ABSTRACT

Policies are usually initiated in response to specific circumstances, but they do not become effective unless they are embedded in operating institutions. Understanding the historical process through which policies evolve is essential for assessing their character and their consequence. This study is a detailed history of the US bioweapons program from its inception to the present. It is an original analysis based on archival documents and scientific reports. The issue is, does the application of national security measures such as the classification of scientific programs improve biodefense?

Initial organization of the US bioweapons program as a secret, military program that performed threat assessment work (1941-1969) led to the development and stockpiling of biological weapons for deterrence, but few medical defenses. A strategic review in 1969 concluded that bioweapons were not useful for legitimate military missions and did not enhance US deterrence. It also concluded that proliferation threatened the US. To reduce proliferation, the US destroyed its
bioweapons arsenal and enforced the norm against bioweapons acquisition by signing the Biological and Toxin Weapons Convention (BWC) in 1972. Subsequent organization of the US biodefense program was as an unclassified military medical research program. This work at the US Army Medical Research Institute of Infectious Diseases (USAMRIID) improved medical countermeasures without a concomitant classified, offensive program. However, in response to the terrorist attacks of 2001, the US is again imposing secrecy over important aspects of its biodefense work, including its threat assessment work. Based on the analysis here, current policy will increase the risk to US security by both enlarging the threat space and reducing defensive options.
STRUCTURING BIODEFENSE: LEGACIES AND CURRENT POLICY CHOICES

By

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Dissertation submitted to the Faculty of the Graduate School of the University of Maryland, College Park, in partial fulfillment of the requirements for the degree of Doctor of Philosophy 2007

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“The unleashing of the power of the atom has changed everything but our modes of thinking, and thus we drift toward unparalleled catastrophes.”

-- Einstein
Dedication

For my sons
Acknowledgements

So much intervenes in the course of developing an idea, especially when that course takes years. Something about the process requires time spent in speculation, misery, inspiration, and plain hard work – possibly in equal amounts. As Virginia Woolf wrote, “If anything comes through in spite of all this, it is a miracle, and no book is born entire and uncrippled as it was conceived.” For the miracle of this dissertation’s completion, I want to thank those who helped shape my thinking and those who tempered the hard times with their friendship.

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indebted to my sister, Sheryl, who has lifted me up out of my doubts – usually with a really good laugh! My brother, Ken, set the intellectual bar high. And my father, Brian, has always kept alive the question, “why?” – sustaining the curiosity one must have to pursue any topic.

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Chapter 1: Management of the Bioterrorist Threat

“Problems are not solved at the same level of awareness that created them.” – Einstein

Biological weapons are excellent terrorist weapons, but are not effective for legitimate military missions. That was the original judgment of the US in the first few decades of the twentieth century. During World War II, the accuracy of that assessment was challenged through an intense BW R&D program that grew through the 1950s and 1960s. The original justification for the US BW program was defense against presumed enemy BW programs: the result was a stockpile of biological weapons. Driving this outcome was the argument that understanding of offensive BW potential was critical to development of defenses. However, when the US terminated its BW program in 1969, it had not produced or stockpiled adequate medical countermeasures. Current policy in reaction to the 2001 terrorist events is applying the same logic – and expecting the opposite outcome. Instead, US policy should evolve out of the predominantly open and defensive medical research program that has existed since 1969 to manage the bioweapons threat. That was a robust, unclassified scientific R&D program based at the US Army Medical Research Institute of Infectious Diseases (USAMRIID): it developed important medical defense against the most virulent bioagents known.

The Approach

This study reviews the history of the US policy with respect to biological weapons. It is a review of the judgments made in 1942 that led the US to create its
own biological weapons program and those made in 1969 when the judgment was made to terminate the offensive part of the program and restrict itself to defensive investigations. There is very little published about the policy discussions that led to the origins of the US BW program. There is less about the US biodefense program after 1969.

No information that is not publicly available was used in this study. The information presented to establish the context for the US BW program’s origins comes from the archives of the National Academy of Sciences (NAS). There are extensive records from the NAS committees that argued for the establishment of the US BW program.

The work at USAMRIID after 1969 receives at most a passing reference in every work published about the US biodefense program. There is literally no published history of this effort that describes the work performed at USAMRIID despite the fact that it was unclassified. A major effort was made to locate a complete set of Annual Reports published by USAMRIID: these contain summaries of the scientific work done there. There is no single library outside of USAMRIID that has every published year and the USAMRIID library is not accessible without a security clearance and does not lend materials to other libraries. There is reason, therefore, to believe that the nearly complete set assembled for this dissertation (only FY1983 and FY1984 are omitted because they were not published) is the only one of its kind outside of Ft. Detrick. The extensive summaries provided in Appendix A and the tables in Appendices B, C, and D are based on an exhaustive reading of every
scientific report in every USAMRIID Annual Report from 1969-1990. This is, therefore, the first detailed account of USAMRIID’s work ever published.

Through these original contributions, a more informed discussion about biodefense policy is now possible.

**Broader Issues**

Fundamentally, this study deals with the benefits and risks of secrecy and of transparency in security policy. Biotechnology is currently a prominent area of concern, but it is only a single example of the kinds of security challenges countries will face as technologies evolve seemingly ever more rapidly. Practices in other areas are adjusting to the way technology is altering their operations. How we understand the requirements of national security may also require changes in practice.

In the midst of an evolving global dynamic, policymakers are faced with the need to make security decisions under complexity – a circumstance recognized years ago.¹ This complexity is extended in the case of biotechnology because of its application to predominately medical research and not to the creation of novel weaponry. Decisions about how to manage scientific work will affect the development of both areas of research and, ultimately, the security of the US.

Wolfgang Reinicke explored the need for public policy to evolve in ways commensurate with the challenge. In his book, *Global Public Policy*, he characterizes the challenge well:

> Dual-use technologies are not “destabilizing” in themselves – their military application is. A regime based on denial has to assume that dual-use technologies are destabilizing because it has no means of verifying how they

are used. Although this was a reasonable policy maxim during the era of bipolar conflict, the end of the cold war and in particular the advent of globalization have made a denial-based regime, except with regard to a few highly sensitive technologies, unrealistic and unwise in light of other pressing global issues. Rapid technological evolution, borderless communication, and continued liberalization of international economic transactions make efforts to establish criteria to proscribe transfers a waste of time and resources, creating a false sense of security. As an alternative, disclosure-based regulation coupled with public-private partnerships represents a regime in and of itself, and not just a data support structure, although such a structure is no doubt an important element in overcoming information asymmetry – an issue that regulation by denial does not even attempt to address. Under a disclosure-based regime, dual-use technologies would be “stabilized” by disclosing their application, ensuring adherence to agreed-upon standards. In addition, stability established through disclosure is qualitatively superior to stability established through denial. Disclosure-based regulation not only has a better chance of preventing market failure (that is, proliferation), but also permits other interests – public and private – associated with dual-use trade to be realized, thus responding to their changing weight in framing the debate on this global policy issue.²

This approach to security is not generally understood. Much of what is generally believed about national security still rests on notions of deterrence and models based on the interaction of sovereign states seeking security through the protection of their own borders. Globalization is challenging the ability of states to ensure their own security through traditional methods of national security. One definition of globalization is, “an ‘unbundling’ of the relationship between sovereignty, territoriality, and state power.”³ Given the transformational changes in the way the world operates, strategies going forward must adapt. Recently, more studies of the effects of and policy responses to globalization have been published.


The notions of national and military security are undergoing a significant transformation as the emphasis shifts to cooperative or multilateral defence and security mechanisms, and the security agenda expands to incorporate a multiplicity of threats from the environmental to the cultural. This reflects the increasingly overlapping fortunes of national communities of fate.4

The importance of globalization for security policy is clear. Biotechnology can be used by any state for the improvement of its medical research. It can also be used to create weapons. Preventing the military application of biotechnology is crucial and likely requires the cooperation of all states.

Crafting an appropriate policy response to the threat of biological weapons requires an understanding of the history of policy in this area, the characteristics of the threat, and the characteristics of the tools that will likely work to prevent catastrophe.

Since overwhelming retribution cannot be inflicted on an opponent that cannot be identified, it has to be assumed that the problem escapes the bounds of deterrent policy. The natural threat certainly does. Moreover, since access cannot be restricted by standard methods of security classification and physical isolation, direct preventive protection has to be based on different methods than those developed for nuclear materials and chemical agents.... In combination, natural and deliberate sources of biological threat constitute powerful pressure for innovation in security policy.5

Generally, a balance needs to be struck between the conduct of military and law enforcement operations and the control of infectious diseases generally. There are actually sharp trade-offs between the policy principles and institutional practices of these two communities. Starkly put, the alternatives are secrecy vs. transparency.

4 Ibid., 136.

National security measures are designed to clearly identify a threat and countermeasures to it. The nature of the work lends itself to secrecy. Intelligence collection and threat assessment work generates information that is classified. Revelation of such would compromise national security by revealing the very dangers the US seeks to prevent. Such information, unfortunately, does not remain secret forever. At numerous times in US history, highly consequential information has been acquired by foreign governments. Nonetheless, the tendency is to seek to control access to materials and information and to identify vulnerabilities. The logic of this approach to national security is simple and intuitive. In the case of biological weapons, however, it has been shown to be counterproductive.

The basis for classifying information has been at the prerogative of the Executive Branch. There is no clear statutory authority for the protection of military and state secrets. Instead, “the authority of the Presidency itself has been the principal basis for the entire network of security classification, with the possible exception of the Atomic Energy Act.”6 President Franklin Roosevelt is credited with developing the specific criteria used to classify documents: he also gave the order to found the classified US BW program in 1942.

Greater transparency is the alternative to standard national security methods. Transparency is not meant here to be understood as making all information generated available to every curious soul on an instantaneous basis. There are degrees of transparency. However, programs structured under the presumption of transparency are markedly different from those structured under principles of secrecy.

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Transparency serves important purposes in international relations. Perhaps its most important role is in providing a level of reassurance among states and for the institutionalization of norms of behavior. This approach was embraced by the US after 1969 because of the need to prevent the proliferation of biological weapons.

Biotechnology is the specific case here, but other technologies, e.g. nanotechnology, will pose very similar problems for policymakers in the nearest future. Articulating the general challenge and providing this historical analysis, it is hoped, will help researchers understand how to conceptually grasp the most important security challenges of the future. These kinds of security threats are widely dispersed, rapidly evolving technologies that have important beneficial applications, but which could be misused by small numbers of individuals to put whole cities – and possibly whole countries – at risk.

How we choose to structure biodefense policy will heavily influence the kinds of results we can expect. The relevant history is a guide.

_A Brief History Lesson_

Prior to World War II, biological weapons were considered potentially powerful, but too unmanageable for standard military operations. Major Leon Fox wrote in 1933, “at the present time practically insurmountable technical difficulties prevent the use of biologic agents as effective weapons of warfare.” In addition, there was a basic repulsion to using BW, “The use of germs as a method of warfare is

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prohibited by a national law and would be generally regarded as a practice of the
grossest inhumanity." As late as 1940, the consensus of both the US National
Institute of Health and the US Chemical Warfare Service, was that biological
weapons were most useful as sabotage weapons. Reports of possible Japanese and
German BW programs, however, prompted a complete reversal of opinion, leading to
the establishment of a large, secret BW program housed in a civilian agency and
devoted to surveying the full range of potential BW agents and the development of
weapons and countermeasures.

The question for the first BW committee – a group of scientists assembled by
the US National Academy of Sciences – was whether biological weapons were
technically feasible. It was asserted that biological weapons would be a potent
addition to the US arsenal if they could be manufactured with predictable effect that
the military could control and that its use would not be governed by moral
considerations or international agreements. Based on the recommendations of the
committee and Secretary of War Henry Stimson, President Roosevelt approved the

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9 J.E. Mills, Memo to Commanding Officer, Edgewood Arsenal (October 26, 1921). National
Academy of Sciences Archive, Committees on Biological Warfare Series 1, Box 2, “Report: Exhibit
B.”

10 The progress report prepared by CWS (August 28, 1939) is cited in “Progress Report No. 54:
Biological and Bacteriological Warfare” (August 15, 1941), National Academies of Science Archive:
Committees on Biological Warfare Series 1, Box 1: “Organization and Administrative Liaison: 1941-
1942.” The NIH opinion is reported in a Letter from R.E. Dyer, Chief, Division of Infectious
Diseases, NIH to The Surgeon General, USPHS, Bethesda, Maryland, December 16, 1940. National
Academies of Science Archive: Committees on Biological Warfare Series 1, Box 1: “National Institute
of Health: 1941.”

11 “Report of the W.B.C. Committee” (February 19, 1942), National Academy of Sciences Archive,
Committees on Biological Warfare Series 1, Box 2, “Report: Feb 1942.”
creation of the War Research Service (WRS) in 1942. All information about WRS was classified: even the order for its creation was never written down.\(^\text{12}\)

Although civilian scientists pushed for the creation of a BW program and oversaw the initial basic research, the Chemical Warfare Service (CWS) asserted exclusive control of promising biothreat agents for the purpose of weaponization. In 1944, the civilian advisory group was almost completely excluded from the BW program, relegated to a minor role and then disbanded a few years after the end of the war. After another review in 1949/1950, the Chemical Corps – formerly the CWS – decided to invest in the creation of biological weapons for deterrence based on promising advances in technology. The original rationale of investigating the offense for the purposes of creating better defenses was abandoned and deliberate acquisition of offensive potential was pursued for its own sake.

Within a few years after the standardization of its first biological weapon, US policy shifted from retaliation-only to first-use of lethal BW. Incapacitants were added to the arsenal in the 1960s to provide more options for use in limited war scenarios. Hundreds of field tests were conducted to assess the US vulnerability to sabotage and to create better models for BW use. By the close of the 1960s, the US had standardized eight anti-personnel biological weapons and five anti-plant weapons. Thousands of pounds of biothreat agents were stockpiled. “During the

1960s... the Army showed that it had met the challenge of large-scale attack, at least by its own calculations.\textsuperscript{13}

What is notable is the lack of physical and medical countermeasures to the very BW agents the US weaponized. Only the vaccines for tularemia and yellow fever were both safe and effective against the BW form of the agent. The LVS for tularemia is still an IND vaccine. The anthrax vaccine – although licensed by the FDA – was not considered effective against all virulent strains,\textsuperscript{14} such as Ames, and required numerous initial shots and annual boosters. The VEE and Q fever experimental vaccines then in the US stockpile were known to induce unacceptably high levels of undesirable reactions, unacceptable side-effects, or both. The botulinum toxoid was not effective against all seven types of botulinum toxin, and stockpiles were depleted by the early 1980s. For SEB and Brucellosis, there is still no human vaccine available, and immunity acquired through natural exposure to SEB does not provide complete protection from an aerosol challenge.\textsuperscript{15} Furthermore, in the event of a massive exposure, it remains unknown how effective any of these vaccines would have been.

When the US chose in 1969 to unilaterally terminate its offensive BW program, it was not because of the imbalance in the BW offense vs. defense. Key

\textsuperscript{13} Davir R. Franz, Cheryl D. Parrott, and Ernest T. Takafuji, “the U.S. Biological Warfare and Biological Defense Programs,” in Frederick R. Sidell, Ernest Takafuji, and David R. Franz, eds., \textit{Medical Aspects of Chemical and Biological Warfare} (Washington, DC: Office of the Surgeon General, 1997), 430.

\textsuperscript{14} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1985} (Frederick, Maryland: USAMRIID), Work Unit D807 AA 011.

\textsuperscript{15} US Army Medical Research Institute of Infectious Diseases, \textit{Medical Management of Biological Casualties Handbook, Fifth Edition} (Frederick, MD: USAMRIID, 2004), 92-95.
documents from the 1969 review remain classified. However, one scientist testified that the influential Presidential Scientific Advisory Committee (PSAC) concluded, “our biological weapons program was a substantial threat to our own security.”\textsuperscript{16} An Office of Systems Analysis review, DOD, concluded that lethal BW added nothing to the US nuclear deterrent at the strategic level and BW proliferation reduced US power when held in even small quantities by adversaries.\textsuperscript{17} Essentially, after over twenty-five years of work that finally established the technical feasibility of BW, the weapons were determined to hold no military value. BW were only useful for sabotage operations and putting powerful states at extreme risk.

Instead, BW nonproliferation became a primary goal and upholding the international norm against such weapons the means. The US associated itself with the international agreement to ban biological weapons, signed the Biological and Toxin Weapons Convention (BWC) in 1972, and ratified the Geneva Protocol in 1975, the same year the BWC entered into force. The US biological weapons stockpile was destroyed by 1972 and all work after 1969 was restricted to defensive investigations.

To carry forward US biodefense work, the US Army Medical Research Institute of Infectious Diseases (USAMRIID) was created out of the old Army Medical Research Unit. Volunteers from the Seventh Day Adventists had provided most of the personnel used for human testing of BW vaccines for the previous

\textsuperscript{16} Testimony of Matthew Meselson, Global Spread of Chemical and Biological Weapons. Hearings before the Committee on Governmental Affairs and its Permanent Subcommittee on Investigations. US Senate 101\textsuperscript{st} Congress First Session. May 17, 1989. Dr. Meselson was an author of the PSAC report. (Emphasis in text is mine.)

\textsuperscript{17} Han Swyter, “Political Considerations and Analysis of Military Requirements for Chemical and Biological Weapons,” Proceedings of the National Academy of Sciences of the United States of America 65, no. 1 (Jan. 15, 1970): 261-270
decade. However, that group was no longer available after the end of the Vietnam War. USAMRIID, therefore, limited itself to basic research for the development of vaccines and therapies for biothreat agents and virulent emerging infectious diseases like Ebola, Marburg, other viral hemorrhagic fevers, and Rift Valley Fever. This period of US biodefense work is not well understood because most interest in the past US BW program was on the classified, offensive work in the pre-1969 period. Interest after 1969 typically shifts to international efforts to craft and enforce the terms of the BWC.

USAMRIID’s work is informative and instructive about the effectiveness of a biodefense program that is unclassified. Over time, the USAMRIID research program evolved toward greater openness and international collaboration. Scientific publications increased from an annual rate of about 20 to approximately 120. USAMRIID’s unique biosafety facilities made it possible for scientists to study the most virulent and infectious pathogens in order to develop defenses. For this period of time, the annual, detailed scientific reports published by USAMRIID were consulted. These were available from 1969-1990.

In broad terms, USAMRIID’s work was similar to that of the medical research community and followed general principles of public health. The Institute of Medicine defines public health as follows:

Public health is what we, as a society, do collectively to assure the conditions in which people can be healthy. This requires that continuing and emerging

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19 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report (Frederick, Maryland: USAMRIID) for each fiscal year.
threats to the health of the public be successfully countered. These threats include immediate crises, such as the AIDS epidemic; enduring problems, such as injuries and chronic illness; and impending crises foreshadowed by such developments as the toxic by-products of a modern economy.\textsuperscript{20}

USAMRIID did all of these things in the context of biodefense: scientists reacted to immediate crises, such as responding to outbreaks of novel diseases like Ebola, Marburg, Lassa Fever, Legionnaire’s, and T-2 mycotoxins. In addition to reacting to sudden outbreaks of exotic diseases around the world, scientists at USAMRIID maintained efforts to develop vaccines and therapies to “classic” biothreat agents like anthrax, Q fever, VEE, and botulinal toxins. Vaccines and therapies developed at USAMRIID were sometimes field tested in or shared with other countries where the diseases occurred naturally. Finally, USAMRIID maintained the research base to counter future crises such as novel biothreat agents, by continuing to adapt new technologies to further its work and by investing in basic research to understand pathogenesis and mechanisms of human immunity.

The record of work at USAMRIID is largely one of success despite an extremely small budget – approximately $23 million annually in current (2006) dollars. Most of its effort – approximately 60% of its funding and time was spent on vaccines and therapies. Between 1969-1990, numerous new, experimental vaccines were under development and some achieved IND status (full FDA licensing is typically not feasible because the clinical human trials to establish their safety, potency, and effectiveness cannot be performed). New therapies, including the antiviral drug ribavirin and the interferon-inducer poly-ICLC were tested and found

effective against a wide variety of biothreat agents. This work was helped by a strong program of basic research which consumed approximately a third of USAMRIID’s funding and work. Only ten percent of USAMRIID’s work was, however, devoted to the creation of better detection devices.

This was an unfortunate choice because it was discovered, over time, that most biothreat agents and EID are susceptible to some form of therapy if they can be accurately detected or diagnosed within a short period after the initial infection occurs – often before clinical signs are present. Accurate diagnoses are difficult: even in the midst of the anthrax attacks some victims died because of erroneous initial diagnoses.

A contributing factor to USAMRIID’s success was its work in and with other countries. Clinical trials with the antiviral drug ribavirin were done in collaboration with Chinese scientists in China to treat an endemic viral hemorrhagic fever. The work also allowed USAMRIID scientists to test their diagnostic tests under field conditions. Work with Lassa fever victims in Liberia allowed scientists to collect and purify human plasma for the treatment of the disease. The vaccine for VEE was useful in countering two outbreaks of the disease – and allowed for a large-scale testing of the vaccine’s safety and efficacy. A joint UN project with scientists in Argentina led to the development of a vaccine against Junin – a viral hemorrhagic fever that is on the NIH Category A list.

The unclassified nature of the US biodefense program was designed to enhance confidence in US commitments to the BWC. The open, beneficial, and defensive nature of USAMRIID’s work was repeatedly emphasized both in public
testimony\textsuperscript{21} and in public statements about the US Biological Defense Research Program (BDRP) itself: “While the detailed threat analyses provided by the intelligence community are classified, ALL WORK CONDUCTED UNDER THE BDRP IS UNCLASSIFIED.”\textsuperscript{22} The emphasis is in the text.

In reaction to the terrorist events of 2001, the US is shifting its biodefense policy in important ways in an attempt to better defend itself against the threat of bioterrorism. New regulations are in place to control access to select agents that excludes some foreign participation in domestic or international projects. New orders have extended the number of agencies with classification authority and expanded the kinds of information considered for classification. This is being applied in an ad hoc way.\textsuperscript{23} Significant amounts of new funding is allocated to biodefense: both for medical countermeasures at the National Institute for Allergy and Infectious Disease (NIAID) at NIH and for other biological countermeasures at the new Department of Homeland Security (DHS), including work on threat assessments and red teaming. Red teaming work is designed to test the vulnerabilities of the US in order to anticipate potential threat scenarios.

The US published its strategy in April 2004, “Biodefense for the 21\textsuperscript{st} Century.” Strategy rests heavily on development of security measures to predict,

\begin{itemize}
\item \textsuperscript{21} Colonel David L. Huxsoll, former Commander, USAMRIID, testimony before the Committee on Governmental Affairs and its Permanent Subcommittee on Investigations. US Senate 101\textsuperscript{st} Congress 1\textsuperscript{st} sess., May 17, 1989.
\item \textsuperscript{22} U.S. Army Medical Research and Development Command (USAMRDC), \textit{Biological Defense Research Program: Final Programmatic Environmental Impact Statement} (Frederick, MD: USAMRDC, 1989): 2-2.
\end{itemize}
prevent, and respond to bioterrorist acts on US soil. New facilities are being created by NIAID to work with the most dangerous human pathogens to further the creation of medical countermeasures to known agents and to provide the scientific base to respond to novel pathogens whatever their source. In addition a major new entity is being created in DHS for the purpose of biological threat assessment – and its work is largely classified.

NBACC – the National Biodefense Analysis and Countermeasures Center – was established in 2002. It is comprised of four major entities: a Biodefense Knowledge Center organized at Los Alamos National Lab, a Biological Threat Characterization Center (BTCC) and BioForensics Analysis Center (BFAC) that is being created near USAMRIID, and an Agricultural Biodefense Center for research on animal diseases. Officials have repeatedly stated that the purpose of BTCC is to understand potential bioterrorism agents, that is, the “next-generation of biological threats.” It is asserted that such work is only for defensive purpose and will not violate the terms of the BWC. However, it is not at all clear that the work will not be in violation of the international treaty.

As of the end of 2006, the US has three major structures in place with relatively distinct responsibilities. The Defense Department remains responsible for countermeasures and protective devices for military personnel. This includes some threat assessment work and intelligence collection. The Department of Health and

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Human Services became responsible for monitoring biosafety and biosecurity at labs that handle Select Agents, for purchasing medical countermeasures to WMD threats under Project Bioshield, and for R&D on new medical countermeasures to biothreat agents and certain EID. DHS is working on detection systems for domestic surveillance, threat characterization, red teaming, and threat attribution.

Current biodefense strategy is an attempt to impose fairly standard security practices onto medical research and public health organizations. These are two very different institutions with very different practices: It is an uneasy fit. HHS has not been comfortable with the law enforcement requirements thrust upon it under the Select Agent rules. NIAID cannot make Bioshield purchases until DHS completes material threat assessments – but the recent assessments are classified and cannot be released, which has slowed purchasing of medical countermeasures to a standstill for over a year.26 And new rules that require that scientists be “vetted” for participation in international conferences to ensure that sensitive information is not divulged goes against the tradition of open scientific exchange which has been standard practice.27

To stem the rising tide of government control, editors of scientific journals voluntarily imposed a review process to prevent the inadvertent publication of information potentially useful for bioterrorists.28


27 See Letter to Secretary Tommy Thompson, HHS, from Henry Waxman, Ranking Minority Member, Committee on Government Reform (June 24, 2004).

Secrecy vs. Transparency

Structuring a policy response to a security threat (i.e. biotechnology) that also serves important beneficial purposes – in this case, medical research – is not at all clear. The tendency, again as before, is to impose classification on information and activities that are not usually subject to such controls.

Such measures, particularly threat assessments, are deemed especially important for the threat of bioterrorism. A recent article made an important case for threat assessment work based on what is technologically feasible and not based on confirmed threats. The authors argued that high-quality intelligence cannot be acquired about state or terrorist BW capabilities, so “a biological threat characterization program would enable US biodefense to anticipate and prepare for emerging threats, bringing the defense closer to parity with the threat curve.” This is a simple and direct application of the general national security approach to the specific area of biodefense. It is a natural reaction to a security threat, but has not been demonstrated to be true. Rather, the history of proceeding under this presumption leads to precisely the opposite outcome: more threats and inadequate defenses.

The public health and medical research communities, on the other hand, typically work on a variety of actual disease agents. For example the list of Category A agents that guides NIAID’s biodefense R&D is composed of equal numbers of DoD biothreat agents (those believed to have been weaponized) and virulent EID that

are endemic to parts of the world, but pose potentially great harm to all. It remains very challenging to develop vaccines and therapies to known pathogens. Policymakers hope that developing the scientific base and infrastructure for dealing with highly pathogenic agents can be used to combat future threats as they emerge. That is, instead of working on medical countermeasures to future biothreat agents that the US creates, scientists are attempting to develop a scientific infrastructure that is capable of adapting to new threats as they emerge.

There is a tendency in the public health community toward transparency and collaboration to combat disease. Scientific publications rapidly spread information and advanced techniques. Networks to monitor disease have existed for years: the influenza monitoring network operates under the World Health Organization (WHO) and was instrumental in detecting and containing the first outbreak of highly pathogenic avian influenza strain H5N1 in 1997 and continues to inform annual flu vaccines. More recently, international scientific collaboration organized under WHO was critical to the quick response to the SARS outbreak in 2002-2003. During that crisis, the nearly real-time sharing of information and materials was essential to the containment of that deadly disease.

Historically, public health has also been understood as a common good, making possible invasive vaccination campaigns and access by international teams during outbreaks. Public health depends, ultimately, on trust and openness about the motivations for their efforts. When organizations designed to improve public health operate responsibly, the results can be tremendous because they can gain access to countries that would otherwise close their doors. The successful smallpox eradication
campaign is an example. Measures by states that erode this trust can destroy its core mission, putting the health of all at risk. For example, unfounded suspicions about the motivations behind the polio vaccinations caused entire regions to forego the life-saving vaccine and threatened the polio eradication program.

Transparency, therefore, makes possible international collaboration based on reassurance and the institutionalization of norms. Security is gained through information sharing and activities that enhance verification. Because a norm of openness is established, deviations are noteworthy.

Secrecy, on the other hand, denies the possibility of open collaborations. Instead the burden of ensuring security is placed on the control of information and access, infiltration, and the accurate assessment of the threat. It is risky, because bioterrorism is a low probability but high consequence event. In several scenarios, efforts to respond to bioterrorist use of transmissible pathogens quickly spiral out of control – and that is when the individuals knew they were participating in an attack exercise. Structuring a policy based predominantly on secrecy denies the benefits that can accrue from organizing policy based on transparency.

Application of what measures, therefore, best addresses the threat of biological weapons? There are actually two threats: those from dedicated state programs and those from terrorist groups. It is likely that terrorists will adopt and use established biothreat agents because they are known to be relatively inexpensive, technologically feasible, and reliable – and US countermeasures – physical, medical,

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or organizational – are clearly not adequate. For example, the use of contaminated letters to anonymously spread biological agents was noted as a potential threat over fifty years ago.\(^{31}\) Furthermore, the anthrax in the 2001 attacks bore uncomfortably close resemblance to the anthrax the US BW program had weaponized decades earlier.

However, dedicated state programs do possess the resources to create novel biological threats.\(^{32}\) As an example, the USSR’s program applied recombinant technology in attempts to combine pathogens or make detection more difficult. This is the major source of future danger. Classified US work on threat assessments and Red Teaming at DoD and NBACC possibly pose the greatest threat of all because such work enlarges the threat space, provokes suspicion, and gives legitimacy to emulation. The experience derived from over fifty years of biodefense work suggest that competitive biothreat programs at the state level is the most significant source of advanced biothreats to the US.

The original US BW program was founded based on fears of being unprepared for the products of more sophisticated enemy BW programs. Threat assessment work was integral to the highly classified program, and yet it led to a highly imbalanced outcome: large stockpiles of potent bioweapons and smaller stockpiles of vaccines of questionable effectiveness and utility. In seeking to


understand the maximum extent of danger, the US was producing the most advanced forms of it.

By contrast the lessons of post-1969 period is that biodefenses can be created in an unclassified program without concomitant offensive investigations. International collaboration enhanced US medical countermeasures and, importantly, identified the need for much more work on detection systems and quick, sensitive, and simple diagnostic assays.

Those are the two relevant legacies and the unavoidable conclusions about them. More worrisome is the fact that technology in the biosciences is now truly revolutionary. That concern about technology has always been used in the past to justify offensive investigations to characterize the threat over the horizon. It will be difficult to restrain the desire to push the technology to its limits in the name of threat characterization. But it must be resisted. There is in biology an observation called the “Red Queen Effect,” that states that in tightly coevolved interactions, evolutionary change by one species could lead to the extinction of others. Or, as the Red Queen herself says, “Now, here, you see, it takes all the running you can do to keep in the same place.” Defense must evolve as rapidly as the offense. However, if the US proceeds to develop the next generation of bioweapons in the name of defense, it will be placing the seeds of its own destruction into the hands of the next generation of terrorist. And it will take legions of far swifter scientists working hard on actual defenses to preserve even the inadequate offense-defense balance that the last round of US offensive work left us with.
Chapter 2: Creating the Logic of Biodefense

“While we perfect a biological weapon, we perfect the defense against it, thereby destroying the weapon. Would that all weapons of war could be liquidated from the earth as simply as this.” -- George Merck, 1946

Modern biological weapons originated in World War II: previous attempts to deliberately spread disease were neither as organized or sophisticated. Although infectious diseases had a significant impact on the course of wars past, biological agents were never studied so extensively with the purpose of creating militarily useful weapons. Much of how we still think about biological weapons was shaped during that war, when the US established its own BW program.

By request of the War Department, the National Academy of Sciences was intimately involved in establishing the BW program. NAS documents were declassified twenty years ago, providing a reasonably full account of the initiation of the US BW effort. Unfortunately, most documents of the Chemical Warfare Service (CWS) are not publicly available, leaving the history incomplete. This is nonetheless an important starting point to a small but significant military program.

Pre-War BW Attitudes

Before scientific understanding of microbes advanced sufficiently in the nineteenth century, biological warfare methods were crude and infrequently employed. When attempts were made to spread disease, it is uncertain how much

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any affect was due to the methods employed and how much to natural spread.\textsuperscript{34} Devastating epidemics that changed the course of wars and civilizations were mostly unintended and attributed more to divine interference rather than human calculation. Therefore, the potential of bioweapons has long been recognized, but little investment was made to harness it until the twentieth century.

In World War I, Germany had a program to research, develop, and produce biological agents and used several biological agents in sabotage operations against animals. The US, UK, and France knew of this history but took rather different attitudes towards the military implications of BW in warfare. The UK remained largely skeptical as to the practicality of biological weapons until the late 1930s, when concern over Germany’s intentions generated the need to begin preparations for a war.

Within the US military, differences of opinion as to the likely future use of BW existed. A 1922 lecture on BW (later reprinted) by Dr. L. Georges, Surgeon Inspector of the Army – reminding his audience of German use of anthrax and glanders in France and Romania – recommended a better understanding of BW to begin preparations for a defense.\textsuperscript{35} It is unknown how widely Dr. George’s opinion was shared within the Army medical community at the time. Within the Chemical Warfare Service, the opinion was strongly against the notion that BW would be


employed because it was not militarily useful. Technical Director, J.E. Mills wrote to the Commanding Officer of Edgewood Arsenal in 1921,

My own feeling is that it is not advisable at the present time to undertake work of this character [research and development regarding the offensive and defensive problems of germ warfare]. I do not believe that germ dissemination was used in the past war nor that it has ever been used in the past in any war. The use of germs as a method of warfare is prohibited by a national law and would be generally regarded as a practice of the grossest inhumanity and could not possibly be confined in its results to combatants.36

Little is known about the details of the French BW program. Their knowledge of German activities in WWI led them to engage in some BW work prior to the signing of the 1925 Geneva Protocol for the Prohibition of the Use in War of Asphyxiating, Poisonous or Other Gases, and of Bacteriological Methods of Warfare. The Geneva Protocol was signed largely in response to the repulsion of chemical warfare during World War I.

The Protocol prohibited the use of biological weapons, but not research, production, or possession. The US was instrumental in introducing and gaining agreement on the Geneva Protocol, but the Senate refused to ratify it then. (It did so in 1975.) While military figures supported it, veterans groups opposed ratification.37 Nonetheless, forty-two nations were adhering to it by World War II, with several states reserving the right to retaliate in kind.38

36 J.E. Mills, Memo to Commanding Officer, Edgewood Arsenal (October 26, 1921). ). National Academy of Sciences Archive, Committees on Biological Warfare Series 1, Box 2, “Report: Exhibit B.”


38 When the US ratified the Geneva Protocol in 1975, it did so with the right of retaliation in kind.
Nearly a decade later, opinions in the US and UK about BW began to shift. In 1933, US Major Leon Fox wrote an influential article, arguing that biological weapons would be used if they could be made to work. Yet he concluded, “I consider that it is highly questionable if biologic agents are suited for warfare. Certainly at the present time practically insurmountable technical difficulties prevent the use of biologic agents as effective weapons of warfare.”

The skepticism in the US was shared by the UK, but their higher probability of being a target of Nazi Germany led them to take action earlier than the US. A UK subcommittee on Biological Warfare submitted two reports, in 1937 and 1938, expressing concern, but no decision was taken to conduct any experiments. Instead, to reduce the likelihood of civilian casualties, they took two other enduring actions: the creation of the Emergency Hospital Service and the Emergency Public Health Laboratory Service. The former would form the framework for nationalized health care after the war. The latter, a network of laboratories to aid in the diagnosis and treatment of civilian casualties, became the Public Health Laboratory Service. That is, efforts in the UK were directed first toward providing better protection for the civilian population, and only after to experimentation in the area of bioweapons.

In November 1939, the British Medical Research Council assessed the value of BW as less effective than orthodox methods of warfare. However, because the Committee’s views were based on general considerations and not actual laboratory


41 Paul Fildes, “Bacterial Warfare” (undated), National Academies of Science Archive: Committees on Biological Warfare Series 1, Box 2: “Meetings: June 1942.”
study, it was decided in September 1940 to grant resources and laboratory space at Porton Down for Paul Fildes to test the feasibility of BW. Because Fildes did not wish to report to the Armed Forces, and the Medical Research Council did not want direct association with his work, it was agreed that his team would report (through a subcommittee of experts) to a Committee of the War Cabinet under Colonel Sir Maurice Hankey. Their work was classified and much of it remains as such. From information shared with the US, however, it is clear that they pursued work on *Bacillus anthracis* and *Clostridium botulinum* toxin A, among other bacterium.

Even after the outbreak of war in Europe, the opinion in both the US military and public health organizations dismissed BW as a weapon of war and did not urge any testing to confirm that opinion. An August 1939 study for the US Chemical Warfare Service (CWS) concluded that it was possible to disseminate bacteria by airplanes, but such methods would be of doubtful military value. Instead, there were significant possibilities for using BW as sabotage weapons. Similarly, in response to a request to review the issue of biological warfare in December 1940, the consensus at the US National Institute of Health was, “That though there are a few definite possibilities, the field of bacteriological warfare … offers possibilities

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43 The progress report prepared by CWS (August 28, 1939) is cited in “Progress Report No. 54: Biological and Bacteriological Warfare” (August 15, 1941), National Academies of Science Archive: Committees on Biological Warfare Series 1, Box 1: “Organization and Administrative Liaison: 1941-1942.”
decidedly less potent than generally assumed, and would for the most part be futile, having only a slight nuisance value.”

However, just over one year later, a single committee of scientists convened by the National Academy of Sciences (NAS) and National Research Council (NRC) reversed that prevailing view of BW. Their assessment led to the quick establishment of a secret and comprehensive biological warfare program.

Paradigm Shift: The WBC Committee and the Feasibility of Bioweapons

In the summer of 1941 concern in the Army CWS and Surgeon General’s office (SGO) about evidence of enemy preparations to use BW led to requests for action. On July 15, 1941, the Army Surgeon General asked the Division of Medical Sciences, NRC, to form a special committee of civilian scientists to survey all phases of the BW problem and provide advice. It was the Surgeon General’s opinion that “since the primary function of the Medical Department is to preserve life rather than to destroy it, its efforts should be directed solely toward prevention and cure.” This opinion was not shared by the CWS.

CWS Progress Report No. 54 (August 15, 1941) recommended that either CWS pursue both offensive and defensive research, or that it confine itself to offensive research and assign defensive work to the Medical Corps. That month a new, secret research project was submitted to the National Defense Research

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44 Letter from R.E. Dyer, Chief, Division of Infectious Diseases, NIH to The Surgeon General, USPHS, Bethesda, Maryland, December 16, 1940. National Academies of Science Archive: Committees on Biological Warfare Series 1, Box 1: “National Institute of Health: 1941.”

45 R.C. Jacobs, Memorandum for H.H. Bundy, Special Assistant to the Secretary of War (August 18, 1941), National Academy of Sciences Archive, Series 1, Box 1, “Beginning of Program.”
Committee (NDRC) by the Army: “CWS-20: Study of Bacteriological Warfare Methods and Means.”

The problem therefore, is to work out by actual tests after thorough study of the literature means and methods for the most effective use of bacteria, toxins and insects as a means of waging a war against an enemy country with a view of later working out adequate defense measures in our own country against such means and methods.⁴⁶

To resolve the issue, Harvey Bundy, Special Assistant to Secretary of War Henry Stimson convened a conference on biological warfare on August 20, 1941. The division of offensive/defensive responsibility recommended by CWS was basically endorsed, but a single civilian committee of experts was appointed to look at the whole issue of biological warfare.⁴⁷ Oversight of the research remained more in the hands of the civilian committee and CWS: the Surgeon General did not receive formal authority to oversee defensive research until 1944.

At the time, knowledge in the US about the potential BW threat was extremely limited. There were reports indicating German interest in the toxins produced by *Clostridium botulinum*, the existence of Japanese bacteriological warfare battalions, and Japanese use of plague in China and interest in the yellow fever virus. To protect against the latter, the Medical Corps asked the Rockefeller Institute to keep a supply of yellow fever vaccine for US troops serving in tropical regions. There was agreement “that despite exceedingly scanty information and previously held belief

⁴⁶ Letter from H. W. Dix to the National Defense Research Committee (August 25, 1941), including a copy of the index sheet for CWS-20. (emphasis mine) NAS Archives, Committees on Biological Warfare Series 1, Box 1, “Organization, Administration, Liaison, 1941-1942.”

⁴⁷ “Conference on Biological Warfare” (August 20, 1941), National Academy of Sciences Archive, Committees on Biological Warfare Series 1, Box 1, “Beginning of Program.”
that the possibility of enemy use of such agents is very remote, the whole subject is potentially of great importance and should be given careful study.”

In response to Bundy’s recommendations, Secretary of War Henry Stimson formally requested that the NAS and NRC appoint a committee, “to survey the present situation and the future possibilities.” A secret committee, the WBC Committee, was convened and held its first meeting on November 18, 1941. The WBC Committee existed for less than a year, but in that short time it overcame the major obstacle to military investment in BW: it established the presumption that biological agents could be employed as military weapons on a large scale.

In its first report of February 19, 1942, the WBC Committee stated that “Biological Warfare is regarded as distinctly feasible…. The majority of the authors conclude that biological warfare is entirely possible, even probable, and that in the future, its use will be governed by the likelihood of military effectiveness rather than by any moral considerations or international agreements.” The report gives little indication of the reasons for their confidence. It can be inferred, however, that three factors influenced their conclusion: perception of the threat, potential effectiveness of a biological weapon, and technical feasibility.

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48 “Conference on Biological Warfare” (August 20, 1941), National Academy of Sciences Archive, Committees on Biological Warfare Series 1, Box 1, “Beginning of Program.”

49 Letter from Henry Stimson, Secretary of War to Dr. Frank B. Jewett, President, National Academy of Sciences. October 1, 1941. National Academy of Sciences Archives, Committees on Biological Warfare Series 1.Box 1, “Organization of Committee”.

Establishing the Threat

The known threat was not at all clear, and at that time seemed limited to a few organisms: anthrax and glanders against animals, and yellow fever, plague, and botulinum toxin against humans. Intelligence reports about Japanese and German interest in BW were scanty, but worrying. In addition, the WBC Committee learned of Germany’s use of anthrax and glanders in Romania, France, and the US to infect animals in World War I. Some WBC members also believed that cholera was also used by Germany to contaminate water supplies in the Balkans. Their concerns about the potential of BW were reinforced by early interaction in December 1941 with the Canadian BW group, M-1000.

A survey of the literature convinced them that the threat was even more significant. “It is evident from this review of the literature that biological warfare has been the object of extensive investigation by scientists throughout the world.” Dr. Georges’ 1922 article, considered one of the most comprehensive by the Committee, gave a false sense of the ease by which bioweapons could be created, “[Microbial poisons] lend themselves to very rapid, I might almost say instantaneous, manufacture, in large quantities.” Enemy intentions and the literature survey


52 J.H. Defandorf, Lt. Col., Sanitary Corps, “Memo for file: First Meeting of the WBC Committee” (November 19, 1941), National Academy of Sciences Archive, Committees on Biological Warfare Series 1, Box 1, “Meetings: Nov 1941.”


indicated interest in bioweapons and both then begged the more fundamental question of whether biological weapons could actually be produced.

Technical Feasibility

The question of effectiveness is subordinated to the first question of feasibility. In an undated memo, “Bacterial Warfare” from early in 1942, this issue is set forth:

The answer to the last question [is BW technically feasible] is the real crux of the matter, since, if bacterial warfare is possible, one must assume that the enemy will consider its use, and history offers plenty of evidence as to the effectiveness of epidemic diseases. In our opinion, the potential effectiveness of such a weapon is so great that, even if it seems a long shot, the subject merits the most careful appraisal. We believe that the technical advances of the last few years point the way to the solution of those problems which in the past have made bacterial warfare seem improbable.\(^5\)

This is an important document in that it concisely sets forth the considerations favoring the pursuit of biodefense at that time. In summary:

- As an agent of total war, bacterial warfare could powerfully affect civilian morale; History contains many instances in which disease was the deciding factor in war.
- There is a general reluctance by experts in military hygiene, medical bacteriology and epidemiology to seriously consider BW based on largely humanitarian grounds;
- Technical advances and present involvement with an unscrupulous enemy make it highly desirable that the entire subject be examined in detail.
- Any examination must include factors bearing on offensive use as well as on the defense, if the defensive picture is to be realistic.

\(^5\)“Bacterial Warfare” (undated), National Academy of Sciences Archive, Committees on Biological Warfare Series 1, Box 3, “General: 1942.” This secret document is referred to in a note from Caryl Haskins of OSRD (April 21, 1942), National Academy of Sciences Archive, Committees on Biological Warfare Series 1, Box 1, “Office of Scientific Research and Development: 1941.” It is one of five documents listed as having been received by Caryl Haskins and to be shown to Frank Jewett and Harvey Bundy. None of the other documents listed are in the OSRD file.
• To be suitable as an offensive weapon, a disease must incapacitate or kill; be highly infectious via channels which favor its spread to epidemic proportions (respiratory); and for which effective control measures are available – but not known or promptly available to the enemy.

• Frozen-dried (lyophilized) cultures might be especially suitable for spreading air-borne diseases: the effective spread of air-borne diseases could be a simple matter.

• Use of chemotherapeutic drugs is preferred for cost reasons, because they may be effective against new diseases, and because immunization of an entire population is difficult and would forfeit the advantage of surprise.

From the beginning it seems that biological weapons are envisioned primarily as strategic terror weapons. There is no indication in this document or in any WBC Committee reports that BW would work well for standard military operations. It is stated that BW could be made to be militarily effective, but there is no document indicating the conditions under which its use would be effective other than “as an agent of total war”.

Choosing a Response

The WBC Committee performed a wide-ranging, largely theoretical assessment of every candidate agent as to its possible methods of producing a harmful effect on man, animals, plants, and food supplies. They received 110 survey reports in just 3 months. The core presumption was, “It is obvious that preparation for defense necessitates a knowledge of the offense and, if this knowledge is not available from experience, it must come from the results of careful investigation.”

The appendices to the February 1942 are exhaustive discussions of the range of microbes that could be made into a threat and possible methods of use. Curiously, despite the lengthy discussion of possible offensive applications, the

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56 “Report of the W.B.C. Committee” (February 19, 1942), National Academy of Sciences Archive, Committees on Biological Warfare Series 1, Box 2, “Report: Feb 1942.”
recommendations are modest and largely defensive: development of vaccines, pest control, disease surveillance, protection of water and milk supplies. Only a few deal with mass cultivation, use of vectors, and methods of dissemination.

