, testing in human subjects might be required to give a military planner confidence in genetically engineered biological agents.

| Modified Toxins and Bioregulators

Another potential threat from the biotechnological revolution is the development of new toxin-warfare agents. Known protein toxins, such as botulina1 and ricin, deteriorate in response to environmental factors such as temperature and ultraviolet radiation, and thus rapidly lose toxicity after dissemination. Although genetic engineering is unlikely to increase the potency of naturally available toxins, it might conceivably be applied to modify the chemical structure of toxins to:

- increase the stability of toxins so that they can better be disseminated as an aerosol;
- alter the antigenic structure of toxin molecules, rendering them insusceptible to existing antitoxins or antibody-based diagnostic techniques;
- develop "chimaeric" toxins (combinations of two different toxin molecules, such as ricin and diphtheria toxin) that are more capable of penetrating and killing target cells; and
- design novel peptide toxins (possibly consisting only of the biologically active region of a protein toxin) that are as poisonous as nerve agents but are small enough to penetrate the filters currently used in masks and protective garments.[124]

BIOREGULATORS

Recombinant-DNA research may also lead to the development of more effective incapacitants. With genetic engineering, even the body's own natural substances might be utilized as warfare agents. "Bioregulators' are small, physiologically active peptides (chains of amino acids smaller than proteins) that are normally present in the body in minute quantities and that orchestrate key physiological and psychological processes. They are active at very low concentrations and influence the full spectrum of life processes, both physiological and mental. Bioregulators govern, for example, hormone release, control of body temperature, sleep, mood, consciousness, and emotions. An important subgroup of the bioregulators are the opioid peptides, which can induce analgesia and euphoria. Since these naturally occurring peptides are active in the body in trace amounts, the application of larger quantities might induce euphoria, fear, fatigue, paralysis, hallucinations, or depression, giving them some potential as nonlethal incapacitating weapons.[125]

Bioregulators might be modified chemically to enhance their physiological activity, stability, or specificity. For example, the modification of the peptide hormone LHRH (leutenizing hormone

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124 External Affairs and International Trade Canada, Verification Research Unit, *Novel Toxins and Bioregulators: The Emerging Scientific and Technological Issues Relating to Verification of the Biological and Toxin Weapons Convention (Ottawa:* External Affairs, September 1991), p. 47.

12S Swedish National Defense Research Institute, *Genetic Engineering and Biological Weapons*, Report No. PB88-210869 (Umea, Sweden: National Defense Research Institute, November 1987; translated for the Office of International Affairs, National Technical Information Service, May 1988), p. 58.

Table 3-3—implications of Genetic Engineering for Biological and Toxin Warfare

Capability	Possible now	May be possible In 5 years	May be possible In 10 years, If ever
Shorter incubation time		X	
Temperature stability	Xa		
UV stability		Xb	
Drying/aeosol stability			X
Antibiotic resistance	x c		
Controlled persistence			X
Immunological modification	x c		
Host specificity			X
Cloning of toxin genes	X		
More stable toxins		X	
Novel toxins			X

a For certain protein toxins
b For bacteria
c In some cases, not all

SOURCE: Office of Technology Assessment, 1993.

releasing hormone) by substituting a single amino acid yielded a product 50 times more potent.[126] Even so, it would be difficult to disseminate peptides through the air in a militarily effective way. The ability of a peptide to diffuse across the mucosa1 membranes of the respiratory tract depends on its molecular size. Although attempts to deliver the small peptide hormone ADH (antidiuretic hormone) with a nasal aerosol have been successful, similar efforts with insulin have failed because of the molecule's relatively large size.[127]

The possible implications of genetic engineering for biological and toxin warfare are summarized in table 3-3. Although the potential for the misuse of genetic engineering to develop new and militarily more effective BTW agents currently appears limited, this emerging threat clearly deserves to be monitored carefully. *Advanced* genetic-engineering capabilities are still rare in the developing world, but most of the larger countries in the Middle East already have the technical capability to selectively breed microbial strains with enhanced virulence, survivability, and antibiotic resistance. In According to one analyst, "If you can identify the gene you want to move, it is possible to do so. ' [129]

For at least the medium-term, BTW proliferants are likely to produce proven agents such as anthrax and botulin toxin, rather than invest large amounts of time and money on experimentation with genetically engineered microorganisms. Eventually, however, technologically sophisticated proliferants might try to modify standard agents to make them more stable during dissemination or more difficult to detect or to defend against.

126 Government of the United States, op. cit., footnote 44, p. 29.

127 Zelicoff, Op. cit., footnote 29.

128 Anthony H. Cordesman, *Weapons of Mass Destruction in the Middle East* (London: Brassey's (UK), 1991), p. 77.

129 Zelicoff, Op. cit., footnote 29.