On March 24, 1942 Secretary Stimson submitted the WBC’s first report to the General Staff. When he reported the results of their deliberations to President Roosevelt on April 29 he indicated the reluctance of the General Staff to being directly associated with BW work.\textsuperscript{57} The official history explains:

The formation of a civilian agency to take charge of b.w. presented certain definite advantages over direct participation by some branch of the armed forces. In the summer of 1942 the entire subject of b.w. was in a purely theoretical stage and civilian scientists formed the principal sources of knowledge and advice. The United States had never undertaken any experimental work in the field. The fact that b.w. was “dirty business” as the Secretary of War expressed it, implied possible dangerous grounds for public criticism of the armed forces if they should be found actively engaged in such work, while the activities of a civilian agency might be more readily disavowed officially. Furthermore, a civilian agency secretly housed in the Federal Security Agency would have the advantage of working under a cover that would readily protect it from enemy detection.\textsuperscript{58}

Secretary Stimson’s request for a civilian agency was approved the next month: the War Research Service (WRS) was established with George W. Merck as its director. Over its first year of work, WRS received $685,000 from the President’s Special Emergency Fund.\textsuperscript{59} From the beginning, all biological weapons-related work was classified as secret. Even presidential orders for the formation of WRS were


\textsuperscript{59} Historical Report of the War Research Service, November 1944-Final. National Academy of Sciences Archive, Committees on Biological Warfare Series 4, Box 5, p.82.
never written, but given in oral directives. Work was moved to “Top Secret” on April 8, 1944 when fears increased about possible enemy use.\(^\text{60}\)

When WRS was created a scientific advisory committee was also established. The ABC Committee replaced the WBC Committee, although its membership remained largely similar. Just prior to its dissolution to make way for the ABC Committee’s formation, the WBC Committee issued its final report in June 1942. Its research suggestions are more offense-oriented, influenced perhaps by reports from Paul Fildes of Porton Down.

As noted earlier, the UK began its BW investigations before the US. When Paul Fildes visited the US early in 1942, he shared information about the more advanced UK BW program with the US counterparts. The UK work was focused, it seems, on anthrax and botulinum toxin as retaliatory weapons.

The presumption of the need for offensive investigations is repeated in both Fildes report and Col J.H. Defandorf’s summary of his visit to England in the spring of 1942. Lord Hankey wrote that, “it is only by a full examination of the methods of attack that we can develop effective means of defense” and so work at Porton “has been directed almost entirely to the exploration of offensive possibilities to supply evidence on which defensive action can be taken, and on a means of retaliation if required.”\(^\text{61}\) Because viruses could not be safely handled, experimentation at Porton was confined to bacteria and toxins. Furthermore, research was focused on anti-


\(^{61}\) J.H. Defandorf “Bacterial Warfare: A Summary of Dr. Paul Fildes’ Report” (May 21, 1942) and Paul Fildes, “Bacterial Warfare” (March 11, 1942). Both are attached to the “Fifth Meeting of the W.B.C. Committee (June 1 and 2, 1942). National Academy of Sciences Archive, Committees on Biological Warfare Series 1, Box 2, “Meetings; June 1942.”
personnel and anti-animal weapons because anti-crop weapons were not considered to be feasible.

British experimentation at Porton were influenced by intelligence reporting the use of lice to spread typhus in Poland, production of botulinum toxin as a powder of high potency by Germany (although the proposed method of use was considered impossible), Japanese attempts to spread plague in China, and outbreaks of foot and mouth disease in Ireland and potato disease in England.

Two major hurdles were the mass production of agent and the preservation of virulence. When they began, the British had little evidence of the practicality of spreading BW by inhalation. “When attempting to use bacteria as a ‘toxic dust’ we have… dispersed the bacteria as finely as possible, but it may be found that coarser particles are more efficient, since it is not known certainly that penetration into the lung is necessary and, further, the finest dispersion leads to greater losses in viability of the bacteria.”

Progress after less than two years at Porton Down were as follows:

- Methods for the mass production and storage of anthrax.
- Methods for distributing anthrax against cattle (linseed cakes).
- Methods for protecting cattle against anthrax (1 c.c. of a spore vaccine containing 9 million spores).
- Experiments to disperse anthrax as a ‘toxic dust’.
- Methods to mass produce botulinum toxin type A with a potency never before recorded reliably (average lethal dose for man of 0.01mg by injection and 40-50mg by mouth). This overcame the earlier assumption that inhalation of botulinum toxin was impractical.
- Early experimentation with an anti-toxin indicating the likelihood of methods for active immunization for man.

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62 Paul Fildes, “Bacterial Warfare” (March 11, 1942), National Academy of Sciences Archive, Committees on Biological Warfare Series 1, Box 2, “Meetings; June 1942.”
• Sabotage uses of botulinum toxin by ingestion (superior to chemicals because onset of symptoms is delayed and hence the circumstances of initial exposure are more readily concealed).
• Mass production of house flies to contaminate food.
• Unsuccessful attempts to store non-sporing bacteria in bulk, although methods of drying some bacteria in various suspensions investigated.
• Experimentation with Glanders, Swine Fever, Rinderpest, and \textit{Brucella melitensis} against animals.

Fildes concluded his report with a request to the US for help on experimentation along the same lines on a larger scale.

Given the British information and, having spent the previous four months devoting “much time and thought to a study of the possible application of biology to war…. The possibilities of biological warfare, the conditions under which it is most likely to succeed and the end results that may be expected have been discussed…”63 the WBC Committee’s June 1942 suggestions were as follows:

1. Investigations be started on specific human, animal and plant diseases (e.g. anthrax, botulism, cholera, bacillary dysentery, plague, typhus, rinderpest, foot-and-mouth disease, late blight of potato, stem rusts of cereals, certain insects and insect vectors).
2. Measures be taken to secure laboratories and personnel to produce and preserve mass cultures of the various harmful agents that may be used in biological warfare.
3. That authority and funds be provided to carry forward the work on biological warfare.

Interaction with the Canadian and British BW committees seems to have shifted the WBC Committee’s focus. When it was formed, it was given both the Army Surgeon General’s request for a BW survey to identify necessary defenses and the CWS request for the investigation of offensive applications. It can be concluded that they believed that the former role was fulfilled by recommendations for the

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63 “Report of the W.B.C. Committee,” (June 18, 1942), National Academy of Sciences Archive, Committees on Biological Warfare Series 1, Box 2, “Report: June 1942.”
safeguarding of food and water supplies. Henceforth, it seems that the WBC Committee’s recommendations were more focused on supporting the CWS R&D requests. That is, offense first and defense after:

One of the tasks which face the WBC Committee is to develop a program of Biological Warfare, to develop new agents and to devise methods of distribution. This will require large-scale production and large-scale dissemination of these microorganisms. The important point to consider is the possibility of using new diseases or combinations of diseases and new techniques of distribution of these diseases.⁶⁴

Until the ABC Committee was formed in the fall of 1942, WRS initiated research projects based largely on the WBC Committee’s recommendations.

*The War Research Service (1942-1944)*

WRS had three major areas of responsibility: Research and Development; anti-biological warfare program; and Intelligence and Information. The anti-biological warfare program was defined as “the institution of countermeasures against enemy attempts to use bacterial or chemical agents in a civilian or military population.” Measures included the protection of the nation’s water supply against sabotage; protection of food supplies; the protection of biological products; and a special program in the Hawaiian islands and Panama Canal zone. Military personnel in Hawaii were vaccinated against yellow fever and a supply of vaccine was sent for the civilian population in Hawaii in the event it was considered necessary. (Large-scale immunization in 1942 with yellow fever vaccine, however, was not without risk. It resulted in the appearance of over 28,000 cases of serum jaundice, with 62

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deaths, shown later to be due to contaminated human serum used as an agent to stabilize the vaccine potency.\textsuperscript{65}

Research and Development

E.B. Fred, former chairman of the WBC Committee, headed the Research and Development department of WRS. (The ABC Committee provided scientific advice to WRS, but it is unclear how influential they were. It met only four times over two years.) The purpose of WRS was as follows\textsuperscript{66}:

1. To develop practical methods for large-scale production of agents potentially adaptable to B.W.
2. To develop practical methods for safeguarding the personnel engaged in the production, testing and handling of all B.W. agents, and of military and civilian personnel who may be exposed to such agents.

There is no desire on our part to suggest the use of such agents in warfare, but in order to study protection, it is necessary to investigate the offensive as well as defensive operations. In this way, it should be possible to keep ahead of the enemy and avoid a bacteriological “Pearl Harbor.”

CWS was assigned full responsibility for projects relating to weapons development. Other projects first began under WRS supervision and were later transferred to CWS authority when sufficiently promising results were generated that required development beyond the laboratory stage. All projects were transferred to CWS in 1944 when the War Department took over all BW work and abolished both WRS and the ABC Committee.


\textsuperscript{66} “A.B.C. Committee: Second Meeting.” (November 18, 1943), National Academy of Sciences Archive, Committees on Biological Warfare Series 2, Box 3, “Meetings: Nov 1943.”
WRS work was influenced in several ways by the UK BW work. First, Fildes told Merck that “My instructions are that H.M. Government feels that (1) a study of offense is an essential preliminary to the study of defense, (2) offensive studies are valueless until they are taken to a stage of complete realism…. In similar fashion, WRS and CWS actively pursued studies on offense.

In November 1942, Dr. Fildes requested help from the US in the mass production of anthrax spores, better bomb designs for the dispersal of anthrax, and production of botulinum toxin (“three kilo dried X”), among other items. In response, Merck indicated that work along these and other lines was underway. He requested that CWS work on: a) development of effective means of dissemination of agents using simulants; b) development of effective dispersal of anti-animal agents; c) determination of the most effective design, size and tactical use of a bomb for the dispersal of anthrax when dropped from aircraft. Although Fildes was probably also working on ricin (“W”) – “likely to be used by us [UK]” – no request for help

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69 George W. Merck, Director, WRS, “Letter to Dr. Fildes” (December 5, 1942), National Academy of Sciences Archive, Committees on Biological Warfare Series 4, Box 5: “U.S.-U.K. Cooperation: 1942-1944.”


with developing this was made. However, by 1944, “freshly prepared potent ‘X’ mud” was packed and ready for shipment to Porton.\textsuperscript{72}

The actual work of the WRS is hard to define clearly. It existed under the informal direction of Secretary Stimson through his Special Assistant and although it was involved in the direction and coordination of nearly all BW matters, it issued no orders or directives to the armed services, who were never officially connected with WRS. WRS initiated some BW projects, but was only consulted on others. “Its object was to serve mainly as a catalytic agent; to initiate broad general policies, and to act chiefly as a liaison between various government departments and branches of the Service…”\textsuperscript{73} The major actors are listed in figure 1 below.

At the ABC Committee’s first meeting in February 1943, they discussed the issue of protecting against the importation of plant diseases and insect pests and recommended special protective efforts. Upon their recommendation, and with WRS support, the Department of Agriculture secured $469,000 from the President’s Emergency Fund for a plant and insect pest quarantine program.\textsuperscript{74}

\textsuperscript{72} William B. Sarles, letter to Paul Fildes (January 24, 1944), National Academy of Sciences Archive, Committees on Biological Warfare Series 6, Box 7, “Fildes, P.: 1942-1944.”

\textsuperscript{73} \textit{Historical Report of the War Research Service, November 1944-Final}. National Academy of Sciences Archive, Committees on Biological Warfare Series 4, Box 5, p.7.

\textsuperscript{74} \textit{Historical Report of the War Research Service, November 1944-Final}. National Academy of Sciences Archive, Committees on Biological Warfare Series 4, Box 5, p.31.
At the second meeting of the ABC Committee in November 1943, WRS provided a summary of all projects and the committee was given a tour of Camp Detrick. While there, Colonel Chittick requested the Committee’s advice on how new construction at the camp could serve both the immediate BW needs and still remain adaptable to whatever future use was required after the war. A subcommittee recommended a continuation of its BW work to provide general scientific knowledge and to remain current on biological warfare.

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Once projects were moved into the direct military oversight of CWS, little information was given back to the civilian scientists in the ABC Committee. This happened infrequently because projects were only moved to CWS when they approached the prospect of pilot plant production. “Thus, as experiments progressed, scientific investigation moved increasingly from civilian to military supervision.”

This led to some tension between the civilian and military groups. Civilian scientists did not believe they could adequately advise WRS without knowledge of the projects conducted at CWS.

As of the termination of WRS, total annual salaries and expenses were $107,566. Work on BW projects sponsored by WRS totaled $176,200 for 1944-45, for a total of $283,766. (That is approximately $3 million in 2006 dollars.) Table 1 below is a chronological account of WRS projects. Those studies that were exclusively defense-oriented are highlighted.

Table 1: WRS Research Projects 1942-1944

<table>
<thead>
<tr>
<th>BW Agent</th>
<th>Code</th>
<th>Date Started</th>
<th>Status as of May 1944</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rinderpest</td>
<td>GIR-1</td>
<td>July 1942</td>
<td>Established a vaccine plant, stockpiling of</td>
</tr>
</tbody>
</table>

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76 Historical Report of the War Research Service, November 1944-Final. National Academy of Sciences Archive, Committees on Biological Warfare Series 4, Box 5, p.9. See also Memorandum from Commander W.B. Sarles, USNR to George W. Merck (20 April 1944). See Historical Report of the War Research Service, November 1944-Final. National Academy of Sciences Archive, Series 4, Box 5: “In the United States the tendency is to have the CWS and the Naval Unit attached to the CWS, responsible for research and development of biological warfare projects after they pass the stage of preliminary experimentation in projects sponsored by WRS.”


78 Information gathered from “The Research Program of the War Research Service” (May 15, 1944), National Academy of Sciences Archive, Committees on Biological Warfare Series 2, Box 3, “Meetings: Jun 1944,” and “A.B.C. Committee Second Meeting” (November 18, 1943), National Academy of Sciences Archive, Committees on Biological Warfare Series 2, Box 3, “Meetings: Nov 1943.”
<table>
<thead>
<tr>
<th>Disease</th>
<th>Letter Code</th>
<th>Month</th>
<th>Year</th>
<th>Event Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhus Fever</td>
<td>YE</td>
<td>Aug</td>
<td>1942</td>
<td>Growth on yolk sacs of developing chick embryos developed. Methods to preserve it in dry form being investigated. Objective was exclusively offensive: develop methods of (1) growing virulent strains; (2) drying &amp; preserving; and (3) dissemination.</td>
</tr>
<tr>
<td>Dysentery</td>
<td>Y</td>
<td>Sep</td>
<td>1942</td>
<td>Mass cultivation achieved, large-scale production recommended. WBC recommended it for offensive use.</td>
</tr>
<tr>
<td>Foot &amp; Mouth Disease</td>
<td>OO</td>
<td>Sep</td>
<td>1942</td>
<td>Work discontinued for lack of a suitably isolated location, cost, and relevance.</td>
</tr>
<tr>
<td>Blight of Potatoes</td>
<td>LO</td>
<td>Sep</td>
<td>1942</td>
<td>Evidence provided for the possibility of an epidemic disease of potatoes under favorable weather conditions.</td>
</tr>
<tr>
<td>Preservation of Rickettsiae</td>
<td>RI</td>
<td>Oct</td>
<td>1942</td>
<td>Lacking material for work.</td>
</tr>
<tr>
<td>Coccidioides</td>
<td>OC</td>
<td>Oct</td>
<td>1942</td>
<td>Virulent strain selected with characteristics suitable for biowarfare.</td>
</tr>
<tr>
<td>Anthrax</td>
<td>N</td>
<td>Nov</td>
<td>1942</td>
<td>Selected virulent strains, optimized conditions for mass cultivation of spores, devised method for drying; unknown effect of dried spores on man. Spores could be stored in a suspension, dried, or added to an inert carrier for weaponization. SPD Mk I bomb produced (not filled); penicillin effective if given early.</td>
</tr>
<tr>
<td>Botulism</td>
<td>X</td>
<td>Nov</td>
<td>1942</td>
<td>One type A strain selected for large-scale cultivation. Determined ideal conditions for maximum toxin production and process to preserve dried, purified toxin. Toxoid in use provided active immunity in humans.</td>
</tr>
<tr>
<td>Fowl Plague and Newcastle Disease</td>
<td>O/E</td>
<td>Nov</td>
<td>1942</td>
<td>Method for mass cultivation of viruses dvlpd, Developed a stable formalin killed vaccine (25,000 doses/day capacity), preparation and storage of active immune sera (dried). (Permit was required to import the virus.)</td>
</tr>
<tr>
<td>Cholera</td>
<td>HO</td>
<td>Dec</td>
<td>1942</td>
<td>Work suspended. Difficulty obtaining infective material and lack of method for assaying virulence. WBC Committee recommended for offensive use.</td>
</tr>
<tr>
<td>Anthrax immunization (2 projects)</td>
<td>MN</td>
<td>Jan</td>
<td>1943</td>
<td>Penicillin effective in the chemotherapy of anthrax in mice. No effective active or passive immunization for humans.</td>
</tr>
<tr>
<td>Brucellosis</td>
<td>US</td>
<td>Jan</td>
<td>1943</td>
<td>Virulent strain of <em>Br. suis</em> selected, mass culture medium developed, infectivity</td>
</tr>
<tr>
<td>Project Description</td>
<td>MB</td>
<td>Monthly</td>
<td>Date</td>
<td>Details</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>-----</td>
<td>----------</td>
<td>---------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Greenhouse and field experiments</td>
<td>CO</td>
<td>Jan 1943</td>
<td></td>
<td>Studied, dissemination methods developed. Recommended as one of the most promising war weapons in 1942.</td>
</tr>
<tr>
<td>Sclerotium Rolfsii</td>
<td>CO</td>
<td>Jan 1943</td>
<td></td>
<td>Greenhouse and field experiments established the viability and effectiveness of this fungus for anti-crop use.</td>
</tr>
<tr>
<td>Blood studies</td>
<td>LT</td>
<td>Apr 1943</td>
<td></td>
<td>No positive results from tests of prisoners of war. Tested for yellow fever, botulinus antitoxin, anthrax, plague, cholera, typhus, Shiga dysentery, Typhoid-paratyphoid.</td>
</tr>
<tr>
<td>Mass culture of spores</td>
<td>AU</td>
<td>May 1943</td>
<td></td>
<td>No detection of any deliberate spread of plant diseases or plant pests. ($469,000 effort)</td>
</tr>
<tr>
<td>Pest prevention and plant surveys</td>
<td>ER</td>
<td>June 1943</td>
<td></td>
<td>Completed. Work on mass production of bacterial spores of simulants for anthrax.</td>
</tr>
<tr>
<td>Tularemia (Foshay and Downs)</td>
<td>UL</td>
<td>Sep 1943</td>
<td></td>
<td>Working on mass cultivation and vaccine development.</td>
</tr>
<tr>
<td>Rice Diseases</td>
<td>II</td>
<td>Sep 1943</td>
<td></td>
<td>Work incomplete, but destructive possibilities indicated.</td>
</tr>
<tr>
<td>Mussel Poisoning (2 projects)</td>
<td></td>
<td>Jan 1944</td>
<td></td>
<td>Initial work promising, but preliminary. Purpose was to determine the chemical structure of the poison and to work out methods for the synthesis of it</td>
</tr>
<tr>
<td>Plant Growth Regulating Substances</td>
<td></td>
<td>Jan 1944</td>
<td></td>
<td>Large scale work begun.</td>
</tr>
<tr>
<td>Glanders</td>
<td></td>
<td>Mar 1944</td>
<td></td>
<td>No report.</td>
</tr>
<tr>
<td>Plant Growth Regulating Substances (chemical)</td>
<td></td>
<td>May 1944</td>
<td></td>
<td>No report.</td>
</tr>
<tr>
<td>Psittacosis</td>
<td></td>
<td>Suggested</td>
<td></td>
<td>Suggested</td>
</tr>
<tr>
<td>Rift Valley Fever</td>
<td></td>
<td>Suggested</td>
<td></td>
<td>Suggested</td>
</tr>
</tbody>
</table>

Given WRS’s heavy emphasis on offensive R&D, two exclusively defensive projects are noteworthy as exceptions to WRS’s general orientation.

**Rinderpest:** This is the first approved BW project and predates the establishment of the WRS. Rinderpest is a disease of cattle. After its December 1941 meeting – which included members from the Canadian M-1000 committee – the

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WBC Committee urged work on the production and storage of rinderpest vaccine. As a result, after many months of discussion, a joint, US-Canadian commission was appointed by Secretary of War Stimson on July 10, 1942. All its initial work was exclusively defensive in nature: (a) establishment and equipment of a plant at Grosse Ile, Canada for the production of tissue-type vaccine of known effectiveness; and, (b) research on a better vaccine.

Within two years, the plant was fully established and maintained a stockpile of at least 5,000 doses of finished vaccine. In addition, unprocessed tissue was maintained for the production of another 100,000 doses of vaccine. Research also led to the production of a new vaccine whose methods allowed for much quicker vaccine production and which induced full immunity more swiftly. In addition, the new vaccine could be dried without loss of potency for a long period of time.

Only after these accomplishments on the major problems of defense was there consideration of a request to investigate the offensive potential of the rinderpest virus. This was discussed in the fall of 1944.

*Tularemia:* This was not recommended for study in the June 1942 WBC Committee report. However, another study written in 1942 concluded that this agent “has much to recommend it to those seeking potential agents of bacterial warfare…. It is easily cultivated and its virulence can be maintained undiminished for long periods.”

WRS initiated work in 1943 towards mass cultivation, preservation, and vaccine development. Part of the reason given to Lee Foshay – who was

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investigating it without any WRS encouragement – was that tularemia was rumored to be “one of the most likely organisms to be used by the enemy.”81 Initial vaccine results looked promising.

On June 8, 1944, President Roosevelt approved the transfer of all BW work to the War Department. Secretary Stimson’s reasoning was as follows:

When War Research Service was first established, the primary considerations were research, and secrecy so far as military participation was concerned. Therefore, this activity was placed in a civilian agency for more perfect cover. The immediate urgency now is one of military development, planning and preparation. This leads up to the conclusion that the responsibilities for biological warfare should now be unified and centralized within the military establishment.82

George Merck was soon thereafter appointed a consultant to Secretary Stimson. Between June-August 1944, responsibility for all WRS projects were transferred to the Special Projects Division (SPD) of CWS based at Camp Detrick. The Surgeon General was instructed to collaborate with CWS on defensive aspects. The SGO accepted responsibility for the procurement, storage and issue of biological products designed to protect troops against BW agents that the enemy might use and for supervision of research on immunology and therapeutics. To provide policy guidance, a War Department Committee on Biological Warfare (USBWC) was created with Merck as its chairman.

The basis for US actions consolidating BW work within the War Department was primarily a belief that Nazi Germany was planning to use botulinum toxin in


long-range bombs. It was a fear based mostly on faulty intelligence, but enhanced by domestic research on the possibility of doing so. Because US research indicated that they could create such a weapon, it assumed that the Nazi government already had.

Intelligence

An initial survey of the intelligence collected was done in January 1943 by J.P. Marquand. Only four reports were from sources considered reliable: three (accurately) dealt with Japanese BW experimentation and only one indicated Nazi interest in botulinum toxin. This single report of Nazi BW preparations was itself not wholly convincing. The Surgeon General’s office commented that, “While this report is obviously inaccurate in many details, it contains certain points which may be of significance.”

This paucity of intelligence about BW preparations by Nazi Germany was repeatedly attributed to impenetrable secrecy. The possibility of there being no sophisticated BW program at all is not given consideration. Instead, more intensive requests for intelligence on BW were made, including visits to theater commanders to educate them on what to look for. Perhaps as a result of this activity, more reports about Nazi BW activity arrived in the latter half of 1943.

The increase in intelligence reports heightened concern about an attack with botulinum toxin. However, in a November 1943 memo, the Army Surgeon General reported that no antibodies to yellow fever, botulinun toxin, anthrax, cholera toxin, bacillary dysentery, typhus and ricin were found despite regular tests done on

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German, Italian, and Japanese prisoners of war. This is a significant finding because the only way then (and since) to provide immunity to botulinum toxin requires several shots over many weeks. However, this finding did not weigh as heavily as other reports coming in from Europe.

Fear, scientific advances in the US program, and the new reports essentially drove the US BW program from civilian laboratories into military production. Concern in the Office of Strategic Services (OSS) about Nazi intentions to use BW led to high-level meetings in December 1943 within the US that quickly transformed the BW program into an exclusively military enterprise:

Experiments of our own scientists and of the Canadians, though not wholly conclusive, were beginning to indicate that “X” in powdered form could be made in great quantities and preserved in an active state, and had highly lethal qualities as a weapon against animals or man. It was the opinion of OSS that the possible use of “X” or “N” in the German secret weapon was sufficiently serious to be called to the attention of the JCS….84

This drive to military control is interesting for two reasons. First, US analysts regularly presumed that Nazi Germany was ahead of the US in its work on BW: “It is safe to assume that the Germans have made much further advances in the field of experimentation with anthrax and that they developed more advanced bombs and other methods for its dissemination.” Also, regarding botulinum toxin, “…we have means of producing this in large quantities and it is safe to assume that the Germans have perfected their technique much further.”85 It is a common error to apply self-referential thinking.

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85 “Probable Biological Agents which may be Employed by the Enemy” (1 January 1944). See *Historical Report of the War Research Service, November 1944-Final*. National Academy of Sciences Archive, Series 4, Box 5, Section II, p.149.
The second point is the controversial nature of the US intelligence analysis. Many reports pointed vaguely to Nazi intentions to use some kind of biological weapon. Few indicated the actual biological agent they were presumed to be working with. Even when the strong likelihood indicated botulinum toxin, there were serious reasons to doubt it. Even the British did not think botulinum toxin a useful weapon and doubted that the Germans had produced enough to pose a tactical or strategic threat.  

*The Chemical Warfare Service*

Carrying the WRS R&D forward, and to fulfill British BW requests, CWS established Camp Detrick in April 1943. Work at Camp Detrick was organized in four main divisions: offensive, defensive, engineering, and safety. Four biological agent production plants were started. In addition to smaller pilot plants to study other antipersonnel, antianimal, and antiplant agents, Camp Detrick established the following:

Pilot Plant No. 1 (Activated October 1943): production of botulinum toxin;
Pilot Plant No. 2 (Completed March 1944): production of anthrax simulant *Bacillus globigii* and actual anthrax spores;
Pilot Plant No. 3 (Completed February 1945): production of plant pathogens;
Pilot Plant No. 4 (Completed January 1945): production of bacteria that cause brucellosis and psittacosis in embryonated eggs.

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As of September 25, 1944, CWS had the following major projects underway. In 2006 dollars, the total cost was approximately $500 million.

1. Camp Detrick: Frederick, Maryland (544.33 acres): $12,963,584. Construction began in April 1943 and continued through 1944. The purpose was research and development.

2. Horn Island (Jackson Project): Pascagoula, Mississippi (1969 acres): $444,886.47 spent. Construction began in July 1943 and was completed in November. It was established to serve as a proving ground for agents, munitions, and defensive measures. It was used for nine months to conduct 23 trials with botulinum toxin slurry dispersed from the Mk I bomb, none of which were successful.  

3. Granite Peak Project: Tooele, Utah (250 sq mi): $1,474,885 spent. Construction began in July 1944. The purpose was to provide additional facilities for testing agents, munitions, and defensive measures.

4. Vigo Plant, CWS: Terre Haute, Indiana (6100 acres): $29,778,846 spent. Construction began in April 1944. The purpose was the production of botulinum toxin and anthrax spores on a large scale. The Special Projects Division, CWS, had ordered one million Mk I bombs to be mass produced and filled with anthrax – half of which would go to the British.

The Army Surgeon General’s Office

In contrast to this frenetic activity by CWS, the Surgeon General’s requests were more modest. After the transfer of all responsibility for BW to the War Department, responsibility was shared between CWS and SGO. Secretary of War Stimson more clearly defined the SGO’s role in the defense program against BW in January 1944. This directed the Surgeon General to collaborate with the Chief, CWS in defense against BW. By CWS request, the Surgeon General agreed to be responsible for “the procurement, storage, and issue of biological products designed to protect troops against various biological agents that might be employed offensively.

88 Dates and costs of construction are from William B. Sarles, “DEF Committee: Meeting of 12 October 1944 – National Research Council,” (October 12, 1944), National Academy of Sciences Archive, Series 5, Box 5: Meetings: Oct 1944.”


90 Ibid, 70.
by the enemy.”

On January 25, 1944, a liaison officer was appointed by the SGO to provide closer relations between SGO and the Special Projects Division, Camp Detrick.

Because of the increased concern about possible German use of botulinum toxin, the SGO requested a meeting in January 1944, wanting to know what recommendations WRS had about protective measures. Camp Detrick scientist Fothergill described work on the Type A toxoid, estimating that 200 to 400 gallons of toxoid could be produced weekly – sufficient to immunize 200,000 men – without interfering with its other work. With the addition of alum precipitated material, toxoid to immunize 400,000 men could be made. However, it would be necessary to ship the toxoid to a biological manufacturing plant for bottling.

By May 1944, Camp Detrick was producing large amounts of botulinum type A toxoid in response to the SGO request. Gilliland Laboratories in Pennsylvania agreed to bottle the toxoid and the Biologics and Control Division, NIH performed the final control tests. By August 31, 1944, 101,196 vials of toxoid was sent overseas, sufficient to protect 1,250,000 men according to an injection schedule of two 1 ml doses. Toxoid production was then moved from Camp Detrick to a commercial company using methods of production developed at Camp Detrick.

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92 William B. Sarles, “Memorandum: Meeting in Dr. Fred’s office” (January 10, 1944), National Academy of Sciences Archive, Series 4, Box 5: “Meetings: Surgeon General’s Meeting on Defense: Jan 1944.”

93 Karl Lundeberg, “Memo for Dr. W.M. Clark (ABC Committee)” (15 May 1944), National Academy of Sciences Archive, Series 2, Box 3: “Chairman: Clark W Mansfield: 1944.”
At the January meeting Dr. Baldwin asked about the production of large quantities of penicillin for therapy against anthrax. It was agreed that “there were insufficient facts” available to comment on the production of penicillin.

**DEF Committee: 1944-1948**

Secretary of War Stimson, acting on the June 1944 recommendation and final report of the ABC Committee, requested a new NAS-NRC committee be established to carry on the work of the ABC Committee, providing advice to the War Department when requested. The reasons for having such a committee was not only to harness the best scientific advice possible, but also because, given the risks of mass hysteria and misunderstanding, “those responsible for conducting the war need the backing of a group of scientists outside the War Department.”

By August 1944, the DEF Committee was formed.

Because the USBWC would settle questions of policy, the DEF Committee’s main purpose was to provide advice only on scientific matters. The first meeting was held in October 1944. Members were told that the War Department requested DEF Committee help in the following matters: (1) reaching a decision on whether biological warfare is as good or better than existing methods of warfare; (2) developing improvements in defense against possible enemy use of biological warfare; (3) planning the postwar program of work on biological warfare.

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By June 1945, the DEF Committee was still discussing these questions. It was of necessity theoretical, no biological weapon having been actually produced. At the time, the War Department did not permit CWS to manufacture and stockpile BW munitions (the Vigo plant was completed but still being proofed, the Mk I, 4-lb bomb was in production). In response to the first question, they recommended that since the US would be reluctant to use BW even in retaliation, “possibly more time and effort should be expended on therapy of infectious diseases which may be naturally encountered or due to enemy actions.” In response to the second matter, they urged more work on dissemination studies, an expansion and intensification of studies on defense and therapy, and more work on crop destruction agents. There remained no consensus on the third point, post-war research. General Porter, Chief, CWS did provide his own conclusion, that, “if we have learned anything in this war, it is that military and naval research must not only be continued and supplemented, but must have general direction that comes from the intimate knowledge of new things.”

Prior to the full Committee taking up these issues, a panel of the DEF committee took up the issue of the control of publications and concerns over the production and testing of anthrax (leakage and spread). On December 12, 1944, it recommended controls on publications in the field of plant hormones (substances that could be used for the destruction of crops). Just two weeks later, Dr. Jewett informed the DEF Committee that he had requested the NAS-NRC’s Advisory Committee on Scientific Publications to initiate the controls recommended by the

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96 “DEF Committee Meeting: 1:45-4:15pm” (June 13, 1945), National Academy of Sciences Archive, Series 5, Box 6: Meeting Jun 1945.”

97 “DEF Committee Meeting: 10:00am” (June 13, 1945), National Academy of Sciences Archive, Series 5, Box 6: Meeting Jun 1945.”
panel. By January 1945, the editors of ten scientific journals, nine agricultural experiment stations, and two USDA officials were advised of the decision to place such publications under control.98

Upon War’s end, the DEF Committee turned its attention to other publication issues: what and when scientific information developed under the secret BW program could be released. It would also make recommendations on the practicality of BW and the need for future research. This is taken up in the next chapter.

**War’s End**

George Merck issued his Report to Secretary Stimson on January 3, 1945.99 An abridged version was released publicly in 1946. He praised the BW program as an important contribution to science and national security. Among its accomplishments, Merck listed the following:

- Development of methods and facilities for the mass production of microorganisms and their products;
- Development of methods for rapid and accurate detection of minute quantities of disease-producing agents;
- Significant contributions to knowledge of the control of airborne disease-producing agents;
- Production and isolation, for the first time, of a crystalline bacterial toxin, opening the way for the preparation of a more highly purified immunizing toxoid;
- Development and production of an effective toxoid in quantities sufficient to protect large scale operations should it be necessary;
- Significant contributions to knowledge concerning the development of immunity in humans and animals against certain infectious diseases;
- Important advances in the treatment of certain infectious diseases of human beings and animals, and in the development of effective protective clothing and equipment;

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98 “DEF Committee: Meeting of the Executive Committee” (February 13, 1945), National Academy of Sciences Archive, Series 5, Box 5: “Executive Committee: Meeting Feb 1945.”

99 George W. Merck, “Biological Warfare: Report to the Secretary of War” (January 3, 1945), National Academy of Sciences Archive, Series 5, Box 6: “Merck Report to the Secretary of War.”
• Development of laboratory animal propagation and maintenance facilities for strains of experimental animals;
• Applications of special photographic techniques to study airborne microorganisms;
• Information on the effects of more than 1000 different chemical agents on living plants;
• Studies of the production and control of certain diseases of plants.

The US program was certainly successful in establishing the study of biological agents as weapons of war and defenses against it. After enormous investments of time, money (at least $44,945,968 for WRS salaries and construction of Camp Detrick, Vigo, Granite Peak, and Horn Island), and manpower (approximately 3900 people in the Special Projects Division of CWS alone), Merck drew an important conclusion, “It is important to note that, unlike the development of the atomic bomb and other secret weapons during the war, the development of agents for biological warfare is possible in many countries, large and small, without vast expenditures of money or construction of huge production facilities.”

At the end of the war, the US had acquired much of the knowledge and some capability to wage biological warfare. No agents were extensively stockpiled and no weapons were ready for use. Its immediately useful medical contribution was in the development of a new rinderpest vaccine. In the public version of Merck’s summary of the wartime BW work, he spends time discussing the rinderpest project.

The work resulted in building up an adequate defense. It has made that particular agent impotent as a weapon in Biological Warfare. (One might give a thought to this paradox: While we perfect a biological weapon, we perfect the defense against it, thereby destroying the weapon. Would that all weapons of war could be liquidated from the earth as simply as this.)

The rinderpest project was the first and only one that was designed exclusively for the pursuit of adequate defenses (vaccines) for production in mass
quantity. In this it was immensely successful. However, no rinderpest weapon was ever investigated or developed. No biological weapon of any kind was assembled, much less perfected. The future of the US biowarfare project was very much in doubt.

**Conclusion**

Civilian scientists played a pivotal role in the establishment of the US biowarfare program. Their assessment that advances in technology made bioweapons feasible and that they would be effective military weapons convinced a skeptical military to invest in their development. Once established, the biowarfare program soon became dominated by the military: civilian scientists were useful for basic R&D, but policy and information about weaponization was withheld from civilian oversight.

Several important presumptions about bioweapons became established. First, that it is necessary to extensively investigate the offense in order to prepare appropriate defenses. This is the most important conclusion shared by the US, UK, and Canadian BW groups from the very beginning – it continues to shape US BW policy today. This led to extensive investigations of offensive applications, but fewer of defensive remedies (medical or mechanical). However it was not uniformly practiced: development and production of a rinderpest vaccine was pursued prior to any investigation of potential offensive use. The rinderpest project was exceptional because it was carried out by an independent commission and resulted in the development of an effective vaccine. In general, projects overseen by the military tended towards weaponization in no small part because that is what the military’s
structure is designed for: the development and effective use of weapons to win wars. The military is not structured to develop and manufacture medical therapies either for itself or the civilian population. Thus, manufacture of a botulinum toxoid and yellow fever vaccine were ultimately carried out by non-military organizations, even when the R&D and processes were developed at Fort Detrick (as with the botulinum toxoid).

Second, many believed it necessary to keep the BW program strictly secret. The reasoning was that only in this way could the weapon be useful: knowledge of any BW possession would both allow an enemy to prepare adequate defenses (vaccines, therapeutic drugs) and provoke emulation – putting the US at risk. Although the need for a deterrent to enemy BW use was the rationale behind developing a US BW capability, the inherent contradiction of having a highly secret BW program coupled to the deterrent rationale was not examined.

Third, little is known about how bioweapons would have been employed for standard military operations. Rather, they clearly held significant potential as “agents of total war” – i.e. strategic terror weapons designed for use against civilian populations.

Fourth, many assumed that the use of BW would not be governed by moral considerations or international agreements. This was expressed in the very first report of the WBC Committee. The extended strategic implications of having a bioweapon and threatening its use was not fully appreciated at the time.

Fifth, fear of technological surprise and the lack of ability to collect useful intelligence about adversary BW programs created a need for a BW threat
assessment. World War II generated enormous concerns about enemy intentions that helped override pre-war assessments about biowarfare. Fears about a Nazi BW program led the US and UK to invest in a more sophisticated biowarfare program than those founded either by Japan or Germany. In performing a threat assessment, the US expanded the list of potential bioweapons based on a self-referential cycle: i.e., if we can do this, the enemy must either be pursuing the same path or already have done it. Worries about being behind caused the US to create the most advanced BW program in the world.

Sixth, on a large scale, defense against biological weapons is typically much more complicated than offensive development. Defense involves monitoring, detection, surveillance, intelligence collection, production and stockpiling of medical therapies and physical protective gear, and an organizational capacity to deliver them when needed. Offense involves R&D to mass produce, stabilize, enhance the virulence of, and disseminate an agent and an appropriate delivery device. Medically, creating safe and effective vaccines for humans that will work against all varieties of potential BW agents takes longer than creating the biological weapon itself because of the complexity of human immunity. Organizationally, providing medical protection to every potential victim of a BW attack is more complicated than that required to put them at risk.

As the US biowarfare program expanded over the next two decades, many of these presumptions and their consequences remained unexamined.
Chapter 3: Developing the Offense

“The military exploitation of massive amounts of highly infectious agents through unusual portals of entry creates new problems for which these [preventive medicine] were not designed and against which no experience has been developed.” – LeRoy Fothergill, M.D., Ft. Detrick scientist

US choices about BW development diverge from those made by the UK after WWII. Unlike nuclear weapons, which the UK chose to acquire, the US was alone in its relentless pursuit of a biological weapons capability during this time. The BW program in the former USSR would not begin in earnest until after 1970. Yet by 1969, the US had the ability to initiate the use of weapons we could not confirm as being in the arsenal of any other state. This chapter reviews the history of debate and motivation for the US BW effort.

In the twenty-four years between World War II and the decision to terminate the US offensive BW program, biological weapons were produced, agents stockpiled and tested, and a policy for first-use replaced that of retaliation. This effort consumed tens of millions of dollars. It is therefore remarkable that despite acquisition of a BW capability in the 1950s and 1960s, the US chose to terminate its entire biowarfare program in 1969, destroy all its bioweapons, and restrict military BW activities to primarily defensive R&D thereafter.

The Late 1940s

In the immediate post-war years, opinions diverged over the potential effectiveness of bioweapons. In a complete reversal of opinion, many civilian scientists who urged the initiation of the US BW program were skeptical about such
weapons at war’s end. In contrast, the military that had reluctantly embraced the promise of bioweapons in 1942 became committed to preserving the program. This debate played out in the discussions of the DEF Committee regarding publication issues and over the future of the BW program at Camp Detrick. The military view prevailed and launched a new stage of BW research, production, and development in the 1950s and 1960s.

The DEF Committee: Publication Issues

By CWS request, the DEF Committee – the third BW scientific advisory committee – provided advice on two major issues in 1945 and 1946. The first was on publication of scientific knowledge acquired in the course of BW research at Camp Detrick. The second was an assessment of the BW program for the CWS.

In early 1945, the war in Europe clearly favored the allied powers. Anticipating the end, scientists in the Special Projects Division (SPD), Camp Detrick, asked the DEF Committee to make a recommendation on the publication of their work. The problems the SPD scientists faced were several: all BW information was classified as secret or top secret; papers would reveal the scientists’ association with Camp Detrick – revealing its purpose; and publication of results by associated universities or institutes under WRS or CWS sponsorship would indicate their interest in BW studies. However, much of the work was of fundamental scientific value and not directly related to BW.

After meeting in February 1945, the DEF Committee recommended that it take responsibility for accepting, dating, and filing copies of papers that might be submitted for scientific publication. It proposed working with the NAS Committee
on Publications to determine whether papers could be published and – if they could not be published for security reasons – the authors would be notified and a copy kept.\footnote{Excerpt from Minutes of the Executive Committee Meeting” (13 February 1945), National Academies of Science Archives, Committees on Biological Warfare, Series 5, Box 5: “Research Papers: Review of Permission to Publish.”} It is not known whether this process was adopted, as publication restrictions on biological warfare-sponsored research were relaxed later in 1945.

The revised policy was “to retain security restrictions on the military developments resulting from our research and to make available for the benefit of humanity, information of scientific value.”\footnote{Colonel H.N. Worthley, Chief, SPD, Letter to Frank B. Jewett (27 December 1945), National Academies of Science Archives, Committees on Biological Warfare, Series 5, Box 5: “Joint Security Control: 1944-1948.”} Some defensive aspects remained secret when “military necessity” demanded it, but otherwise was unclassified when no direct reference to any connection with BW work was made. Also unclassified were “agents, techniques, and apparatus for the destruction of living plants, when not identified with classified military activities.”\footnote{JCS Policy Memo 32 (19 November 1945) “Revised Classification of Matter Concerning Biological Warfare,” National Academies of Science Archives, Committees on Biological Warfare, Series 5, Box 6: “Meetings: Report & Follow-up: Jun 1946.”} Within six months nearly 100 papers were accepted for publication in scientific journals.\footnote{William B. Sarles, “Report on DEF Committee Meetings, June 17-18, 1946,” National Academies of Science Archives, Committees on Biological Warfare, Series 5, Box 5: “Joint Security Control: 1944-1948.”} All offensive investigations and military developments were to remain secret. Despite this – in addition to George Merck’s revelation of the offensive BW program early in 1946 – two remarkable publications came out in 1947 and another in 1949.

Theodor Rosebury published his June 1942 study of possible biowarfare agents for WRS in 1947. The title is unambiguous, “Bacterial warfare, a Critical
Analysis of the Available Agents, Their Possible Military Applications, and the means for Protection against Them.”

As introduction, the editor wrote,

Publication of this compilation of data pertaining directly or indirectly to bacterial warfare seems desirable not only for the sake of its value per se, but also as a contribution to an informed discussion of the portentous moral and political issues involved. As the authors became associated with the government’s biological warfare project, they are not in a position to incorporate into the review data published since 1942…. It will not escape the informed reader, that in a striking number of cases, technical developments discussed as possibilities in this paper have already become realities as evidenced by recent publications.

The second publication in 1947 – also by Theodor Rosebury – directly linked him with Camp Detrick (the co-author of the book), and hence Camp Detrick with offensive BW work. Experimental Air-Borne Infection is a detailed account of the methods and types of experimentation done at Camp Detrick on a range of agents, documenting the new field of aerobiology. Later in 1947, possibly in reaction to many news accounts speculating about the US BW effort, the Army banned publications on biological weapons. However, Rosebury then published Peace or Pestilence: Biological Warfare and How to Avoid It two years later. It is a fairly detailed account of offensive and defensive studies done at Camp Detrick during the war, including scientific references. Rosebury wrote, “we need not doubt that BW is
capable of taking its place beside the atomic bomb and other major weapons adaptable to mass destruction.”

It is clear from these publications that the US had assembled the scientific base for research on and development of biological weapons. The US still lacked a usable weapon, however, and a clear sense of how such a weapon would be used should one become operational. This was discussed by the DEF Committee, but even they could not come to a consensus as to the usefulness of the BW program.

The DEF Committee: The Future of BW Work in Peacetime

After the war, Major General Waitt, Chief, CWS, asked the DEF Committee – later the Advisory Committee on Biological Warfare – for a thorough review of the research and development work of the CWS Special Projects Division (SPD). Knowledge about the US BW program had become public, but no details were released, leading to speculation. Rather than deny its existence, CWS wanted to increase support for the BW program. However, members of the DEF Committee were more skeptical about the military usefulness of BW.

At a meeting in June 1946 to review the SPD’s work, the question of how to respond to an editorial in The New Republic on June 10, 1946 was raised. The New Republic wrote:

The War Department, in releasing the Smyth Report, gave the world and the average man a glimpse of the epochal achievement behind the atomic weapon and emphasized its fateful meaning for the human race. There is, however, no Smyth Report for biological warfare. The statement made through the War Department by George Merck, head of the project, was most inadequate… The subject remains veiled as if it were still wartime and as if this revolution

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in warfare were as legitimate a secret as the minor invention of a new
gunsight. What our leaders actually may be keeping secret is the nature of the
next war. So man walks along the precipice, and will not know the depth of
the abyss until the fatal plunge.\footnote{“Germ Warfare,” The New Republic 114, no. 23 (June 10, 1946): 821.}

General Waitt thought that an authoritative paper should be published to
respond to such charges and Dr. Woolpert, scientific director of SPD, recommended
was done.) Members of the DEF Committee disagreed, however, on the necessity of
a thorough report on BW. Dr. Dyer, USPHS, worried that “such a report would have
to be so conservative in its claims – if it adhered strictly to known facts – that it might
potential biological warfare agents were released, but nothing of the military
accomplishments of the US BW program.

After the meeting, Dr. W. Mansfield Clark wrote to the chair of the DEF
Committee, “… you may if you judge wise, quote me as advocating more publicity of
the war-time activities at D [Camp Detrick]. Between you and me and the doorpost, I
am disturbed by the apparent fear on the part of the Generals that the truth will cut the
budget.”\footnote{W. Mansfield Clark, Letter to Perry Pepper (June 25, 1946), National Academies of Science Archives, Committees on Biological Warfare, Series 5, Box 6: “Meeting: Report & Follow-up: Jun 1946.”} Dr. Clark, a civilian scientist, had served on all prior BW advisory
committees and had a thorough knowledge of the wartime work. He believed BW work should continue, but with qualification.

Specifically, Dr. Clark was skeptical about pursuing further work on anthrax, and worried about a lack of military direction. He and others thought the military should tell the scientists what they wanted from BW agents. General Waitt told them that the military planners don’t know what they want because they are not aware of the potentialities of BW weapons. There was a difference of opinion as to which was the cart and which the horse.

At this point, after millions of dollars of investment, the scientific advisors did not have a sense from the military of what was expected of the biological weapons they were instructed to develop. The reason seems to be clear: the military wanted a BW capability, but had not yet defined how such weapons would be incorporated into its arsenal. Strategy for bioweapons was to emerge after a weapon was developed, but, logically, scientists found it difficult to develop weapons without knowing the applications for which they were intended.

Because of this lack of direction – a lack of indication as to military utility – and the failure to create any usable weapon at all after several years of intensive effort, many of the scientific advisors recommended terminating the US BW program.

Dr. Clark closed his letter to Dr. Pepper, Chair of the DEF Committee, on a very skeptical note, “I am disturbed…by the general attitude that what was started

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during the war must go on with the plea that it will contribute to general well being and medicine in particular…. It doubtless would not do to say so but I would look sympathetically to a decision to grease and close D. and farm out some problems…”

Another long-time scientific adviser, Ernest Goodpasture, was even more skeptical. “It has seemed to me that we have been all along actuated more by an emotional reaction than by a very critical analysis of the situation…. I am still not clear about the potentialities of using such biological weapons against personnel of the enemy.” Similarly, Dr. Hagan wrote, “I would emphasize again that although progress toward the development of offensive B.W. agents has been somewhat disappointing…We should go far enough into the subject, now that we have the facilities for doing so, to satisfy ourselves and generations to come that there is no practical use for such agents in warfare…” The most emphatic opposition to further BW work came from Dr. J. Howard Mueller:

I am extremely skeptical about the probably efficacy of BW as an offensive weapon in relation to other known military devices. Certainly nothing which has come out of the work thus far offers a practical plan for an offensive use against human beings…. I am convinced that a large part and perhaps all of the bacteriological work which is in progress at Detrick or which is projected could be carried out better in well organized civilian medical research institutes. The objective of all this work differs in no way from investigations which will be carried out in every such medical institution in this country and in foreign countries. … They are the problems of every day life and will

113 W. Mansfield Clark, Letter to Perry Pepper (June 25, 1946), National Academies of Science Archives, Committees on Biological Warfare, Series 5, Box 6: “Meeting: Report & Follow-up: Jun 1946.”

114 Ernest W. Goodpasture, M.D., Letter to Perry Pepper (October 16, 1946), National Academies of Science Archives, Committees on Biological Warfare, Series 5, Box 5: “Advisory Committee on BW 1946-1948.”

continue to be studied intensively and I can see no excuse for putting any of them into the hands of a segregated group of individuals back of a barbed wire fence.\textsuperscript{116}

Dr. Pepper provided a summary of the Committee’s views to Dr. Jewett, NAS. Only Dr. Ira Baldwin expressed strong support for the continuation of BW work. Dr. Pepper reported support for continued BW work; but also that certain members suspected that BW would never prove as efficacious an offensive weapon as other known military devices. He recommended efforts to increase the virulence of all agents under study and research on other agents (in part because anthrax would not be an effective weapon without an increase in its virulence) and the continuance of an advisory committee.\textsuperscript{117}

Dr. Jewett then forwarded the report and Dr. Pepper’s letter and wrote to General Waitt, Chief of CWS, expressing his view that “If the Military are to get full value out of whatever there may be in b.w. it is essential that the theoretical possibilities be developed to the limit unhampered by preconceived notions of an engineering or military character.”\textsuperscript{118}

Gen. Waitt responded that skepticism about the practicability of BW was a minority opinion (which it was not). “I am firmly convinced that this is a weapon capable of achieving decisive effect against an enemy.” He believed that R&D on therapies be kept at Detrick, but that the responsibility for production and use of

\textsuperscript{116} J. Howard Mueller, letter to Perry Pepper (June 24, 1946), National Academies of Science Archives, Committees on Biological Warfare, Series 5, Box 6: “Meeting: Report & Follow-up: Jun 1946.”

\textsuperscript{117} Perry Pepper, Letter to Frank B. Jewett (July 2, 1946), National Academies of Science Archives, Committees on Biological Warfare, Series 5, Box 6: “Meeting: Report & Follow-up: Jun 1946.”

\textsuperscript{118} Dr. Frank B. Jewett, Letter to Major General Alden Waitt (July 9, 1946), National Academies of Science Archives, Committees on Biological Warfare, Series 5, Box 6: “Meeting: Report & Follow-up: Jun 1946.”
biological and chemical preparations developed for prophylactic purposes be the responsibility of the SGO.119

The contract of the DEF Committee – then renamed the Advisory Committee on BW – with the government expired in 1946. General Waitt did not see any further need for the committee, which met little after the summer of 1946, and so he terminated it in January 1948. One scientist wrote back, “There can be no question concerning the wisdom of the discharge of the Academy Advisory Committee on Biological Warfare. Many of us have felt for some time that we were not serving a very useful purpose.”120

**BW Policy Evolution**

In 1947, the BW R&D program was placed under the authority of the Research and Development Board of the Office of the Secretary of Defense.121 A Chemical and Biological Warfare committee was created to advise the Secretary of Defense and in 1948 issued a report that concluded that the US was susceptible to covert attacks. This led to the creation of the Special Operations division at Camp Detrick the next year to investigate this threat. However the JCS still considered biological weapons in the research and development stage in 1949, that is, weapons

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119 Major General Alden Waitt, Letter to Frank B. Jewett (September 17, 1946), National Academies of Science Archives, Committees on Biological Warfare, Series 5, Box 5: “Advisory Committee on BW 1946-1948.”

120 M.C. Winternitz, M.D., Letter to Dr. A.N. Richards, NAS (February 17, 1948), National Academies of Science Archives, Committees on Biological Warfare, Series 5, Box 5: “Advisory Committee on BW 1946-1948.”

on which there was insufficient reliable information to base military plans or to develop new military applications.

This JCS view of BW was challenged by an ad hoc committee chaired by Caryl Haskins. The committee included the most active supporters of BW work – Dr. Ira Baldwin and General Waitt – and none of the skeptics. Secretary of Defense Forrestal asked the committee to “undertake a full examination of all the technical and strategic possibilities of biological warfare. The subject should be approached, moreover, from a highly imaginative, although of course also realistic, point of view…” Again, as in 1942, reluctance and skepticism by the military – this time at the highest levels – about investments into BW was overcome by the persuasion of scientists and the CWS promising that impressive bioweapons were technically feasible given major advances in technology and science.

In introducing the report Dr. Haskins wrote, “It was the feeling of the committee that a brief report dealing with the subject on a broad policy basis and in a forward-looking manner and suggesting early action in certain major areas would be more important and of more assistance to you than would extensive technical studies…. The Report deals with the present capabilities of CEBAR weapons and emphasizes their future potentialities.” The major findings of the 1949 report were as follows:

1. Biological weapons are at present potent, but not decisive.

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122 Letter from Secretary of Defense Forrestal to Dr. Caryl Haskins (16 March 1949), in Report of the Secretary of Defense’s AD HOC COMMITTEE ON Biological Warfare (11 July 1949), Papers of Harry S. Truman President’s Secretary’s Files, Harry S. Truman Library.

123 Caryl P. Haskins, Letter to Louis Johnson Secretary of Defense (11 July 1949), in Report of the Secretary of Defense’s AD HOC COMMITTEE ON Biological Warfare (11 July 1949), Papers of Harry S. Truman President’s Secretary’s Files, Harry S. Truman Library.
Biological weapons are characterized by: versatility of application, psychological impact; direct action on man and his living resources, and non-destruction of material; capacity for self-propagation beyond the initial target area; difficulty of detection; high ratio of effect-to-weight; simple and inexpensive mass production. BW can cause considerable incapacitation or loss of life, and widespread destruction of animals and plant crops. BW are particularly well suited for sabotage against both key and mass targets.

2. Biological warfare is in its infancy.
Foreseeable improvements in the production and distribution of existing biological weapons would increase their effectiveness by a large factor. Medical and biological sciences are on the threshold of great new advances comparable to those in the atomic field. New biological weapons more potent than any now known may be expected – for example, weapons causing epidemics, glandular or hereditary changes, or other biological “chain-reactions.”

3. CEBAR attacks on the United States could today be serious and in the future might be disastrous.

4. Present defenses are not adequate.

5. No operating agency of our Government now has the clearly defined responsibility for the protection of the civil population against CEBAR attack.

This report echoes past reports in that the most promising application of BW was for sabotage purposes. No clear military utility is indicated. Rather, there is a strong sense of vulnerability to a BW attack and hence recommendations for better defenses. At the time, the Soviet Union was consolidating its power over the East European countries and war was about to erupt in Korea. There was no evidence of an actual BW threat to the US.

Instead, fear of potential enemy capability in 1949 led the US to again invest heavily in its BW program just as it had in the 1942 in response to a feared Nazi bioweapon. The start of the cold war gave rise to concerns about being technologically inferior to a Soviet BW program, launching the next phase of BW
development in the US. “The United States, although it enjoys atomic superiority… does not necessarily possess a corresponding superiority in the field of biological warfare – in fact, the situation might be the reverse.”

The Haskins Committee’s first recommendation was that the US military adopt an adequate program of defense against CEBAR weapons, including research on defensive measures, public information, and measures to coordinate the defense activities of various government agencies. It urged the organization of an alert civil defense organization and new methods for the prevention and cure of infectious diseases by way of a broad and vigorous research program. Because most military BW efforts were in weapons development that the committee supported – and because the Chemical Corps was not intended to deal with the protection of the civilian population – the Committee recommended that a separate program be performed by a separate civilian research organization with some liaison with then Camp Detrick. It later recommended that strategic planning for the possible future use of BW be undertaken without further delay.

In 1949, the US military was concerned about BW use as sabotage weapons just as it had been in 1939 – ten years earlier. (This is perhaps due to BW being recognized as a particularly effective sabotage weapon – and little else yet: “the Committee has noted a paucity of military thinking in the strategic employment of CEBAR weapons, particularly as elements in a weapon system.”) And just as before, a NAS committee saw much greater strategic potential for BW. However, while the

124 Report of the Secretary of Defense’s AD HOC COMMITTEE ON Biological Warfare (11 July 1949), Papers of Harry S. Truman President’s Secretary’s Files, Harry S. Truman Library, 6.

125 Report of the Secretary of Defense’s AD HOC COMMITTEE ON Biological Warfare (11 July 1949), Papers of Harry S. Truman President’s Secretary’s Files, Harry S. Truman Library, 25.
WBC Committee had initiated BW work in 1942 on the basis of the presumption that producing BW was technically feasible, the Haskins Committee urged work on the presumption of its potential effectiveness *despite* the fact that BW had not proven technically feasible at all.

At the time of the report, the US still had no bioweapons ready for use. Instead, the committee emphasized that “medical sciences are on the threshold of great new advances. Within the next decade, man’s new knowledge of biology, biochemistry, biophysics, and physiology may well place in his hands powers both of healing and of destruction not even contemplated today.”

Again, support for developing BW was based in part on what new knowledge and technology made possible – and not any military need that no other weapon could fill.

Following the Committee’s recommendation, the Secretary of Defense convened a second committee to study chemical, biological, and radiological weapons together. The Ad Hoc Committee on CBR Warfare in 1950 investigated the technical and strategic aspects of BW. It led to significant investments in BW: establishment of a BW production facility, field tests of BW agents and munitions, and an expansion of all aspects of BW research. The perceived need for a BW program was further heightened by the Korean War.

Despite the emphasis in the Haskins report on the need for more effective defense, the focus in the 1950s shifted to emphasize the threat of punishment. Brigadier General William Creasy, then head of the Research and Engineering

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126 Report of the Secretary of Defense’s AD HOC COMMITTEE ON Biological Warfare (11 July 1949), Papers of Harry S. Truman President’s Secretary’s Files, Harry S. Truman Library, 6.

Command, CWS and later Chief, CWS, wrote in 1952, “although the development of adequate protective measures against CBR attack is an important part of Chemical Corps work, the development of superior offensive potential is believed to be an even greater deterrent to possible aggressors.” Here again, although defenses were determined to be inadequate, the thrust of the CWS work was on weapons development. How a secret, superior offensive BW capability was intended to deter a BW sabotage attack (i.e. not attributable) is not addressed. There also does not seem to be consideration of the inferiority of a strategic BW weapon as compared with nuclear weapons.

After a thorough review in May 1954, a revision in BW policy in 1956 was made, “to the effect that the US would be prepared to use BW or CW in a general war to enhance military effectiveness. The decision to use BW or CW would be reserved for the President.” The timing of the change was linked to a speech by Soviet Marshall Georgiy Zhukov in February 1956 that seemed to indicate Soviet capability and intention to use biological weapons in the future.

*Bioweapons Development and Testing: 1950-1969*

Between 1945-1949, the US did not achieve any BW production. The Vigo plant was sold and all field test sites were shut down (other than Dugway Proving Ground). Vulnerability tests were conducted to assess the threat of sabotage with BW

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beginning in 1949. Repeatedly over these years, concerns about the vulnerability of the civilian population was expressed and used to justify military testing, although the military was not responsible for civil defense. After the 1949 and 1950 reviews, this lull in the BW program ended and a new intensive period began.

Small scale pathogenic field testing resumed in 1950 at Dugway. The first anti-animal BW test was conducted in July 1951, but the anti-animal program was discontinued in 1954 because it lacked military worth. The first anticrop bomb was “developed, tested, and placed in production for the air force” in 1951, providing the first limited BW retaliatory capability. Research and development on anticrop BW expanded, was discontinued in 1958, then restarted in 1959. Five BW anticrop agents were standardized and three anticrop biological agents were produced and stockpiled: stem rust of wheat and rye and rice blast. The total amount of anti-crop material in the US inventory in 1969 was 160,510 pounds, mostly at Rocky Mountain Arsenal, but also at Ft. Detrick and Beal Air Force Base.

During the 1950s, the Chemical Corps “concentrated on standardizing the agents investigated during World War II and weaponizing them at Fort Detrick” with the highest priority placed on anti-personnel agents. Eight antipersonnel agents were standardized. Rather than continue an open-ended research program, the JCS tasked the Army to develop specific BW agents in September 1951. Work on the

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creation of Pine Bluff Arsenal (PBA), a new BW agent and munitions production center, was completed in 1953 at a cost of $90 million. By early 1954, Pine Bluff achieved production readiness.\textsuperscript{134} In the spring of 1954, PBA began producing its first BW agent: \textit{Brucella suis}. A year later, large scale production of \textit{Pastuerella tularensis} began.\textsuperscript{135} PBA was the only facility operated for large scale production of antipersonnel BW agents (known later as the Directorate of Biological Operations, DBO). It had the capacity to produce bacterial, viral, and rickettsial agents and also toxins and the capacity to grow and infect mosquitoes with viral agents.

Between 1954 and 1967, the facility produced the following biological agents and toxins: \textit{Brucella suis}, \textit{Francisella tularensis}, \textit{Q fever rickettsia}, \textit{VEE}, \textit{Bacillus anthracis}, botulinum toxin, and staphylococcal enterotoxin. Bulk agents and antipersonnel munitions filled with these various agents and toxins were produced and stored at DBO as a deterrent capability.\textsuperscript{136}

The Army’s BW effort was boosted in 1960. The reason for the increase was not necessarily based on the military usefulness of the BW program, but possibly because Herbert York, Director of Defense Research and Engineering, wanted to compensate the Army for having eliminated them from competition for strategic intercontinental ballistic missiles and for the transfer and consolidation of military space programs in the Air Force and NASA.\textsuperscript{137} The bioweapons program was reorganized and the structure of its operations are shown in figure 2 below.

\textsuperscript{134} Department of the Army, \textit{U.S. Army Activity in the U.S. Biological Warfare Programs}, Volume I (24 February 1977), 3-2.

\textsuperscript{135} Department of the Army, \textit{U.S. Army Activity in the U.S. Biological Warfare Programs}, Volume I (24 February 1977), 4-1.

\textsuperscript{136} Department of the Army, \textit{U.S. Army Activity in the U.S. Biological Warfare Programs}, Volume II (24 February 1977), D-2.

Figure 2: Concept of Munitions Command (1962)

**Commanding General**

**Chief of Staff**

**Coordinating and supporting Staff:**

**Chemical-Biological-Radiological Agency**

**Chemical & Radiological R&D Lab**
- Army Cml Ctr, MD
  - R&D, Pilot plant design and operation:
    - Toxic Chemical Agents
    - Chemical Munitions
    - Smoke & flame incend.
    - CBR protective items
    - Chemotherapeutic msrs.

**Biological R&D Laboratory**
- Fort Detrick, MD
  - R&D, Pilot plant design, and operation:
    - Lethal BW Agents
    - Incapacitating BW Agents
    - Anti-Crop Agents
    - Biological Munitions
    - BW Biosafety Measures

**CBR R&D Assessment Laboratory**
- Dugway, Utah
  - Research, Field Assess., Development testing:
    - Toxic Chemical Agents
    - CBR Munitions
    - Ecology
    - Micro-Meteorology

**Pine Bluff Arsenal**
- Pine Bluff, Arkansas
  - Mfc, prod, filling, assembly, stdby plants, industrial mobil. planning:
    - Biological Munitions
    - Smoke Munitions
    - Incendiary Munitions
    - Toxic Cml Munitions
    - Riot Control Munitions

**Rocky Mtnn Arsenal**
- Denver, Colorado
  - Mfc, prod, filling, assembly, stdby plants, industrial mobil. planning:
    - Nerve Gas Munitions
    - Mustard Gas Munitions
    - Incendiary Munitions
    - Incapacitating Cml Mnts
    - Anti-Crop Munitions

**Edgewood Arsenal**
- Army Cml Ctr, MD
  - Mfc, prod, filling, assembly, stdby plants, industrial mobil. planning:
    - Protective Masks
    - Collective Protectors
    - Smoke & Incendiary Mnts.
    - Mustard Gas Munitions
    - Riot Control Munitions

**CBR Engineering Activity**
- Army Cml Ctr Center, MD
  - Process, product, and maintenance engineering, cataloging & standard’n:
    - Chemical Agents
    - Biological Agents
    - Weapon Systems
    - Semi-Works Plants

**CBR Material Mgt Activity**
- Army Cml Ctr Center, MD
  - Supply control, procure’t, quality assurance:
    - Detection & Iden’n Equip
    - Decontamination Equip
    - Protective Equip
    - Munitions Components
    - Radiological Waste Disp.

**CBR GOCO Plants**
- Niagara Falls Plant
  - Decontaminating Cmls
- New Cumberland Plant
  - Clothing Treatment
- Marshall Plant (Wva)
  - Smoke Chemicals
- Muscle Shoals Plant
  - Nerve Gas Cmls
- Newport Plant (Indiana)
  - Chemical Agent
Upon taking office, the Kennedy Administration ordered a reassessment of BW: the JCS was to “evaluate the potentialities of BW/CW, considering all possible applications; and, prepare a costed plan for development of an adequate BW/CW capability.” In their response, the JCS relied upon a 1960 report of the Biological and Chemical Defense Planning Board (DOD). Part of their recommendation was for increased emphasis on bioweapons that would incapacitate because “the advent of limited war and small scale conflict evoked a need for weapons which could assist in controlling conflict with minimum casualties.”

Instead of agents of maximum destruction, BW was promoted as “agents of minimum destruction” because they did not destroy infrastructure and facilities. “Biological warfare weapons have unique potentialities which could very well make them most attractive to an enemy bent upon subjugation but not total destruction.”

The new Chief Chemical Officer, Maj. Gen. Marshall Stubbs, described the new approach to BW nonlethals:

If we recognize the fact that conflict has a spectrum, that it is not a matter of holocaust or surrender, then it would be foolish to assume that the infinite military variety offered by chemical and biological weapons has not been carefully scrutinized by a potential aggressor… we must provide our own forces with the same form of measured response. That we possess this form of response is extremely important. First, because it gives us a possible deterrent for that level of conflict. Second, it gives us the ability to engage in that level of conflict on an equal footing. It gives us the means to avoid stronger measures which could escalate the form of conflict. In short, CBR is

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synonymous with the flexibility required by a concept of measured and restrained response…\textsuperscript{141}

Plausible on the face of it, this reasoning was flawed as regards bioweapons. BW, like nuclear weapons, offered little flexibility in the way of a spectrum of conflict. Any detected use of BW during war would likely escalate the conflict to the level of a nuclear response. This reasoning was not recognized at the time, but was later a major factor in the elimination of the program in 1969.

Upon review of the JCS recommendations, the DDRE concurred that bioweapons “had great potential; however, he felt that they could be considered operational only in the most limited sense and that the task of measuring their impact accurately still had to be done.”\textsuperscript{142} However, by 1960, numerous tests had already been done: 85 series of field tests with biological simulants; 10 field tests of non-biological simulants; and at least 40 series of field tests with pathogenic agents (\textit{Coxiella burnetii}, psittacosis virus, \textit{Brucella suis}, \textit{Pasteurella tularensis}, \textit{Bacillus anthracis}).\textsuperscript{143}

Yet at this point, the US still felt more work with agents was necessary. By contrast, after a series of sea trials between 1948-1955, the UK abandoned their plans to develop an offensive BW capability. Early BW munitions work was terminated. This decision was not based on the inutility of bioweapons. Rather, the sea trials had confirmed the results of earlier testing: that BW was feasible, that it was likely to be


\textsuperscript{142} Department of the Army, \textit{U.S. Army Activity in the U.S. Biological Warfare Programs}, Volume I (24 February 1977), 5-3.

relatively cheap and certainly many times more ‘toxic’ on an agent weight basis than any chemical weapons agent.\textsuperscript{144}

As a result of the UK trials with agents (and after 1955, with simulants alone) the consensus that emerges by the 1970s is that, “the trials consistently showed the feasibility of BW at sabotage, tactical and strategic levels and demonstrated the extreme vulnerability of the UK…. Perhaps, above all, the trials emphasized the relative simplicity and economy of BW.”\textsuperscript{145}

This conclusion was shared by the JCS in the US by the late 1960s after an intensive series of tests with both live agents and simulants. To carry out the testing, the DOD created a new task group: Project 112 Working Group. The Army’s BW budget was increased in the early 1960s to support an expansion of testing. A major reorganization of the CWS followed: the BW program was centered at Fort Detrick and BW testing was assigned to a Testing and Evaluation Command.

According to one estimate, at least fifty trials took place under Project 112 from December 1962 – 1970, the majority of which was designed to test the offensive capacity of biological weapons.\textsuperscript{146} A single trial often involved hundreds of bomb tests.

During the last 10 years of the offensive research and development program, many scientific advances were made that proved that biological warfare was clearly feasible, although dependent on careful planning, especially with regard to meteorological conditions. Large-scale fermentation, purification,


concentration, stabilization, drying, and weaponization of pathogenic microorganisms could be done safely.\textsuperscript{147}

By the end of the Johnson Administration – after over twenty-five years of investment and hundreds of millions of dollars spent – an offensive bioweapons capability was finally at hand. “During the 1960s… the Army showed that it had met the challenge of large-scale attack, at least by its own calculations.”\textsuperscript{148}

\textit{Medical Defenses against BW}

When President Nixon terminated the offensive program in 1969, the US had an arsenal of biological weapons at the ready and enough knowledge to use them effectively either in a theater of war or for sabotage. However, it still lacked the ability to adequately protect all its soldiers and, more importantly, the civilians who were considered even more likely targets of a BW attack. As of the mid-1970s, the US still lacked BW field detectors: research on detection, warning, decontamination, and protection was still largely at an exploratory, conceptual stage. Experimental vaccines had been developed for a large number of agents, but the SGO and USPHS still lacked the policies and procedures to stockpile large quantities of these agents. Furthermore, some vaccines were effective, but involved multiple inoculations and had serious adverse reactions.

The Army Surgeon General’s Office was involved in BW discussions from the beginning of the program and continued to have responsibility for medical

\textsuperscript{147} Davir R. Franz, Cheryl D. Parrott, and Ernest T. Takafuji, “the U.S. Biological Warfare and Biological Defense Programs,” in Frederick R. Sidell, Ernest Takafuji, and David R. Franz, eds., \textit{Medical Aspects of Chemical and Biological Warfare} (Washington, DC: Office of the Surgeon General, 1997), 430.

defenses. In 1952, the Armed Forces Medical Policy Council reported that there was no scientific data to assess human vulnerability to biological agents (as opposed to tests with simulants).149 The risk for humans was being extrapolated from animal tests. That was remedied with the initiation of human testing. The rationale was given by LeRoy Fothergill, senior Ft. Detrick scientist:

A number of unique medical problems might be created when man is exposed to an infectious agent through the respiratory route rather than by the natural portal of entry…. In some instances a different clinical disease picture may result from this route of exposure, making diagnosis difficult. In tularemia produced by aerosol exposure, one would not expect to find the classical ulcer of “rabbit fever” on a finger… I cannot emphasize the following point too forcefully. May I point out that the marvelous techniques of preventive medicine were developed over the years for dealing with naturally occurring infectious disease. The military exploitation of massive amounts of highly infectious agents through unusual portals of entry creates new problems for which these procedures were not designed and against which no experience has been developed.150

The term “Operation Whitecoat” refers to Army plans to use human volunteers in field testing with biological agents. Authority for the first field test to conduct dose-response data on Q fever was granted in January 1955 under project CD-22. It was a two-year project and considered successful enough to warrant the creation of the US Army Medical Unit (USAMU) at Camp Detrick in June 1956. USAMU was given the Army Medical Department’s research responsibilities to provide a BW defense.151 In 1958, USAMU was assigned to the US Army Medical

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Research and Development Command (USAMRDC) and in 1969, USAMU became the US Army Medical Research Institute of Infectious Diseases, USAMRIID.

After CD-22, the first research project involving human volunteers was designed to identify the infectious dosages of *Pasteurella tularensis*. The second involved Venezuelan Equine Encephalitis (VEE). In 1964, the immunization requirements for VEE and tularemia were “reasonably established.”¹⁵² Human testing continued and expanded to include testing of Q fever vaccines. (All three were also weaponized.)

In the summary provided by the army in 1977 (Annex K: Research Projects Involving Volunteers 1954-1969), there are accounts of experimentation with the tularemia and VEE organisms, but no accounts of human testing with any other kinds of organisms. The results of many of these tests were published in the open scientific literature, including findings of the effects of aerosol age on the infectivity of airborne *P. tularensis* and the effects of *P. tularensis* on blood chemistry when acquired by the respiratory route.

Despite advances in medical knowledge about the effects of bioweapons and medical defenses, efforts to organize the civil defense plans were not nearly as advanced. In 1954, responsibilities were delegated to the Department of Health, Education, and Welfare for a Public Health Service Civil Defense Program.¹⁵³ That included planning a national program for the protection and emergency restoration of community facilities essential to health; for the protection of humans against BW,


CW and other public health hazard (including communicable diseases); and to provide a reservoir of trained professional personnel who could be swiftly deployed in areas damaged by enemy attack. In addition, HEW was made responsible for immunization studies and development of vaccines of enhanced effectiveness; the detection, prevention, and control of airborne diseases, and laboratory techniques for rapid identification of biological agents, among other duties. These responsibilities were reiterated in the “National Biological and Chemical Warfare Defense Plan” of October 1959. There is no mention of responsibilities for actual production or stockpiling of medical therapies against biological weapons.

It was recognized within the military that defense against large-scale application was improbable: “Biological warfare stresses the value of vaccinations, but it is dangerous to depend too greatly on them. Logistically, it is impossible to develop vaccines against all the diseases which might be used as BW agents and have them in the right places at the right times. Furthermore, there is no solid immunity against many agents, particularly when the disease results from relatively massive attacks…”

Table 2 below is a crude summary of the initial status of work with agents as of 1969, when the offensive program was terminated. Because it is unknown what agents were stored in which munitions, those munitions are listed separately.

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Vaccines for all agents were experimental as of 1969. Supplies were very small and intended solely for military use.

### TABLE 2: Biological Weapons (1944-1969)\(^{156}\)

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Anthrax (<em>Bacillus anthracis</em>)</td>
<td>Vaccine and Skin Test developed (<em>Bacillus anthracis</em>): Research years 1959-1968</td>
<td>220 lb (dry-lethal)</td>
</tr>
<tr>
<td>Botulinum toxin (<em>Clostridium botulinum</em>)</td>
<td>Toxoid: <em>Clostridobotulinum</em> A, B, C, D, E</td>
<td>23,000 Cartridges and other special devices.(^{157})</td>
</tr>
<tr>
<td>Tularemia (<em>Francisella tularensis</em>)</td>
<td>Vaccines developed: <em>Pasteurella tularensis</em> (attenuated and irradiated); <em>Pasteurella tularensis</em> (425); Skin Test developed: Research years 1960-1969 (industrial sized lots produced)</td>
<td>804 lb (dry-lethal)</td>
</tr>
<tr>
<td>Venezuelan Equine Encephalomyelitis (VEE)</td>
<td>Vaccines developed: VEE, EEE: Research years 1960-1974 (Industrial sized lots produced-VEE)</td>
<td>4,991 gal (wet-incapacitant); 334 lb (dry-incapacitant)</td>
</tr>
<tr>
<td>Q fever (<em>Coxiella burnetii</em>)</td>
<td>Vaccines developed: Q Fever Rickettsia and Q Fever and RMSF (combined): Research years 1960-1974</td>
<td>5,098 gal (wet-incapacitant)</td>
</tr>
<tr>
<td>Brucellosis (<em>Brucella suis, abortus &amp; melitensis</em>)</td>
<td>Vaccine and Skin Test developed: <em>Brucella suis</em></td>
<td>Standardized in 1954: used with the M114 4-lb anti-personnel bomb that held 320mL of Brucella suis. 108 M114s were clustered in the M33 500-lb cluster bomb.</td>
</tr>
<tr>
<td>Typhus Fever (<em>Rickettsia prowazeki</em>)</td>
<td>Vaccine developed: Typhus Rickettsia</td>
<td></td>
</tr>
<tr>
<td>Coccidioidomycosis (<em>Coccidioides immitis</em>)</td>
<td>Vaccine developed: <em>Coccidioides immitis</em></td>
<td></td>
</tr>
<tr>
<td>Plague (<em>Pasteurella pestis</em>)</td>
<td>Vaccine developed <em>Pasteurella pestis</em>: Research years 1965-1974</td>
<td></td>
</tr>
<tr>
<td>Dysentery (Shigella dysenteriae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholera (<em>Vibrio cholera</em>)</td>
<td>Vaccine developed: <em>Vibrio comma</em></td>
<td></td>
</tr>
</tbody>
</table>

\(^{156}\) Information on the 1945 agent list comes from “The Research Program of the War Research Service” (May 15, 1944), National Academies of Science Archives, Committees on Biological Warfare, Series 2, “Meetings: June 1944”. Information on BW agents and munitions stockpiled comes from Memo for the President from Dr. Edward E. David (6 July 1970); White House Title Folder Vol. 1 (1969); Box 1; WHCF; SMOF David; Nixon presidential Materials, National Archives. Vaccine information comes from Department of the Army, *U.S. Army Activity in the U.S. Biological Warfare Programs*, Volume II (24 February 1977): G-4.

\(^{157}\) Michael A. Guhin, “Memorandum for Dr. Kissinger,” (December 18, 1969), NSC Files, Subject Files, Box 310, Chemical, Biological Warfare (Toxins, etc), vol. 1. NARA, Nixon Presidential Materials. Downloaded from www.gwu.edu/~nsarchiv/NSAEBB/NSAEBB58.
<table>
<thead>
<tr>
<th>Disease/Agent</th>
<th>Vaccine/Agent</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psittacosis</td>
<td>Vaccine developed</td>
<td></td>
</tr>
<tr>
<td>Melioidosis (Malleomyces pseudomallei)</td>
<td>Vaccine developed: <em>Malleomyces mallei</em></td>
<td></td>
</tr>
<tr>
<td>Mussell Poisoning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ricin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow fever</td>
<td>Vaccine developed: Yellow Fever virus</td>
<td>Standardized with a mosquito vector in 1959.</td>
</tr>
<tr>
<td>Staphylococcal enterotoxin B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rio Bravo virus</td>
<td>Vaccine developed: Rio Bravo virus</td>
<td></td>
</tr>
<tr>
<td>RMSF</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>Vaccine developed: <em>Salmonella typhi</em></td>
<td></td>
</tr>
<tr>
<td>Chikungunya</td>
<td>Vaccine developed: Research years 1969-1974</td>
<td></td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>Vaccine developed: <em>Mycobacterium tuberculosis</em></td>
<td></td>
</tr>
<tr>
<td>Influenza virus</td>
<td>Vaccine developed</td>
<td></td>
</tr>
<tr>
<td>Bolivian Hemorrhagic Fever Virus</td>
<td>Vaccine developed</td>
<td></td>
</tr>
<tr>
<td>Anti-animal agents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rinderpest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fowl Plague and Newcastle Disease</td>
<td>Hog cholera and Newcastle virus tested as antianimal BW at Plum Island (Ft. Detrick program terminated in 1954)</td>
<td></td>
</tr>
<tr>
<td>Foot-and-Mouth Disease</td>
<td></td>
<td></td>
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<tr>
<td>Anti-plant agents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late Blight of Potato</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sclerotium Rot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cereal rusts (Puccinia)</td>
<td></td>
<td>Wheat rust: 158,684 lb (1955) for use primarily against cereal crops. Used with the M115 500-lb antiplant bomb. Rice blast (?) 1,865 lb</td>
</tr>
<tr>
<td>Rice Diseases (Piricularia oryzae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Munitions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 1 (toxin &amp; simulant filled)</td>
<td></td>
<td>4450</td>
</tr>
<tr>
<td>M 2 (toxin, biological, simulant)</td>
<td></td>
<td>71,696</td>
</tr>
<tr>
<td>M 4 (biological &amp; simulant)</td>
<td></td>
<td>21,150</td>
</tr>
<tr>
<td>M 5 (simulant only)</td>
<td></td>
<td>90</td>
</tr>
<tr>
<td>M 32 (biological &amp; simulant)</td>
<td></td>
<td>168</td>
</tr>
</tbody>
</table>
Total lethal agent in filled munitions was 737.5 pounds. Total anti-crop munitions was: 1,856 lbs (Ft. Detrick); 153,463 lbs (Rocky Mountain Arsenal); 5,191 lbs (Beal Air Force Base, CA).\textsuperscript{158}

Throughout the US effort to weaponize these agents and develop vaccines, there was almost no knowledge of what the USSR had in the way of bioweapons. That is, the rationale for acquiring a BW capability was based more on the potential effectiveness of such weapons (as described in the 1949 and 1950 reports) rather than as a response to reliable intelligence about enemy BW capability.

By 1969, the militaries of the US and UK seemed to be in agreement about the likely effectiveness of bioweapons – and the US possessed both a stockpile and the capacity to produce them on a large scale. After all this effort and investment on the part of the US, however, President Nixon chose to suddenly terminate the offensive program within the first year of his term. There remains no clear rationale for the decision, only speculation.

Many consider that it was for solely political reasons, albeit that a rational connected with uncontrollability was stated. This rationale had not hitherto been considered during the several decades of the US BW capability. Its validity at the time of Nixon’s statement has been questioned and it has been refuted in recent years, but not, probably, by US official sources.\textsuperscript{159}

At the heart of the debate is the effectiveness of bioweapons. This was played out between reports prepared by several interdepartmental groups established to provide an assessment of BW and CW. On one side was the President’s Scientific


\textsuperscript{159} Peter Hammond and Gradon Carter, \textit{From Biological Warfare to Healthcare: Porton Down 1940-2000} (Houndsmills, UK: PALGRAVE, 2002), 57.
Advisory Board (PSAC) and the Interdepartmental Advisory Group. On the other
was the report of the Joint Chiefs of Staff (JCS). Again, as before, civilian scientific
and military groups perceived the feasibility and effectiveness rather differently at a
crucial decisionmaking moment. This time civilian scientific advice prevailed
through a bureaucratic process that omitted the military’s case for bioweapons.

The 1969 Choice to Disarm

On April 30, Defense Secretary Melvin Laird asked National Security Advisor
Henry Kissinger to review CW and BW policy. In response, Morton Halperin, an
NSC staffer, wrote that a draft National Security Study Memorandum (NSSM) was
being prepared. On May 28, 1969, Henry Kissinger issued NSSM 59 to the
Secretaries of State and Defense, the Director of Central Intelligence, the Special
Assistant to the President for Science and Technology, and the Director, ACDA.
NSSM 59 stated that the President directed “a study of U.S. policy, programs, and
operational concepts with regards to both chemical and biological warfare and
agents” be undertaken to “examine present U.S. policy and programs on CBW, the
main issues confronting that policy, and the range of possible alternatives thereto.”

In response, three interdepartmental groups were formed by the NSC staff:
one evaluated foreign chemical and biological warfare capabilities and was staffed by
the intelligence community; the second examined military options for CBW
employment, with emphasis on establishing their military utility; and the third
explored diplomatic options for the President (ratification of the Geneva Protocol and

160 “National Security Study Memorandum (NSSM) 59,” Downloaded from
www.gwu.edu/~nsarchiv/NSAEBBV/NSAEBB58. Source: FOIA Request.
negotiation of additional arms control agreements).\textsuperscript{161} NSC staff also asked the President’s Science Advisory Committee (PSAC) to write a report on chemical and biological weapons technology.

The PSAC report recommended that the US forfeit its BW capability while maintaining a vigorous defensive research program; ratification of the Geneva Protocol; renunciation of first use of lethal and incapacitating lethal CW; and continuation of research on the synthesis of toxins.\textsuperscript{162} Biological weapons, according to the PSAC report, posed a potential long-term danger because of the potential for mutation into an unknown or uncontrollable pathogen. A strategic analysis from the Office of Systems Analysis in the Defense Department was also critical of bioweapons and their possible value as either a deterrent or coercive instrument.\textsuperscript{163} In contrast, the report submitted by the Joint Chiefs of Staff argued for maintenance of existing biological capabilities: it was more optimistic about the reliability of BW and the ability to control it in the field and included almost no mention of the technical drawbacks of BW mentioned in the PSAC report.\textsuperscript{164}

To resolve the sharp discrepancy, Defense Secretary Laird decided to withdraw the JCS paper and ordered a new report from the Office of International


Security Affairs, who cribbed heavily from the PSAC report. His motivation for this is unknown. At the time, the US was under heavy international pressure to act on biological and chemical weapons both internally from Congress because of reported incidents relating to testing, transportation and overseas storage of CW and BW and externally because of US use of riot control agents in Vietnam. At the Eighteen-National Disarmament Committee, the UK tabled a Draft Convention on Biological Weapons on July 10, 1969. At the UN, the UN Secretary-General issued a report on CBW that drew attention to the danger of proliferation of such weapons if they were not effectively banned.

The Interdepartmental Political-Military Group (IPMG) drew from the IG reports and submitted its report to the NSC on November 10, 1969. As to Soviet BW capability, it wrote “Useful intelligence on actual production, weaponization and stockpiling is nonexistent.”165 In considering US BW capabilities, the report states: “No large inventory of dry (powdered) anti-personnel lethal or incapacitating biological agents is maintained and only eight aircraft spray disseminators are in the inventory. No missile delivery capabilities are currently maintained for delivery of biological agents, although a bomblet-containing warhead for the SERGEANT missile has been standardized, but not produced in quantity. Small quantities of both lethal and incapacitating biological agents are maintained in special warfare devices.”166


Clearly, the IPMG report presents a dismal picture of US BW capabilities. While no large inventory was maintained – possibly because of issues relating to loss of virulence in storage – the numbers of people potentially held at risk from BW was rather large – the area potentially affected by a bioweapon was estimated to be greater than the US atomic bomb dropped on Hiroshima.\textsuperscript{167} Again, no results of the numerous field tests were included. Defense against BW was presented as primitive: “No biological detection system is presently deployed or in prospect. There is no effective prophylaxis for large-scale or multi-agent biological attacks.”\textsuperscript{168} Funding for the BW program was insignificant: at its height (FY64), the US spent $39 million.

At the November 18 NSC meeting to consider future CW and BW options, General Earl Wheeler presented the original JCS position, but was opposed by other members of the NSC who already knew Secretary Laird’s position.\textsuperscript{169} The Joint Chiefs position on three major policies was overruled. The NSC instead endorsed a US renunciation of biological weapons, the renunciation of first use of lethal and incapacitating chemical weapons, and a resubmission of the Geneva Protocol to the Senate.\textsuperscript{170}

A week later, President Nixon issued National Security Decision Memorandum 35. In it, the US renounced the use of lethal and other methods of

\textsuperscript{167} Table 2.5, “Estimates of the relative potencies of CB and non-CB weapons,” in SIPRI, \textit{The Problem of Chemical and Biological Warfare volume II} (Stockholm: Almqvist & Wiksell, 1973): 134.
biological warfare, announced the destruction of existing BW stocks, restricted its biological programs to R&D for defensive purposes, and associated the US with the UK draft BW convention.\(^{171}\) The US also renounced the first use of lethal and incapacitating chemical weapons (excluding riot control agents or herbicides). A few months later, President Nixon included toxins in his unilateral ban according to National Security Decision Memorandum 44. Curiously, a consensus among the leaders of State, Defense, the JCS, the UN Secretary General, and WHO – and shared by the President’s science advisor – was that toxins were chemical weapons.\(^{172}\)

Destruction of all biological weapons stockpiles began in 1971. Demilitarization of antipersonnel agents was completed in January 1972. Anticrop agents stored at Rocky Mountain Arsenal, Beale Air Force Base, and Ft. Detrick were destroyed by October 1972.\(^{173}\) Ft. Detrick entered a period of uncertainty as to its future operation as 75% of it was shut down by mid-1971. USAMRIID and the US Naval Unit were the only two small units that remained at Ft. Detrick performing BW R&D.

As a result of the decision to terminate the program, much information about the once highly secret program became publicly available. In 1973, the Stockholm International Peace Research Institute published its six-volume study, *The Problem of Chemical and Biological Warfare*, a standard in the field that included many details


about CB agents, their utility, and national policies and programs.\textsuperscript{174} In that same year, the USSR launched its program to modernize its bioweapons program and founded Biopreparat.\textsuperscript{175}

\textit{The Biological Weapons Convention}

On April 10, 1972, President Nixon signed the “Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction,” known commonly as the Biological Weapons Convention (BWC). It is a brief document, lacking enforcement or verification activities. The first article is the most important: it intends to encompass all relevant activity then and henceforth:

Each State Party to this Convention undertakes never in any circumstances to develop, produce, stockpile or otherwise acquire or retain:

(1) Microbial or other biological agents, or toxins whatever their origins or methods of production, of types and in quantities that have no justification for prophylactic, protective, or other peaceful purposes;

(2) Weapons, equipment or means of delivery designed to use such agents or toxins for hostile purposes or in armed conflict.

The BWC entered into force in 1975. Efforts to enhance verification of its terms stalled in recent years. One problem is the dual-use nature of research related to biological weapons: nearly all basic research on defense against BW can be employed on behalf of offensive work. Any attempt to monitor all relevant work runs up against concerns over commercial proprietary information and national security.

\textsuperscript{174} Stockholm International Peace Research Institute, \textit{The Problem of Chemical and Biological Warfare} (Stockholm: Almqvist & Wiksell, 1973).

\textsuperscript{175} Ken Alibek with Stephen Handelman, \textit{Biohazard} (New York: Random House, 1999), 41.
Without a reliable deterrent or monitoring ability, the US nonetheless continued its biodefense research at USAMRIID.

All research at USAMRIID was unclassified from 1969 - 1990. The impact of this decision and the medical contributions of the program are important pieces of the US biodefense history that is not told in the policy literature.

**Conclusion**

Several notable features emerge about the US BW program from 1942-1969. First, the US never limited its work to agents it had strong reason to believe was being investigated by other states. This was true from the earliest years, and continued to be true throughout the course of the offensive biowarfare program. Knowledge of enemy capabilities and intentions were inferred based on what the US was itself pursuing. The US never had reliable intelligence of any specific enemy BW work in this period of time.

Second, while the initial justification for the program was defense, the US created several offensive bioweapons without ever developing an adequate defense. Threat assessment activities identified US vulnerabilities and methods to exploit them, but not the organizational, medical, or technical capacity to protect either military personnel or civilians. As a single notable example, a National Intelligence Estimate (NIE) from 1951, warned that “contaminated letters may be sent directly to the intended victims, without risk of detection.”\(^\text{176}\) Fifty years later there was nothing in place to reduce that threat.

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The military had the institutional capability to efficiently produce weapons and protective gear, but lacked the internal structure to produce and stockpile vaccines. Protection of the civilian population was assigned to the Public Health Service, but they, too, lacked the organization and authority to produce and stockpile vaccines and drugs and the structure to quickly deliver them should the need have arisen.

Third, civilian scientific advice was instrumental in initiating and terminating the US biowarfare program. In the interim, civilian scientific advice waned as military programs to develop and stockpile BW weapons dominated the program’s focus.

Fourth, the decision to terminated the offensive BW program seems to have been made without some significant information about the military effectiveness of bioweapons. In the end, broader political considerations and bureaucratic maneuvering contributed to the termination of the program. The reasoning applied by the Office of Systems Analysis demonstrated convincingly that BW simply could not be employed on any level when one considered the likely reaction of an enemy: major escalation of a conflict to the level of nuclear warfare.

Fifth, for years the need to possess bioweapons was justified by deterrence: to prevent enemy use, the US needed to be able to threaten retaliation in kind. The problem, however, with this logic was the competing desire to keep the entire BW program secret. A BW deterrent could not be effective if the enemy did not know the US possessed an effective bioweapon. However, revealing the existence of such weapons could spur an arms race and reduce its potential impact by indicating which
agents were weaponized – and hence allowing an enemy to stockpile medical and physical protections. Deterrence could, in theory, work for nuclear weapons because revelation of their possession does not diminish the punitive power of a nuclear strike – there are no defenses in the event of a detonation. Possession of BW – because it must be kept secret to preserve its utility – is only for offensive, surprise uses.

Sixth, the presumption that bioweapons were easy for small states to create pushed the investigation of sabotage possibilities and contributed to the fear of the US vulnerability to such attacks. Clearly, creating effective bioweapons for strategic use was neither cheap nor easy. Scientists overcame significant hurdles in developing the agents for offensive use and their delivery systems. These then required expensive and extensive testing. Testing of simulants was itself risky and detected across a large area. Testing of actual agents required large, isolated spaces to prevent accidental exposures and yet accidents did happen that led to the revelation – and contributed to the termination – of the US biowarfare program. Yet because imitation is swifter and surer than creation, there is something to the observation by Matthew Meselson, a scientist who contributed to the PSAC reported, that by pursuing a BW capability, the US was pioneering threats to its own security.
Chapter 4: Relying on Defense

“Obviously, defensive measures will lag behind a given biothreat; however, the same technologies that make these new potential threats possible will also help counter them.”
—USAMRIID Commander, David Huxsoll (1986)

After nearly thirty years spent pursuing a predominantly offensive program for the development of biological weapons, the US military had to radically alter course and limit itself to defensive research. Between 1969-1990, USAMRIID pursued important work on medical defenses against bioweapons at a very small fraction of a percent of the DOD budget. Work at USAMRIID incorporated new technologies and new agents as potential threats were identified. During this time, foreign scientists worked at USAMRIID and US researchers cooperated on international projects to test new therapies and vaccines that aided both foreign countries where diseases were endemic and the US biodefense research program.

After 1969, there were two major changes to the way biodefense work was conducted: it operated without an offensive program and it operated at an unclassified status. This was stated repeatedly and emphatically reaffirmed in the Biological Defense Research Program in 1989:

While the detailed threat analyses provided by the intelligence community are classified, ALL WORK CONDUCTED UNDER THE BDRP IS UNCLASSIFIED. Those results which impinge on the national security may be classified in accordance with Army Regulation 380-86. 177

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The emphasis is in the text. This decision to keep all work under the BDRP unclassified came despite the 1979 Sverdlovsk anthrax release, reports of deaths from T-2 mycotoxins in Southeast Asia, and other reports indicating the existence of biological weapons programs in other states. Therefore, in the midst of revelations of an evolving threat, a deliberate choice was made to operate the US Biodefense program under basic transparency rules and to retain the defensive focus of its work.

To gather policy implications from this history, it is important to assess the biodefense work done at USAMRIID by examining the capability created in several key areas:

1. Ability to assess new threats (utility of their threat assessments) – both agent-based and technology-based;
2. Ability to respond to novel threats (based on IC information and technology-enabled threats) and incorporate new technology to develop better vaccines and therapies against threats new and old (medical countermeasures); and,
3. Ability to develop useful detection devices – both for environmental samples and for clinical use.

There are two critical questions: how did the unclassified nature of the work impact national security, and were the results of the biodefense work heavily dependent on findings made under the offensive program? These are taken up at the end of the chapter. If a credible judgment can be made that the unclassified US biodefense program was essentially successful since 1969, then one has to question whether any recent changes in the security environment warrants its adjustment.

**USAMRIID: 1969-1990**

The US Army Medical Unit was renamed USAMRIID in 1969 and continued the work of creating medical defenses against biological agents. As USAMRIID
began operations, the disposition of stockpiles of biological weapons occurred quickly over the course of the early 1970s.

Any description of USAMRIID’s research will necessarily be incomplete. USAMRIID compiled Annual Reports from FY1969-FY1990. Two of these years (1983 and 1984) were said to never have been published.\textsuperscript{178} However, references to these documents were made in subsequent Annual Reports. It is likely that administrative changes led to the disruption in publishing these reports.\textsuperscript{179} No Annual Reports are available after FY1990 because there was no further requirement for them from the Army Surgeon General. The first war in Iraq and concerns after the terrorist and anthrax letter attacks of 2001 led to a reconsideration of government policy: USAMRIID now implements biosecurity policies that limit its transparency and openness.

Many of the early years of the program included detailed descriptions of the research work. These became more abbreviated in the 1980s. Overall it provides a reasonable sense of the scope of the research performed. Not all work was published in the open scientific literature, although USAMRIID scientists regularly published many of their findings internally.

The information in this chapter is brief summary compiled from hundreds of individual work units from 1969-1990. Over that time work at USAMRIID was reorganized several times and the coding for the work units changed (1977, 1981, 1985). Every attempt was made to accurately track individual research projects

\textsuperscript{178} Email communication with Denise Lupp USAMRMC Command Librarian/USAMRIID Library Director 1425 Porter St. Ft. Detrick, MD 21702-5011 (Wednesday, 11 May 2005).

\textsuperscript{179} Personal communication, September 27, 2006.
through time (FY69-FY90), but a few errors may have been made. Dramatic changes in FY81 and FY85 made further sequential tracking impossible.

In addition, the three subcategories of research described – pathogenesis, vaccines and therapy, and detection – were artificially imposed. In only a few years was work categorized in this way (FY1969 – FY1976). In more recent years, there were numerous sub-categories. Finally, judgments were made as to where a work unit that encompassed more than one category fit best. In general, if work on pathogenesis led to a new therapy or method of detection, it would go into one of the latter categories.

A fuller description of the work is in Appendix A and a list of all work units is attached as Appendices B-D: these include information organized by category of research, along with a title of the work unit, the PI(s), total funding, professional man years committed, and total number of publications.

Funding

Funding at USAMRIID increased regularly between 1969-1989. Figure 3 below shows growth from just over $1 million to nearly $20 million by FY87. There are two jumps in funding: in FY73 and FY85. (The increase was likely in FY84, when a reorganization led to the initiation of numerous new work units.) The reasons for these shifts are not known. (If one converts these sums to 2006 constant dollars, the average spending at USAMRIID was approximately $23 million per year.)
Generally, the majority of effort was on the development of vaccines and therapies for agents that posed a biowarfare or medical threat to soldiers. Less was spent on general pathogenesis studies, and the least was spent on detection devices.

<table>
<thead>
<tr>
<th>Research Area</th>
<th>Funding</th>
<th>%</th>
<th>Man Years</th>
<th>%</th>
<th>Publications</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccines/Therapies</td>
<td>$119,491,399.00</td>
<td>62%</td>
<td>814.3</td>
<td>55%</td>
<td>943</td>
<td>53%</td>
</tr>
<tr>
<td>Pathogenesis</td>
<td>$56,082,200.00</td>
<td>29%</td>
<td>508.25</td>
<td>34%</td>
<td>559</td>
<td>32%</td>
</tr>
<tr>
<td>Detection</td>
<td>$16,784,700.00</td>
<td>9%</td>
<td>167.9</td>
<td>11%</td>
<td>260</td>
<td>15%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>$192,358,299.00</td>
<td>100%</td>
<td>1490.45</td>
<td>100%</td>
<td>1762</td>
<td>100%</td>
</tr>
</tbody>
</table>

New agents were periodically added to the list as new diseases emerged and also in reaction to possible indications of Soviet BW work (T-2 mycotoxins and the Sverdlovsk anthrax release). Research focused on certain Bunyaviruses, Togaviruses, Flaviviruses, and Arenaviruses, in addition to toxins. There were other smaller
projects on Ebola and AIDS. The majority of effort throughout this time was spent on arboviruses, hemorrhagic fever viruses, and toxins. Rickettsia were actively studied in the early part of this time, but most research was terminated or transferred to WRAIR in the late 1970s, as more effort was devoted to anthrax, hemorrhagic fever, and toxin studies.

Over this twenty-year period, numerous experimental vaccines and chemical therapies were developed and tested, but only two received FDA approval: a vaccine for anthrax; and the antiviral drug ribavirin for treatment of respiratory syncytial virus.

No experiments required use of dry aerosolized agents. Most notably, in only two cases was a vaccine that was shown effective against the wild type or parent strain of an agent not shown effective against a challenge with more virulent strain or different portal of entry. For anthrax, later tests in more appropriate animal models established the effectiveness of the vaccine against all strains. Only the plague vaccine was ever known to be ineffective against an aerosol challenge.

Again, all research at USAMRIID was unclassified. This did not mean all results were published or that all results are still available. However, it did allow USAMRIID to host foreign scientists and to engage in important collaborative research projects.

There are references to certain agents being “acquired” – for example, dried powders of virulent anthrax (Vollum 1B) and streptomycin-resistant tularemia (SCHU-S5). Because all stockpiles of agents were reported as destroyed by 1972, it is assumed that these were not produced at USAMRIID, but under contract for
specific research purposes. The methods of doing such are likely classified and therefore are not referenced in the annual reports.

An Overview of USAMRIID’s Work

USAMRIID’s unclassified work involved the following kinds of experimentation with potential bioweapons agents:

- Vaccine and Adjuvant Development
- Antiviral Drug Development / Drug Screening
- Assay Development
- Susceptibility to Current Rx
- Aerosol Dynamics
- Epidemiology (Modeling)
- Genetic Engineering
- Environmental Stability
- Bioregulators/Immunomodulators
- Host Range Studies
- Aerosol Animal Model Development
- Basic Pathogenesis

Much of the basic research into pathogenesis, host range studies, aerosol animal model development, bioregulators/immunomodulators, and genetic engineering was applied to further the advancement of drug discovery, vaccine development, and the creation of assays to detect agents. Taken out of context or in isolation, some of the work could be characterized as potentially offensive work – such is the nature of most scientific exploration. However, the application of the information gained was clearly defensive. This can be seen from the heavy emphasis on vaccine development (and adjuvants) and on the development and testing of antivirals and assays for detection. Development and validation of new medical therapies depended on this integrated approach.
USAMRIID spent the vast majority of its funding and manpower – (62% and 55%, respectively) – on the development of vaccines and therapies to major biothreat agents. As of 1969, only the anthrax and tularemia vaccines were considered to be both safe and effective against various forms of their respective diseases (the licensed plague vaccine was safe but not effective against aerosolized Y. pestis). Even then, tests from the 1960s and 1980s indicated that the anthrax vaccine was not effective against all virulent strains, such as Ames, that were labeled as “vaccine-resistant.” (That concern was diminished after 2002.) Therefore, as of 1969, the biodefense researchers could be certain that only a single vaccine – the LVS for tularemia – was both safe for widespread use and effective against a virulent aerosol based on challenge tests in humans performed in the 1960s. LVS is still an IND vaccine.

For all the other agents that had been weaponized, the vaccines in existence were known to induce unacceptably high levels of undesirable reactions, or unacceptable side-effects, or both. Scientists therefore developed new, experimental vaccines – using new technologies and building on new knowledge that biotechnology made possible. The following is a brief summary of USAMRIID’s work in the area of vaccine development on major biothreat agents. A full description of the work, including citations, is provided in Appendix A.

Anthrax. When researchers found that the anthrax vaccine might not be reasonably protective against an aerosol spore challenge, they applied rDNA techniques to clone the expression of the PA gene in order to produce a more effective vaccine. Site-specific mutagenesis allowed the creation of a variant of the
PA protein that deleted the six amino acids spanning the cleavage site, allowing for the creation of a possibly less toxic anthrax vaccine. In all, five new live anthrax vaccines developed using rDNA technology entered preclinical safety and efficacy testing by FY1989.

Q fever. A similarly extensive effort was made to find a new, less reactogenic vaccine for Q fever. In addition to a new, whole cell vaccine that entered safety and immunogenicity testing in FY1979, USAMRIID researchers developed a CMR vaccine in FY1985, and two subunit vaccines. Again, rDNA technology was used in their research, especially to identify and clone immunogenic proteins as subunit vaccine candidates. The safe and effective induction of nonspecific resistance by an immunomodulatory complex of C. burnetii was identified in two vaccine candidates: they reduced susceptibility to lethal infections by other agents. A similarly broad research program (live, inactivated, and subunit vaccines) to find a new vaccine for RMSF was underway at USAMRIID in the 1970s, but work was transferred to WRAIR in 1979 under a mandate to reduce work on rickettsiae.

VEE. The TC-83 (attenuated) vaccine was developed in the 1960s to replace an old, formalin-inactivated vaccine. Despite having protective efficacy in man, it produced a febrile illness, with virus shedding in 15-30% of recipients, had abortogenic and/or teratogenic potential and was not tested in children. It might also be pancreotropic. The C-84 formalin-inactivated vaccine was prepared to avoid these side-effects, but it was not as protective against an aerosol challenge with virulent VEE. To improve its effectiveness, poly-ICLC and tilorone HC1 (analog 11,567) were found to be good adjuvants when given in combination with inactivated VEE
vaccine in lab rodents and rhesus monkeys. USAMRIID researchers developed a metabolized lipid emulsion, an effective adjuvant with the inactivated VEE vaccine, and submitted it for a government patent. The lipid emulsion adjuvant demonstrated high effectiveness at potentiating the immunologic response to VEE, RVF, and WEE inactivated vaccines.

**Rift Valley Fever.** The RVF inactivated vaccine was developed prior to 1969, but in FY1977, it was reported that it did not meet modern safety standards for use in man. USAMRIID scientists therefore prepared a new inactivated vaccine and began work on an attenuated vaccine. In addition, basic research that identified vaccine-relevant epitopes on a small sequence of the RVF G2 surface viral glycoproteins, provided an excellent model to test the feasibility of the synthetic peptide approach to vaccine development. This was pursued through the use of a bacterial plasmid expression system and *E.coli* and also through the creation of recombinant vaccinia viruses. Researchers also determined that Avridine combined with the inactivated RVF virus vaccine, effectively reduced or prevented aerosol-acquired encephalitis.

**Hantaan:** The Salk Institute was producing two vaccine candidates for USAMRIID in FY89. One was a vaccinia-Hantaan recombinant containing both the S and M segments. Another was based on a vaccinia-Hantaan recombinant that included only the M segment.

**Machupo:** USAMRIID began work in 1971 after an outbreak of Bolivian hemorrhagic fever. Work included attempts to prepare a killed vaccine and subunit vaccines; on defining the efficacy of prophylaxis and treatment with immune serum; and a joint study with the Middle America Research Unit on a candidate attenuated
virus vaccine. By FY1975, a dosage of BHF immune globulin (human origin) that protected monkeys against severe disease was determined. Studies in monkeys showed that the Junin virus protected against experimental lethal challenge with Machupo virus.

**Junin:** Work on a new Junin virus vaccine was performed under a joint program with Argentina under the auspices of a UN Development Program that resulted in a new vaccine for Argentine Hemorrhagic Fever. (An IND submission was made to the FDA.) USAMRIID scientists demonstrated that the new vaccine induced a protective response in guinea pigs and monkeys against a significant airborne challenge of virulent virus.

**Lassa:** USAMRIID scientists developed Lassa-vaccinia virus recombinants in an attempt to create a vaccine for Lassa fever. In addition, during outbreaks in Liberia, human immune serum was collected that provided the US with a potential therapy in case of infection.

**Botulinum toxin:** USAMRIID had some pentavalent toxoid stockpiled that had been developed in 1958 by Parke, Davis & Co. under a contract with Ft. Detrick. However, it was considered reactogenic and stockpiles were diminishing. New lots were prepared with similar reactogenicity, but which enhanced the immune response to the type B toxin. USAMRIID researchers worked on developing a new, heptavalent toxoid that would be effective against all types of botulinum toxin (A,B, C, D, E, F, G). Two RFPs were issues – one for a hexavalent toxoid and one for a heptavalent toxoid in FY1985.
**SEB:** As of 1969, USAMRIID had a toxoid to staphylococcal enterotoxin B stockpiled that retained immunogenicity for 18 months. Work at USAMRIID was on a new, polyvalent toxoid that included enteroroxins A, B, C, and D. Although gram amounts of purified toxin (SEA, SEB, and SEC) were prepared, as of FY1982 when work on SEB was curtailed, no polyvalent toxoid was available.

**Ricin:** Work on ricin is reported as of FY1989. Active and passive immunization protected mice from lethal effects of aerosolized ricin, but pulmonary lesions were still detected. USAMRIID scientists developed a bivalent vaccine consisting of whole ricin and saxitoxin that reacted with anti-saxitoxin and anti-ricin antibodies.

**Drug Development and Drug Screening**

For many biothreat agents, chemotherapeutic drugs were developed. What is notable about many of these post-exposure therapies is the heavy dependence on rapid diagnosis. If it could be quickly determined that an individual has an infection, there are drugs that can mitigate or completely reverse the course of the disease despite the lack of a vaccine.

For example, a two week course of antibiotics is effective post-exposure to tularemia when given within 24 hours. Similarly, Rocky Mountain Spotted Fever is fatal without prompt and appropriate treatment, but is susceptible to antibiotic therapy if it can be diagnosed accurately. Antibiotics can also successfully treat exposures to aerosols of anthrax – again, only if it is diagnosed quickly. In 2001, six of eleven individuals with inhalational anthrax survived with modern medical care. Finally,
antibiotic therapy is used to treat Q fever infections, but are most effective if begun with three days after the appearance of symptoms.

In 1972, USAMRIID began to actively investigate antivirals. It was recognized that vaccines could not be relied upon because they were usually virus-specific and ineffective after the onset of infection. (For example, botulinum antitoxin is effective when given prior to onset of clinical symptoms, but gives no protection against respiratory failure once symptoms present.) In 1980, USAMRIID established a major drug-screening program both in house and under contract to The Swiftwater Laboratories. This program identified new antivirals with efficacy against potential biothreat agents. Two of the most promising antivirals were Ribavirin and poly-ICLC, an interferon-inducer.

**Ribavirin:** Ribavirin is a nucleoside analogue with a close structural resemblance to guanosine. USAMRIID scientists demonstrated that Ribavirin significantly inhibits a broad spectrum of both DNA and RNA viruses. Ribavirin was effective in the treatment of the following viruses: type A and B influenza virus infections when administered in small particle aerosols, Yellow Fever virus, Hantaan, Machupo, Junin, Lassa, Rift Valley Fever, and VEE. When combined with amantadine and rimantadine, Ribavirin’s effects were enhanced against RVF and VEE. Treatment with Ribavirin could begin as late as day 3 against RVF. In man, intravenous treatment with Ribavirin during the first six days of illness decreased mortality from Lassa fever from 73% to 8%. Prophylactic treatment with Ribavirin completely prevented the onset of clinical disease of Machupo completely. Ribavirin is the only antiviral with FDA approval for therapy against respiratory syncytial virus.
Poly-ICLC: Poly-ICLC is an interferon-inducer and is effective in the prophylaxis and early treatment of Yellow Fever, Japanese Encephalitis, and VEE. However, it enhanced Machupo virus infections.

Other antivirals tested: Amantadine and Rimantadine were shown to have prophylactic and therapeutic effectiveness against influenza. Tilorone hydrochloride and three analogs were effective antiviral agents against VEE by a mechanism that did not involve the synthesis or release of interferon. Finally, Avridine, in addition to being an effective adjuvant with RVF virus vaccine, reduced mortality in hamsters in the early stages of RVF infection through day six post-infection.

Detection

A significantly smaller share of USAMRIID’s resources was spent on assays to detect biothreat agents – whether in environmental samples or during infection. By FY1990, USAMRIID had developed IgM antibody-detection assays for each agent. Researchers had found that assays for antigen, and, in some cases, early IgM antibody, can be used to rapidly diagnose patients presenting with a number of militarily relevant diseases. As of FY1990 when rapid diagnosis teams were deployed in support of Operation Desert Shield, the diagnostic capability included 11 assays for naturally occurring viral, rickettsial, and bacterial disease threats, and four assays for potential biological warfare agents.

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180 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1990* (Frederick, Maryland: USAMRIID), Work Unit D809 EA 005.
This capability resulted from years of basic research to understand the pathogenesis of the various agents and the application or adaptation of new technologies like PCR biotechnology, ELISA, and solid-phase radioimmunoassay.

Basic Research

Approximately thirty percent of USAMRIID resources were spend on basic research over the twenty years of this study. Much work done was in support of new vaccines or assays. Nonetheless, a variety of work was performed on pathogenesis, strain cultivation and production, the development of appropriate animal models, genetic engineering of microbes, and tests of environmental stability and aerosol dynamics. Because so much of the work is described in detail in Appendix A, the summaries here are intended to highlight only some of the work done.

Pathogenesis. Some effort was spent on understanding the immune system response to various agents, including distinctions between humoral and cell-mediated immunity. These helped identify appropriate interventions. In addition, pathogenesis studies of the action of T-2 mycotoxin led to the discovery that virtually all tricothecenes bound to the same site on the ribosome as T-2, so if protection at that stage could be devised, it would cross-protect from a large number of tricothecenes.

Pathogenesis studies of anthrax led to a fuller understanding of the basis for virulence. An understanding of precisely where the PA toxin of anthrax was cleaved led to the production of an inactive PA for experimental anthrax vaccines. Other studies led to an understanding of exactly what combinations of its toxins was lethal. While it became possible to genetically alter anthrax strains, simple filter matings demonstrated the ease with which antibiotic-resistant characteristics could be donated.
between organisms, leading to tetracycline-resistant and streptomycin-resistant anthrax strains.

In another study of the virulence factors of Q fever, researchers studied the mini-chromosomes involved in transforming avirulent strains into virulent ones in an effort to identify the DNA involved in the attenuation of virulent strains.

**Cultivation and Production.** Methods and specific media to cultivate large quantities of agents was developed and detailed in the USAMRIID reports. Similarly, methods to generate large amounts of toxin production by agents were developed as well as methods to purify toxins. By the late 1980s, methods to synthesize large amounts of toxin were applied to test the potential of new technology to produce toxins in quantities that were not previously possible. This significantly expanded the list of toxins USAMRIID researchers were working with.

**Animal Models.** Animal models were developed for all agents to establish LD50s and to test vaccines and therapies for safety and efficacy. USAMRIID carefully monitored the health of all animals and established a breeding program for animals that were difficult to purchase. In order to gather the information required, animals were challenged by various routes – including aerosol exposures – to lethal and incapacitating agents, vaccines, and therapies.

**Genetic Engineering.** Genetic engineering of organisms was done to create new vaccines (mostly vaccinia recombinants), create gene libraries, and to develop assays. The genetic engineering of the vaccinia virus was to produce a single vaccine
that could confer immunity to several hazardous viral diseases.\textsuperscript{181} Some genetic engineering was done to alter pathogenesis as described above. As the knowledge and technology advanced over the course of the 1970s and 1980s, USAMRIID scientists applied new techniques to study the various agents.

**Environmental Stability and Aerosol Dynamics.** Over the course of this period of time, no aerosol survival/environmental stability tests were reported for anthrax, tularemia, Q fever, VEE, and botulinum toxins. These were presumably done under the offensive program, as they were weaponized then. However, environmental stability tests were done for many agents. Some involved artificially stabilizing the agent.

Aerosol studies of \textit{P. pseudomallei} in FY1980 showed that even with no effort to stabilize it, \textit{P. pseudomallei} cells survived the stresses of aerosol dissemination and persisted as airborne particles long enough to constitute a potential hazard in all environmental conditions studied. However, Junin virus, while transmissible in SPA, exhibited a biological half-life of approximately 28 minutes.

Aerobiological studies with the virulent strains of Machupo and Lassa fever virus (Carvallo and Josiah, respectively) were done in 6200-L aerosol chambers. These established that while Lassa virus was more stable in aerosol than Machupo, both were relatively stable in aerosol and both were highly infectious and moderately lethal for cynomolgus monkeys by the airborne route.

\textsuperscript{181} U.S. Army Medical Research and Development Command (USAMRDC), \textit{Biological Defense Research Program: Final Programmatic Environmental Impact Statement} (Frederick, MD: USAMRDC, 1989), 2-5.
Environmental stability and aerobiological studies were also done with T-2 mycotoxins. Researchers determined that it was very stable in a dry state for a long time and could be aerosolized (wet) with a common solvent with a particle size of 3 microns. Studies established the increased potency of an aerosol exposure to T-2.

The potential for aerosol transmission of RVF isolates was studied in FY1979. It was also shown in mice that the inactivated vaccine did not provide complete protection against RVF virus disseminated in small particle aerosols. Other studies investigated a variety of potential vectors for RVF virus.

**Epidemiology.** USAMRIID researchers sought to understand the basis for natural disease outbreaks caused by viruses such as Hantaan (HFRS), Lassa, Machupo, Ebola, and RVF. Efforts to find natural reservoirs of these agents led to a better understanding of possible factors associated with outbreaks in some cases. For others, like Ebola, no natural reservoir was found.

Perhaps the best epidemiological modeling was done for RVF. Because it is carried by specific strains of mosquitoes, tracking the conditions that led to dramatic increases in the population of those vectors helped researchers predict RVF outbreaks and, in some instances, prevent them. USAMRIID researchers used data from satellites to track conditions relevant to RVF virus activity in fifteen regions in five countries in sub-Saharan Africa. It was also postulated that such information would help distinguish natural from artificial outbreaks.

*Biodefense Work Under Transparency Rules*

Over time, working under a biodefense strategy that was unclassified and limited predominantly to defensive studies led to more scientific publications and
enhanced international cooperation on natural disease threats that were also potential offensive biothreat agents. This scientific exchange both enhanced the US biodefense program and provided assistance to countries that lacked the resources to tackle exotic disease outbreaks on their own. Working under a strict defensive mandate also meant the end to large, open-air tests in public areas.

Scientific publication at USAMRIID

Results of the research at USAMRIID could be classified. However, the work itself as it was being performed was unclassified. Over time, the number of scientific publications in independent, peer-reviewed journals rose steadily to the point where the number of articles published consistently exceeded the number of “professional work years” at the institute – basically, more than one article per PI each year, as demonstrated in figure 4 below.

Figure 4: USAMRIID Work Years and Publications
This output was used as evidence of the scientific merit and relevance of the work done at USAMRIID. In addition to publishing actively, scientists made numerous presentations of their work at scientific conferences and meetings. There were no reports of which, if any, research results were classified. Some aerobiology results were published, but very little of the work with anthrax was made public in the general scientific literature – especially not the disappointing results of the challenge studies with the MDPH vaccine against aerosolized anthrax spores. These results were published in a more limited way.\footnote{Bruce Ivins, Patricia Fellows, and Gene Nelson, “Efficacy of a standard human anthrax vaccine against Bacillus anthracis spore challenge in guinea pigs,” (Ft. Detrick: Army Medical Research Institute of Infectious Diseases: 16 August 1993). NTIS publication # ADA269134.}

International Cooperation: Validation of Technologies and Medicines

Scientific exchanges brought foreign scientists into USAMRIID laboratories to work. International cooperation also brought USAMRIID scientists and experimental vaccines and drugs to countries that needed assistance in controlling local outbreaks.

As a result of this work, diagnostic assays were tested under actual field conditions, vaccines and therapies were tested in humans, and sometimes the readiness of US biodefenses was enhanced by the acquisition of human immune plasma for rare, lethal diseases. The following are examples of international cooperative efforts:

- In 1969-1970 there was a severe outbreak of VEE in Central America. USAMRIID scientists provided the attenuated, live virus vaccine and technical support to create an immune barrier to prevent the further spread
of the virus. This was successful, and also provided researchers with greater confidence in the vaccine: a 96% conversion rate was noted and no reversion to virulence.

- In 1987, an extensive epidemic/epizootic of RVF in Mauritania and Senegal offered researchers an opportunity to validate their rapid diagnosis assays under field conditions. The new procedures were significantly faster and used less equipment: it was not as sensitive, but was 97% as specific.

- In 1979, USAMRIID researchers worked with the Israeli Defense Force to define alternative immunization schedules for the RVF vaccine, because it was not available in quantities sufficient in many scenarios for its use.

- In FY1986, USAMRIID scientist John Huggins demonstrated that Ribavirin could treat Hantaan virus infections. This led to a clinical trial of Ribavirin in the PRC, where Hantaan virus infections occur naturally each year. Assays for Hantaan virus were also successfully field-tested during that study.

- In FY1990, other collaborative research in Yugoslavia led to the awareness of an outbreak of HFRS there. A field study was established to test the efficacy of ribavirin and USAMRIID’s diagnostic tests.

- In the 1970s, work on a new vaccine for Argentine Hemorrhagic Fever was done under a UN Development project, jointly conducted by US and Argentine investigators. The live, attenuated Junin vaccine was taken to final product in 1982 in compliance with vaccine requirements for the US and Argentina. Researchers also tested the effectiveness of ribavirin in a field trial against AHF.

- During outbreaks of Lassa fever in Liberia in the late 1980s, human immune plasma was harvested from recovering patients and used to treat infected ones. In the process, USAMRIID collected several hundred, high-quality Lassa-immune plasma units for future treatment of the disease.

In FY1986 alone, NRC postdoctoral fellows came to work at USAMRIID, from the UK, Republic of Korea, India, France, Sweden, the PRC, Japan, Senegal, and Finland. USAMRIID also hosted international conferences on filovirus

research. As a result of USAMRIID’s leadership, a “comprehensive, collaborative research program was organized with the Institut Pasteur and implemented in the Central African Republic. The innovative international program, a pioneering first, allowed previously separate and independent laboratory and field efforts to be combined, closely integrated, and efficiently focused on Ebola and Marburg viruses.”

It is difficult to imagine that the US government would have allowed foreign scientists into its biodefense labs if the work was classified. It is even more difficult to imagine USAMRIID scientists being given permission by foreign governments such as China to administer experimental US drugs to foreign human subjects if those drugs came out of a classified US biodefense program. The orientation of the program toward medical therapies, the willingness to publish and share information, and the lack of security classification, therefore, made international cooperation possible which, in turn, enhanced the US biodefense program.

No Open-Air Testing in the Public Domain

The US conducted a series of tests in the 1940s and 1950s to test the vulnerability of the US to covert attacks with biological weapons. Various delivery devices were tested in public areas, such as subways, ventilation systems for buildings, and for large-area tests. These established that US citizens were vulnerable to sabotage and covert delivery of BW agents on very small and very large scales. Another round of tests was done in the 1960s to test the military utility of BW. These

\(^{184}\) Commander’s Forward, USAMRIID, Annual Progress Report FY1986 (Frederick, MD: USAMRIID), pp. ix – x.
established the conditions under which BW could have effect for military purposes. After 1969, no further vulnerability tests are reported. All testing was later confined to Dugway Proving Ground, and then only to validate new technologies or equipment.

Most testing of biological defense material was in the laboratory: “Laboratory studies requiring the use of aerosols are conducted only in response to specific equipment or material testing requirements and in the larger context of the goals of a particular project.”185 Nearly all testing was done with simulants. Outdoor testing was extremely limited and only after preparation of NEPA documentation. Outdoor testing was done to validate detectors, masks, protective clothing and other protective devices, and decontamination systems.186

At Ft. Detrick, aerosol tests were done, but with wet agents. These were done both to understand any differences in pathology when an agent was presented by aerosol and to check whether vaccines or therapies worked against an aerosol challenge. Novel delivery methods were not investigated at Ft. Derick nor were novel pathogens created.

Limiting Threat Assessment

Threat assessment types of work was limited at Ft. Detrick to determining whether an agent posed an aerosol threat – as a wet agent. It was not considered

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necessary to run tests with dry agents. Much of the work was, by its nature, dual use. These included aerosol stability tests, the identification of resistant strains, large-scale production of agents and toxins (and purification methods), and animal challenge tests to determine LD50s. However, no work was done to deliberately enhance virulence:

“Use of recombinant DNA procedures with pathogenic organisms and toxins is closely controlled at all locations, both within and outside the government. Development of a more virulent strain of a pathogen is specifically prohibited under any circumstance, and is not the goal of any BDRP effort. In fact, BDRP uses of recombinant techniques are with the goal of producing a less virulent strain which may be more safely used in the laboratory or for vaccine development.”\(^{187}\)

The veracity of this statement is confirmed by the work reported annually from 1969-1990. Methods to isolate particularly virulent strains were used for the purpose of finding good candidates for attenuated vaccines.

Perhaps the most unusual threat assessment work was done to establish the effectiveness of vaccines after radiation exposures, i.e. in a nuclear warfare environment.\(^{188}\) Otherwise, the work performed was predominantly protective: threat assessment was done in the context of a medical therapy being developed and not simply to evaluate vulnerabilities. When aerosol stability tests were done without a candidate vaccine to test, these were in response to information from the intelligence community (e.g. T-2 mycotoxins).


\(^{188}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1976* (Frederick, Maryland: USAMRIID), Work Unit 096 01 010.
National Security and the Question of Past Offensive Work

What is striking from a review of work on the agents in Appendix A is that the vast majority of work was on the development of vaccines and therapies. Very little work was done that could be characterized as “threat assessment” or potentially offense-oriented. It is possible to argue that the reason for the absence of that kind of activity was the prior existence of the offensive program. That is, threat assessment work had already been done for the agents on which USAMRIID was working during the 1969-1990 period. While that would be true for nearly all the agents, there were additional agents studied as they emerged as potential threats: T-2 mycotoxins, Hantaan virus, Ebola and Marburg.

It is difficult, therefore, to conclude that the work of the defensive program depended heavily on information from the offensive program. Rather, it is possible to argue that insufficient attention was given to developing appropriate and adequate defenses under the pre-1969 program: vaccines were not always as effective as previously thought, not usable for the general population, and stockpiled in quantities insufficient for general use. Furthermore, it is not necessary to know the exact behavior of an agent under various environmental conditions in order to create defenses: the effectiveness of vaccines could be tested in animal models with wet aerosols. That is, it was not necessary to bring a biothreat agent to the doorstep of weaponization (or to weaponize it at all), in order to develop medical countermeasures.

The answer to the question of whether national security was harmed by the unclassified nature of USAMRIID’s work depends on whether one concludes that the
scientific work was enhanced by close international collaboration or not. Foreign scientists were working in USAMRIID laboratories and on joint projects because the program was open. As discussed above, it is possible to conclude that international collaborations enhanced US biodefense in several important ways (vaccine development, antiviral testing, field detection devices). Furthermore, decisions on whether to publish results clearly were made and it is possible that information was classified in order to protect national security.

Conclusion

The overall record of USAMRIID is largely one of success: a focused research program on major biothreat agents yielded new vaccines and therapies, contributions to scientific understanding of disease processes, and detection systems. What is surprising is the level of achievement with extremely limited funding. Over twenty years, only $465 million (2006 dollars) was spend at Ft. Detrick for the development of medical countermeasures. That averages to approximately $23 million each year.

There are two areas that clearly needed improvement: production capability and diagnostic capability. Only a few lots were produced for any given vaccine. These were often depleted. The vaccine for tularemia was produced in the 1960s and depleted by the 1980s. New lots were produced in the late 1980s by more modern methods, requiring a whole new round of safety and efficacy testing. The Q fever vaccine was also depleted and needed replacement in the early 1980s: that is, there was little stockpiled until a new experimental vaccine was produced. The RMSF vaccine stockpile was also depleted in the early 1970s, however a new vaccine was
needed as this 1939 vaccine was determined to not provide total protection against RMSF in the 1970s. The Chikungunya vaccine stock was also depleted in 1970, requiring the production and evaluation of new vaccine lots. Finally, stocks of the botulinum toxoid was also diminishing as of the early 1980s and the only producer of the equine antitoxin notified the CDC that they would end production in 1978.

There was apparently no accounting done to ensure that stockpiles were kept adequate to research needs and for potential outbreaks. Production of new lots of experimental vaccines for exotic diseases is not likely to be done for commercial interests. Therefore, a better system for procuring and stockpiling needed vaccines is required.

Finally, only about ten percent of USAMRIID’s efforts went towards detection systems. These were both field detection systems and diagnostic assays of clinical samples. USAMRIID scientists did make significant contributions to the development of faster, more sensitive and simple assays. However, more attention is required here because, as described in this chapter, many biothreat agents are susceptible to known therapies if and only if they can be accurately diagnosed early – often prior to the onset of symptoms.

Looking back at how much was done, the conclusion that much more should have been invested is difficult to avoid. Much more funding is going into biodefense today, but the current trend has shifted toward greater secrecy and more limited opportunities for international collaboration. The risks of doing so should be measured against this successful record at USAMRIID’s record.
Chapter 5: The Regression of BW Strategy

“Scrupulous adherence to the BWC on the U.S. side, coming to the bar with clean hands, is of course an absolute prerequisite to the moral platform of BW prohibition. There is no more powerful instrument for that credibility than self-inspection.” – Joshua Lederberg

A common reaction to uncertainty is to seek control of whatever can plausibly be expected to submit to the exertion of power. September 11, 2001 and the subsequent anthrax attacks initiated a major reaction within the US government. As part of that response, agencies that never before had classification authority acquired it; a new department was created within the government; and new controls were placed on scientists and scientific research, along with new funding. At the same time, the US government chose to withdraw from international agreements and cooperative approaches, relying instead on its own power and initiatives. All this is reflected in the current biodefense strategy, “Biodefense for the 21st Century,” issued by the White House in April 2004.

Controlling access to information, individuals, technology, and materials and gaining a greater belief of control of the threat through threat assessments is a reactionary posture that is eroding US strategy to address the problem of bioterrorism. Fortunately, significant amounts of new funds are being invested for the development of medical countermeasures (including new high-containment laboratories) through NIH. However, the current approach is a major departure from the past thirty years. It is, fundamentally, a return to the US BW strategy as originally conceived in World War II.
Several key events in the past fifteen years made policymakers think more immediately about the threat of biological weapons – both from states and from non-state actors. The consistent response and continuing trend is that of the government attempting to exert more and more control over as many aspects of the bioterrorist threat as possible.


From the early 1990s until the US Biodefense Strategy was announced in 2004, biodefense policy was evolving in reaction to domestic and international events. Fear of WMD terrorism motivated new investments, mostly into domestic preparedness. After the anthrax attacks in 2001, however, billions of dollars were allocated for medical countermeasures. Therefore, many important aspects of the current biodefense strategy were developed over this period of time.

Eighteen years after the US signed the BWC (and sixteen years after ratifying it), the US Congress passed the “Biological Weapons Anti-Terrorism Act of 1989.”¹⁸⁹ It is the implementing legislation that binds individual US citizens by the terms of the treaty, with the stated purpose being to “protect the United States against the threat of biological terrorism.”¹⁹⁰ However, international efforts to negotiate a Protocol to the BWC that would have included verification and enforcement measures came close to completion, but was suddenly abandoned by the US in 2001.


Critical Events and US Responses

Vladimir Pasechnik defected from the USSR to the UK in 1989, bringing with him stunning information about a massive Soviet investment in biowarfare under the guise of a legitimate scientific organization, Biopreparat. More information came subsequently from Ken Alibek, who later published an account of his involvement in Biopreparat in *Biohazard* (1999). In addition, in the midst of the historic transformations within the USSR, reciprocal visits were arranged for Russian, American, and British scientists. The Americans and British were surprised at the extent of the offensive activities they viewed at several sites in the then-Russian Republic.

In January 1992, Russian President Boris Yeltsin admitted to having programs in violation of the BWC. In April 1992 he issued a decree stating, “It shall be established that the development and implementation of biological programs in breach of the Convention … is not being permitted in the territory of the Russian Federation.” This was not verified by the US because planned visits to Russian military sites were not permitted, although Russian scientists were allowed to visit USAMRIID and sites associated with the former US offensive program.

In addition to these revelations, concerns about Iraqi WMD and the ability of inspections to reveal the extent of those programs – especially BW programs – was

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ongoing in the 1990s. In its Comprehensive Report in 1999, The UN Special Commission stated the following:

Since the adoption of Security Council resolution 687 in April 1991 and until July 1995, Iraq denied that it had had any proscribed biological warfare (BW) activities. Based on the results of its inspection and verification activities, the Commission assessed and reported to the Council in its report of April 1995, that Iraq had not provided an account of its proscribed biological programme nor accounted for materials and items that may have been used or acquired for such a programme. The Commission stated that with Iraq's failure to account for the use of these items and materials for legitimate purposes, the only conclusion that can be drawn is that there is a high risk that they had been purchased and used for a proscribed purpose - acquisition of biological warfare agent. Iraq was provided with evidence collected by the Commission. On 1 July 1995, Iraq, for the first time, acknowledged that it had had an offensive BW programme but still denied any weaponization. Subsequently, in August 1995, after the departure from Iraq of Lt. Gen. Hussein Kamel Hassan, Iraq admitted that it had weaponized BW agents and deployed biological weapons for combat use.194

Subsequent declarations by Iraq about its BW program were declared inaccurate or incomplete and inspections could not verify all aspects of the dismantlement of the Iraqi BW program

In addition to concerns about state-sponsored BW programs in the former USSR and Iraq, terrorist activities were raising alarm. In February 1993, terrorists detonated a car bomb under the World Trade Center. Then on March 20, 1995 the Aum Shinrikyo cult released sarin gas, a nerve agent, on the Tokyo subway, killing 12 and sickening several thousand others.195 The cult had used sarin gas in a prior attack and investigators later learned of their interest in biological weapons such as


botulinum toxin. Only a month later, on April 19, 1995 domestic terrorists bombed the Alfred P. Murrah Federal Building in Oklahoma City, killing 168 people – 19 of them children.

In response to these major terrorist acts, the Clinton Administration issued a secret Presidential Decision Directive (PDD-39) in June 1995. It stated that “the United States would give the highest priority to developing effective capabilities to detect, prevent, defeat, and manage the consequences of nuclear, biological, or chemical materials or weapons use by terrorists.” In this directive, the FBI was designated as the lead agency for crisis management and FEMA the lead for consequence management. The next year, Congress passed the Nunn-Lugar-Domenici WMD bill that required Federal agencies to put systems into place to protect the public from terrorism. As a result, the United States Army's Chemical and Biological Defense Command (CBDCOM) began delivering domestic preparedness training nationally to emergency responders in 120 cities. These programs were developed and executed as a partnership among six federal agencies (DOD, DOE, FBI, FEMA, PHS, and EPA) and the emergency response community. Concerns about bioterrorism were mounting.

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In November 1997, Defense Secretary William Cohen held up a five-pound bag of sugar on national television and stated that if such an amount of anthrax was dispersed over a city the size of Washington, DC, half the population would die.  

According to one expert, the prominent and sustained attention given to the threat of bioterrorism by the US prompted at least one terrorist organization, al-Qaida, to become interested in BW. Dr. Ayman al-Zawahiri, an al-Qaida member, wrote in April 1999:

“… we only became aware of them [biological weapons] when the enemy drew our attention to them by repeatedly expressing concerns that they can be produced simply with easily available materials.”

In 1998, President Clinton issued Presidential Decision Directive 63 to protect America’s critical infrastructures. It created a National Coordinator to deal with critical infrastructure protection, foreign terrorism, and domestic mass destruction (including biological weapons). He added $1 billion for chemical and biological defense to the Five-Year Defense Plan and announced the selection of ten states whose National Guard units would receive special training to manage the consequences of a WMD attack. Some funding was also made available for medical countermeasures and a national pharmaceutical stockpile.

Within one week after the September 11, 2001 attacks, anthrax-laced letters were postmarked to news media organizations. More anthrax letters were later sent to

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congressional offices. In all, eighteen people in five states contracted anthrax, five of whom died of inhalational anthrax. A total of 33,000 people were give post-exposure prophylaxis.\textsuperscript{203} The perpetrator’s decision to identify the powder as anthrax contributed to a quick and appropriate medical response. Terrorism – and bioterrorism in particular – became a far more immediate and public threat than ever before in US history.

In 2002, a new department was created to deal with threats to the US, the Department of Homeland Security (DHS).\textsuperscript{204} The stated mission of DHS is to prevent terrorist attacks within the United States; reduce the vulnerability of the US to terrorism; and to minimize the damage, and assist in the recovery, from terrorist attacks that do occur within the United States.

The dominant response to the perception of the WMD terrorist threat focused on enhancing domestic response capabilities at all levels of government. Additional, modest efforts were also being made to specifically address the bioterrorist threat. These included access controls to biological agents and oversight proposals for dual use research.

Access Controls on Select Agents

In May 1995, Larry Wayne Harris acquired plague bacteria from the American Type Culture Collection under false pretenses. Harris was charged with three counts of mail fraud. Harris’s fraudulent acquisition of plague bacteria


prompted concern in the government about the ease with which individuals (i.e. terrorists) might be able to acquire the most virulent pathogens. Therefore, in 1996, Congress passed the “Antiterrorism and Effective Death Penalty Act”\(^2\) that made it a federal crime to even threaten to use a weapon of mass destruction, enlarged the definition of biological weapons, and required HHS to promulgate regulations covering certain biological agents.

CDC became responsible for the Laboratory Registration/Select Agent Program in early 1997: it developed the list of 42 agents covered by the program, issued rules requiring that laboratories have appropriate controls for handling biological agents in place, and required notification to the CDC of any transfers. This program was expanded in 2002 under the “Public Health Security and Bioterrorism Preparedness and Response Act”\(^3\) by increasing security measures, adding screening of entities and personnel, and creating a comprehensive national database of select agents and toxins.

More legislation was being considered by Congress to limit possession of biological agents, but was not enacted until after September 11, 2001. Within weeks of the September 11 and anthrax attacks, the House and Senate both passed legislation that required registration of anyone possessing select agents, criminalized unsafe handling (and required certain standards and procedures for proper handling),


and banned their possession by a group of “restricted persons”. The President signed the USA Patriot Act into law on October 26, 2001. 207

For the first time, the US government would know the location of every institution handling select agents and likely have performed background checks on the researchers performing work with them. In addition, the US sought to expand its control of research. The US began requiring that institutions outside the US institute the same kinds of physical security in order to engage US scientists in joint research projects. This led to the curtailment of joint research projects because other institutions outside the US did not have the funding to upgrade their physical security. 208 In addition, new rules about which scientists could attend international conferences were instituted. 209 Therefore, in addition to defining who could work with select agents in the US, the government was also beginning to define what researchers could do and say about their work.

Oversight of Research

Four different initiatives to institute an oversight process for research with particularly virulent pathogens are in place. The first began in the early days of recombinant engineering in the 1970s. The Asilomar conference of 1975 was the first major attempt by scientists to voluntarily impose oversight mechanisms over their work to prevent accidental releases or exposures. It resulted in the creation of


209 See Letter to Secretary Tommy Thompson, HHS, from Henry Waxman, Ranking Minority Member, Committee on Government Reform (June 24, 2004).
Institutional Biosafety Committees (IBCs) at institutions doing recombinant work and the Recombinant Advisory Committee (RAC) within the NIH. This is a precedent for compliance with biosafety guidelines. (As of 2002, two categories of research now require approval of the HHS Secretary according to new regulations that implement the 2002 bioterrorism bill. \textsuperscript{210})

The second oversight process evolved in the late 1990s during debates at WHO over the destruction of the last known stocks of variola virus. The WHO Orthopoxvirus Committee had recommended the destruction of all variola virus stocks as of 1996, but in May of that year the deadline was extended to June 1999. \textsuperscript{211} However, the US reversed its support for destruction in 1999, and instead WHO authorized a smallpox research program in return for another delay (until 2002). \textsuperscript{212} An Advisory Committee on Variola Virus Research was soon convened: it defined the areas of research, vetted research proposals, and annually reviewed progress. All work done under the WHO Variola Virus Research Committee is publicly available. In 2002, the decision to destroy the variola virus stocks was delayed indefinitely and research with the virus is ongoing. This is a precedent for the international oversight of a particularly virulent virus.

\textsuperscript{210} P.L. no. 107-188 (June 12, 2002) “The Public Health Security and Bioterrorism Preparedness and Response Act of 2002”. The Final Rule for Possession, Use, and Transfer of Select Agents and Toxins was published on March 18, 2005. See Federal Register v.70 no.52: 42 CFR parts 72 and 73 and 42 CFR Part 1003. For the Secretary’s role in approving certain types of experiments with Select Agents, see 42 CFR part 73.13, “Restricted Experiments.”


The third initiative is still being developed within the US: the National Science Advisory Board for Biosecurity (NSABB). It was convened in response to a call from a National Academy of Sciences report, *Biotechnology Research in an Age of Terrorism*. In that report, the NRC Committee recommended the creation of a committee to provide advice, guidance, and leadership to implement a system of review and oversight. In March 2004, NSABB was created within HHS to provide advice regarding biosecurity oversight of dual use research. According to its charter, NSABB is charged with the following activities:

- Developing criteria for identifying dual use research and research results.
- Developing guidelines for the oversight of dual use research, including guidelines for the risk/benefit analysis of dual use biological research and research results.
- Providing recommendations on the development of a code of conduct for scientists and laboratory workers, and development of mandatory programs for education and training in biosecurity issues.
- Advising on national policies regarding the conduct of dual use biological research, including policies governing publication, public communication, and dissemination of dual use research methodologies and results.
- Advising on national policies governing local review and approval processed for dual use biological research.
- Advising on criteria and processes for referral of classes of research or specific experiments by IBCs to the NSABB for guidance.
- Responding to requests for interpretation and application of the guidelines to specific research proposals in instances where a proposal has been denied by an IBC.
- Recommending strategies for fostering international collaboration for the effective oversight of dual use biological research.

Working Groups are still meeting and focusing their recommendations. Unlike the NIH RAC oversight process, this is a possible precedent for oversight of a

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215 See webcast downloaded from www.biosecurityboard.gov/
much broader range of research by the government. The rationale behind the NAS proposing such an oversight group was so that it could both identify consequential work and find ways to instill responsibility in advance of carrying out such research.

Finally, the fourth oversight arrangement in place deals not with the conduct of research, but the publication of dual-use research results. An experiment published in 2001 that transformed a mild poxvirus into a lethal one attracted the attention of scientists and policymakers because it generated a broader realization that pathogens more virulent than would otherwise evolve in nature could be created using biotechnology. Concerns about the misapplication of biotechnology were generating discussion in scientific circles.

In response, the editors of the *Proceedings of the National Academy of Sciences* included a commentary justifying their decision when they published an article with potential bioweapons implications. The article was by Ariella Rosengard on how the vaccinia virus evades the human immune response. In the accompanying commentary, P.J. Lachmann wrote, “The experiments of Rosengard *et al.* are an illustration of how the exploitation of microbial genomics can allow studies of the biology of viruses that cannot themselves be studies safely – or at all. The

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work is far more likely to stimulate advances in vaccinology or viral therapy than it is to threaten biosecurity.\textsuperscript{218}

Just a few months later, Eckhard Wimmer published the results of his lab’s chemical synthesis of the polio virus.\textsuperscript{219} *Science* included no separate commentary in the print version in which it appeared, but one blistering commentary did appear in the prior issue (Wimmer’s article was published online on July 11, 2002). Steven Block wrote the following about Wimmer’s work:

> [It] amounts to little more than a stunt – and not a particularly cheap stunt at that, given that the effort was reportedly bankrolled by hundreds of thousands of dollars from DARPA….It’s critically important to hold a national dialog among biologists, health care experts, politicians, and the general public about the future of biological work with biological weapons implications. But publishing research like this is a poor way indeed to open the conversation.\textsuperscript{220}

Wimmer’s piece received wide press coverage expressing concern about the implications for the control of bioweapons in general and poliovirus, in particular.\textsuperscript{221}

In an effort to be proactive, thirty-two of the world’s leading journal editors and scientist-authors called for vigilance and personal responsibility whenever potentially dangerous research is submitted for publication. They issued a joint statement that was reprinted in the major scientific journals in February 2003 in which they urged scientists and journals to consider establishing processes for reviewing risky papers and either modify or decline to publish articles if the potential


risks outweighed the benefits. However, as the technology continues to evolve, more research articles will continue to explore the creation of new and genetically altered microbes and their products.

Government policy on scientific information has shifted. According to National Security Decision Directive 189 (NSDD-189) from 1985, the National Policy was that products of research were to remain unrestricted to the maximum extent possible. “If national security requires control of that research, then it will be controlled through classification.” However, Executive Order 13292 (signed in March 2003) expanded the kinds of information that can be classified to “(e) scientific, technological, or economic matters relating to the national security, which includes defense against transnational terrorism…. or (h) weapons of mass destruction.” (The expanded items are underlined.)

**Current Strategy**

The official biodefense strategy was articulated in Homeland Security Presidential Directive 10, *Biodefense for the 21st Century*. The four major pillars of current biodefense strategy are summarized here:

**Threat Awareness.** This includes biological warfare-related intelligence and Red Teaming Efforts “to understand new scientific trends that may be exploited by our adversaries to develop biological weapons and to help position intelligence

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collectors ahead of the problem.” It also includes periodic assessments of the BW threat as it evolves, and an effort to anticipate and respond to emerging BW threats – including the development of safe and effective countermeasures. DHS was given the lead for conducting assessments of the threat and HHS was given the lead to develop countermeasures.

**Prevention and Protection.** “Preventing biological weapons attacks is by far the most cost-effective approach to biodefense. Prevention requires the continuation and expansion of current multilateral initiatives to limit the access of agents, technology, and know-how to countries, groups, or individuals seeking to develop, produce, and use these agents.” For protection, the government proposed vulnerability assessments to better protect critical infrastructure and gave the lead to DHS to develop and deploy biodetection technologies and decontamination methods.

**Surveillance and Detection.** DHS was given the lead to develop a domestic attack warning system and the capability to perform technical forensic analysis through the creation of a National Bioforensic Analysis Center within the National Biodefense Analysis and Countermeasure Center.

**Response and Recovery.** In response to an attack, plans are being created to integrate response efforts at every level of government. HHS was given the lead to coordinate a public health response, including the distribution of medicines and the development of medical countermeasures.

To implement this strategy, the US government poured an enormous amount of new funding into the NIH for the development of medical countermeasures.

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including public health preparedness and support to the Strategic National Stockpile. Significant amounts are also being spent by DHS for biothreat countermeasures, including the creation of a new facility, the National Biodefense Analysis and Countermeasure Center.

Overall, funding for civilian biodefense rose from approximately $295 million in FY2001 to $5.24 billion in FY2006.\(^2\) Total federal funding – including DOD – for bioweapons prevention and defense jumped from $1.6 Billion in FY2001 to nearly $8 billion in FY2006.\(^2\) The agencies receiving funding for biodefense-related work are: USDA, Commerce, DOD, DOE, HHS, DHS, State, Veterans Affairs, EPA, and NSF. DOD’s budget in this area increased modestly: the majority of new funding has gone to HHS for medical countermeasures and to improve state and local capacity and to DHS for biological countermeasures. The work of these three major agencies are discussed below.

Department of Defense

DOD has a long history of work in the chemical and biological defense area. The National Defense Authorization Act for FY1994 mandated that DoD coordinate, consolidate, and integrate the chemical and biological (CB) defense requirements into a single program, the Chemical and Biological Defense Program (CBDP). Four agencies play key roles in the CBDP: The Defense Threat Reduction Agency, the Defense Advanced Research Projects Agency, the Defense Logistics Agency, and the


Defense Intelligence Agency. A significant portion of the budget goes to DARPA’s BW Defense Program. It works on “anticipating threats and developing novel defenses against them…” However the CBDP overall “continues to be a threat-driven program, not technology-driven…. [O]ur programs and technologies are driven by validated threat assessments and user mission requirements, not by technologies.” In addition to DARPA’s approximately $150 million in spending each year, the CBDP will, beginning in FY06, invest more that $1.5 billion over five years to develop broad-spectrum medical countermeasures against advanced bioterror threats, including genetically engineered intracellular bacterial pathogens and hemorrhagic fevers.

Health and Human Services: Response, Recovery & Countermeasures

The 2006 budget for HHS includes more than $4 billion for biodefense. This includes approximately $1.7 billion for NIAID for new and improved vaccines, diagnostic tools, and therapies against potential bioterrorism agents; $1.6 million for the CDC (surveillance and detection and training local response teams), and $600 million for the Strategic National Stockpile. Funding for the work at HHS – the

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The majority of which goes to NIAID – has been consistently higher than that for DHS. Since FY2003, funding for HHS has been approximately $4 billion per year, while funding for all biological countermeasures at DHS has been approximately $400 million.231

The National Institute of Allergy and Infectious Diseases reacted swiftly to the September 11 attacks. It developed a Strategic Plan for Biodefense Research and NIAID Research Agendas for CDC Category A, B, and C agents. NIAID’s mission “is to carry out the research needed to understand the pathogenesis of these [agents of bioterrorism] microbes and the host responses to them, and to translate this knowledge into useful interventions and diagnostic tools for an effective response.”232

Agents were placed on the list based on shared characteristics: high morbidity and mortality, potential for person-to-person transmission, directly or by vector, low infective dose and high infectivity by aerosol; ability to contaminate food and water supplies; lack of a specific diagnostic test and/or effective treatment; lack of a safe and effective vaccine; potential to cause anxiety in the public and in health care workers; and potential to be weaponized. Accordingly, the six areas of work were listed as follows: biology of the microbe, host response, vaccines, therapeutics, diagnostics, and research resources.


Among the current accomplishments are listed the following:\textsuperscript{233}

- More than 300 million doses of smallpox vaccine available; a “next generation” vaccine (MVA) in advanced testing; and oral cidofovir in advanced product development for use in a smallpox attack;

- A new vaccine (rPA) for anthrax tested and procured under Project Bioshield (75 million doses), and development of novel antitoxins to neutralize anthrax toxin.

- Vaccines for Ebola in human trials at NIAID Vaccine Research Center; and

- A new vaccine under development for botulinum toxin and also candidate antibody treatments in development.

NIAID is also building National Biocontainment Laboratories to BL4 standards; nine Regional Biocontainment Laboratories with BL3 facilities; and funding ten Regional Centers of Excelleece for Biodefense and Emerging Infectious Diseases Research to provide the “human infrastructure” for biodefense research.\textsuperscript{234} NIH also has an important role in purchasing medical countermeasures under funding provided under Project Bioshield.

Congress passed legislation in 2004 authorizing the procurement of biodefense vaccines by establishing a Special Reserve Fund of $5.6 billion to make purchases for the Strategic National Stockpile over ten years.\textsuperscript{235} The intent was as follows:

To provide protections and countermeasures against chemical, radiological, or nuclear agents that may be used in a terrorist attack against the United States


by giving the National Institutes of Health contracting flexibility, infrastructure improvements, and expediting the scientific peer review process, and streamlining the Food and Drug Administration approval process of countermeasures.

However, the process requires that the Secretary, DHS, perform a material threat assessment of current and emerging CBRN agents before countermeasures can be purchased. Only four material threat assessments have been made (anthrax, smallpox, botulinum toxin, and radiological/nuclear devices), resulting in only four contracts totaling $1 billion of the $3.418 billion allotted through 2008. Two of those were for anthrax vaccines and the others for countermeasures against a radiological or nuclear event. No further material threat assessments have been released as of summer 2006 because they are classified.

Department of Homeland Security: Threat Awareness & Surveillance

Established in 2002, the Department of Homeland Security is designed to protect the territory of the US from acts of terrorism and assist in the response to an attack. It is the third largest cabinet department in the Federal government. In the area of biological countermeasures, DHS has the lead role in threat awareness, environmental surveillance and threat attribution. Work on biosensors has been coordinated with the EPA and CDC. The goal is the creation of a national biosurveillance capability.

The other major area of work involves threat awareness and attribution. To accomplish these missions, DHS created the National Biodefense Analysis and

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Countermeasures Center (NBACC). The mission of NBACC is “to provide the nation with the scientific basis for awareness of biological threat and attribution of their use against the American public” by:\textsuperscript{238}

- Understanding current and future biological threats, assessing vulnerabilities, and determining potential impacts to guide the development of biodefense countermeasures; and

- Providing national capability to conduct forensic analysis of evidence from bio-crimes and terrorism to attain a “biological fingerprint” to identify perpetrators and determine the origin and method of attack.

The stated mission is to preserve homeland security through knowledge of the potential threat, prevention of surprise, and attribution of use. The NBACC program is intended to complement the capabilities of the existing USAMRIID, national labs, and other contract research organization.\textsuperscript{239} The NBACC program has four “pillars”

\begin{itemize}
    \item \textbf{BTCC – Biological Threat Characterization Center:} Provides laboratory-based, scientific data from the analysis and assessment of biological threats to human health and agriculture. Develops and applies models, materials, and validation processes to evaluate vulnerabilities and define risk.
    
    \item \textbf{BFAC – BioForensics Analysis Center:} Provides definitive forensic examination of biothreat agents and related evidence and serves to integrate the forensic requirement for law enforcement, national security, and homeland security. (Five years after the anthrax letter attacks, the FBI has no strong leads as to who the perpetrator was despite knowledge of the agent and delivery methods.\textsuperscript{240})
\end{itemize}


\textsuperscript{240} “Grassley Seeks Anthrax Briefing,” \textit{The Washington Post} (October 26, 2006): A06.
BKC – Biodefense Knowledge Center: Evidence-based subject matter expertise to integrate, analyze and distribute critical information assembled from multiple sources through a clearinghouse center. The Biodefense Knowledge Center (BKC) of NBACC was dedicated on September 10, 2004 and is located at Los Alamos National Laboratory (DOE). BKC, along with the future BTCC, will develop material threat assessments and formal risk assessments of select pathogens.\(^{241}\)

ABC – Agricultural Biodefense Center: Advances research-based solutions for prevention, detection, diagnosis and response to high consequence foreign animal diseases. It is established at the Plum Island Animal Disease Center.

NBACC will be the first DHS laboratory focused specifically on biodefense. DHS officially broke ground for NBACC at Ft. Detrick in June 2006. Construction is estimated at $128 million. The 160,000-square-foot facility will house the Biological Threat Characterization Center (BTCC) and the National Bioforensic Analysis Center (NBFAC).\(^{242}\) It will include over 70,000 square feet of laboratory space, 20% of which will be built to BL-4 standards. Officials have repeatedly stated that the research is designed to advance scientific understanding of potential bioterrorism agents, that is the “next-generation biological threats.”\(^{243}\) Doing this, it is asserted, is


\(^{242}\) Center for Infectious Disease Research & Policy, “DHS starts work on $128 million biodefense center,” (June 27, 2006). Downloaded from www.cidrap.umn.edu/cidrap/content/bioprep/news/jun2706dhs.html.

\(^{243}\) Charles McQuerey Remarks before the 7\(^{th}\) Annual Executive Symposium on Emerging Business Opportunities in Photonics,” (November 13, 2003). Downloaded from www.dhs.gov/xnews/speeches/speech0142.shtm.
only for defensive purposes and will not violate the terms of the BWC. However, it is not at all clear that the work will not be in violation of the international treaty.

The argument for performing threat assessment work based on what is technologically feasible and not necessarily based on an actual threat was described in a recent article by Seth Carus and James B. Petro. The authors make two important assertions:

- “In the absence of high-quality intelligence, the use of capabilities-based assessments will increase the number of potential BW scenarios incorporated into existing assessments; thus, a methodology for characterizing and prioritizing these ‘threats’ will be required to increase the likelihood that biodefense expenditures address those that pose the greatest risk to the U.S.”

- The termination of the US offensive BW program left gaps in the US ability to identify organisms or toxins potentially within foreign BW programs. “Empirical data and resulting technical assessments are needed to effectively address questions of technical feasibility raised by intelligence and some openly published research reports.”

Basically, the authors state the need to investigate the offense in order to prepare better defenses because intelligence collection alone cannot be relied upon to accurately identify the evolving threat. Development of potential threat agents and scenarios for their use (red teaming) are advocated as essential to biodefense. This is


dangerous ground to walk. The whole purpose of “Red Teaming” is described in a DoD report as follows:

In general, red team challenges can help hedge against surprise, particularly catastrophic surprises. It does this by providing a wider and deeper understanding of potential adversary options and behavior that can expose potential vulnerabilities in our strategies, postures, plans, programs, and concepts. This role (to explore technically feasible and responsive threats) has become increasingly important as a complement to the more traditional intelligence-based threat projections (capabilities-based versus threat-based planning).

BTCC will therefore investigate, novel threats, novel delivery of threats, novel packaging, and perform red teaming exercises to provide high resolution pictures of future biothreats. The work is not intended to be unclassified, but subject to some level of security restriction.

**Characteristics of the Current Strategy**

What the US has in place are three structures with relatively distinct responsibilities. DoD develops countermeasures and protective devices for military personnel. DHS develops surveillance and response systems for use within the US homeland for the protection of the US civilian population and for the ability to identify the perpetrator of any terrorist act. HHS’s responsibility is in the development and stockpiling of medical countermeasures for the civilian population in addition to improving public health response capabilities. All three departments

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have some responsibility for scientific investigations of current and future biothreat agents. However, while DoD and DHS attempt to define future threats through experiments designed to create them, HHS works on medical countermeasures based on an agent’s known characteristics (i.e. without significant modification).

HHS moved quickly to define its work prior to the creation of DHS and has worked in the absence of any enlarged biothreat list from DHS. HHS’s criteria are based on the characteristics of the pathogens themselves and potential for widespread harm. This kind of information is readily available and has not traditionally been classified. That is, researchers do not need to first demonstrate that the pathogen can be weaponized in order for it to be on a high priority research list: over half the agents on the Category A list are not DoD threat agents (i.e. DoD does not believe they have ever been weaponized). See table 4 below.

<table>
<thead>
<tr>
<th>Table 4: Category A Agents (NIAID list)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DoD Threat Agents (weaponized)</strong></td>
</tr>
<tr>
<td>Bacillus anthracis</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em></td>
</tr>
<tr>
<td><strong>Yersinia pestis</strong></td>
</tr>
<tr>
<td>Vaiola major and other pox viruses</td>
</tr>
<tr>
<td><em>Francisella tularensis</em></td>
</tr>
<tr>
<td>Ebola</td>
</tr>
<tr>
<td>Marburg</td>
</tr>
</tbody>
</table>

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Work at HHS is in large measure an expansive version of the post-1969 biodefense program at USAMRIID. However, the current US biodefense strategy as it is being carried out by DHS is, on the other hand, a much more expansive version of the pre-1969 US BW program – lacking only the explicit approval of offensive work (although some of the work recently revealed has pushed the limits of the BWC).\textsuperscript{252} The military’s pre-1969 approach to the threat – surveillance, detection and threat assessments – has essentially been duplicated at DHS. In summary, the current strategy emphasizes the following:

- BW are feasible, powerful, inexpensive, and easily hidden (useful for sabotage).
- Advances in technology are making new bioweapons possible.
- Intelligence collection cannot be relied upon to characterize the extent of the BW threat.
- In order to have a more complete understanding, investigations into the offensive potential of current and novel pathogens must be done in order to develop appropriate defenses.
- It is also necessary to thoroughly test the ways in which the US is vulnerable to attack (Red Teaming) in order to develop better strategies for protection.
- BW work requires classification – of basic research and possibly of publications.
- BW work requires access controls.
- BW use is not governed by moral considerations or international agreements.
- Deterrence is not feasible: work must focus on prevention and response

The \textit{National Strategy for Homeland Security} released in 2002, states that, “The expertise, technology, and material needed to build the most deadly weapons

\footnote{\textsuperscript{252} William Broad, Judith Miller, Stephen Engleberg, \textit{Germs: Biological Weapons and America’s Secret War}, (New York: Simon \& Schuster, 2001p).}
known to mankind – including chemical, biological, radiological, and nuclear weapons – are spreading inexorably. If our enemies acquire these weapons, they are likely to try to use them.”

The US response, therefore, focuses on law enforcement methods to control access to the US and to pathogens in order to apprehend terrorists before they are able to strike and the forensic ability to ensure attribution in the event a crime is committed. Very little in the strategy relies on cooperative efforts with other states (except in sharing law enforcement information). There is not even a passing mention in either the *National Strategy for Homeland Security* nor in *Biodefense for the 21st Century* of the Biological and Toxin Weapons Convention nor the Geneva Protocol. The omission of the BWC reveals the extent to which the current US strategy has departed from the past thirty years.

In 1969, the US forfeited its bioweapons capability in favor of a strategy to support the convention – the norm – against BW as having any place in any arsenal in the world. It became a party to the BWC in order to reinforce this notion. The considered judgment in 1969 was that biological weapons provided no strategic value in the hands of a well-armed state like the US and reduced US power in the hands of others even if held in small quantities. It was enough to perform unclassified biodefense work on medical and mechanical countermeasures.

Thirty-seven years after the termination of the US offensive BW program, the agents the US fears most are still those it weaponized. The anthrax used in the 2001 attacks bore uncomfortably close resemblance to the anthrax the US military produced and stockpiled for potential use. Pushing to stay at the leading edge of the

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threat curve brought the threat itself to being in an unpredictable fashion. The fear of precisely this outcome led the US to the 1969 decision. However, this reasoning has been forgotten.
Chapter 6: Choice and Consequence

“We can choose to save the world for ourselves and our children with science as our servant, helping us to restore and to build…Or we can choose the easier road, the road of hate and fear that would lead us to destroy our neighbors because we don’t like the way they live and because we are sure they are threatening to destroy us.” – Theodor Rosebury, 1949

The US is currently applying logic that bears close resemblance to that which drove the War Department to invest in a new BW program during World War II. Fear and technology are entwined once again to form a strong plea for offensive work in the name of defense. The proclaimed enemies this time are believed to be determined terrorists armed with WMD employing sabotage techniques to cripple the US at home as well as states seeking a weapon with deterrent value. The enemy sixty years ago was feared to be the same. Then as now there are active discussions about technological advances making possible more potent bioweapons. Now, however, the claim is unfortunately more true than ever before.

There are both potential benefits and risks to the strategy as currently conceived. Investigations that enhance scientific understanding of disease processes and human immunity can lead to better medicines. Funding for detection devices can improve the basic technology such that diagnoses of common and exotic diseases are quick and reliable.\(^{254}\) That is the potential positive interaction that could improve public health and biodefense preparedness. The risk comes from the pursuit of threat assessment and red teaming activities – and their classification. Such work invites

emulation and gives all governments legitimacy to pursue similar programs in similar fashion. This is likely to increase suspicion and fuel a dangerous cycle of increasing threat and fear.

As an alternative to the current approach, the US could build more upon the strengths of the post-1969 biodefense program: cooperative, international, unclassified research restricted to defensive investigations and designed to improve the capacity to identify and respond to outbreaks wherever and whatever their source. It may seem paradoxical, but more security can possibly be obtained through increased interaction and cooperation with the rest of the world rather than through predominantly restrictive security measures that attempt to control and contain the threat to areas outside the US.

*The Past as Future?*

Table 5 below compares the presumptions of the three periods of time in US BW policy. These key presumptions formed at the end of World War II about the BW threat are analyzed here in the context of past and current policy.

<table>
<thead>
<tr>
<th>Table 5: Comparison of Presumptions</th>
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<tbody>
<tr>
<td>BW are feasible, powerful, inexpensive &amp; easily hidden</td>
</tr>
<tr>
<td>Advances in technology make new BW possible</td>
</tr>
<tr>
<td>Intelligence collection not reliable for BW</td>
</tr>
<tr>
<td>Offensive investigations necessary for defense</td>
</tr>
<tr>
<td>Perform realistic testing of US vulnerabilities</td>
</tr>
<tr>
<td>Classify BW work</td>
</tr>
<tr>
<td>Classify BW publications</td>
</tr>
<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>Biosecurity access controls</td>
</tr>
<tr>
<td>BW use not governed by moral considerations or international agreements</td>
</tr>
<tr>
<td>Deterrence requires ability to respond in kind (BW)</td>
</tr>
</tbody>
</table>

BW are Feasible, Powerful, Inexpensive and Easily Hidden

Current US strategy assumes that bioweapons are all of the above: this has been demonstrated. The US created and stockpiled dangerous bioweapons and, more importantly, the USSR did as well. Indeed, the enormous Soviet BW program ballooned in the years after the BWC was signed, demonstrating the ease with which such a program could be hidden. It was not until Vladimir Pasechnik defected to the UK in 1989 and Ken Alibek followed that the US was able to determine the scale of the Soviet BW program – and the exotic kinds of research it pursued. Both were a significant shock to the US intelligence community.

While the US military studied the possible applications of BW in war-fighting environments, the consistent observation from 1941 to now is that bioweapons are most useful for sabotage purposes. They are – and have always held the most potential as – strategic terror weapons.

Advances in Technology make new BW possible

Another major theme that echoes down through the decades is that advances in technology make new, potentially more fearsome BW possible. This was a major argument made by the WBC committee to press for the initiation of the BW R&D program in 1941. The same argument was then employed by the Haskins Committee
in 1949 to resuscitate a BW program after several years of indifference. Today, the same argument is again employed to make the case for investigations into the characteristics of future BW agents in a classified facility (NBACC). However, allowing the military exploration of this space is a major risk: it leads to the development of novel weapons.

Technology is enabling the creation of novel pathogens and the large-scale production of exotic toxins. Synthetic genomics is making possible the creation of agents that resemble and have attributes of select agents, but which cannot be identified as such based on their sequence. It also provides the capability to produce novel pathogens of equal or greater danger than select agents.\textsuperscript{255} Recent experiments have deepened this concern: avoiding the genesis of such weaponry by any state or group should be a major concern.

Intelligence Collection not Sufficient

The intelligence community (IC) does not have a strong record of success in the BW area. That is largely due to the first point: a BW program is readily hidden because it carries no unique signatures. During World War II, the IC looked actively in the European theater for evidence of a BW program. Although significant evidence pointed away from any such program (no antibodies detected in German and Italian prisoners of war), the strong belief that Germany had the intent to use BW led to a dismissal of this information and a more intensive hunt for any BW information. In addition, although strong evidence pointed toward Japanese BW investigations and

use, the US did not pursue that information until the end of the war – perhaps because the government did not believe that the Japanese could develop such a program.

The limits of the IC in the BW area were known during the post-1969 period, but the biodefense program nonetheless relied upon the IC to provide lists of validated threats to guide their research. As new threats were identified (e.g. T-2 mycotoxins, Ebola etc.), work at USAMRIID shifted or expanded.

However, the recent lack of sufficient information from the IC about the actual BW threat has led to the adoption of a seemingly logical, but actually the most dangerous presumption of all: that investigation of the offense is necessary to develop appropriate defenses. This was first argued in 1941 by the Chemical Weapons Service. It was abandoned after 1969, but is being resurrected today. The logic is not supported by historical experience.

Investigation of the Offense is Necessary to prepare Defenses

NBACC was founded in 2002 to perform threat characterization and bioforensic work. Just as in 1941, the need to understand the implications of technological advances drives the need for up-to-date threat assessments. Thus an old argument is being repeated: in order to develop appropriate defenses, a thorough investigation of the offensive potential of BW is necessary.

In the 1941-1969 period, this guiding logic led to the creation of potent bioweapons and pitifully few defenses. That is, the theory was tested and proven wrong. Despite this relevant historic example, rather the opposite result is expected today. “A biological threat characterization program would enable U.S. biodefense to anticipate and prepare for emerging threats, bringing the defense closer to parity with
the threat curve.” There is no reason to believe, as the authors and many others do, that the outcome will be exactly opposite to that already experienced by the US from its 1941-1969 threat assessment work.

Threat assessment based on technological potential will enlarge the threat space, rather than provide a realistic characterization of it. Among a myriad of possibilities, it is unlikely that the US will develop just those that an adversary is pursuing. It has also been demonstrated that such work is not actually necessary for development of appropriate defenses.

USAMRIID’s work resulted in the development of new vaccines and therapies for both DoD threat agents and for emerging infectious diseases (EID) of particular virulence. It made rather limited investigations of the offense – and then only what was necessary to validate defenses. Proceeding in this way allowed for the development of the scientific base to respond to novel threat agents and EID.

Necessary to Investigate the Nature and Extent of US Vulnerability

It is the current policy that it is not only necessary to investigate the characteristics of future bioweapons, it is also necessary to test the ways in which such novel organisms could threaten the US. Again, a reasonable assumption is to find vulnerabilities in order to minimize them. The problem is that many ways in which bioweapons make US citizens vulnerable are already known because of extensive testing in the past. That knowledge combined with more sophisticated epidemiological models should be sufficient on which to base estimates of novel BW

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effects. Furthermore – as with the creation of novel pathogens – the generation of new information about how to effectively disseminate them cannot be kept secret forever from every potential terrorist. History bears this out.

In the late 1940s, the usefulness of BW for covert and clandestine operations was recognized and many tests done to assess this utility. Ira Baldwin, former scientific director at Camp Detrick, wrote a “Report on Special BW Operations” in October 1948 that emphasized the vulnerability of the US to secret BW attacks. What ensued was a series of tests in public areas with simulants, including subway and ventilation systems. In 1951, a US National Intelligence Estimate concluded, “Possible methods of introducing and disseminating biological weapons are almost limitless.” As one example, “Contaminated letters may be sent directly to the intended victims, without risk of detection.” So it was written, so it was done over fifty years later.

**BW Research and Publication Requires Classification & Access Controls**

As noted in the previous chapter, a recent Executive Order expanded the kinds of scientific information that could be subject to classification to include information about WMD and transnational terrorism. Numerous other actions by Congress have imposed biosecurity controls on work with Select Agents. Editors of scientific journals now voluntarily monitor their own publications for bioweapons implications. In addition, most of the work on biological countermeasures at DHS is subject to

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some level of security restriction. This is causing significant delays in the purchasing of medicines under the BioShield Project, because new purchases are dependent on prior DHS material threat assessments, which are currently classified and cannot therefore be released as written. Information about the exact quantity of medicines in and location of the Strategic National Stockpile is also not publicly available.

This is all very similar to the way in which the US BW program was carried out from 1941-1969. It operated under strict secrecy, with a brief window of openness between 1945-1949. Much of the work remains classified, but that has not prevented the inadvertent spread of information relevant to weapons application. Simply knowing that it is possible to use a pathogen as an efficient and effective biological weapon is enough to provoke emulation.

Operating a secret biodefense program invites emulation and suspicion. It also reduces opportunities for international collaboration on research with important pathogens. This lack of transparency can lead to dysfunctional behavior. Disease outbreaks have long been considered natural until proven otherwise. This allows for quick public health interventions to save lives – often involving international responses. If the reverse were presumed, efforts to collect and preserve “evidence” could interfere with an appropriate public health response.

BW Use Not Governed by Moral Considerations or International Agreements

In its very first report, the WBC committee clearly stated its belief that BW use would not be governed by moral considerations or international agreements. At the time, the US had signed but not ratified the 1925 Geneva Protocol banning the use of BW in war. Their reasons for this belief are not stated, although the events of
World War II undoubtedly affected their perceptions: the second meeting of the WBC committee was the day after the attack on Pearl Harbor.

National Strategy today implicitly adopts the same posture. There is no mention of either the Geneva Protocol nor the BWC in the US Biodefense strategy. International cooperation is limited to the sharing of law enforcement information and the interdiction of prohibited technologies. The US scuttled attempts to create a Protocol to the BWC in 2001 because it did not believe in its purpose. Current policymakers simply do not see any worth in or power to be derived from supporting the norm against BW or international agreements towards that end. The only power on which it believes it can rely is military strength.

Deterrence Not Feasible

The original BW program began with the goal of creating bioweapons in order to have a deterrent to enemy BW use. The idea of deterrence in kind later shifted to a notion of measured response during the Vietnam War to allow for the first use of non-lethal BW in an attempt to avoid a full escalation of the conflict. That provoked a very strong response. In 1969, it was determined that as a deterrent on a strategic level, BW were redundant and not usable against a nuclear-armed state because any BW use – even nonlethal BW – would escalate the conflict to nuclear exchange.

Today, the US is more concerned about terrorist use of BW and less so with state BW programs because the US nuclear arsenal is still a deterrent against states. The conclusion – again implicit – is that terrorists using WMD cannot be deterred. They must either be physically prevented from carrying out their crimes, or the US must be able to act quickly and effectively to minimize the damage and later
prosecute the offenders. If the strategy is to be characterized as one of deterrence, it is through the denial of ends sought through effective domestic preparedness. However, it is difficult for the US to argue both that it can fully defend itself against BW and that technology is rapidly making novel and more potent BW possible.

Which History?

This study shows that the fork in the road the US is currently following has already been well trodden. It is a dangerous path, if history is any guide. It is not too late to walk further down the road less traveled by because the prospects are actually better there.

A National Program

The 1941-1969 period is instructive not only for the biodefense logic it created, but also for the consequences that followed. The fear was always that the US might be behind the threat curve, even as it was actually pushing the leading edge of it outward. Offensive BW investigations generated lethal and nonlethal biological weapons that the US held in substantial quantity. Within this period of time, the US military changed its attitude toward BW from one of skepticism, to one of support: policy shifted from deterrence to first-use. Housing the secret program within the military establishment after WWII encouraged the weaponization of BW, because the creation of effective weapons is part of the military’s mission. A major oversight was the placement of the R&D for medical defense within the same organization. However, that was deemed logical because of the secrecy surrounding the entire BW program.
In the end, the application of this strand of logic to biodefense resulted in a significantly imbalanced situation: the US possessed BW weapons, but not adequate defenses against even the major threat agents it had weaponized. Table 6 below shows the quantities of biological threat agents the US had stockpiled in its arsenal as of 1969 and the kinds of vaccines available.

### Table 6: Balance of Readiness as of 1969

<table>
<thead>
<tr>
<th>1969 Weapons Program</th>
<th>1969 Safety Program</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthrax 220 lb (lethal)</td>
<td>Vaccine – 1960s*</td>
</tr>
<tr>
<td>Botulinum Toxin 23,000 cartridges</td>
<td>IND Vaccine *</td>
</tr>
<tr>
<td>Tularemia 804 lb (lethal)</td>
<td>IND Vaccine</td>
</tr>
<tr>
<td>VEE 4,991 gal + 334 lb (incapacitant)</td>
<td>IND Vaccine *</td>
</tr>
<tr>
<td>Q Fever 5,098 gal (incapacitant)</td>
<td>IND Vaccine *</td>
</tr>
<tr>
<td>Brucellosis M114 4-lb bombs</td>
<td>Vaccine*</td>
</tr>
<tr>
<td>SEB Stockpiled</td>
<td></td>
</tr>
<tr>
<td>Yellow Fever Standardized 1959 (mosquito vector)</td>
<td>Vaccine</td>
</tr>
<tr>
<td>Plague Stockpiled</td>
<td></td>
</tr>
</tbody>
</table>

The list above does not include agents in weapons, because there is no published list available that indicates which agents were placed into which bombs.

Also, an important qualifier to the list of vaccines is that they were not all considered effective (these are starred). At the time, the anthrax vaccine was not considered effective against certain strains labeled as “vaccine-resistant” (including the Ames strain) and required numerous injections and annual boosters. The Q fever and VEE vaccines both induced unacceptably high levels of side effects. The botulinum toxoid also produced undesirable reactions and was effective against only five of the seven types of toxin. For brucellosis, an IND vaccine was reported to have been developed, but as of 2004, no human vaccine is licensed or available for use. For plague, a vaccine was available, but it was known to be ineffective against an aerosol exposure. Therefore, only the vaccines for tularemia and yellow fever were known to
be safe and effective for use in man against the respective weaponized forms of the agent as of 1969.

Biodefense policy can continue to reenact this early history or choose to build upon the history after 1969. In very broad terms, the choice is between applying national security-type measures to the biodefense problem or those of public health. The consequences of furthering the path the US is now on should be clear. Building instead upon the post-1969 program is not as certain, but carries fewer risks and potentially greater benefits.

**International Cooperation**

The post-1969 program spent several decades attempting to create more effective vaccines for agents the US had previously weaponized and new vaccines and therapies for highly pathogenic EID and new threat agents. As described in chapter 5, the program was modestly successful, despite extremely limited funding. The average annual budget for USAMRIID’s work was approximately $23 million in current (2006) dollars.

Work done under the Biological Defense Research Program was unclassified and restricted to defensive work. This sometimes required limited investigations of what could be considered offensive work, such as tests of environmental stability, aerosol dynamics, and selecting and cultivating particularly pathogenic strains, and purification of toxins. However, careful inventories were kept of quantities of toxins produced for research purposes and for the production of toxoids. In addition, aerosol tests with virulent agents were done with wet aerosols – not dry aerosols as would be more appropriate for offensive use. That is, knowing how a pathogen performed in a
wet aerosol was sufficient to test the efficacy of vaccines and therapies in animal models.

Overall, the limited types of offensive studies were consistent with the overall purpose of the program and done without classified status. Furthermore, USAMRIID scientists appreciated the implications of recombinant DNA technology, but chose to apply it primarily toward novel vaccine development rather than use it as a reason to develop novel pathogens. The need to perform experiments in a fairly transparent organization that sometimes included foreign researchers possibly helped keep the kinds of experimentation away from exotic threat assessment work.

Orienting the program in this way was designed to enhance confidence that work at USAMRIID was consistent with its obligations under the BWC. In addition, it made possible international collaborations that enhanced confidence in its biological countermeasures: detection systems were field tested during outbreaks of viral hemorrhagic fevers in other countries, the efficacy of the antiviral drug Ribavirin was evaluated in humans during natural outbreaks in China and Yugoslavia, and other important knowledge about experimental vaccines and therapies was gained.

Current biodefense work at NIAID most closely resembles the scientific work done at USAMRIID from 1969-1990. However, NIAID’s budget is significantly larger – nearly $1.7 billion per year. Medical countermeasures are the core of any strategy to deal with pathogens – whether used deliberately as a weapon or naturally occurring. Detection systems are also important: development of this technology and its domestic implementation is largely the responsibility of DHS.
Therefore, the recent shift in approach must reflect either an assessment that the post-1969 program was inadequate in some fundamental way or that a change in the security environment warranted such a reversion. The three significant features that are transforming the post-1969 program are: a dedicated BW threat assessment effort conducted under NBACC; significant amounts of classified work; and significant amounts of funding.

A major failure of the post-1969 program was the ability to track developments in other state BW programs. Threat assessment is intended as the remedy. Historically, it has been shown that threat assessments did reveal US vulnerabilities, but still could not reduce them nor enhance intelligence collection. Knowing what adversaries might hypothetically be working on will do little to describe what they actually are working on. It is akin to the story of the drunk man who lost his wallet in a dark alley but searched for it under the street lamp because the light was so much better there.

Thus, although threat assessments are defended as necessary to gain parity with the actual, emerging biothreats – there must be another reason for the change in policy. It is more likely that concerns about WMD terrorism after 2001 prompted a need to do something the US was comfortable with, such as meeting a national security threat with national security measures.

*The Road Ahead*

The history of US engagement with the problem of biological weapons is instructive both for the unanticipated results of the 1941-1969 period and the forgotten era of defensive work (1969-2001). The early period produced weapons
before strategy – and when strategy was carefully considered, the weapons were considered dangerous for the US to have. The latter period produced defenses against natural and biothreat agents in the absence of a weapons strategy, but rather under the commitment to the international BW ban.

Therefore the most important elements of a strategy to deal with the BW threat is quick detection, organizational response capability, and medical countermeasures under a program that operates under transparency rules. Preventing the military application of technological advances for the investigation of offensive applications is of the utmost primacy. Part of the solution is enforcing the norm against such activities – which requires that the US not actively violate it. There is no defensible justification of such work. Refusing such work would enforce the norm articulated in the BWC. It would improve US security by both restricting any offensive work to that only necessary to improve defenses (as was done at USAMRIID) within a transparent, defensively-oriented program. That transparency is key to providing reassurance and avoiding unnecessary, provocative investigations.

Another area of work is to improve detection capabilities. Detection of pathogens is now most advanced within the US, but the US would be in a better position if detection of virulent pathogens was possible before being released on US soil. That is, an international network of detection systems would enhance US security if it was designed for both EID and potential BW agents. It would require investments in training laboratory workers and building laboratory capacity – transferring materials and knowledge to improve scientific capabilities in areas of greatest need.
Such networks are not without precedent. Networks to monitor compliance with the Comprehensive Test Ban Treaty is already in place although the treaty itself has not entered into force. Monitoring of disease activity depends on the sharing of information and individuals with the experience and training to know what is natural and what suspicious. This can and should be strengthened because it is more than likely that more can be known through inadvertent revelations through sanctioned contacts than through attempts to infiltrate select programs. For example, some of the former USSR BW secrets were gained while US and UK scientists were on an approved visit: one Russian scientist did not realize his comment about work with smallpox was highly unusual.

As the US invests heavily in the scientific base to work with the most virulent pathogens, it is important to emphasize that the work be done as transparently as possible to diminish international concerns and avoid the proliferation of classified BW work. After all, the medical benefits of such work cannot legitimately be restricted to any one country. The development of a vaccine or therapy that can save lives has not and should not ever be considered as information relevant to national security. In the past, an argument was made to this effect: that the US should not reveal its defenses – past stockpiles of vaccines were classified – because it would invite attack. It is a curious argument. Currently, the inadequacy of US medical countermeasures is public: USAMRIID’s Medical Management of Biological Casualties is a description of major biothreat agents and the current state of medical
defense and therapy.\textsuperscript{259} Knowing the effectiveness and limits of a vaccine is essential public health information and should not be classified.

Instead, international cooperation enhances US biodefenses by allowing for the field testing of systems and therapies against many biothreat agents that occur naturally in other parts of the world – over half of the NIH Category A list is endemic overseas. It builds some measure of trust, as well, that the US is not simply interested in the advancement of its own interests at the expense of others – but that the health of all can be pursued together and that it has its own worth. WHO efforts are instructive in this way: WHO scientists have gained access to countries even in the midst of wars in order to better the health of local populations. The successful smallpox campaign is an instructive example.

International cooperation is even more imperative today in the fight against terrorism. In this fight, the US cannot depend only on its military strength. It must find ways to reduce the sources of terrorism abroad by investing in the health and well-being of other countries. It is true that in doing so, the US builds local capacity and transfers knowledge. However, because all medical research is potentially consequential work and the only defining factor is intent, then it is even more important that the US shape the intentions of the scientists, making them contributors to the shared goal of international health and security and not providing incentives to put it at risk.

Unfortunately, current US policy works in precisely the opposite direction, nationalizing policies and granting currency to other governments to conduct

\textsuperscript{259} US Army Medical Research Institute of Infectious Diseases, \textit{USAMRIID’s Medical Management of Biological Casualties Handbook, Fifth Edition} (Fort Detrick, MD: August 2004).
classified BW threat assessments. Oversight is today being directed to identifying dangerous experiments in order to perform them rather than to induce caution. The NAS committee that proposed an oversight system (NSABB) did so for the purpose of minimizing the potential for the misuse of biotechnology: “The key issue is whether the risks associated with misuse can be reduced while still enabling critical research to go forward.”\textsuperscript{260} That is the key challenge and the answer will likely require the participation of all countries to prevent the deviation of any one.

David Huxsoll, former commander of USAMRIID, wrote the following: he captures the essence of what the history recounted here teaches:

An open, transparent biomedical defense program is the only type of program that truly supports US national policy, which is based on the provisions of the BWC. If the medical defense program were shrouded by secrecy of any degree, it would be incompatible with program acceptance, execution, and accomplishment. Selective openness is unacceptable: being “open” or “transparent” implies access to information relating to all stages of the research and development cycle. I also firmly believe that a laboratory or institute that can lay claim to internationally recognized scientific excellence has an open program. Transparency is the responsibility of everyone who has any connection with the issues, either as part of his job, or as a consulting expert (whether scientifically recognized or self-appointed). In other words, transparency is everybody’s business.\textsuperscript{261}


Appendix A: USAMRIID Research Summary

Below are the summaries for the agents on which USAMRIID spent significant time and effort. Some agents not included in this summary, but on which researchers did report work are: Japanese encephalitis B, St. Louis encephalitis, alphaviruses other than VEE (EEE, WEE, Mayaro, Chikungunya, O’nyong-nyong), Legionnaire’s disease, Ebola, Dengue, *Pseudomonas aeruginosa*, HIV, Malaria, nor studies of low molecular weight peptides (neuropeptides and monokines), nor many other toxins studied (e.g. marine toxins like saxitoxin and tetrodotoxin; toxin in snake venoms) that was initiated in the mid-1980s. The summaries are intended to provide a very broad overview of the types of experimentation done and results recorded. They are not intended to be exhaustive nor authoritative.

*Anthrax*

The anthrax bacillus is a gram-positive, sporulating rod that was weaponized during the US offensive program and more recently by other states. Historically, mortality for the inhalational form of anthrax exceeded 85%. A vaccine (Anthrax Vaccine Adsorbed (AVA) Biopost, Lansing, Michigan) was licensed in 1970. It is derived from sterile culture fluid supernatant taken from an attenuated (non-encapsulated) strain. The vaccination series consists of six subcutaneous doses over 18 months followed by yearly boosters.\(^{262}\) For post-exposure therapy, intravenous antibiotic treatment is recommended.

The Department of Defense mandated the vaccination of all US service members beginning in 1998. However, production of the vaccine was suspended in 1998 when the only production facility was closed for renovations designed to meet FDA regulations.

The Institute of Medicine reviewed the AVA, as licensed, and concluded the following:

AVA, as licensed, is an effective vaccine to protect humans against anthrax, including inhalational anthrax. Moreover, because the vaccine exerts its protection via an antigen crucial to the action of the bacterium’s toxins, AVA should be effective against anthrax toxicity from all known strains of *B. anthracis*, as well as from any potential bioengineered strains.\(^2\)

During this period of USAMRIID’s history, there was considerably more doubt about the effectiveness of the US anthrax vaccine. After the 1979 accidental anthrax release in Sverdlovsk, USSR, more work at USAMRIID went into research on the anthrax bacillus. By FY1990, it was known that in order to develop an optimal anthrax vaccine, the mechanisms of virulence and vaccine resistance of *B. anthracis* needed to be known. In experimental animals, the toxigenic (Tox+) and encapsulated (Cap+) Ames strain could overwhelm immunity induced by toxin-based vaccines more readily than other virulent strains.\(^3\) The findings of the USAMRIID researchers in these years about the potentially limited protection afforded by the US anthrax vaccine contradict the later conclusion by the Institute of Medicine (above). The reason for the discrepancy is the differences between the results with the lab

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\(^3\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1990* (Frederick, Maryland: USAMRIID), Work Unit A91C LA 133.
animals tested at USAMRIID (the guinea pig and mouse models) and those the IOM committee relied upon (macaque and rabbit): the latter models were considered more accurate representations of anthrax infection in humans.

Anthrax Toxin Studies

For eight years, USAMRIID performed virtually no work on anthrax (FY69-FY77). For reasons that are not elaborated, basic research began in FY78 to elucidate the mechanism of intoxication of anthrax toxin alone and to define its relationship to the overall course of anthrax infections.²⁶⁵ Part of the reason given was that prior with involved challenge studies using spores of virulent strains (1962), with very little work on the toxin itself (1968) because interest was centered on the organism and establishment of infection. Research on the anthrax toxin began because while antibiotic therapy can eradicate the organism, death could still result as a direct consequence of toxin elaboration. The prime goal of the work was to produce a standard antigen as a vaccine and to improve the anthrax vaccine in use.

In FY79, production of the anthrax toxin components (lethal factor (LF), protective antigen (PA) and edema factor (EF)) in vitro began using the Sterne strain for production of LF, and V770 for production of PA alone. (The V770 strain is avirulent, noncapsulated, and nonproteolytic. It was used to develop the Wright human vaccine. It produces the PA antigen, but lacks the ability to produce the other

²⁶⁵ US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1978 (Frederick, Maryland: USAMRIID), Work Unit A841 00 059.
two antigens.) Unconcentrated crude supernatants alone contained enough LF and PA to kill rats in 1-2 hours.\textsuperscript{266}

Within another year, satisfactory conditions were established for the in vitro production of anthrax toxins. LF and EF, when isolated, lack biological activity. Only when combined in a specific ration with PA, do they have biological activity.\textsuperscript{267} Using purified components of the anthrax toxin, it was found that the combination of PA and EF caused rapid, profound, and reversible increases of intracellular levels of camp, a result identical to that caused by cholera toxin. PA appeared to bind to surface receptors and enable EF to penetrate cells. EF was determined to be an adenylate cyclase enzyme active only within animal cells.\textsuperscript{268} PA mixed with LF was found lethal to rats with a minimum time to death of 60 minutes.

USAMRIID already had a standard purification procedure that it developed for the isolation of PA from culture supernatant. PA is antigenic and affords some protection in animals. Guinea pigs immunized with this partially purified antigen survived an IM challenge of 9200 LD50 of the Vollum strain anthrax spores.\textsuperscript{269}

In FY1982, the three protein components of anthrax toxin were purified and characterized. Yields, purities, and subunit molecular weights were, respectively:\textsuperscript{270}

\begin{itemize}
\item \textsuperscript{266} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1979} (Frederick, Maryland: USAMRIID), Work Unit A841 00 059.
\item \textsuperscript{267} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1980} (Frederick, Maryland: USAMRIID), Work Unit A841 00 059.
\item \textsuperscript{268} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1981} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 133.
\item \textsuperscript{269} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1981} (Frederick, Maryland: USAMRIID), Work Unit A871 BA 150.
\item \textsuperscript{270} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 133.
\end{itemize}
PA: 50mg, >95% pure, 85,000 daltons  
LF: 10mg, >90% pure, 83,000 daltons  
EF: 2 mg, >90% pure, 89,000 daltons

The heat-stable eukaryotic substance required by EF to express its latent cyclase activity was shown to be calmodulin, a protein ubiquitous in eukaryotes. Purified calmodulin fully activated EF and the dependence on calmodulin by EF is absolute. Data suggested that EF is not recognized by the experimental host as a foreign protein.

When LF was purified using a synthetic medium developed at USAMRIID (R medium) instead of medium 1095, the LF was ten times more toxic than reported in FY1981. Only 2 micrograms of LF mixed with 100 micrograms of PA killed Fisher 344 rats in 120 minutes. However, the mechanism of action of LF was not determined.

Further studies continued to find ways to ensure the production of enough anthrax toxins for research purposes. Initial recombinant work on anthrax toxin focused on cloning the gene for PA. A fragment of the pX01 plasmid was cloned into E. coli plasmid vector pBR322 and production of biologically active PA was demonstrated.

In FY1986, the genes for PA, EF and LF were sequenced. It was found that PA binds to a receptor and is cleaved by a trypsin-like enzyme in the membrane, exposing a site to which LF or EF binds. In vitro, the cleavage of PA by trypsin is at

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271 Ibid.

272 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1985 (Frederick, Maryland: USAMRIID), Work Unit A91C 00 131.
arginine-167 in a unique sequence of four contiguous basic amino acids. This was a novel find: the mechanism of action of the three toxin components were not previously understood. PA therefore had two binding sites. It was hypothesized that alteration of the cleavage site by directed mutagenesis could produce an inactive PA for use in future anthrax vaccines.

Large numbers of monoclonal antibodies to all three toxin proteins were obtained. Monoclonal antibodies that neutralize PA bind to the C-terminal half of the protein and prevent toxin binding to cells.

The Role of Plasmids

In FY1982, plasmids were discovered that regulate all the anthrax toxin components (PA, LF, EF). A literature survey that year revealed no published reports on the role of plasmids in B. anthracis. Plasmids were then isolated from various strains of B. anthracis: the Sterne and V770 strains contained both large and small molecular weight plasmids, while the encapsulated virulent strains contained an additional large molecular weight plasmid. In FY1986, analysis of the gene library of the pX01 plasmid showed that it contained the genes for all three toxin proteins.

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273 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1986* (Frederick, Maryland: USAMRIID), Work Unit BS12 AB 002.

274 Ibid.

275 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1982* (Frederick, Maryland: USAMRIID), Work Unit BS10 AO 199.

276 Ibid.

277 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1986* (Frederick, Maryland: USAMRIID), Work Unit BS12 AB 002.
Researchers determined that the basis for virulence in the anthrax bacillus resulted from two factors: a polyglutamic capsule and a three-component protein toxin. In FY1985, a US contractor and a Japanese researcher working independently both found that virulent *B. anthracis* strains contain a second large plasmid, pX02, which codes for synthesis of the polyglutamic acid capsule.278

By FY1990, researchers found that strains cured of pX01 had reduced virulence for mice, but some, such as D Ames-1 were still lethal at low doses. Virulence was partially mediated by pX02. Mutants that produced no detectable capsule were avirulent while mutants that produced more capsule than the parent strain were more virulent. This indicated that plasmid pX02 contributed significantly to the virulence of *B. anthracis*. However pX02 loci unrelated to capsule production also appeared to contribute to virulence. Results suggested that pathogenesis depends on a complex interaction of plasmid- and chromosome-encoded factors of *B. anthracis* and is modulated by the genetic background of the host.279

Virulence Testing

In FY1981, “a vial of lyophilized *Bacillus anthracis*, Vollum 1B strain, was obtained” for research purposes.280 Spores obtained were suspended and used to reestablish the validity of lab animal models for use in candidate anthrax vaccine evaluations. Dose-response values for oral exposure had not been reported

278 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1985* (Frederick, Maryland: USAMRIID), Work Unit A871 AC 132. The articles reporting this finding were published in the *Journal of General Microbiology* and *Infection and Immunity* in 1985.

279 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1990* (Frederick, Maryland: USAMRIID), Work Unit A91C LA 133.

280 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1981* (Frederick, Maryland: USAMRIID), Work Unit A870 BB 069.
previously, but was determined in guinea pigs to be equivalent to that by aerosol challenge, approximately 5 log 10 viable spores.

In another study in FY 1985, researchers tested the hypothesis that 3% calcium “potentiated” the virulence of the Vollum 1B strain. The addition of calcium did not enhance the virulence of the culture. This experiment may have been done because a significant finding in the [unpublished] FY1984 annual report was that the calcium ion greatly stimulates phosphorylation and that PA causes the incorporation of 32P from gamma-labeled ATP into macrophage proteins. In later studies, researchers found that calcium is absolutely required for cell killing by lethal toxin. Calcium appeared to be required at several stages, but most importantly at a step after toxin binding and internalization. Verapamil, a calcium channel blocker, protected cells against lethal toxin, suggesting its potential therapeutic value in anthrax intoxication.

Genetic manipulation of anthrax bacillus strains was done to find transposons. Simple filter matings of S. fecalis DC16C2 with B. anthracis VNR-1 (a derivative of Vollum 1B that has been cured of the pX02 plasmid) yielded tetracycline-resistant B. anthracis colonies that donated their tetracycline resistance when mating with streptomycin-resistant Sterne strain colonies.

281 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1985 (Frederick, Maryland: USAMRIID), Work Unit D807 AL 020.

282 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1985 (Frederick, Maryland: USAMRIID), Work Unit A871 AC 132, p.207.

283 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1989 (Frederick, Maryland: USAMRIID), Work Unit A871 AA 130.

284 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1985 (Frederick, Maryland: USAMRIID), Work Unit A871 AC 132.
In FY1985, it was found that the virulence of anthrax spores depended on the growth conditions used in their preparations. For example, spores grown on NBY agar appeared to kill CBA/J mice more slowly while being no different in their potency toward A/J mice.\textsuperscript{285}

Vaccine Studies

The human vaccine against anthrax consists of an alum precipitate of the culture supernatant of Vollum 770 strain \textit{B. anthracis}. (Note: D807-AA-011-1985 says the vaccine is from the Sterne strain.) The primary constituent for this vaccine is the PA component of the tripartite toxin. However, it was generally agreed that live attenuated vaccine used by veterinarians for vaccination of livestock provide better protection.\textsuperscript{286}

In FY1981, the sera of over 100 USAMRIID personnel receiving anthrax immunizations were evaluated. Those who had received extensive immunizations in earlier years but no boosters for 5-10 years were found to be essentially seronegative. This led to numerous individuals receiving boosters at one-year intervals.\textsuperscript{287}

Also in FY1981, USAMRIID researches began applying recombinant DNA techniques to the anthrax bacillus in order to clone the expression of the PA gene with

\textsuperscript{285} Ibid.

\textsuperscript{286} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit A871 BB 149.

\textsuperscript{287} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1981} (Frederick, Maryland: USAMRIID), Work Unit A871 BA 150.
the goal of producing a more effective vaccine that would produce a long-lasting, high level of immunity.288

Because live, attenuated vaccines are more effective, work progressed on finding a more potent and safer anthrax vaccine. Several PA fusion vaccines were investigated. In FY1986, the PA gene was cloned into *B. subtilis* with pUB110 as the vector. The clones, PA1 and PA2, produced PA at levels equivalent to or greater than that produced by the parent *B. anthracis*. They gave protection to rats against anthrax toxin challenge (?!?) and guinea pigs against virulent anthrax spore challenge, even when the spores came from a “vaccine-resistant” strains.289 Thus, these two clones were the first prototype, “new generation” live vaccines against anthrax. Further work confirmed the safety and efficacy of this vaccine.290

Later, site-specific mutagenesis yielded a variant of PA protein in which the six amino acids spanning the trypsin cleavage site were deleted. This variant PA was expressed in *B. subtilis* and purified. The deleted PA was non-toxic in rats and in cell culture. Guinea pigs were immunized with this PA in combination with LF and EF. This induced antibody titers equal to those obtained with native PA. When the immunized guinea pigs were challenged with virulent *B. anthracis*, they were

288 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1981* (Frederick, Maryland: USAMRIID), Work Unit BS10 AO 199.

289 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1982* (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131 and Work Unit A871 AD 133.

290 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1987* (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131.
protected just as well as those receiving native PA. Thus the deleted PA could serve as a substitute for PA in any subunit vaccine.\textsuperscript{291}

Dose-range experiments in FY1987 showed that the number of organisms required to produce immunity for the clones exceeded that for the veterinary vaccine, but that the immunization schedule is similar to the veterinary vaccine and produces similar results.\textsuperscript{292} The recombinant also protected mice resistant to the Sterne spore vaccine from challenge.

As an alternative vaccine, aromatic amino acid requiring strains of \textit{B. anthracis} were identified. These mutant strains were expected to be avirulent.\textsuperscript{293} These protected guineas pigs challenged by virulent \textit{B. anthracis}.\textsuperscript{294}

In another study, PA was tested in combination with the adjuvant, “Tri-mix” as a prototype vaccine in guinea pigs. Tri-mix was a new immunological adjuvant developed from Ribi Immunochem Research. A single injection of the mixture provided complete protection ten weeks later to a challenge of 7,000 \textit{B. anthracis} Ames spores and ELISA titers greater than 20,000 were elicited.\textsuperscript{295}

In addition, the antigens EA-1 and EA-2 were evaluated for their ability to protect guinea pigs against challenge with virulent \textit{B. anthracis}. Despite high post-

\textsuperscript{291} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit BS12 AB 002.

\textsuperscript{292} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1987} (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131.

\textsuperscript{293} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1987} (Frederick, Maryland: USAMRIID), Work Unit BS12 AB 002.

\textsuperscript{294} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1988} (Frederick, Maryland: USAMRIID), Work Unit BS12 AB 002.

\textsuperscript{295} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1988} (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131.
immunization titers to these antigens, the guinea pigs were not protected. Further studies suggested that these antigens are on the surface of non-encapsulated organisms and are probably masked during infection with virulent encapsulated organisms. Because of this, these antigens were probably not involved in protection.296

By FY1989, five new live anthrax vaccines entered preclinical safety and efficacy testing: two transposon-mutagenized strains of *B. anthracis*, and three recombinant strains of *B. subtilis* carrying the PA gene of *B. anthracis*. These were compared with the veterinary Sterne and human MDPH-PA vaccines. All five recombinants protected the sensitive A/J mouse from lethal challenge and high doses of the recombinants were non-lethal to these mice. As before, the MDPH-PA vaccine failed to protect mice from “vaccine-resistant” strains such as Ames. However the five recombinants were as effective as Sterne in protecting mice against Ames (approximately 80% survival). The mutagenized *B. anthracis* strains were the first vaccines tested that were able to protect highly susceptible A/J mice against virulent challenge. These five vaccine candidates also demonstrated substantial protection to guinea pigs against an IM challenge from *B. anthracis* spores. Finally, a single immunization of chemical vaccines consisting of PA + either “Tri-Mix” or “DeTox” provided complete protection to guinea pigs against Ames spore challenge 24 weeks later. These vaccine candidates also appeared to result in significant immunity in a

296 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1987* (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131, p.118.
shorter time than the currently-used vaccines and generally safer than the live veterinary vaccine.\textsuperscript{297}

In FY1990, the PA gene of \textit{B. anthracis} Sterne strain was cloned into baculovirus and vaccinia virus. Immunization with baculovirus-PA conferred full protection to guinea pigs when challenged with Ames spores. Vaccinia-PA conferred partial protection enhanced by emulsion of the vaccine in TriMix adjuvant.\textsuperscript{298} Guinea pigs received a single dose of vaccine and were challenged eight weeks later with 2000 LD50 Ames spores. Protection was: TriMix + PA (95%); monophosphoryl lipid A (MPL)+MDPH (60%); MDPH (50%); and MPL+PA (40%).

Production

The anthrax vaccine that is licensed for use in the US is produced under conditions that support the production and release of substantial quantities of PA by strain V770, an avirulent strain. Knowledge of this was published in 1963.\textsuperscript{299} By FY1981, all three protein components of the anthrax toxin were produced and purified to states approaching homogeneity.\textsuperscript{300}

In FY1982, highly purified and soluble PA was converted to a more potent immunogen by adsorbing it onto an aluminum hydroxide gel adjuvant. Soluble and

\textsuperscript{297} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131.

\textsuperscript{298} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1990} (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131.

\textsuperscript{299} Puziss, “Large-scale production of protective antigen of \textit{B. anthracis} in anaerobic cultures,” \textit{Applied Microbiology} 11: 330-334.

\textsuperscript{300} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1981} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 133.
adsorbed PA preparations were evaluated as inducers of protective humoral antibody in the standard model, the guinea pig.\textsuperscript{301}

In FY1981, USAMRIID scientists grew the avirulent Sterne strain in 10- and 20-liter batches in a small fermenter.\textsuperscript{302} In FY1982, 50-liter anthrax fermenter cultures were harvested using tangential flow filtration, a safer method.\textsuperscript{303}

Challenge Tests

In FY1982, tests with guinea pigs gave worrisome results about the effectiveness of the PA-based anthrax vaccine (the one currently used). Previously reported data showed that >80\% of the Michigan Department of Public Health (MDPH)-vaccinated guinea pigs were protected against an IM challenge containing 100-200 LD50s of virulent Vollum 1B strain.\textsuperscript{304} Surprisingly, there was no data available on the efficacy of the MDPH vaccine to protect guinea pigs against an aerosol challenge of anthrax spores.

Outbred Hartley strain guinea pigs were vaccinated with the MDPH vaccine using the standard regime of 3 sc injections of 0.5ml each given at 2-week intervals. They were presented with an aerosol dose of 152,000 anthrax spores (the LD50 for guinea pigs averages 60,000 spores). All controls died by day 4.5. Only 50\% of the vaccinated guinea pigs survived, with death ranging from 3-7 days, suggesting that

\textsuperscript{301} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1982 (Frederick, Maryland: USAMRIID), Work Unit A871 BA 150.

\textsuperscript{302} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1981 (Frederick, Maryland: USAMRIID), Work Unit A91C 00 133.

\textsuperscript{303} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1985 (Frederick, Maryland: USAMRIID), Work Unit D807 AA 011.

\textsuperscript{304} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1982 (Frederick, Maryland: USAMRIID), Work Unit A870 BB 069.
the protective antigen-type vaccine (MDPH) was not as protective against aerosol spore challenge as against IM challenge.305

In a further study, guinea pigs were vaccinated either with the Sterne vaccine or the MDPH vaccine and then challenged either by aerosol exposure or by IM injection with virulent anthrax spores. All Sterne-spore-vaccinated guinea pigs survived both the aerosol and IM challenges. MDPH-vaccinated animals had an 88% survival rate against the IM challenge, but only 38% survived the aerosol of anthrax spores.306

In FY1985, it was reported that the PA vaccine did not provide complete protection against 9 of 25 B. anthracis challenge strains. Those strains that overcome PA vaccination were termed, “vaccine-resistant” strains, of which the NH and Ames strains were examples. British investigators confirmed that Ames and NH challenge overcame immunization with chemical vaccines. Testing was planned to see if the same vaccine-resistant phenomenon was observed when PA-vaccinated animals were challenged by the aerosol route.307

When presented with an aerosol challenge of virulent B. anthracis, the non-encapsulated Sterne strain consistently induced greater protection in guinea pigs as compared to PA products. At 39 weeks after vaccination, 50% of Sterne-vaccinated

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305 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1982 (Frederick, Maryland: USAMRIID), Work Unit A870 BB 069.

306 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1982 (Frederick, Maryland: USAMRIID), Work Unit A870 BB 069.

307 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1985 (Frederick, Maryland: USAMRIID), Work Unit D807 AA 011.
animals survived, compared with only 30% of the PA-vaccinated guinea pigs.308 However, in another study, guinea pigs were challenged with 10 LD50 of virulent Vollum 1B strain six weeks after a booster (at 12 weeks). No differences in protection were noted: all vaccinated animals survived challenge. This suggested that insufficient time elapsed between vaccination and challenge for the differences to become apparent.309

In the same work unit in FY1985, researchers noted that “reports from the 1950s and 1960s indicate that immunization with PA vaccines may not provide protection against all virulent strains of B. anthracis. These reports have been confirmed in studies reported in the USAMRIID Annual Report, 1984.”310 The possibility of genetically manipulating the Sterne strain for use in humans was evaluated. The Sterne strain was developed over fifty years ago for use in livestock. It was reported that a Sterne-type strain (STI) was used in several million persons in the USSR with apparent efficacy.

Detection

In FY1982, researchers found that the Sterne strain could be easily detected at 100,000 CFUs when opsonified with specific antibody.311 However this method had little applicability for field use.

308 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1985 (Frederick, Maryland: USAMRIID), Work Unit D807 AL 020.
309 Ibid.
310 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1985 (Frederick, Maryland: USAMRIID), Work Unit D807 AA 011, p.45. This is one of several references to the 1984 and 1983 USAMRIID Annual Reports that were reported as never published.
311 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1982 (Frederick, Maryland: USAMRIID), Work Unit. BS10 AR 196.
Also in FY1982, researchers isolated N-acetylglucosamine galactose polysaccharide from \textit{B. anthracis} that seemed unique to it, opening the possibility of using this sugar to quickly identify anthrax spores from other closely related by nonpathogenic spore formers.\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1981} (Frederick, Maryland: USAMRIID), Work Unit A871 BB 149.}

In FY1990, PCR biotechnology and a study of the DNA sequences of the six genes involved in virulence allowed researchers to develop a detection protocol for virulent isolates. Similarly, a method to distinguish vaccine-sensitive and vaccine-resistant strains of \textit{B. anthracis} was developed based upon the relative binding of congo red dye.\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1990} (Frederick, Maryland: USAMRIID), Work Unit BS12 AB 002.}

\textit{Tularemia}

Tularemia is caused by \textit{Francisella tularensis}, a bacteria first identified in 1911 in Tulare County, California (formerly known as \textit{Pasteurella tularensis}). As few as 10 to 50 organisms can cause disease if inhaled or injected intradermally. Case fatality rates are as high as 35\% in untreated typhoidal tularemia (the form most likely after an aerosol BW attack). There is an investigational (IND) live-attenuated vaccine that prevents typhoidal tularemia. A two-week course of antibiotics is effective post-exposure when given within 24 hours.\footnote{USAMRIID, \textit{USAMRIID’s Medical Management of Biological Casualties Handbook, Fifth Edition} (Fort Detrick, MD: August 2004): 52-64.} USAMRIID performed fairly extensive work on the tularemia bacterium from 1969-1990.
Vaccine Interactions

Mice simultaneously immunized with TC-83 (VEE) and tularemia (LVS) vaccines showed increased mortality over that observed with the vaccines when given singly. Those that survived, however, were more solidly protected against virulent P. tularensis, (typically 15-20% receiving the LVS vaccine alone succumb to tularemia challenge). In monkeys, deaths occur with little enhancement of immunity (FY1969).\textsuperscript{315}

In FY1969 researchers also studied the effects of combining the TC-83 (VEE), LVS (tularemia), and EV 51 F plague vaccines in guinea pigs. The combined vaccine delayed but increased the antibody titer to plague.\textsuperscript{316} The LVS vaccine was then tested for any adjuvant effect. Others had shown that LVS in combination with anthrax protective antigen (APA) had markedly improved the protective efficacy of APA in guinea pigs. No similar effect was seen with LVS in combination with SEB toxoid in monkeys (FY1969).\textsuperscript{317}

In FY1973 researchers tested the effectiveness of combining a pentavalent vaccine (composed of formalin-treated vaccines for WEE, EEE, RVF, Chikungunya, and Q fever) with the LVS of F. tularensis. Protection by the attenuated vaccine was neither enhanced nor impaired by the pentavalent combination. After this

\textsuperscript{315} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1969} (Frederick, Maryland: USAMRIID), Work Unit 096 01 401.

\textsuperscript{316} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1969} (Frederick, Maryland: USAMRIID), Work Unit 096 01 004.

\textsuperscript{317} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1969} (Frederick, Maryland: USAMRIID), Work Unit 096 01 004.
experiment, studies on combined antigens were discontinued, in part because of lack of specific military direction for such combinations.\textsuperscript{318}

In other studies, the LVS vaccine did not lose antigenic potency in humans after nine years in storage.\textsuperscript{319}

Skin Test

In FY1974, approximately 300,000 human skin test doses were bottled for human use. (The Foshay skin test antigen is a 1:1000 dilution of the phenol-killed Foshay tularemia vaccine, first manufactured in 1943.) It is considered reasonably effective in identifying individuals previously infected with \textit{F. tularensis}. For example, between 85-93\% of persons who had previous vaccination with LVS of \textit{F. tularensis} tested positive for as long as 3.5 years later.\textsuperscript{320}

Other Vaccine Studies

In 1974, three nonliving vaccine preparations from the attenuated strain of \textit{F. tularensis} were tested for protective activity against three virulent strains of \textit{F. tularensis}. Neither whole-cell extracts nor extracts prepared from \textit{S. aureus} offered significant protection against lethal challenge with \textit{F. tularensis}, strains 503, 425, of SCHU S4.\textsuperscript{321} In FY 1975, RNA-rich phenol extracts of a broken-cell supernatant

\textsuperscript{318} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1973} (Frederick, Maryland: USAMRIID), Work Unit 096 02 004.

\textsuperscript{319} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1974} (Frederick, Maryland: USAMRIID), Work Unit 096 02 002.

\textsuperscript{320} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1974} (Frederick, Maryland: USAMRIID), Work Unit 096 02 002.

\textsuperscript{321} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1974} (Frederick, Maryland: USAMRIID), Work Unit 096 02 106.
from strain LVS protected mice against lethal challenge with strain 425, but not against strain SCHU-S4.\textsuperscript{322}

Studies of Modes of Vaccination

One study in FY1978 stated that protection against airborne tularemia ideally would be via immunoprophylaxis; much work was reported on the protective effect of parenteral vaccination. More information was required, however, to define optimal methods of vaccination to induce the most effective immune defense mechanisms of the host, particularly against aerosol challenge. One novel approach was to deposit antigen locally in the respiratory tract by IN instillation to stimulate local immunity, but there was little data on how effective these immune responses would be in protecting against subsequent aerosol challenge. Studies were initiated to study relationships between host immune responses and protection against subsequent infections.\textsuperscript{323}

In FY1977, rats were vaccinated with \textit{F. tularensis}, LVS, by SPA, IN, or IP routes. LVS was recovered from the lungs of the SPA and IP animals within 24 hours to 4 days, and only at 7 days from the IN group. Humoral agglutininins were measured for the three vaccinated groups at 7 days, with the IP-vaccinated rats having the highest titers.\textsuperscript{324}

\textsuperscript{322} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1975} (Frederick, Maryland: USAMRIID), Work Unit 096 02 106.

\textsuperscript{323} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A91C 003.

\textsuperscript{324} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit 096 02 416 / A841 003.
In FY79, Fischer 344 inbred rats were vaccinated with either the LVS or inactivated Foshay-type vaccine of *F. tularensis*. After treatment with cyclophosphamide to severely depress the production of humoral antibodies, the rats survived aerosol and intraperitoneal challenge with virulent *F. tularensis* while all unvaccinated controls died. This was the first direct demonstration that serum antibody is not a critical component of the immune system for total protection against lethal tularemia. Other studies showed that the LVS vaccine administered by SPA inhalation, intranasal instillation or parenteral routes (IP, SC, or IM injection) provided practically total and equal protection to the rat against respiratory tularemia (SCHU S4).\(^{325}\) Rats vaccinated with LVS aerosols or by IM injection with LVS were fully protected against aerosol challenge for at least two years.\(^{326}\) In addition, passively administered *F. tularensis* whole antiserum or the total immunoglobulin fraction protected F-344 rats against lethal tularemia.

**Drug Interactions**

In another study (FY78), the effect of nonspecific stimulation on macrophages on infection was studied in rats treated with glucan prior to infection with SCHU S4 *F. tularensis*. Mortality was significantly reduced when glucan was administered IN and rats were infected by IP inoculation or SPA exposure.\(^{327}\)

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\(^{325}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1979* (Frederick, Maryland: USAMRIID), Work Unit A841 00 003.

\(^{326}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1981* (Frederick, Maryland: USAMRIID), Work Unit A871 BB 149.

\(^{327}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1978* (Frederick, Maryland: USAMRIID), Work Unit A841 056.
Clinical Trials (LVS)

In FY88, eighteen volunteers participated in an initial safety and efficacy trial of the *F. tularensis* vaccine (live, TSI-GSD-213, Lot 1R). All volunteers who received the vaccine developed a characteristic local lesion at the inoculation site and experienced a rise in antibody titers. However, three of the nine who received the vaccine also had evidence of transient livery dysfunction, resulting in the suspension of the trials. The LVS strain of *F. tularensis* has been in use as a vaccine since the early 1960s. The FY88 testing was for new lots of the vaccine produced by more modern technology to replenish stocks because the older lots became depleted. The clinical trials were resumed in FY89 with thirty new volunteers.

Studies of volunteers who received the *F. tularensis* vaccine showed a rise in IgG, IgM, and IgA titers, with the IgA response being the most remarkable. IgA antibodies appeared within fourteen days after vaccination, with the largest overall titer increase of the three antibody classes tested. IgG significantly bound to non-vaccinees, suggesting the existence of IgG-binding proteins in *F. tularensis*. Thus, vaccinating human volunteers with *F. tularensis*, LVS, stimulates antigen-specific lymphocyte proliferation activity significantly.

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328 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1988* (Frederick, Maryland: USAMRIID), Work Unit D809 AN 002.

329 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1988* (Frederick, Maryland: USAMRIID), Work Unit D809 AM 003.

330 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1990* (Frederick, Maryland: USAMRIID), Work Unit. D809 AM 003.
Pathogenesis

In FY1969, researchers studied the effect of Pasteurella tularensis on serum Fe and Zn in humans. Half of the volunteers were exposed to 2500 viable SCHU-S4 strain P. tularensis and half to 25,000 organisms. Early significant decreases in serum Fe and Zn levels with a concomitant rise in serum Cu were demonstrated in humans infected with either acute bacterial (tularemia) or viral (sandfly fever) infections. In FY77, multiple zinc treatments enhanced survival incidence during the early post-infection period in rats infected with F. tularensis or S. pneumoniae. This was explained in part by zinc’s ability to modify certain aspects of the host’s defense mechanism and zinc’s inhibition of bacterial proliferation. At the subcellular level during tularemia infection, only changes in the endoplasmic reticulum were seen.

In FY77, an assay for the specific bactericidal activity of murine peritoneal macrophages was adapted to tularemia studies. The bactericidal capability of these macrophages correlated with resistance of the donor to tularemia infection. In FY79, researchers tried to determine the mechanism that permits the virulent strain of F. tularensis SCHU S4 to survive and grow within macrophages and concluded that

331 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1969 (Frederick, Maryland: USAMRIID), Work Unit 096 01 001.
332 FY1971: W.U. 096 03 010.
333 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1977 (Frederick, Maryland: USAMRIID), Work Unit A91C 138.
334 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1972 (Frederick, Maryland: USAMRIID), Work Unit 096 01 803.
335 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1977 (Frederick, Maryland: USAMRIID), Work Unit BS03 00 002.
none of the known microbicidal mechanisms of phagocytes are effective in killing *F. tularensis*. The difference between SCHU S4 and LVS strains appeared to reside in their ability to effectively perform anabolic functions within the acid environment of the PM lysosome. In FY80, a developing principle was that the attenuation of intracellular parasites like *F. tularensis* alters the pH optima of the pathogen for macromolecular synthesis and growth. Immunization acidifies the macrophage lysosomes below pH 4.5, leading to a less favorable environment and thereby to more rapid denaturation and death of such microorganisms.

These kinds of studies of biochemistry, structure and function of phagocytes and their organelles in normal and diseased animals were done to disclose how microorganisms, by altering normal cellular physiology, mitigate host defense mechanisms, promote cellular dysfunction and enhance host susceptibility to infectious diseases. Studies on microbial killing and digestion in phagocytes were in their infancy then, but it was important to conceive and characterize the ways in which microorganisms avoid being killed and digested because of their application to development of vaccines, understanding of persistent and latent infections, design of new therapeutic agents and the ability to deal with any new microbes that periodically arose.

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336 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1979* (Frederick, Maryland: USAMRIID), Work Unit A91C 155.

337 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1980* (Frederick, Maryland: USAMRIID), Work Unit A91C 144.

338 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1979* (Frederick, Maryland: USAMRIID), Work Unit A91C 155.
Acquisition of Host Resistance

Mice were immunized with the vaccine strain, LVS, of *F. tularensis*. Passive transfer of either 28-day serum from inbred LVS-vaccinated mice or spleen cells from 12-day LVS vaccines that were simultaneously challenged with streptomycin-resistant *F. tularensis* SCHU S5 showed a significant delay in the appearance and reduced the rate of growth of this strain for 48-72 hours in spleens of streptomycin-treated inbred recipients. Therefore, this data contrasts with then-current concepts on the mechanism of acquired immunity to tularemia. It suggested that humoral as well as cell-mediated responses contribute to early resistance in the highly susceptible mouse.\(^{339}\) However, based on studies with the murine tularemia model in FY77, it was later stated that CMI has primary responsibility: humoral immunity has never been shown to provide protection against infection with fully virulent strains of *F. tularensis* SCHU S4 and SCHU S5R.\(^{340}\) With increasing time after vaccination, AKR mice became less resistant to challenge with SCHU S4.\(^{341}\)

Nonspecific Resistance

Nonspecific host resistance to *L. monocytogenes* and *S. typhi* in the AKR/J mouse by live tularemia and DPT vaccines suggested that effective host resistance to infectious diseases of military significance may be induced rapidly and effectively by

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\(^{339}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1973* (Frederick, Maryland: USAMRIID), Work Unit 096 02 103.

\(^{340}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1977* (Frederick, Maryland: USAMRIID), Work Unit BS03 00 002.

\(^{341}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1978* (Frederick, Maryland: USAMRIID), Work Unit A841 00 060.
vaccines in current medical use.\textsuperscript{342} When it was tested in FY1977, it was not found viable for \textit{F. tularensis} or \textit{S. pneumoniae} infections. The specific immune response to live tularemia vaccine is unaffected by concurrent administration of preparations that induce nonspecific protection against Listeria or Salmonella. The researchers concluded that it appears likely that the spectrum of infectious diseases which can be prevented or attenuated by induction of nonspecific resistance (NSR) may be very limited and not include potential BW agents; and that the duration of induced NSR is short and variable and adoptive transfer is probably not possible.\textsuperscript{343}

Passive Transfer

Under appropriate conditions, passively transferred spleen cells from mice immunized with LVS ensured high-grade protection (survival approaching 100\%) to nonimmune recipients against IV, IP, or SC challenge with fully virulent \textit{F. tularensis}.\textsuperscript{344} In similar studies in FY1976, it was found that the model systems developed permitted definitive studies on mechanisms involved in effective control of infections caused by highly virulent and potentially antibiotic-resistant, facultative intracellular bacteria.\textsuperscript{345}

In FY1977, further work on understanding the host response using the murine tularemia model involved immunization with live vaccine or adoptive transfer of

\textsuperscript{342} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1976} (Frederick, Maryland: USAMRIID), Work Unit 096 02 103.

\textsuperscript{343} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit. A841 00 005.

\textsuperscript{344} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1975} (Frederick, Maryland: USAMRIID), Work Unit 096 02 103.

\textsuperscript{345} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit 096 01 103
immune splenocytes. These two protected against death, but not against systemic infection with fully virulent *F. tularensis*. Evidence suggested that macrophages, but not ATS-sensitive cells (T-cells), are essential for natural defense against attenuated strains, but immune defense against fully virulent strains is considerably more complex and is affected by antitularemia serum, antithymocyte and antilymphocyte sensitive cells.\textsuperscript{346}

In FY78, it was found that passive transfer of protection against lethal infection with fully virulent strains (SCHU S5R) in the murine tularemia model can be effected by transfer of syngeneic immune splenocytes mixed with killed bacterial antigen. There seemed to be a requirement for highly activated populations (recently boostered donors). Also, while the immune lymphocyte dose is the most critical factor for protection, immune macrophages are also required: both T- and B-lymphocyte activity is required. (While only sensitized T-cells or immune serum alone is effective against strains of lesser virulence.)\textsuperscript{347}

Detection

Work on the detection of small numbers of biological agents using luminescence began in FY1978.\textsuperscript{348} Within a year, a workable chemiluminescent immunoreactive assay for *F. tularensis*, LVS was established.\textsuperscript{349} In FY80, *F.*

\textsuperscript{346}US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1977* (Frederick, Maryland: USAMRIID), Work Unit. 096 02 103 / A841 00 002.

\textsuperscript{347} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1978* (Frederick, Maryland: USAMRIID), Work Unit A91C 002.

\textsuperscript{348}US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1978* (Frederick, Maryland: USAMRIID), Work Unit A841 00 060.

\textsuperscript{349} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1979* (Frederick, Maryland: USAMRIID), Work Unit A841 00 060.
tularensis could be detected at concentrations of 10-100 thousand organisms per ml by ELISA and chemiluminescent (CL) response of polymorphonuclear leukocytes was found valuable in the early diagnosis of tularemia infections. The sensitivity of the CL response of polymorphonuclear leukocytes to F. tularensis was the detection of 100,000 bacteria in FY82.

Q Fever

Q fever is caused by a rickettsia-like organism, Coxiella burnetii, that is highly infectious via aerosols and can persist in the environment. Inhalation of a single organism can lead to infection. It has a low mortality rate (less than 3%), but is a debilitating illness. A single dose of a licensed Q fever vaccine is available in Eastern Europe and Australia that provides complete protection against naturally occurring Q fever and 95% protection against aerosol exposure that lasts for five years. In the US, a formalin-inactivated whole cell IND vaccine is available for at-risk personnel.

Phase II and Phase I whole cell vaccines

In 1960, a Q fever vaccine was prepared at WRAIR. It was derived from the 22nd egg passage of the Henzerling strain of C. burnetii, a phase II seed stock. By FY1978, researchers who tested the Henzerling strain vaccine in humans concluded

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350 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1981 (Frederick, Maryland: USAMRIID), Work Unit A870 BC 068.

351 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1982 (Frederick, Maryland: USAMRIID), Work Unit BS10 AR 196.

that it was highly reactogenic and led to an unacceptable incidence of local reactions, including sterile abscesses especially among recovered or previously immunized individuals.\textsuperscript{353} Supplies of the phase II vaccine were reduced over the years and required replacement in the early 1980s.

In 1964, researchers showed that phase I vaccine preparations were more effective immunogens, have lower dose requirements, lower levels of contaminating host material, and possibly less frequent or milder adverse reactions. An industrial-sized quantity (five lots) of the phase I vaccine (NDBR-105) was prepared in 1972 as an alternative to the then-current vaccine and underwent evaluation and testing prior to testing in man in the US.\textsuperscript{354} The phase I antigen had already been tested successfully as a vaccine in man in Czechoslovakia in 1974 and Romania in 1973.\textsuperscript{355}

In FY1976, a protocol was prepared for investigating the protective efficacy and other properties of a new phase I Q fever vaccine (NDBR-105) to replace the phase II vaccine.\textsuperscript{356} This experimental vaccine evoked a humoral and CMI response in guinea pigs and conferred resistance to subsequent challenge presented as SPA to simulate a natural respiratory exposure.\textsuperscript{357} A single injection (12 micrograms protein) of the soluble phase I antigen of \textit{C. burnetii} completely protected guinea pigs against

\textsuperscript{353} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 001.

\textsuperscript{354} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit A841 00 053.

\textsuperscript{355} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A841 00 047.

\textsuperscript{356} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1976} (Frederick, Maryland: USAMRIID), Work Unit 096 02 304.

\textsuperscript{357} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit 096 01 303.
10,000 medial infectious doses of *C. burnetii*. Even 0.12 micrograms provided partial protection, even while no phase I complement fixation antibody was detectable prior to challenge. In FY 1978, a cynomolgus monkey model was developed for Q fever that closely resembled Q fever in man.

In FY1979, a new protocol, “Clinical Evaluation of Formalin-Inactivated, Dried, Henzerling strain, phase I, Q fever Vaccine, NDBR 105 (IND 610) for safety and immunogenicity” was developed and submitted to a blue-ribbon panel of prominent rickettsiologists. Progress on this was delayed due to a prolonged illness and subsequent resignation of the PI on the project in FY1980.

After several years without any research reported on a new Q fever vaccine, studies re-commenced in FY1985. Mass-scale purification and chemical extraction procedures for the commercial production of Q fever vaccine were established to meet the conditions for large volume operations. Pilot lots of Q fever vaccine were produced. In that year it was reported that adverse reactions during vaccine trials with phase I whole cell vaccines prevented widespread and unconditional use of these vaccines. Attempts to remove those components that induced adverse reactions had only limited success.

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358 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1976* (Frederick, Maryland: USAMRIID), Work Unit 096 02 301.

359 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1978* (Frederick, Maryland: USAMRIID), Work Unit A91C 00 007.

360 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1979* (Frederick, Maryland: USAMRIID), Work Unit A841 00 001.

361 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1985* (Frederick, Maryland: USAMRIID), Work Unit D809 AC 001.

362 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1985* (Frederick, Maryland: USAMRIID), Work Unit D807 AB 012.
However, in FY1986, 72 volunteers were evaluated prior to vaccination with the IND 610 vaccine. Immunization resulted in no adverse reactions.\textsuperscript{363} By FY1987, 150 volunteers had participated.\textsuperscript{364} Post-vaccination mean values were significantly greater than pre-vaccination values for both humoral antibody and cell-mediated immune responses. However, there was a low conversion rate that was likely due to the low tolerable dose of the phase I vaccine.\textsuperscript{365}

By FY1988, over 180 volunteers participated in the study of the IND 610 vaccine. No adverse reaction and no cases of lab-acquired disease occurred among vaccinated persons. Also, there was no correlation apparent among skin-test results and measurements of cellular and humoral immunity, before or after immunization.\textsuperscript{366}

In FY1989, immune responses of 142 persons vaccinated with IND 610 phase I Henzerling vaccine were assessed by skin test reactions against 20ng of antigen, specific antibody response by ELISA, and cell-mediated immunity by lymphocyte proliferation tests. If immune responses were marginal, individuals were considered candidates for vaccination. However, a 30 microgram dose of the vaccine did not elicit a measurable, consistent immune response. Doses greater than 30 micrograms could not be given because of the potential for severe side reactions.\textsuperscript{367}

\textsuperscript{363} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1986} (Frederick, Maryland: USAMRIID), Work Unit D809 AC 001.

\textsuperscript{364} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1987} (Frederick, Maryland: USAMRIID), Work Unit D809 AC 001.

\textsuperscript{365} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1987} (Frederick, Maryland: USAMRIID), Work Unit D809 AC 001.

\textsuperscript{366} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1988} (Frederick, Maryland: USAMRIID), Work Unit D809 AN 002.

\textsuperscript{367} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit D809 AC 001.
In FY1989, USAMRIID researchers concluded that the current level of protection against infection by \textit{C. burnetii} after vaccination of animals and humans is inadequate because of the following: (1) the potential for immunopathological damage; (ii) partial protection which does not adequately restrict growth of the microorganism in the phagolysosome; (iii) the elicitation of immune responses against several immunodominant, non-protective antigens.\textsuperscript{368} Only partial protection is afforded because growth of the organism on the phagolysosome was not adequately restricted. The small cell of the \textit{C. burnetii} developmental cycle evades host immunity through antigenic variation, creating a need for an efficacious subunit vaccine prepared from the small cells. (USAMRIID used the small cells of \textit{C. burnetii} for infectious challenge by either aerosol or IP injections to test potential vaccine materials.) It was discovered that the determinants of either spores or small cells were not detected by antibodies obtained from infected or immunized humans.\textsuperscript{369}

Because of the considerable reactivity and substantial disadvantages of immunological screens and skin tests before vaccination, the phase I whole-cell vaccine was considered inapplicable to military use.\textsuperscript{370}

\textsuperscript{368} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit D807 AB 012.

\textsuperscript{369} Ibid.

\textsuperscript{370} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1990} (Frederick, Maryland: USAMRIID), Work Unit D809 AC 001.
CMR Vaccine

In FY1985, work on a chloroform-methanol extracted residue (CMR) vaccine began to replace the phase I whole cell vaccine. The CMR vaccine was shown to be efficacious and non-reactogenic, but difficult to produce from infected yolk sacs. It was also found to be non-toxic at high concentrations (>100 micrograms/ml) in vitro. By FY1988, this new Q fever candidate vaccine was produced (TSI-GSD 217, Lot 1-1-88) and plans were made to enter phase I human trials after animal safety testing. The CMR vaccine is a lyophilized produce derived from phase I whole cell C. burnetii. Bacteria were harvested from the yolk sacs of embryonated specific pathogen-free chicken eggs infected with phase I, Henzerling strain C. burnetii. The CMR vaccine is 99% phase I LPS.

However the CMR vaccine was only scheduled to be delivered to USAMRIID in January 1989 to enter phase I safety testing. In FY1989, the CMR vaccine passed animal safety and immunogenicity tests. In FY1990, animal tests showed that the CMR and NDBR-105 were equally efficacious at protecting A/J mice against a lethal challenge. Unlike NDBR-105, the CMR vaccine resulted in milder and

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371 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1985* (Frederick, Maryland: USAMRIID), Work Unit D807 AB 012

372 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1988* (Frederick, Maryland: USAMRIID), Work Unit D807 AB 012.

373 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1990* (Frederick, Maryland: USAMRIID), Work Unit D809 AC 001.

374 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1988* (Frederick, Maryland: USAMRIID), Work Unit D809 AC 001.
transient liver lesions and mild splenic lesions. USAMRIID researchers then proceeded on tests in humans.\textsuperscript{375}

Subunit Vaccines

Because of concerns about the NDBR-105 vaccine, there was a need to develop a subunit vaccine that could be administered safely and at a dosage level that would consistently elicit measurable immune responses and evidence of protection without pre-screens.

In parallel with tests of the phase I Q fever vaccine, researchers were also working on a possible human subunit vaccine based on the soluble phase I antigen of \textit{C. burnetii}. This antigen was found to be stable to lyophilization, to storage at 4C in liquid or dry state and even autoclaving; able to induce a cellular immune response; and 100X less skin-reactogenic than the Merrell National Labs particulate, phase I Q fever vaccine (NDBR 105).\textsuperscript{376}

Research proceeded to prepare a subunit vaccine by cloning the DNA of virulent \textit{C. burnetii} and screening the clones for production of immunogenic proteins. Studies proceeded in A/J mice that showed that the phase I lipopolysaccharide (LPS) was nontoxic at a dose of 100 micrograms and elicited significant antibody titers and lymphocyte responses against LPS, and phase I and phase II cells. A single injection

\textsuperscript{375} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit D809 AC 001.

\textsuperscript{376} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1980} (Frederick, Maryland: USAMRIID), Work Unit A841 00 053.
of 2.5 micrograms gave complete protection against virulent aerosol challenge without a detectable pre-challenge immune response.\textsuperscript{377}

In FY1989, phase I non-toxic LPS and two surface proteins (P1 and P2) were evaluated as possible candidates for development of subunit, multivalent, and diagnostic reagents. Purified LPS I and protein one (P1) were demonstrated to have protective efficacy in mice. P1 was more effective than LPS I in reducing the number of infectious \textit{C. burnetii} in spleens of challenged mice.\textsuperscript{378}

Vaccine Combinations

In FY1970, experimental pentavalent vaccines containing inactivated WEE, EEE, Rift Valley Fever (RVF), Chikungunya, and Q fever were prepared. It met the USPHS criteria for safety, but was hypertonic.\textsuperscript{379} Each component was compared to its analogue in monovalent form by a standard challenge with the virulent homologous strain. Researchers found that WEE, EEE, RVF, and Q fever vaccines were as effective in a pentavalent vaccine as when given alone. Attempts to compare the Chikungunya vaccine were delayed by a depletion of vaccine and the need to produce and evaluate a new vaccine lot.\textsuperscript{380}

\textsuperscript{377} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1988} (Frederick, Maryland: USAMRIID), Work Unit D807 AE 015.

\textsuperscript{378} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit D807 AB 012.

\textsuperscript{379} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1970} (Frederick, Maryland: USAMRIID), Work Unit 096 02 004.

\textsuperscript{380} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1971} (Frederick, Maryland: USAMRIID), Work Unit 096 02 004.
When the pentavalent combination was given with simultaneous immunization with the TC-83 vaccine, the Q fever vaccine component was enhanced.\textsuperscript{381}

Immunology

*C. burnetii* infections and killed vaccines induce non-specific resistance to challenge by various bacterial and viral pathogens. (Prior immunization of mice or guinea pigs with *C. burnetii* significantly reduced susceptibility to lethal infections by *Listeria monocytogenes*, encephalomyocarditis virus, EL-4 leukemia cells, RVF virus, and Banzi virus.) Studies at USAMRIID centered around an immunomodulatory complex (IMC) of *C. burnetii* and the immune mechanisms induced by this complex. Both the current phase I whole cell vaccine and a chloroform-methanol-extracted residue (CMR) of phase I whole cells contained the IMC. The objective of the research was to describe the interacting components of the IMC for the induction of safe and effective immunomodulation.\textsuperscript{382}

In other studies, it was found that the immunological response to chronic and acute Q fever were rather distinct. Subjects with chronic Q fever recognized more antigens. In patients with acute Q fever, there were few IgA antibodies and none developed antibodies to phase I LPS. This contrasts with findings in guinea pigs, where all infected animals developed antibodies to phase I LPS. Therefore, in

\textsuperscript{381} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1972* (Frederick, Maryland: USAMRIID), Work Unit 096 02 004.

\textsuperscript{382} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1989* (Frederick, Maryland: USAMRIID), Work Unit D807 AE 015.
humans, antibodies to phase I LPS may be diagnostic of chronic Q fever in humans.\textsuperscript{383}

**Virulence Studies**

Virulent strains of *C. burnetii* are characterized by a phase I phenotype identified by a surface coat of smooth-type lipopolysaccharide LPS, a cell wall immunomodulatory complex (IMC), a variant endogenous plasmid, and a variant chromosome. Avirulent strains, designated as phase II, express a truncated LPS molecule, do not express IMC, harbor a variant endogenous plasmid, and harbor a variant chromosome similar to the phase I strains. In FY1990, identification of common deleted segments or mutations that render virulent strains avirulent was studied in order to develop vaccine vehicles and to map the *C. burnetii* chromosome. As part of this study, mini-chromosomes that transform avirulent strains to virulence were studied with the goal of describing the DNA involved in the attenuation of virulent strains.\textsuperscript{384}

*Rocky Mountain Spotted Fever*

Rocky Mountain Spotted Fever (RMSF) is the most severe tick-borne rickettsial illness in the US. It was first recognized in 1896 and the bacterium that causes RMSF, *Rickettsia rickettsii*, was identified in the early 1900s. *R. rickettsii* has long been on the list of potential biowarfare agents. Without prompt and appropriate treatment it can be fatal, but is susceptible to antibiotic therapy if it can be diagnosed

\textsuperscript{383} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1990* (Frederick, Maryland: USAMRIID), Work Unit D807 AE 015.

\textsuperscript{384} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1990* (Frederick, Maryland: USAMRIID), Work Unit D807 AB 012.
accurately. It has been a reportable disease in the US since the 1920s, with about 250-1200 cases annually (it is transmitted by tick bites).\textsuperscript{385} There is no effective vaccine: in 1973, researchers found that the only commercially available vaccine (developed by Cox in 1939) was not actually effective.\textsuperscript{386} USAMRIID researchers worked on producing an improved vaccine that would offer substantial protection against RMSF and perhaps provide protection against all spotted fever members producing disease in man.\textsuperscript{387}

Work was performed predominantly between FY1969-FY1977, at which time guidance from the HASC required that research with \textit{R. rickettsii} be reduced.\textsuperscript{388} Work continued on other rickettsial diseases, and these were subsequently transferred to WRAIR in FY1979.

\textbf{Vaccine Studies}

In FY1969, studies of the commercial killed vaccine (Lederle Laboratories) were used in RMSF studies. Four to six months after immunization, volunteers were inoculated with 10 median guinea pig intraperitoneal infectious doses of the Sheila

\textsuperscript{385} Information on Rocky Mountain Spotted Fever downloaded from the Centers for Disease Control and Prevention: www.cdc.gov.


\textsuperscript{387} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1975} (Frederick, Maryland: USAMRIID), Work Unit A834 02 300.

\textsuperscript{388} Reported in US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit 096 01 300 / A841 00 049.
Smith strain of *Rickettsia rickettsii*. The results were not reported, but symptomatic illness responded promptly to antibiotic therapy.\textsuperscript{389}

In 1971, studies of immunoprophylaxis against the spotted fever group began: a duck-embryo cell grown, formalin-inactivated RMSF vaccine was developed that appeared superior to other RMSF vaccines.\textsuperscript{390} In FY 1972, the Sheila Smith strain was freed of RIF viruses, inactivated, and a lot of vaccine suitable for human use was produced.\textsuperscript{391} In FY1973, a monkey model was developed for the study of RMSF. Those monkeys immunized nine months earlier by either the commercial vaccine (grown in yolk sacs) or one developed at USAMRIID (DEC vaccine) were protected against challenge with virulent rickettsiae, although fewer died when given the DEC vaccination.\textsuperscript{392} Rickettsia grown on yolk sacs were significantly more virulent for monkeys than DEC-grown rickettsia.\textsuperscript{393}

In FY1975, in vaccine studies in monkeys, researchers found that the two embryo cell-culture vaccines afforded relatively better protection than the commercial yolk-sac grown vaccine: two inoculations two weeks apart appeared to be the best schedule.\textsuperscript{394} Also in that year, three cases of aerogenic RMSF in lab workers was

\textsuperscript{389} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1969* (Frederick, Maryland: USAMRIID), Work Unit 096 01 003.

\textsuperscript{390} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1971* (Frederick, Maryland: USAMRIID), Work Unit 096 02 300.

\textsuperscript{391} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1972* (Frederick, Maryland: USAMRIID), Work Unit 096 02 300.

\textsuperscript{392} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1973* (Frederick, Maryland: USAMRIID), Work Unit 096 01 301.

\textsuperscript{393} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1974* (Frederick, Maryland: USAMRIID), Work Unit 096 01 301.

\textsuperscript{394} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1975* (Frederick, Maryland: USAMRIID), Work Unit 096 01 300.
diagnosed and treated. Five thousand human doses of an RMSF vaccine was produced and an application was submitted to the Army Investigational Drug Review Board (AIDRB) for the RMSF vaccine, Inactivated SS Strain, Chick Embryo Cell Origin.\textsuperscript{395} Approval was given and in FY1976 and phase I human testing began.

In FY1978, vaccine trials were conducted with an inactivated RMSF (IND 862) in ten volunteers.\textsuperscript{396} In FY1979, 13 volunteers were given two 0.5ml doses of the Inactivated RMSF vaccine, undiluted (IND 862) SC 28 days apart. There were no systemic reactions. Because the CDC insisted that lab workers be protected against RMSF and because there was a scarcity of the existing lab-prepared vaccine, the USAMRIID Immunization committee proposed that the existing vaccine prepared by Merrill-National IND 862 (lots 1 and 2) be qualified for human use.\textsuperscript{397}

Other studies showed that the USAMRIID CEC-grown RMSF vaccine protected against strains of \textit{R. rickettsii} of diverse geographical origin and that spotted fever “group” protection could be elicited after infection with any one spotted fever group rickettsia.\textsuperscript{398} Three guinea pigs and a monkey were inoculated with \textit{R. Montana}, a spotted fever group rickettsia not known to cause disease in man. Challenge with virulent \textit{R. rickettsii} resulted in a mild illness and prompt recovery in

\textsuperscript{395} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1975} (Frederick, Maryland: USAMRIID), Work Unit 096 02 002.

\textsuperscript{396} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 001.

\textsuperscript{397} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1979} (Frederick, Maryland: USAMRIID), Work Unit A841 00 001.

\textsuperscript{398} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 006.
the monkey, and one completely refractory guinea pig while the other two succumbed.\textsuperscript{399}

In FY1977, researchers established that guinea pigs can be infected with RMSF by SPA, intranasal, conjunctival, intraperitoneal, and subcutaneous routes. The USAMRIID RMSF vaccine protected guinea pigs against SPA, IP, and SC challenges.\textsuperscript{400} In FY1979, a cynomolgus monkey model for RMSF was defined. In this model the USAMRIID-produced RMSF vaccine protected against an aerosol and subcutaneous challenge, with protection demonstrated after one year.\textsuperscript{401}

In other vaccine studies (FY1976), efforts were directed toward the isolation and characterization of components of tick-borne rickettsiae to manufacture new vaccines. At least 32 proteins were detected in the Sheila Smith strain.\textsuperscript{402} In FY1978, attempts were made to separate the protective antigen from suspensions of \textit{R. rickettsii} or from soluble antigen obtained by ether extraction of these suspensions. Vaccines prepared from these preparations protected guinea pigs from challenge with \textit{R. rickettsii}.\textsuperscript{403}

\textsuperscript{399} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A841 00 053.

\textsuperscript{400} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit 096 02 300.

\textsuperscript{401} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1979} (Frederick, Maryland: USAMRIID), Work Unit A841 00 006.

\textsuperscript{402} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1976} (Frederick, Maryland: USAMRIID), Work Unit 096 02 306.

\textsuperscript{403} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A841 00 053.
Immunology

To support vaccine efforts, researchers investigated the role of CMI in hosts infected with rickettsiae to determine how important humoral antibody-producing antigens are.\textsuperscript{404} Studies of lab workers accidentally infected with RMSF led to the tentative conclusion that RMSF is not an immune complex disease and that complement was not critical to development of full-blown disease, a conclusion at odds with the generally accepted hypothesis.\textsuperscript{405}

In FY1977, it was found that prompt and profound suppression of CMI occurred with the onset of febrile disease and the duration of the suppression correlated with the virulence of the infecting organism.\textsuperscript{406} Overall, the pathogenesis of rickettsial diseases were not well understood. Studies of the pathogenesis of \textit{R. rickettsii} was terminated as a result of program changes in FY1979.\textsuperscript{407}

Detection

In FY1977, researchers abandoned attempts to develop a RIA to detect rickettsial antibodies and antigens because the assay had poor sensitivity.\textsuperscript{408}

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\textsuperscript{404} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1976} (Frederick, Maryland: USAMRIID), Work Unit 096 02 305.

\textsuperscript{405} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1975} (Frederick, Maryland: USAMRIID), Work Unit 096 02 104.

\textsuperscript{406} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit 096 02 303.

\textsuperscript{407} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1979} (Frederick, Maryland: USAMRIID), Work Unit A841 00 049.

\textsuperscript{408} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit 096 02 303.
Plague

*Yersinia pestis* is a non-motile, non-sporulating, gram negative bacteria. The US and USSR developed effective methods for the aerosol dispersal of the plague bacillus. Untreated, mortality for pneumonic plague – the result of aerosol exposure – approaches 100%: survival is unlikely if treatment is delayed beyond eighteen hours of the onset of symptoms. A licensed, killed whole cell vaccine was available in the US from 1946 – 1998. It offered protection against bubonic plague, but was not effective against aerosolized *Y. pestis*. USAMRIID is developing an F1-V antigen (fusion protein) vaccine.

Despite its potential as a biological weapon, the US did not stockpile plague as a biological weapon. The USSR did actively investigate plague, however, to possibly develop a more sophisticated and deadly weapon in the years after US defensive work on plague stopped at USAMRIID. Despite the lack of an effective vaccine for pneumonic plague, little research was done on *Y. pestis*. The research years at USAMRIID for plague was between 1969 – 1973, at which time research was transferred to WRAIR.

In FY1969, strains from ten distinct plague outbreaks in the Republic of Vietnam were studied: all strains except 4 showed a striking similarity in respect to physiological and virulence characteristics. The four avirulent strains were negative for the Pesticin I, fibrinolysin, coagulase complex. In that year, researchers were also

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testing an attenuated plague vaccine in guinea pigs: all animals that demonstrated antiplague titers survived challenge with 100 LD50 *P. pestis* 195/P.411

In FY1971, the plague vaccine, USP (E Medium) was evaluated in human volunteers. Each volunteer received a primary dose of 1.0 ml and booster doses of 0.2 ml on days 90 and 270. Primary immunizations resulted in HA antibody responses in 83% of subjects and 93% responded after boosters on day 270.412 It was found that there is no correlation in humans between the quantity of plague vaccine administered and the serological response. That is, after a finite number of boosters, an antibody plateau is reached that is not altered by subsequent immunizations or its absence.413

As for detection, in FY1972, the IHAI test was shown to be an economical, simple, and rapid procedure for the detection of Fraction 1 of *Y. pestis*.414

**Melioidosis and Glanders**

The causative agents of melioidosis and glanders are *Burkholderia pseudomallei* and *Burkholderia mallei* respectively (also known as *Pseudomonas pseudomallei* and *Pseudomonas mallei*). Both are gram-negative bacilli, efficiently spread by aerosol, fatal diseases (without treatment) for humans for which there is no

411 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1969* (Frederick, Maryland: USAMRIID), Work Unit 096 02 102.

412 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1971* (Frederick, Maryland: USAMRIID), Work Unit 096 02 002.

413 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1971* (Frederick, Maryland: USAMRIID), Work Unit 096 02 102.

414 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1972* (Frederick, Maryland: USAMRIID), Work Unit 096 02 102.
available vaccine or reliable therapy.\textsuperscript{415} It is endemic in southeast Asia and is infective by the aerosol route, as well as through skin abrasions, wounds, or ingestion.

Studies at USAMRIID were initiated in FY1977 on \textit{Pseudomonas pseudomallei}. Initial efforts were on establishing a mouse model by enhancing the virulence of two strains in order to induce fatal infections. Serial brain-to-brain passages resulted in enhanced virulence: the LD50 for mice was decreased from greater than 10,000 to less than 180 organisms.\textsuperscript{416}

In FY1978, guinea pigs vaccinated with a killed \textit{P. pseudomallei} were afforded only minimal protection against aerosol challenge with virulent \textit{P. pseudomallei} (increased the mean time to death). Aerosolized tetracycline was used to treat lethal infections in hamsters.\textsuperscript{417}

In FY1980, aerosol studies showed that even with no effort to stabilize it, \textit{P. pseudomallei} cells survived the stresses of aerosol dissemination and persisted as airborne particles long enough to constitute a potential hazard in all environmental conditions studied.\textsuperscript{418}

By FY1982, the work unit on \textit{P. pseudomallei} was terminated. All work to that point suggested that inactivated antigens had little or no value as vaccines.\textsuperscript{419}


\textsuperscript{416} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit A841 00 012.

\textsuperscript{417} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1979} (Frederick, Maryland: USAMRIID), Work Unit A841 00 012.

\textsuperscript{418} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1980} (Frederick, Maryland: USAMRIID), Work Unit A841 00 012.

\textsuperscript{419} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1981} (Frederick, Maryland: USAMRIID), Work Unit BS10 AO 199.
Yellow Fever Virus

There is a single yellow fever vaccine commercially available against yellow fever. It is an attenuated strain based on a wild-type virus, the Asibi strain, which was isolated in Ghana in 1927. A single inoculation confers immunity after ten days and lasts for at least ten years.\textsuperscript{420} Over 400 million doses have been administered worldwide. Recently, however, serious side effects in a small number of cases from the vaccine were noted and is being investigated.\textsuperscript{421} USAMRIID conducted a few studies on the yellow fever virus and the 17D vaccine strain from FY1969 through the mid-1970s.

Vaccine Studies

In FY1969, eighteen humans were inoculated with 17D strain yellow fever virus. Disturbances in normal amino acid periodicity in the absence of clinical symptoms noted.\textsuperscript{422} Mice were irradiated with 400 R 24 hours before immunization with yellow fever, 17D: no protection was induced.

When the yellow fever vaccine was combined with the VEE vaccine (live), monkeys had less response to yellow fever component when compared to monkeys that received 1 vaccine initially, followed by the other after three days.\textsuperscript{423} The interference of the live VEE vaccine (TC-83) and 17D vaccine was further studied in


\textsuperscript{421} CDC, “Yellow Fever Vaccine and Recent Reports of Associated Severe Illness.” Downloaded from www.cdc.gov/ncidod/dvbid/yellowfever/vaccine/index.htm.

\textsuperscript{422} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1969} (Frederick, Maryland: USAMRIID), Work Unit 096 01 009.

\textsuperscript{423} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1969} (Frederick, Maryland: USAMRIID), Work Unit 096 02 409.
1971 in monkeys and it was found to occur through day 28, with the effect lost by day 56. This interaction was further studied in 32 human volunteers in FY73. Simultaneous administration of TC-83 and 17D resulted in a slight enhancement of the titer of YF neutralizing antibody and a depression of the VEE mean titers. The reverse occurred with administration of 17D after TC-83.424

Therapies

Cross-circulation was studied as a mode of therapy for yellow fever in rhesus monkeys: there was a temporary improvement clinically, but it failed to prolong the life of the animal (FY1969).425

Monkeys that were inoculated IV with synthetic polyI-polyC and later infected with the virulent Asibi strain of yellow fever had a longer incubation period, but the clinical illness remained unchanged (FY73).426 Later, Tilorone and its analogs were found effective antiviral agents against yellow fever in mice, but not monkeys. However, a lysine-stabilized polyI-polyC – poly (ICLC) – preparation was shown to be highly effective in both the prophylaxis and early treatment of YF in both animal

424 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1973 (Frederick, Maryland: USAMRIID), Work Unit 096 02 008.

425 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1969 (Frederick, Maryland: USAMRIID), Work Unit 096 02 410.

426 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1973 (Frederick, Maryland: USAMRIID), Work Unit 096 02 406.
models (FY75). Ribavirin was also found to be an effective antiviral agent in vitro against yellow fever.

Basic Studies

The pathogenesis and pathophysiology of yellow fever was studied in mice to collect fundamental information on ultrastructural changes resulting from infection (FY71). Researchers found that the host metabolism of thyroid hormone is accelerated during acute infection but not the febrile phase. It was also discovered that the virus matures on membranes of the rough endoplasmic reticulum and not the smooth endoplasmic reticulum and that the Kupffer cell is the initial cell of infection (FY73).

In FY79, the African green monkey was studied as a possible animal model for yellow fever virus infection that was closer to that seen in man. Subcutaneous inoculation of 6 PFU of the monkey-adapted Asibi strain of the virus led to the development of an acute nonlethal illness. This animal model was intended for study of the metabolic response and effectiveness of nutrient support and antiviral therapy.

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427 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1975 (Frederick, Maryland: USAMRIID), Work Unit 096 02 411.

428 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1976 (Frederick, Maryland: USAMRIID), Work Unit 096 02 411.

429 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1972 (Frederick, Maryland: USAMRIID), Work Unit 096 02 410.

430 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1973 (Frederick, Maryland: USAMRIID), Work Unit 096 02 414.
Also studied was yellow fever-induced changes in tissue concentrations of certain elements.\textsuperscript{431}

\textit{Influenza Virus}

The influenza virus has not been reported as considered for use as a biological weapon in the US. (It is mentioned briefly as a possible biological warfare agent in a 1971 Soviet publication.\textsuperscript{432}) Virulent pandemic strains, however, have spread death and suffering rapidly around the globe in the past (1918, 1957, 1968). The influenza virus was studied sporadically at USAMRIID between FY1973 – FY1985.

Initial work with the influenza virus was on studies to understand respiratory disease mechanisms, including penetration, retention, clearance, and replication of airborne organisms. Mice were challenged and also immunized with a mouse-adapted influenza virus through various routes.\textsuperscript{433} In FY1974, it was shown that Rimantadine was effective in the treatment of influenza infection in mice hours after infection. They also showed that a chemical could be administered by small particle aerosol and have excellent therapeutic effects.\textsuperscript{434} It was also demonstrated that aerosol-immunized mice were better protected than parenterally vaccinated mice against virulent influenza virus. Aerosol-immunized mice were totally immune to rechallenge with 100 respiratory LD50 of virulent virus, while parenteral vaccination

\textsuperscript{431} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1979} (Frederick, Maryland: USAMRIID), Work Unit A841 029.


\textsuperscript{433} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1973} (Frederick, Maryland: USAMRIID), Work Unit 096 01 109; 096 02 415; and 096 02 416.

\textsuperscript{434} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1974} (Frederick, Maryland: USAMRIID), Work Unit 096 02 411.
modified the course of diseased and reduced mortality, but did not prevent reinfection of the respiratory tract.\textsuperscript{435} Studies with influenza were discontinued in 1974.

However, in FY1976, concern over the possible emergence of a new pandemic influenza virus led NIAID to ask USAMRIID to participate in the national program to study management of influenza after the outbreak in early 1976. In its study, 133 healthy Ft. Detrick lab workers were inoculated with Wyeth split-virus A/swine influenza (A/New Jersey/8/76) virus vaccine. Systemic and local reactions were infrequent and mild. Only 28\% of subject 19-24 years developed HI antibody titers \( \geq 1:20 \), while 91-100\% of subjects 25-62 years reached that level. Some reached this level after a single booster dose, while others took two boosters. The split-virus was deemed safe, but poorly antigenic as a primary vaccine in 19-24 year olds.\textsuperscript{436}

Ribavirin appeared to be the most promising broad-spectrum antiviral agent (against both DNA and RNA viruses). In small particle aerosols, it was shown effective in the treatment of type A and B influenza virus infections. Treated patients experienced more rapid defervescence, disappearance of systemic illness, and reduction of viral shedding than patients treated with a placebo.\textsuperscript{437}

USAMRIID researchers also showed the efficacy of a whole virus vaccine in mice as well as the prophylactic and therapeutic effectiveness of amantadine,

\textsuperscript{435} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1974} (Frederick, Maryland: USAMRIID), Work Unit 096 02 415.

\textsuperscript{436} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit 096 02 002 / A841 00 001.

\textsuperscript{437} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1985} (Frederick, Maryland: USAMRIID), Work Unit D809 EA 001.
rimantadine, and ribavirin.\textsuperscript{438} In rhesus monkeys, poly (ICLC), was a good adjuvant when given in combination with swine influenza vaccine.\textsuperscript{439}

Overall, although whole virus and split virus vaccines did not induce antibody formation, good clinical protection was noted. USAMRIID researchers also developed a new technique to measure optical density: as the severity of microscopic lung lesions in mice increased, optical density increased.\textsuperscript{440}

In FY1985, a diagnostic kit developed at USAMRIID included assays for influenza A and B, parainfluenza, adeno and respiratory syncytial viruses was transferred to Brooks Air Force Base to support their “Project Gargle,” a global surveillance program for respiratory diseases among Air Force personnel.\textsuperscript{441}

\textit{Venezuelan Equine Encephalitis (VEE)}

VEE is a mosquito-borne disease that was tested as a BW agent in the US in the 1950s and 1960s. It is an incapacitating disease and rarely fatal. Very few organisms (10-100) are required to cause an infection in humans although it is not readily transmissible among humans. It was isolated from horses in 1936 and in 1952, researchers found that it could cause disease in humans. There were two recent outbreaks: in 1969-1971 in Guatemala, Mexico, and Texas and in 1995 in Venezuela and Columbia. In both, there were thousands of human cases and in the latter

\textsuperscript{438} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit 096 02 415.

\textsuperscript{439} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit 096 02 411.

\textsuperscript{440} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit 096 01 300.

\textsuperscript{441} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1985} (Frederick, Maryland: USAMRIID), Work Unit D809 EA 001.
outbreak 300 died. A live, attenuated vaccine is available as an IND, and a formalin-inactivated, killed vaccine is available for boosters. Work on VEE at USAMRIID was extensive from FY1969 – FY1982, but fell off sharply after that.

Vaccine Studies

There are two IND human unlicensed VEE vaccines that were developed at USAMRIID. The first, TC-83, was developed in the 1960s and is a live, attenuated vaccine produced by the Salk Institute. While not effective against all serotypes in the VEE complex, it has been used to protect thousands against laboratory infections and is licensed for use in equidae. The second IND vaccine (C-84) is prepared by formalin-inactivation of the TC-83 strain and is used as a booster for nonresponders to TC-83.

The attenuated TC-83 vaccine was introduced because of difficulties with the old formalin-inactivated vaccine. TC-83 has protective efficacy in man, but produces a febrile illness, with virus shedding in 15-30% of recipients, has abortogenic and/or teratogenic potential and has never been tested in children. There is evidence that the TC-83 strain may be pancreotrophic, producing carbohydrate intolerance in primates. The C-84 formalin-inactivated vaccine was prepared to circumvent these impediments to widespread use in man.


444 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1977 (Frederick, Maryland: USAMRIID), Work Unit 096 02 002 / A841 00 001.
Vaccine Combinations

It was reported in 1952 that a trivalent vaccine consisting of the three killed vaccines (VEE, WEE, EEE) was successful in guinea pigs. In FY1969, researchers tested the efficacy of a trivalent vaccine combination of attenuated strains (TC-83, Clone 15 strain WEE (AWEE), and small plaque mutant (SPM) of EEE. At 21-27 days postvaccination, guinea pigs were inoculated with one thousand LD50 Trinidad strain VEE, B-11 strain WEE and Texas strain EEE. TC-83 and AWEE gave complete homologous protection. When given in combination with the EEE vaccine, both TC-83 and AWEE had an adjuvant effect. However, the sequence in which the vaccines were given were important to the results: certain sequences depressed rather than enhanced a vaccine’s effectiveness.\textsuperscript{445}

Studies of the VEE and yellow fever vaccines (TC-83 and 17D, respectively) were conducted in human volunteers. Simultaneous as opposed to single administration of TC-83 and 17D resulted in a slight enhancement of the titer of YF antibody and a depression of the VEE mean titers. Administration of 17D after TC-83 resulted in a much greater rise in VEE titer and a depression of YF titer. VEE viremia was greater when TC-83 was administered alone.\textsuperscript{446} In FY1971, monkeys were given the live VEE vaccine and the 17D strain of yellow fever at varying time

\textsuperscript{445} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1969} (Frederick, Maryland: USAMRIID), Work Unit 096 02 402 and 096 02 403.

\textsuperscript{446} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1973} (Frederick, Maryland: USAMRIID), Work Unit 096 02 008.
intervals to assess any interference. Some was noted through day 28, but the effect was lost by day 56.\textsuperscript{447}

In addition, all group A vaccines developed and tested through FY1971 were examined for cross-protection against heterologous virus challenge. No heterologous protection was seen except for the live, attenuated VEE vaccine.\textsuperscript{448}

New Vaccine Testing

In FY1975, USAMRIID submitted an application to the Army Investigational Drug Review Board for VEE vaccine, inactivated, dried, MNLBR 109, Lot No. C-84-1.\textsuperscript{449} The effects of aggregation of virions of formalin-inactivated VEE virus resulting from lyophilization was examined. Prior to freeze-drying, the virions were well dispersed, but after lyophilization aggregation was severe. Prior to lyophilization, the vaccine was 5-7 times more effective.\textsuperscript{450}

In FY1977, the acceptability study of the inactivated VEE vaccine (MNLBR 109) was completed in eighteen volunteers. It was concluded that the C-84 VEE vaccine was safe for human use.\textsuperscript{451} In FY1978, researchers evaluated the response of fifteen individuals who received 0.5ml of the inactivated vaccine; seven of whom received a booster of 0.5ml on day 28 after the initial immunization. There was

\textsuperscript{447} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1971 (Frederick, Maryland: USAMRIID), Work Unit 096 02 004.

\textsuperscript{448} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1971 (Frederick, Maryland: USAMRIID), Work Unit 096 02 403.

\textsuperscript{449} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1975 (Frederick, Maryland: USAMRIID), Work Unit 096 02 002.

\textsuperscript{450} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1975 (Frederick, Maryland: USAMRIID), Work Unit 096 02 424.

\textsuperscript{451} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1977 (Frederick, Maryland: USAMRIID), Work Unit 096 02 002 / A841 00 001.
significant serological response to the vaccine, but not to the degree seen after natural disease or the live TC-83 vaccine.\textsuperscript{452}

In challenge studies, the inactivated VEE vaccine was not as effective as the attenuated VEE vaccine. TC-83 provided complete protection – as measured by mortality – against aerosol and IP challenge of virulent VEE virus (Trinidad strain given at $10^4.72$ LD50). C-84 induced protective responses that varied from partial to incomplete against an aerosol challenge, but gave complete protection against a parenteral inoculation.\textsuperscript{453}

In FY1978, testing of the VEE vaccine, live, attenuated, NDBR-102 (TC-83) (IND 142), continued with the administration of twenty-six immunizations.\textsuperscript{454}

**Adjuvants**

DEAE-dextran was shown to be a potent adjuvant for VEE virus vaccine in rhesus monkeys. However, this effect was not observed in mice, guinea pigs, or hamsters.\textsuperscript{455} Preliminary findings in FY1976 suggested that both human dialyzable transfer factor and stabilized poly-ICLC might be useful adjuvants for the immune response against killed VEE virus.\textsuperscript{456}

\textsuperscript{452} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1978* (Frederick, Maryland: USAMRIID), Work Unit A91C 00 001.

\textsuperscript{453} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1982* (Frederick, Maryland: USAMRIID), Work Unit A870 BB 069.

\textsuperscript{454} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1978* (Frederick, Maryland: USAMRIID), Work Unit A91C 00 001.

\textsuperscript{455} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1975* (Frederick, Maryland: USAMRIID), Work Unit. 096 02 419.

\textsuperscript{456} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1976* (Frederick, Maryland: USAMRIID), Work Unit 096 02 013.
Further studies in FY1976 demonstrated the adjuvant effects of DEAE-dextran and poly-ICLC when given simultaneously with the inactivated VEE vaccine. Poly-ICLC antibody response in monkeys were at levels similar to that resulting from the TC-83 vaccine and persisted 2.5 months.\textsuperscript{457} Neutralizing antibody titers in monkeys were much higher and more persistent than in those given the inactivated vaccine alone. Poly-ICLC (500 micrograms/kg) reduced the median effective dose of IVEE vaccine in mice twelve-fold.\textsuperscript{458}

Further work with poly-ICLC and tilorone HC1 (analog 11,567) showed that both were effective adjuvants when given in combination with inactivated VEE vaccine to lab rodents and rhesus monkeys, even when analog 11,567 was given in doses as low as 15 micrograms/kg. A metabolized lipid emulsion (LE) was prepared for potential use with inactivated aqueous virus vaccines and was an effective adjuvant when given with the VEE vaccine.\textsuperscript{459}

The lipid emulsion (LE) adjuvant was shown to be highly effective in potentiating the immunologic response to RVF, VEE, and WEE inactivated vaccines. In FY1979, a government patent was sought for LE.\textsuperscript{460}

Work with specific IgG and VEE virus resulted in the preparation of a manuscript by FY1977, “Enhancement of the primary immune response and

\textsuperscript{457} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1976} (Frederick, Maryland: USAMRIID), Work Unit 096 02 419.

\textsuperscript{458} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit 096 02 419.

\textsuperscript{459} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 013.

\textsuperscript{460} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1979} (Frederick, Maryland: USAMRIID), Work Unit A841 00 013.
protection by antigen-antibody complexes of an inactivated viral antigen: effect of dosage and inoculation.\textsuperscript{461}

In FY1978, tests were done to compare complete Freund’s adjuvant (CFA) and muramyl dipeptide (MDP) injection. While CFA potentiates both cellular and humoral immunity, MDP favors CMI. MDP also appeared to suppress the primary immune response while potentiating anamnestic responses to VEE C-84 antigens.\textsuperscript{462}

Virulence Testing

Involvement in the control of the 1969-1971 epizootic provided field observations as to the safety and efficacy of the TC-83 vaccine. Back-passage of TC-83 in burros (seven serial passages) gave no evidence of reversion to virulence.\textsuperscript{463} In other studies, it was indicated that the interferon system is important in determining the yield of virus from tissue culture: interferon sensitivity could be a factor to examine when attenuating a virus for vaccine production.\textsuperscript{464}

Vaccine Production

In FY1972, thirty small lots of formalin-inactivated TC-83 strain VEE vaccine was prepared in roller bottle cultures of chick embryo cells (CEC).\textsuperscript{465} In FY1973,

\textsuperscript{461} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit A841 00 016.

\textsuperscript{462} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 010.

\textsuperscript{463} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1972} (Frederick, Maryland: USAMRIID), Work Unit 096 02 007.

\textsuperscript{464} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1972} (Frederick, Maryland: USAMRIID), Work Unit 096 03 402.

\textsuperscript{465} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1972} (Frederick, Maryland: USAMRIID), Work Unit 096 02 407.
preliminary work resulted in a purification procedure for VEE that could be applied
to the large scale production of purified virus.\textsuperscript{466}

Antiviral Testing

In experimental animals, alpha-interferon and the interferon-inducer poly-
ICLC were proven highly effective for postexposure chemoprophylaxis of VEE, but
there was no clinical data to assess its efficacy in humans.\textsuperscript{467}

In FY1972, work on antiviral compounds began, including studies with poly I
– poly C (poly-ICLC).\textsuperscript{468} In hamsters, poly-ICLC delayed the growth of VEE, but
did not protect against late deaths.\textsuperscript{469}

In FY1974, Tilorone hydrochloride and three analogs were found to be
effective antiviral agents of VEE by a mechanism not involving synthesis or release
of interferon.\textsuperscript{470}

In FY1978, researchers found that an altered state of immune responsiveness
to microbial antigens is brought about by cyclophosphamide treatment prior to
immunization, resulting in abolition of humoral antibody responses along with
preservation or potentiation of delayed type hypersensitivity and cellular immune

\textsuperscript{466} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1973}
(Frederick, Maryland: USAMRIID), Work Unit 096 02 417.

\textsuperscript{467} USAMRIID, \textit{USAMRIID’s Medical Management of Biological Casualties Handbook, Fifth Edition}
(Fort Detrick, MD: August 2004), 70.

\textsuperscript{468} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1972}
(Frederick, Maryland: USAMRIID), Work Unit 096 02 411.

\textsuperscript{469} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1973}
(Frederick, Maryland: USAMRIID), Work Unit 096 01 407.

\textsuperscript{470} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1974}
(Frederick, Maryland: USAMRIID), Work Unit 096 02 411.
reactivity. Immunized animals with such altered reactivity show loss of protection upon challenge with VEE virus.\textsuperscript{471}

In FY1979, basic research with the antiviral drug ribavirin in a VEE-infected BHK-21 cell model showed that ribavirin inhibited virus growth by more than 90%. The model suggested that ribavirin does not inhibit viral transcription, but may interfere with translation.\textsuperscript{472} However, ribavirin causes the development of anemia and thrombocytosis in monkeys and man when given in multiple, high doses. It apparently did this by decreasing RBC survival by inhibiting release of RBC from the bone marrow. These effects were fully reversible when treatment was withdrawn.\textsuperscript{473}

Control of an Epizootic

In early May 1969, a severe epizootic of VEE erupted on the Pacific Coastal Plane of Guatemala. From here, the disease rapidly swept westward and northward – through El Salvador, Honduras and Nicaragua before subsiding in October. It reappeared in June 1970 in Mexico and within a month had spread westward 200 miles. USAMRIID provided attenuated, live virus vaccine to control these epizootics for the vaccination of the horse population at the periphery of the epizootic area to create an immune barrier. A conversion rate of 96% was reported.\textsuperscript{474}

\textsuperscript{471} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 004.

\textsuperscript{472} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1979} (Frederick, Maryland: USAMRIID), Work Unit A841 00 065.

\textsuperscript{473} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1981} (Frederick, Maryland: USAMRIID), Work Unit A871 BE 146.

\textsuperscript{474} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1971} (Frederick, Maryland: USAMRIID), Work Unit 096 02 007.
In utero Viral Transmission

Mice infected during gestation with the TC-83 strain had decreased litter sizes, decreased number of live births, and decreased survival until weaning age. Virus levels as high as 2 logs more virus/gram were found in embryos than in maternal blood. The highest virus levels were found in uterine tissues. This work unit was terminated in FY1973 when attempts to localize the viral antigen in fetal and placental tissues of infected dams with fluorescent antibody proved unsuccessful.

Radiation Challenge

Several experiments involved the irradiation of mice (600 R) to test the effect on the immune response of mice to an attenuated strain of VEE. Data suggested that irradiation before or after inoculation with attenuated VEE delays the onset of protection against virulent challenge. When the time interval between irradiation and vaccination was short, onset of protection was delayed longer. However in FY1974, similar experiments in monkeys exposed to 400 R total body irradiation resulted in no effect on either enhancement or delay in clearance of virus.

In FY1976, the implicit rationale for this work was stated explicitly: “This work unit is aimed at investigating interrelationships between acute or chronic

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475 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1969 (Frederick, Maryland: USAMRIID), Work Unit 096 01 403.

476 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1972 (Frederick, Maryland: USAMRIID), Work Unit 096 01 403.

477 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1973 (Frederick, Maryland: USAMRIID), Work Unit 096 01 409.

478 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1971 (Frederick, Maryland: USAMRIID), Work Unit 096 02 010.

479 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1974 (Frederick, Maryland: USAMRIID), Work Unit 096 01 010.
irradiation and immune or disease processes in animal models, so that limitations of protective vaccines or disease in irradiated personnel may be more effectively managed in a nuclear warfare environment.\textsuperscript{480}

Immunological Responses

In FY1975, it was found that VEE turned off leukocytic endogenous mediator (LEM) synthesis while bacterial infections did not.\textsuperscript{481} In other studies of the induction of immunity, an unexpected finding was the demonstration of the potentiation of humoral immunity with a micromolecular leukocyte extract which is known to decrease cellular immunity in vitro. The pooled fraction, which potentiates CMI in vitro, did not affect titer production, but appeared to adversely affect protection since animals so immunized died at greater frequencies despite antibody levels known to be protective in control groups. It was an important observation because it suggested that increased CMI induced by select antigen-adjuvant combinations may adversely affect protection against viral infection.\textsuperscript{482}

Researchers in FY1978 found that the macrophage was the primary replicative cell for virus growth in vitro. Nonimmune donor macrophages convert to an immune donor macrophage state about 28 days post-vaccination.\textsuperscript{483} In a guinea pig model, it

\textsuperscript{480} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1976} (Frederick, Maryland: USAMRIID), Work Unit 096 01 010.

\textsuperscript{481} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1975} (Frederick, Maryland: USAMRIID), Work Unit 096 01 020.

\textsuperscript{482} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit 096 02 013 / A841 00 010.

\textsuperscript{483} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 016.
was shown that the humoral immune system was the protective component of the total response to VEE antigen.  

Detection

In FY1977, opsonized VEE vaccine strain virus and Pichinde virus were shown to stimulate chemiluminescence from human polymorphonuclear leukocyte (PMN) with correlated with the amount of antibody available. The test appeared to be as sensitive – if not more so – than other then-currently employed in diagnostic serology, and could be achieved in less than two hours.

By FY1981, VEE (vaccine strain) could be detected at concentrations of 10-100 thousand organisms/ml by ELISA.

*Rift Valley Fever Virus*

Rift Valley Fever Virus (RVF) is a mosquito-borne disease found in Africa (persisting in Kenya) and is a member of the Bunyavirus family. It was studied extensively at USAMRIID during this period of time, particularly after a new, more virulent strain emerged in the mid-1970s. In 1975, a new clinical spectrum of disease was reported from South Africa: hemorrhagic fever and encephalitis. In 1977, RVF virus entered new territory, Egypt. There were tens of thousands of human cases with the classical acute, undifferentiated febrile illness, but an estimated one percent of the

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484 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1979* (Frederick, Maryland: USAMRIID), Work Unit A841 00 013.

485 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1977* (Frederick, Maryland: USAMRIID), Work Unit 096 03 408 / A91C 00 139.

486 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1981* (Frederick, Maryland: USAMRIID), Work Unit A871 BC 068.
cases were associated with hemorrhagic fever or encephalitis.\textsuperscript{487} Over the next three years, there were an estimated one million human cases. The experimental, first-generation inactivated human vaccine developed by the Army required multiple injections to produce immunity and booster injections to maintain it. It was expensive to produce, in relatively limited supply, and was not standardized from lot to lot.\textsuperscript{488}

Vaccine Research

In FY1971, an inactivated RVF vaccine was evaluated in man by administration of 2 doses of 0.5ml each 28 days apart. Neutralizing antibody was adequate in the majority of subjects up to day 270, but not at day 360.\textsuperscript{489} No further work on this vaccine was reported until FY1977, when it was reported that the existing vaccine did not meet modern safety standard for use in man.

To prepare a better vaccine, work was begun in FY1977 on determining the number, molecular weight and immunogenicity of structural polypeptides of RVF virus.\textsuperscript{490} At the same time, increased demands for the vaccine to treat epidemics of RVF led to the testing of five lots of the inactivated RVF vaccine that had not previously been used in clinical trials (the original protocol was submitted in 1969 to

\begin{footnotesize}
\begin{enumerate}
\item US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 131.
\item US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1980} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 131.
\item US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1971} (Frederick, Maryland: USAMRIID), Work Unit 096 02 002.
\item US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit A841 00 058.
\end{enumerate}
\end{footnotesize}
the Army Investigational Drug Review Board). Safety testing determined that all six lots were identical with respect to reactogenicity.\(^{491}\)

Further testing of the RVF vaccine, formalin-inactivated, tissue culture origin NDBR 103, Lot 1-6 (IND 365) continued in FY1979, along with evaluation of the human response to the administration of RVF vaccine, inactivated, dried, TSI-GSD-200. Immunological responses to two doses of Lot 6 were found to be variable. Only those who received concurrent vaccinations with VEE were found to have a dependably high response.\(^{492}\)

Further testing of the RVF vaccine (NDBR 103) continued under controlled field conditions in FY1979. Lab workers at NAMRU-3 in Cairo were vaccinated and all 108 vaccinees had detectable antibodies.\(^{493}\) Further testing was done at USAMRIID (22 persons) and USDA (83 persons), resulting in adequate PRN titers in all but a handful. No adverse reactions were reported from these and other trials.\(^{494}\)

In that year, work continued on the improved inactivated RVF vaccine (TSI-GSD-200), however, the quantities that were available were unlikely to be sufficient in many scenarios for their use. Therefore, a joint study was initiated with the Israeli Defense Force to define alternative immunization schedules.\(^{495}\) In FY1980, the first

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\(^{491}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1977* (Frederick, Maryland: USAMRIID), Work Unit A91C 00 001.

\(^{492}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1979* (Frederick, Maryland: USAMRIID), Work Unit A841 00 001.

\(^{493}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1979* (Frederick, Maryland: USAMRIID), Work Unit A91C 00 131.

\(^{494}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1980* (Frederick, Maryland: USAMRIID), Work Unit A841 00 001.

\(^{495}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1979* (Frederick, Maryland: USAMRIID), Work Unit A91C 00 131.
human tests of the new vaccine began, but not all lots were found to be of equal potency.\textsuperscript{496}

In FY1986, initial safety and immunogenicity testing of all lots of TSI-GSD-200 were completed in volunteers. There were no adverse reactions and all recipients developed titers which were judged protective.\textsuperscript{497} Potency testing continued with 19 of 20 lots of the vaccine, although potency data would only be available after three years.\textsuperscript{498}

In FY1987, researchers demonstrated that intraperitoneal priming with unmodified RVF vaccine NDBR 103 followed by intranasal boost yielded protective efficacy against aerosol or SC challenge with RVF virus that was superior to that of SC vaccination with RVF vaccine and Avridine (discussed below). Aerosol immunization with the RVF vaccine/Avridine mixture failed to protect any mice when challenged with virulent RVF vaccine by the aerosol or SC routes despite induction of specific IgA and IgG.\textsuperscript{499}

From these studies, it was determined that both peripheral and mucosal immunity are required for complete protection – especially after aerosol exposure.\textsuperscript{500}

\textsuperscript{496} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1980} (Frederick, Maryland: USAMRIID), Work Unit A841 00 001.

\textsuperscript{497} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1986} (Frederick, Maryland: USAMRIID), Work Unit D807 AL 020.

\textsuperscript{498} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1987} (Frederick, Maryland: USAMRIID), Work Unit D809 AK 007.

\textsuperscript{499} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1987} (Frederick, Maryland: USAMRIID), Work Unit D807 AL 020.

\textsuperscript{500} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1988} (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131.
Vaccine Combinations

As reported elsewhere, the inactivated RVF vaccine was combined with the inactivated WEE, EEE, Chikungunya, and Q fever vaccines in a pentavalent vaccine that was found to be physically compatible and met the USPHS criteria to safety. Each component of the pentavalent vaccine was as effective in this form as when given alone.  

Synthetic Peptides for Vaccine Development

In FY1985, studies revealed six distinct vaccine-relevant epitopes capable of eliciting in vitro neutralizing antibodies on the G1 and G2 surface viral glycoproteins. Immunization of mice with G2 gene products expressed in E. coli as well as in recombinant vaccinia viruses containing RVF virus glycoprotein genes protected mice from a lethal challenge of RVF virus.  

Further work found that the vaccine-relevant epitopes were confined to a small sequence on G2, making RVF virus perhaps the best virus model defined to date to test the feasibility of the synthetic peptide approach to vaccine development.  

In FY1985, two approaches were taken. Partial coding sequences of RVF virus glycoprotein genes were incorporated into a bacterial plasmid expression system and introduced into E. coli. These allowed the regulated, high-level expression of

501 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1971 (Frederick, Maryland: USAMRIID), Work Unit 096 02 004.
502 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1985 (Frederick, Maryland: USAMRIID), Work Unit BS12 AC 003.
503 Ibid.
RVFV glycoprotein analogs. Partially purified polypeptides from these systems were used with adjuvants to immunize mice and afforded protection from lethal challenge, although only marginal titers of neutralizing antibody resulted from the immunization. The other method involved creating recombinant vaccinia viruses containing the genes responsible for both RVF virus envelope glycoproteins and evaluated in the mouse-challenge system using various routes for vaccine administration. High-titer neutralizing antibody and high-level protection followed immunization.\(^{504}\)

In FY1987, after animal immunization with a large number of recombinant vaccinia virus constructs, it was clearly demonstrated that any construct expressing mature G2 envelope glycoprotein was protective in the mouse protection assay.\(^{505}\)

In FY1987, researchers found that monoclonal antibodies (MAB) specific for the G1 and G2 polypeptides of RVF virus were completely protective against aerosol challenge when combined together, but resulted in heavy mortalities if used separately. Monoclonal-G2 treated mice died of encephalitis; monoclone-G1 treated mice died of hepatitis.\(^{506}\)

Recombinant vaccinia viruses expressing specific sequences from RVF virus refined requirements for future vaccines. Researchers chose the Connaught strain as the parent vaccinia strain for future vaccinia recombination experiments and

\(^{504}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1985* (Frederick, Maryland: USAMRIID), Work Unit A871 AG 136.

\(^{505}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1987* (Frederick, Maryland: USAMRIID), Work Unit BS12 AB 002.

\(^{506}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1987* (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131.
candidate human virus vaccine development.\textsuperscript{507} In FY1989, vaccinia viral strains from the master seed of the Connaught smallpox vaccine were plaque-purified in certified MRC-5 cells. A single derivative (Connaught 3E-1) was selected as parent for all future recombinants. In addition to RVF genes, sequences from Hantaan, Lassa fever, and VEE were under consideration for expression in vaccinia virus.\textsuperscript{508}

Attenuated Vaccine Studies

In FY1985, three attenuated strains (Caplan, Smithburn, and Moussa) were evaluated as potential vaccine candidates. Each was compared to the formaldehyde inactivated RVF vaccine in mice challenged by aerosol with the virulent ZZ-501 RVF strain. All three performed poorly.\textsuperscript{509}

In FY1986, the T1 strain of RVF virus, isolated from mosquitoes, was used to immunize hamsters by intranasal and subcutaneous routes. Both routes afforded the hamsters total protection from lethal challenge with virulent RVF virus.\textsuperscript{510} Both the wild-type (ZZ-501) and attenuated (T-1) strains of RVF virus were stable in water up to three days. Mice orally exposed to the T-1 strain in their drinking water had a prolonged mean time-to-death versus unvaccinated controls when challenged with

\textsuperscript{507} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1988} (Frederick, Maryland: USAMRIID), Work Unit BS12 AB 002.

\textsuperscript{508} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit D809 AU 011.

\textsuperscript{509} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1985} (Frederick, Maryland: USAMRIID), Work Unit D807 AL 020.

\textsuperscript{510} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1986} (Frederick, Maryland: USAMRIID), Work Unit D807 AL 020.
virulent RVF virus.\textsuperscript{511} The T-1 vaccinated animals recovered from hepatitis, but later died of encephalitis.\textsuperscript{512}

In FY1987 and FY1988, RVF master and production seeds of an attenuated strain (ZA-548 MP12 strain) was produced and a candidate vaccine was in the final test stage at the Salk Institute.\textsuperscript{513} The MP 12 attenuated vaccine candidate was derived from the ZH-548 Egyptian isolate by serial mutagenesis. In FY1989, bovines were inoculated with the RVF vaccine, MP-12 and were fully protected.\textsuperscript{514} In other studies, it was concluded that a reversion to virulence was unlikely and that genetic reassortment with wild-type viruses during a vaccination program in endemic areas would be expected to yield attenuated variants.\textsuperscript{515}

In FY1990, the MP-12 vaccine was tested in rhesus macaques using proposed human doses. Virulent viral challenge by parenteral and aerosol routes were in progress.\textsuperscript{516}

Also in FY1990, an attenuated strain of RVF (C13) was isolated and found to confer solid immunity to virulent challenge. Its attenuation appeared due to the absence of a nonstructural protein (NS8). (It was found that anti-NS8 antibodies are

\textsuperscript{511} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1988} (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131.

\textsuperscript{512} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit A871 AB 150.

\textsuperscript{513} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1987} (Frederick, Maryland: USAMRIID), Work Unit D809 AK 007.

\textsuperscript{514} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit BS12 AB 002.

\textsuperscript{515} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit BS12 AB 002.

\textsuperscript{516} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1990} (Frederick, Maryland: USAMRIID), Work Unit BS12 AB 002.
protective.) If confirmed, this observation identified a method for improving the existing RVF virus vaccines and possibly the basis for pursuit of both subunit and live-attenuated vaccines for other phleboviruses.\textsuperscript{517}

Antiviral Therapies

In FY1978, the antiviral drug ribavirin was shown to be effective in the treatment of RVF virus in mice and hamsters.\textsuperscript{518} In FY1979, ribavirin, poly(ICLC), and antibody were shown to be effective prophylactically and therapeutically in murine RVF models. Successful treatment could begin as late as day 3. It was clear that all three drugs lacked any effect on CNS infection, however.\textsuperscript{519}

Studies in FY1981 showed ribavirin to be effective against Lassa fever and RVF virus in lab animals.\textsuperscript{520} Toxicity studies showed that man and rhesus monkeys develop anemia and thrombocytosis during multiple, high-dose treatments with ribavirin. Apparently, ribavirin induces anemia by decreasing RBC survival and by inhibiting release of RBC from the bone marrow. These effects are dose-dependent and fully reversible when treatment is withdrawn. Ribavirin was evaluated for subacute toxicity when administered orally for 28 days.\textsuperscript{521} In addition, in vitro, combinations of amantadine and rimantadine with ribavirin showed an enhanced

\textsuperscript{517} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1990 (Frederick, Maryland: USAMRIID), Work Unit A91C LA 138.

\textsuperscript{518} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1978 (Frederick, Maryland: USAMRIID), Work Unit A841 00 026.

\textsuperscript{519} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1979 (Frederick, Maryland: USAMRIID), Work Unit A91C 00 131.

\textsuperscript{520} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1981 (Frederick, Maryland: USAMRIID), Work Unit A871 BE 146.

\textsuperscript{521} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1981 (Frederick, Maryland: USAMRIID), Work Unit A870 BE 146.
effect against RVF, VEE, and SFS viruses above that of ribavirin alone against RVF and VEE viruses. These were toxic at the 200 mg/kg level as single drugs or in combination.

In FY1982, further drug screening continued to assess the efficacy of potential antivirals against viruses in tissue cultures and rodent models.

In FY1985, the optimal treatment regimens with the immunomodulator, poly(ICLC) were determined in mice. For prophylactic treatment, three 1 microgram doses over 12 days were found to be effective against RVFV infection. For therapeutic application, three 20 microgram doses were required. However, therapy combining ribavirin and poly(ICLC) was also highly effective: protection was afforded with very low nontoxic doses of these drugs when administered as late as 48 hours after infection. In addition, a new immunomodulatory compound, Picabanil, was also found to be moderately efficacious against RVF virus infection in mice. Picabanil elicited only low levels of interferon, but induced strong cytotoxic activity of NK cells.\textsuperscript{522}

In FY1987, three more immunomodulators, AVS-1968, 1300, and 1018 showed excellent in vivo activity against RVFV, as well as an extract of \textit{C. burnetii} with only a single dose.\textsuperscript{523} Further testing indicated that AVS-1018 was a candidate for clinical trials.\textsuperscript{524}

\textsuperscript{522} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1985} (Frederick, Maryland: USAMRIID), Work Unit D807 AD 014.

\textsuperscript{523} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1987} (Frederick, Maryland: USAMRIID), Work Unit D807 AD 014.

\textsuperscript{524} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1988} (Frederick, Maryland: USAMRIID), Work Unit D807 AD 014.
Adjuvants

In FY1979, the lipid emulsion (LE) adjuvant was shown to be highly effective in potentiating the immunologic response to several inactivated vaccines: RVF, VEE, and WEE. A government patent was being sought at that time. In addition, compound CP 20 (961) was also shown to be an effective adjuvant with the RVF and WEE vaccines.\textsuperscript{525}

In FY1985, mucosal priming with Avridine, an immunomodulator, combined with the inactivated RVF virus vaccine was effective in reducing or preventing aerosol-acquired encephalitis, although it did not prevent hepatitis. Priming by SC injection of the RVF vaccine plus Avridine resulted in mucosal and systemic protective immunity.\textsuperscript{526} Single treatment with Avridine subcutaneously or intradermally significantly reduced mortality in hamsters in the early stages of infection through day six postinfection. During aerosol immunization, Avridine appeared to be well-tolerated by mice.\textsuperscript{527}

In FY1986, mouse studies demonstrated that parenteral and/or enteric vaccination with a mixture of inactivated RVF vaccine and Avridine in liposomes provided significant protection against parenteral or aerosol challenge with virulent

\textsuperscript{525} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1979} (Frederick, Maryland: USAMRIID), Work Unit A841 00 013.

\textsuperscript{526} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1985} (Frederick, Maryland: USAMRIID), Work Unit A871 AJ 138.

\textsuperscript{527} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1986} (Frederick, Maryland: USAMRIID), Work Unit D807 AL 020.
RVF virus 21 days post-vaccination. These studies were done to define the potential complications of high-dose aerosol exposure to RVF.\textsuperscript{528}

Aerosol Tests

In FY1979, work began on evaluating the potential for aerosol transmission of RVF isolates. The physical characteristics of the SPA generated from a virus carrier fluid was studied. The SPA consisted of particles of five microns or less in size, with a median mass diameter of 0.964 micrometers. In young adult ICR mice, the log median lethal doses were determined for four strains of RVF virus: ZZ-501 was 2.54PFU; Entebbe was 1.76PFU; SA-51 was 2.59PFU; and SA-75 was 1.86PFU.\textsuperscript{529}

In FY1980 pathogenesis studies in the rat, it was determined that infection by the respiratory routes caused initial virus replication in the lung. Viremia develops and the foci of infection shifts to the liver, which is fulminating and usually fatal.\textsuperscript{530} The efficacy of the inactivated RVF vaccine was undetermined after tests in mice and monkeys.

In FY1981, it was determined that the Lunyo virus, isolated in 1955 from mosquitoes in Uganda, had a high degree of infectivity following aerosol exposure. This was unexpected because all other RVF virus strains were less virulent for mice when administered by the respiratory route.\textsuperscript{531}

\textsuperscript{528} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1986} (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131.

\textsuperscript{529} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1979} (Frederick, Maryland: USAMRIID), Work Unit A841 00 066.

\textsuperscript{530} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1980} (Frederick, Maryland: USAMRIID), Work Unit A841 00 066.

\textsuperscript{531} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1981} (Frederick, Maryland: USAMRIID), Work Unit A870 BB 069.
Also in FY1981, rats were challenged with ZZ-501 strain RVF after vaccination with either the NDBR-103 vaccine or the TSI-GSD-200 vaccine. Antibody response was dose-related and comparable between the vaccines. However, the data demonstrated that the protection by the TSI-GSD-200 vaccine was less than that against IP challenge. It was unknown what the significance for man was, but it showed that the inactivated vaccine does not provide complete protection against RVF virus disseminated in SPA. 532

In FY1985, aerosol studies suggested that the US should be concerned that hostile nations might weaponize RVF virus as a BW agent. 533

Basic Research

In FY1978, growth, purification, concentration, and radiolabeling techniques were perfected for RVF virus. The South African 1951 isolate gave consistently higher tissue yield than did Entebbe, Zagazig 501 or South African 1975. 534 Other studies in FY1979 that compared four different RVF virus strains that were representative of a broad range of geographic spread, time, and pathogenicity. The primary structure was highly conserved in all despite over 25 years difference in their time of isolation and thousands of miles in their geographic origins. 535

532 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1981 (Frederick, Maryland: USAMRIID), Work Unit A871 BC 148.

533 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1985 (Frederick, Maryland: USAMRIID), Work Unit D807 AC 013.

534 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1978 (Frederick, Maryland: USAMRIID), Work Unit A841 00 058.

535 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1979 (Frederick, Maryland: USAMRIID), Work Unit A91C 00 131.
Also in FY1978, research was initiated to define antigenic and other laboratory characteristics of recent RVF virus isolates to determine if the previously developed vaccine would be effective and also to explain the emergence of severe forms of the disease. The 1977 Egyptian isolate (ZZ501) was found to be 10,000-fold more lethal for rats than other isolates, but had similar pathogenicity for other animals. Convalescent sera from infections with all four strains neutralized ZZ501 and the inactivated vaccine protected mice equally well against ZZ501 and parent (Entebbe strain) virus challenge.\textsuperscript{536}

In FY1979, rhesus monkeys were experimentally infected with the ZZ-501 RVF virus strain, isolated from a fatal case of hemorrhagic fever in Egypt. Hemorrhagic fever had never been reported in nonhuman primates infected with classical RVF strains. Two of eighteen monkeys showed frank clinical signs of hemorrhagic fever.\textsuperscript{537}

Transmission Studies

Studies began in FY1981 to understand the ecologic and intrinsic factors influencing the ability of arthropods to transmit viruses. Research objectives included the determination the of RVF virus vector potential of several species of mosquitoes and sandflies known to feed on humans and domestic animals; and to determine the

\textsuperscript{536} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 131.

\textsuperscript{537} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1979} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 131.
effect of environmental temperature and geographic strain of the vector on the transmission of RVF, Dengue, Chikungunya, and EEE viruses.\textsuperscript{538}

In FY1986, data from the advanced very high resolution radiometer (AVHRR) on the NOAA polar-orbiting meteorological satellites were correlated with a green vegetation index and used to try to predict RVF viral activity in Kenya.\textsuperscript{539} This was then applied to determine the key ecological parameters leading to and associated with a RVF epidemic/epizootic in Senegal and Mauritania in 1987. The AVHRR was being used to monitor and predict the potential for RVF viral activity in fifteen regions in five countries in sub-Saharan Africa. There was a demonstrated link between RVF virus epidemics and periods of exceptionally heavy rainfall.\textsuperscript{540} This work led, in FY1989 to an accurate prediction of an outbreak of RVF in Kenya. In the field, methoprene prevented emergence of adult mosquitoes for two weeks after flooding, preventing initiation of an RVF epidemic/epizootic.\textsuperscript{541}

By FY1990, Data from NOAA LANDSAT, METEOSAT, and SPOT satellites, space-shuttle photography and airborne synthetic-aperture radar were used to determine key ecological changes leading to and associated with RVF epidemics in Senegal, Mauritania, Zambia, and Kenya; and also to YF in Nigeria. Field ecology studies demonstrated that outbreaks of African RVF and other arboviral diseases are

\textsuperscript{538} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit BS10 AP 198.

\textsuperscript{539} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1986} (Frederick, Maryland: USAMRIID), Work Unit A870 AP 131.

\textsuperscript{540} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1988} (Frederick, Maryland: USAMRIID), Work Unit D870 AP 131.

\textsuperscript{541} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit A870 AP 131.
characterized by distinct spatial and temporal patterns directly related to specific environmental parameters. This knowledge could help “distinguish naturally occurring disease outbreaks from those caused by BW.”

**Detection**

In FY1979, researchers developed a solid phase radioimmunoassay (SPRIA) procedure for the quantitation of antiviral antibody and detection of cell surface antigens. In FY1980, the SPRIA for RVF virus antibody demonstrated a 1:1 correlation with the 80% PRNT. Thus this new procedure was near the stage where it could replace the costly PRNT as the primary measure of anti-RVF virus antibody response.

In FY1981, an antigen-specific ELISA was developed for RVF. It held promise for rapid diagnosis, vaccine standardization, and studies of antigen metabolism in infected animals.

In FY1981, new techniques of lymphocyte hybridoma production of monoclonal antibodies to detect and characterize RVF virus antigens was underway. Preliminary lymphocyte hybridoma experiments yielded numerous monoclonal

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542 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1990* (Frederick, Maryland: USAMRIID), Work Unit A870 AP 131.

543 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1979* (Frederick, Maryland: USAMRIID), Work Unit BS03 00 026.

544 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1980* (Frederick, Maryland: USAMRIID), Work Unit BS03 00 026.

antibodies, most of which were directed at nucleocapsid antigens.\textsuperscript{546} Another technique was also pursued in FY1981: antigenic analysis of RVF virus progressed, with the goal of producing RVF virus immunogens using rDNA technology.\textsuperscript{547}

As of FY1982, standardized procedures using ELISA technology allowed complete testing of either RVF of VEE antigens within 5-7 hours. Polyclonal antisera were more sensitive than monoclonal antibodies as capture antibodies for RVF. (A new method was developed in 1979 for RIA using the “antibody capture principal” – later adapted to ELISA – for detection of virus specific IgM antibodies. Such antibodies generally develop shortly after infection, peak rapidly, and decline as specific IgG is produced. This was an improvement on using a retrospective diagnosis of virus infection based on measuring a rise in IgG.)\textsuperscript{548}

In FY1985 a nucleic acid hybridization assay was developed for detecting RVF virus in infected VERO cells and infected cell media by using a cDNA probe. Using this system, researchers detected RVF virus sequences in inactivated vaccine preparations and samples of aerosolized virus collected post-aerosol exposure.\textsuperscript{549}

In October 1987, there was an extensive epidemic/epizootic of RVF in Mauritania and Senegal, offering researchers an opportunity to validate their rapid diagnosis assays under field conditions. The antigen-capture immunoassay was only

\textsuperscript{546} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1981} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 143.

\textsuperscript{547} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1981} (Frederick, Maryland: USAMRIID), Work Unit BS10 AP 198.

\textsuperscript{548} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit A870 BC 068.

\textsuperscript{549} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1985} (Frederick, Maryland: USAMRIID), Work Unit A871 AD 139.
30% as sensitive as the standard virus isolation and identification procedures, but was 97% as specific (few false-positives). The antigen-capture immunoassay took less than three hours and minimal equipment, while traditional procedures required at least one week for isolation and identification, cell cultures and more sophisticated resources.\textsuperscript{550}

\textit{Korean Hemorrhagic Fever Virus (Hantaan)}

Korean Hemorrhagic Fever virus was first recognized in Korea in 1951, but the etiologic agent was not isolated until 1978 in the lungs of a rodent. In FY1979, work progressed on the characterization of the agent that caused Korean Hemorrhagic Fever. It was determined to be a heat-stable, enveloped, cell-associated RNA virus, but it was not yet clear whether it was a Bunyavirus or arenavirus.\textsuperscript{551} In FY1980, it was determined to be a Bunyavirus and was named Hantaan virus.\textsuperscript{552}

In FY1981, Hantaan virus was propagated to high titer in cell culture and a sensitive plaque assay for detecting virus infectivity and neutralizing antibody was developed. These allowed for preliminary characterization of the virus.\textsuperscript{553}

In FY1985, further work continued on Hantaan virus because of their ubiquity in nature and transmission via aerosolization rather than via an arthropod vector. In that year, the medium (M) and small (S) segments of the virus were cloned and

\textsuperscript{550} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1988} (Frederick, Maryland: USAMRIID), Work Unit D807 AL 020.

\textsuperscript{551} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1979} (Frederick, Maryland: USAMRIID), Work Unit A841 00 054.

\textsuperscript{552} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1980} (Frederick, Maryland: USAMRIID), Work Unit A841 00 054.

\textsuperscript{553} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit BS10 AP 198.
partially sequenced. The S segment coded for the nucleocapsid protein and the M segment for the two viral envelope glycoproteins.\textsuperscript{554}

In FY1986, assays for Hantaan virus were successfully field-tested during a collaborative study of an outbreak in the PRC and the efficacy of antiviral drug therapy was monitored with those assays during the China study.\textsuperscript{555}

In FY1989, two vaccine candidates were being produced. At the Swiftwater Institute, a vaccinia-Hantaan recombinant containing both the S and M segments was prepared and was undergoing expression studies and safety tests at USAMRIID. The Salk Institute was preparing a vaccinia-Hantaan recombinant that included only the M segment.\textsuperscript{556}

USAMRIID researchers became aware of a major epidemic of HFRS in Yugoslavia because of their collaborative research there. A field study was established in Sarajevo to attempt an efficacy trial of the antiviral drug, ribavirin and evaluate the diagnostic tests.\textsuperscript{557}

Other work in FY1990 included the testing of specimens from patients obtained from various collaborators worldwide. All attempts to detect hantaviral antigen by immunoassay failed. However, testing of sera obtained during the Korean War confirmed that the hemorrhagic fever described during the conflict was due to

\begin{footnotesize}
\begin{itemize}
\item \textsuperscript{554} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1985} (Frederick, Maryland: USAMRIID), Work Unit A871 AL 140.
\item \textsuperscript{555} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1986} (Frederick, Maryland: USAMRIID), Work Unit D809 EA 005.
\item \textsuperscript{556} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit D809 AU 011.
\item \textsuperscript{557} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1990} (Frederick, Maryland: USAMRIID), Work Unit D809 EA 001.
\end{itemize}
\end{footnotesize}
Hantaan virus and that the IgM antibody-detection assay was the method of choice for diagnosis of acute disease.

*Machupo Virus*

Four arenaviruses are significant human pathogens: Lassa, Junin, Machupo, and Lymphocytic choriomeningitis virus (LCM). All have a demonstrated potential to produce explosive outbreaks under artificial conditions and all are highly infectious by aerosol.558

After an outbreak in 1971, USAMRIID initiated work on Machupo virus, the causative agent of Bolivian hemorrhagic fever, starting with characterization of the gross and microscopic pathologic changes in infected rhesus monkeys and attempts to prepare a killed vaccine, including defining the efficacy of prophylaxis and treatment with immune serum and a joint study with the Middle America Research Unit on a candidate attenuated virus vaccine.559

In FY1975, a dosage of BHF immune globulin (human origin) that protected monkeys against severe disease was determined. As of FY1976, live, attenuated and inactivated experimental vaccines were used to protect monkeys against challenge with Machupo virus. 560

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558 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1985* (Frederick, Maryland: USAMRIID), Work Unit A871 AD 133.

559 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1972* (Frederick, Maryland: USAMRIID), Work Unit 096 03 405; and US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1973* (Frederick, Maryland: USAMRIID), Work Unit 096 01 408 and 096 03 405.

560 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1976* (Frederick, Maryland: USAMRIID), Work Unit 096 02 426.
Cross Protection and Subunit Vaccines

Significant protection against Machupo virus occurred with both Tacaribe and Tamiami viruses and it was found that a combination of two viruses was more protective than one.\textsuperscript{561} In FY1978, work on a single vaccine to protect against both Machupo and Junin virus infection (BHF and AHF, respectively) was underway. Studies in monkeys showed that the Junin virus protected against experimental lethal challenge with Machupo virus. The Junin virus used in this and in guinea pig studies (with similar results), was the clone 3 strain, an attenuated virus used experimentally in 600 volunteers in Argentina as a possible AHF vaccine.\textsuperscript{562}

Briefly in FY 1978, there was work on the glycoprotein and nucleoprotein subunits of Machupo virus as possible vaccine candidates. Guinea pigs inoculated with either subunit antigen survived homologous virus challenge. However, the PI left the institute and the work unit was terminated.\textsuperscript{563}

Therapy

In FY1976, in vitro studies showed that ribavirin was an effective antiviral against Machupo viruses.\textsuperscript{564} Further work showed that rhesus monkeys infected with Machupo virus were treated successfully with ribavirin, even when therapy was

\textsuperscript{561} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1975 (Frederick, Maryland: USAMRIID), Work Unit 096 03 405.

\textsuperscript{562} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1978 (Frederick, Maryland: USAMRIID), Work Unit A841 00 017.

\textsuperscript{563} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1978 (Frederick, Maryland: USAMRIID), Work Unit BS03 00 004.

\textsuperscript{564} US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1976 (Frederick, Maryland: USAMRIID), Work Unit 096 02 411.
delayed until after the onset of clinical illness. \(^{565}\) Ribavirin, in primates, prevented the onset of the hemorrhagic component of the disease.

However, ribavirin, in monkeys, successfully treated systemic, but not CNS components of the disease when therapy was initiated after onset of clinical signs. Prophylactic treatment prevented clinical disease completely: monkeys seroconverted, but failed to develop viremia or any clinical signs of illness. \(^{566}\)

In contrast, lysine-stabilized poly(ICLC) was found to enhance Machupo virus infection. \(^{567}\)

Basic Research

As of FY1977, little was understood about the genetics of arenaviruses, although human pathogenic and nonpathogenic members of the group could be distinguished by complement fixation and immunofluorescence techniques. \(^{568}\) It was known since FY1975 that monkeys that survive acute BHF infection often develop a wasting neurovascular disease. \(^{569}\)

In FY1979, aerobiological research studies were done with the Carvallo strain (#21677) of the Machupo virus and the Josiah strain of the Lassa virus. Under experimental conditions, Lassa virus was considerably more stable in aerosol than

\(^{565}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1978* (Frederick, Maryland: USAMRIID), Work Unit A841 00 026.

\(^{566}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1985* (Frederick, Maryland: USAMRIID), Work Unit D807 AD 014.

\(^{567}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1977* (Frederick, Maryland: USAMRIID), Work Unit 096 02 411.

\(^{568}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1977* (Frederick, Maryland: USAMRIID), Work Unit BS03 00 004.

\(^{569}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1975* (Frederick, Maryland: USAMRIID), Work Unit 096 01 408.
Machupo, although both were relatively stable in aerosol and both highly infectious and moderately lethal for guinea pigs and cynomolgus monkeys by the airborne route.\textsuperscript{570} Experiments were done in 6200-L aerosol chambers. The LD50 for Machupo was estimated at $10^{2.23}$ PFU and the ID50 was less than 10 PFU.

\textit{Junin Virus}

The Junin virus is the causative agent of Argentine hemorrhagic fever (AHF) – an acute, severe disease – and was considered to be a potential BW threat by USAMRIID researchers. Mortality for AHF, like BHF, is approximately 15-30%.\textsuperscript{571} Within endemic regions, 200-400 cases are observed most years, with large epidemics occurring for unknown reasons in some years.\textsuperscript{572} Work on a Junin vaccine was needed because there was no agency or commercial interest in such studies and knowledge of arenavirus vaccine development would be useful because of the existence of related viruses that cause hemorrhagic fevers in many countries.\textsuperscript{573}

\textbf{Vaccine Research}

In FY1979, a known attenuated strain of Junin virus, XJ-44, was selected for development as an attenuated candidate vaccine strain.\textsuperscript{574} A year later, USAMRIID

\textsuperscript{570} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1979} (Frederick, Maryland: USAMRIID), Work Unit A841 00 026.

\textsuperscript{571} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 134.

\textsuperscript{572} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1985} (Frederick, Maryland: USAMRIID), Work Unit D807 AD 014.

\textsuperscript{573} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1985} (Frederick, Maryland: USAMRIID), Work Unit D807 AF 016.

\textsuperscript{574} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1979} (Frederick, Maryland: USAMRIID), Work Unit A841 00 017.
was working with three attenuated strains – all more attenuated than XJ clone 3, an experimental but unsatisfactory vaccine used in Argentina ten years prior. By FY1982, work on a new vaccine had progressed so that researchers estimated that one suitable for use in man would be available by winter 1983.575

Work on a new vaccine progressed under a UN Development Project, jointly conducted by U.S. and Argentine investigators. A vaccine candidate, Candid #1 was taken to final product in 1982 by methods approved for Biologics under GLP and GMP regulations, and in compliance with vaccine requirements for the US and Argentina. In process testing required by the FDA was initiated in 1982 and mostly complete by 1983. Studies in FY1984 continued the development of an optimal freeze-drying menstruum. Working with the Salk Institute – Government Services Division, efforts were made to improve the stabilizer for the dry Candid #1 vaccine – at that time none could enhance the heat stability of the product.576

In FY1985, an IND submission (IND 2257) was made to the FDA for a live, attenuated Junin vaccine. Also in that year, tests were done to study the effectiveness of the Candid#1 vaccine in guinea pigs against an airborne challenge of 10^3.8LD50. These resulted in the conclusion that Candid #1 strain induced a protective response against a significant airborne challenge of virulent virus.577

575 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1982 (Frederick, Maryland: USAMRIID), Work Unit A871 BC 148.

576 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1985 (Frederick, Maryland: USAMRIID), Work Unit D807 AF 016.

577 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1985 (Frederick, Maryland: USAMRIID), Work Unit D807 AL 020.
Phase I clinical trials began in FY1986 for the live, attenuated Candid #1 Junin vaccine to determine vaccine safety and immunogenicity. Fifteen of seventeen vaccines seroconverted after immunization with no adverse local or systemic reactions. Field testing was anticipated for the next 3-5 years.\(^{578}\)

In FY1988, it was demonstrated that the Candid #1 vaccine protected against parenteral virulent Junin and Machupo viral challenge in animal models. It was also effective in protecting monkeys against an aerosol challenge with virulent Junin virus.\(^{579}\)

Cross Protection

Further research in FY1979 in monkeys showed that that Junin virus gave excellent protection against BHF infection. On the basis of this data, USAMRIID began a joint project involving the Argentine Secretariat of Health and the Pan-American Health Organization (PAHO) for the development of an attenuated vaccine against AHF that would offer protection against BHF.\(^{580}\) In FY1980, work progressed with support from the UN Development Program.

In FY1986, basic studies on the pathophysiology and immunology of arenaviruses led to the observation that cross protection among old-world arenaviruses depended more on cross-reactive cytotoxic spleen cells than on humoral

\(^{578}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1986* (Frederick, Maryland: USAMRIID), Work Unit D807 AC 013.

\(^{579}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1988* (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131.

\(^{580}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1979* (Frederick, Maryland: USAMRIID), Work Unit A841 00 017.
antibodies. However, synthetic peptides, prepared as candidate vaccines for LCMV (as a model for LAS), elicited humoral immunity or protection.\textsuperscript{581}

**Therapy**

In the guinea pig model using a virulent strain (Romero) of Junin, ribavirin was administered to test its therapeutic effect. It did not protect against death, but a significant delay in time to death was noted. Ribavirin did not cross the blood-brain barrier.\textsuperscript{582} However, it was presumed that ribavirin may be amplified in human AHF therapy because humans are probably more resistant to the virus.\textsuperscript{583}

To test the effectiveness of ribavirin, up to 80 volunteers were included in a field trial against AHF. (A similar field trial was done in China of a double-blind, placebo-controlled trial of ribavirin for treatment of the Chinese variant of HFRS. Treatment would start within four days after onset of earliest clinical symptoms in up to 200 patients. Two study sites were utilized: Hubei Medical College that say primarily urban cases of the disease and Zong Chang County Hospital, that saw over 800 cases annually of the rural form of the disease.)\textsuperscript{584}

Studies using human immune plasma reduced mortality from AHF and BHF to less than two percent (from fifteen percent). However, while it protects against death during the acute stages of illness, it is complicated by risks associated with

\textsuperscript{581} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1986* (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131.

\textsuperscript{582} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1982* (Frederick, Maryland: USAMRIID), Work Unit A91C 00 132.

\textsuperscript{583} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1985* (Frederick, Maryland: USAMRIID), Work Unit A871 AD 133.

\textsuperscript{584} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1985* (Frederick, Maryland: USAMRIID), Work Unit D807 AL 020.
transfusion-borne diseases, volume overload, and a late-onset neurologic syndrome of obscure etiology observed in ten percent of treated survivors.\textsuperscript{585} Also, this form of therapy was limited to patients whose disease has progressed no further than eight days following symptom onset.

Detection

In FY1986, a rapid diagnosis assay for Junin virus was successfully field-tested in Argentina on samples stored from previous epidemics.\textsuperscript{586} A lymphocyte transformation assay was developed that predicted protection more accurately than conventional serology.\textsuperscript{587} In FY1988, a technique was developed to estimate the numbers of lymphocytes recognizing Junin viral antigen in the blood of human vaccinees.

Basic Research

In FY1982, preliminary experiments allowed partial characterization of the RNA genome.\textsuperscript{588} In that year, a cytotoxicity assay for Junin virus-infected cells using spleen cells from convalescent guinea pigs was developed and a possible model for virulent Junin infection was shown in cyclophosphamide-treated guinea pigs.\textsuperscript{589} In

\textsuperscript{585} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 134.

\textsuperscript{586} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1986} (Frederick, Maryland: USAMRIID), Work Unit D809 EA 005.

\textsuperscript{587} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1986} (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131.

\textsuperscript{588} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit BS10 AP 198.

\textsuperscript{589} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 132.
FY1982, the virus was successfully concentrated and purified and, for the first time, oligonucleotide fingerprints were obtained.\textsuperscript{590} In FY1986, a lymphocyte transformation assay was developed which predicted protection more accurately than conventional serology.\textsuperscript{591} In FY1987, it was demonstrated that the Junin vaccine protected all rhesus monkeys challenged with Machupo viru, in the absence of cross-reactive N-Ab.\textsuperscript{592}

In FY1985, it was reported that Junin virus was shown to be transmissible in SPA and exhibited a biological half-life of approximately 28 minutes.\textsuperscript{593}

\textit{Lassa Virus}

Lassa fever is a severe, often fatal disease of man originally described in 1969 in Nigeria. Fatality rates of 20-40\% were reported in hospitalized patients. Thousands of cases are believed to occur annually in regions of West Africa, particularly Liberia, Sierra Leone, and Nigeria. As of FY1979, management of Lassa infection was largely symptomatic and supportive. Specific treatment using immune serum was attempted in a small number of patients, but was equivocal. If treatment

\textsuperscript{590} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit A91C 00 134.

\textsuperscript{591} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1986} (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131.

\textsuperscript{592} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1987} (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131.

\textsuperscript{593} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1985} (Frederick, Maryland: USAMRIID), Work Unit D807 AL 020.
was delayed until after the onset of clinical signs, only the combination of ribavirin plus immune serum was effective.  

Vaccine Research

In FY1980, work on animal models showed that rhesus monkeys inoculated SC with only 12 PFU were uniformly killed by Lassa virus. Studies resulting in the death of all nine monkeys challenged with aerosols of Lassa indicated that the respiratory LD50 for Lassa was below the lowest exposure dose of $10^{2.7}$ PFU. Monkeys exposed to Junin or Machupo viruses were not significantly protected from Lassa virus challenge.

In FY1989, CDC investigators demonstrated the efficacy of a Lassa-vaccinia recombinant in guinea pigs and monkeys.

Therapy

In FY1980, experiments in strain-13 guinea pigs showed that ribavirin delivered either IM or by aerosol did not protect them from death after aerosol challenge with Lassa virus, although it did significantly extent time-to-death.

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594 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1979* (Frederick, Maryland: USAMRIID), Work Unit A841 00 009.

595 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1980* (Frederick, Maryland: USAMRIID), Work Unit A841 00 009.

596 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1980* (Frederick, Maryland: USAMRIID), Work Unit A841 00 043.

597 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1978* (Frederick, Maryland: USAMRIID), Work Unit A841 00 009.

598 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1989* (Frederick, Maryland: USAMRIID), Work Unit D807 AG 019.

599 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1980* (Frederick, Maryland: USAMRIID), Work Unit A841 00 043.
In FY1982, 385 serum samples were obtained from Liberian patients acutely ill with Lassa fever.\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit A871 BC 148.} By FY1986, collections of Lassa virus convalescent plasma was underway and minimal protective titers against Liberian and Sierra Leone strains were established.\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1986} (Frederick, Maryland: USAMRIID), Work Unit D807 AG 019.} In FY 1988, the collection of Lassa-immune plasma in Liberia was expanded and more plasma units were obtained, bringing the total inventory of high-quality, Lassa-immune plasma unites to 817. After processing, the plasma retained all expected neutralizing activity in vivo, and conferred protection to guinea pigs challenged with either Liberian or Sierra Leone Lassa strains.\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1988} (Frederick, Maryland: USAMRIID), Work Unit D807 AG 019.} In FY1989, a lot of IgG for human use was produced.\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit D807 AG 019.}

Basic Research

Experiments were underway in FY1978 to test the environmental stability of Lassa virus. It was found to retain activity after dilution in tap water.\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A841 00 009.} Also in FY1978, studies showed that the Josiah strain was the most virulent.

In FY1980, researchers demonstrated in vitro that the arenavirus Pichinde inhibits the proliferation of macrophages, a step necessary for the development of cellular immunity. This possibly represented a novel mechanism for a virus to subvert host resistance and possibly explained the immunosuppression observed in

\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit A871 BC 148.}
\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1986} (Frederick, Maryland: USAMRIID), Work Unit D807 AG 019.}
\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1988} (Frederick, Maryland: USAMRIID), Work Unit D807 AG 019.}
\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit D807 AG 019.}
\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A841 00 009.}
human arenavirus infections which occurred with no discernible morphologic damage to the cell. Inhibition was dependent on the dose and duration of exposure to the virus and was blocked by a specific antiserum to PIC.  

In FY1986, Lassa virus RNA was prepared for cloning and sequencing to facilitate construction of synthetic peptide and vaccinia-vectored Lassa virus glycoproteins for use as vaccines.

**Botulinum Toxin**

The botulinum toxins are a group of seven (A-G) related neurotoxins produced by the spore-forming bacillus, *Clostridium botulinum*, and two other *Clostridium* species. These toxins are the most potent neurotoxins known. Botulinum toxins can be delivered as an aerosolized biological weapon: the US weaponized it and there was evidence that Iraq had filled and deployed over 100 munitions with nearly 10,000 liters of botulinum toxin. Symptoms may begin as early as 12-36 hours after inhalation, but may take several days to develop after exposure to low doses of toxin. Botulinum toxins are large proteins that are easily denatured by environmental conditions.

In FY1978, work on all aspects of *C. botulinum* toxins was extended: there is almost no work reported for the botulinal toxins in the years FY1969-FY1977. In FY1979, recognition of the logistical and medical problems with immunizing an

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605 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1980* (Frederick, Maryland: USAMRIID), Work Unit A91C 00 131.

606 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1986* (Frederick, Maryland: USAMRIID), Work Unit A871 AD 131.

entire at-risk population, led to studies of the basic mechanisms of action of bacterial exotoxins to test available drugs or develop new ones with therapeutic potential.\textsuperscript{608} Botulinal toxins were just one of several studied (cholera toxin, pseudomonas exotoxin A, diphtheria toxin).

Toxoids

Animal experiments showed that botulinum antitoxin was effective against aerosolized toxin when given before the onset of clinical symptoms, but offered no protection against respiratory failure once symptoms presented.\textsuperscript{609} Several antitoxins are available: an equine antitoxin from the CDC for treatment of foodborne botulism; a bivalent intravenous antiserum (types A and B) for treating infant botulism (licensed in 2003 by the FDA); two “despeciated” equine antitoxins from USAMRIID; and a commercially prepared heptavalent antitoxin available through USAMRIID and CDC. Human data on the safety and effectiveness of the heptavalent antitoxin is not available and its use is not recommended except under extremely specialized circumstances.

An equine antitoxin was available for the treatment of botulism, but was responsible for adverse reactions in approximately 21% of recipients.\textsuperscript{610} In July 1978,
the only U.S. commercial source of equine antiserum notified the CDC that they would no longer provide the product.\textsuperscript{611}

A pentavalent toxoid to types A, B, C, D, and E is available as an IND for preexposure prophylaxis. It will likely remain an IND because efficacy testing in humans is not feasible. Immunization involves three primary shots followed by a booster at one year. Since 2001, the potency of the available vaccine seemed to be declining.\textsuperscript{612} Several thousand volunteers have received the pentavalent toxoid.

A polyvalent toxoid was prepared in 1958 by Parke, Davis, & Co., under contract to Ft. Detrick. It was made prior to purification of the neurotoxin from the hemagglutinin. It contained five antigens to types A-E neurotoxins. At the time, full knowledge of the neurotoxin was not available. Thus the preparation contained less than 10\% of the desired immunogen: the basic course to produce satisfactory antibody levels required four injections over a period of a year. Although immunogenic, this toxoid had a high reaction rate, creating a need for a new polyvalent toxoid from purified neurotoxin.\textsuperscript{613} As of the mid-1970s, only limited quantities of the toxoid were available – and then to only five (A-E) of the seven (A-G) immunologically distinct types of botulinum toxin. The CDC had responsibility

\textsuperscript{611} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A841 00 020.

\textsuperscript{612} USAMRIID, \textit{USAMRIID's Medical Management of Biological Casualties Handbook, Fifth Edition} (Fort Detrick, MD: August 2004), 86.

\textsuperscript{613} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1977} (Frederick, Maryland: USAMRIID), Work Unit 096 01 802 and US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A841 00 020.
for distribution of the Parke-Davis toxoid, and USAMRIID scientists estimated that at then-current rates, the nation’s supply would be exhausted by spring, 1982.$^{614}$

In FY1979, two lots (MDPH A-2 and MDPH B-1) of a newly bottle pentavalent botulinum toxoid was tested in 52 volunteers and compared to the Parke-Davis produced investigational pentavalent botulinum toxoid. There were no significant differences between the lots and no difference in systemic reactions (a reduction in reactogenicity had been anticipated for the new lots because they contained less formalin).$^{615}$ The immune response elicited in volunteers by both MDPH lots to type B toxin was significantly greater than the response from the Parke-Davis toxoid. As of FY1980, a total of eighty volunteers were immunized with pentavalent botulinum toxoid, adsorbed, pentavalent (ABCDE) (IND 161).$^{616}$

Because of the severe shortage of the Parke-Davis toxoid, USAMRIID responded to a request from CDC by transferring 496 vials (4,960 doses) of botulinum toxoid adsorbed pentavalent (ABCDE), MDPH Lot #A-2 to them. The MDPH toxoids were prepared by a method almost identical to that used by Parke-Davis in the 1950s. The toxoid is not stockpiled at USAMRIID or CDC: it was only a “stop gap” measure until the new and improved USAMRIID-developed neurotoxoids became available.$^{617}$

$^{614}$ US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1981 (Frederick, Maryland: USAMRIID), Work Unit A871 BA 150.

$^{615}$ US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1979 (Frederick, Maryland: USAMRIID), Work Unit A841 00 001.

$^{616}$ US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1980 (Frederick, Maryland: USAMRIID), Work Unit A841 00 001.

$^{617}$ US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1981 (Frederick, Maryland: USAMRIID), Work Unit A871 BA 150.
In FY1982, the conditions for conversion of type A neurotoxin to toxoid were determined and a monovalent type E toxoid, Lot #7007, produced by the MDPH was evaluated in human subjects.\(^{618}\) It proved to be a safe and efficacious product for the induction of substantial titers of neutralizing activity to type E botulinal toxin.\(^{619}\) It was also shown that type E neurotoxin is less potent – and its effect shorter in duration – than type A neurotoxin.

In FY1985, it was reported that botulinum type A toxoid preparations, collected during varying stages of toxin purification and toxoided by formalin treatment, were nontoxic when tested in mice and guinea pigs. Their immunogenic strengths were compared to a known immunogenic monovalent botulism type A toxoid prepared by the MDPH. As the amount of specific toxin protein was increased in the toxoid (purer), resulting titers in immunized guinea pigs showed a significant increase.\(^{620}\)

In FY1985, a solicitation was issued for the development and production of 40,000 doses of a blended product that would contain type A,B,C,D,E, and F toxoids. A second RFP was issued for developmental studies to evaluate the feasibility of producing type G botulinal toxoid and including this toxoid in a final heptavalent (ABCDEFG) product. An option to produce eight million doses of either hexavalent

\(^{618}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1982* (Frederick, Maryland: USAMRIID), Work Unit A871 BA 150.

\(^{619}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1982* (Frederick, Maryland: USAMRIID), Work Unit A871 BA 150.

\(^{620}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1985* (Frederick, Maryland: USAMRIID), Work Unit D807 AI 018.
or a heptavalent toxoid was included in the initial solicitation.\textsuperscript{621} It is unknown what resulted from these RFPs.

In FY1986, for reasons not given, a decision was made to table further development of a heptavalent toxoid.\textsuperscript{622} However work continued on evaluation of the heptavalent toxoid, with approximately 180 persons at Ft. Detrick immunized with the then-current botulinum vaccine.\textsuperscript{623} As a result of these investigations, the institute immunization policy for Botulinum Pentavalent Toxoid was changed.\textsuperscript{624}

**Challenge Studies**

Several conclusions were made in FY1978 as the efficacy of homologous and heterologous (equine) antitoxin in preventing type A botulism in guinea pigs: (a) homologous and heterologous antitoxin are similarly efficacious when administered 2 or 24 hours before, or 2 hours after exposure to lethal neurotoxin; (b) administration of either antitoxin 24 hours before challenge is efficaciously superior to similar doses of antitoxin administered 18 hours after challenge; (c) 14 days after passive immunization, homologous antitoxin is protective and immunologically active at a concentration that is at least 10-fold greater than that of heterologous antitoxin (so the effective half-life of homologous antitoxin is much longer than that of heterologous

\textsuperscript{621} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1985* (Frederick, Maryland: USAMRIID), Work Unit D807 AI 018.

\textsuperscript{622} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1986* (Frederick, Maryland: USAMRIID), Work Unit D807 AI 018.

\textsuperscript{623} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1987* (Frederick, Maryland: USAMRIID), Work Unit D807 AI 018.

\textsuperscript{624} US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1988* (Frederick, Maryland: USAMRIID), Work Unit D807 AI 018.
antitoxin); (d) passive immunization with less than optimal protective amounts of antitoxin can prolong survival time by a factor of at least 2.\textsuperscript{625}

In FY1982, because considerable progress was made in determining the best conditions for the production of highly effective botulinal immunogens, studies were initiated to test the efficacy of current and newly developed botulinal toxoids against an aerosol toxin challenge.\textsuperscript{626}

Toxin Production

Efforts began in FY1978 to purify the neurotoxins of \textit{C. botulinum} types A-G for development of a polyvalent toxoid. In that year, milligram amounts of \textit{C. botulinum} neurotoxin type A were produced and toxoided. When adsorbed to aluminum hydroxide, a satisfactory immunogen for development of antibodies was produced.\textsuperscript{627} Production of the type A toxin in a 50-liter fermenter under various conditions was examined and found to be much quicker than by static cultures of the organism. The Hall strain of \textit{C. botulinum} was found to produce an increased quantity of type A toxin per ml.

In FY1979, new improvements led to a yield of 300 mg of toxin in a single fermentation run, the largest batch obtained. The purification procedures used at USAMRIID appeared in a scientific journal, \textit{Methods in Enzymology}: the authors

\textsuperscript{625} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A841 00 020.

\textsuperscript{626} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit A871 BA 150.

\textsuperscript{627} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1978} (Frederick, Maryland: USAMRIID), Work Unit A841 00 020.
acknowledged that they learned the procedures while on a three-day visit to USAMRIID several years prior.628

In FY1980, the methods for production of type A toxin were adapted to produce increased quantities of type B toxin. The Okra strain produced a concentration of 1 million medial lethal doses/ml in 24 hours in a 50-liter fermenter under optimal conditions.629

In FY1981, the technology was developed for the fermenter-system production of type E botulinal neurotoxin.630 This was the first time type E was cultivated in a fermenter system. Unlike type A toxin, for type E neurotoxin to attain maximum toxicity, it needed to be activated and a method for doing so was established.631

By FY1985, a new and simpler method for the purification of type E neurotoxin was achieved. 30mg of type E neurotoxin had been purified and stored and 35 mg of purified type B neurotoxin had been produced using the rapid and efficient procedure developed for type E neurotoxin.632

628 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1979* (Frederick, Maryland: USAMRIID), Work Unit A841 00 030.

629 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1980* (Frederick, Maryland: USAMRIID), Work Unit A841 00 072.

630 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1981* (Frederick, Maryland: USAMRIID), Work Unit A871 BA 150.

631 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1981* (Frederick, Maryland: USAMRIID), Work Unit A871 BA 150.

632 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1985* (Frederick, Maryland: USAMRIID), Work Unit BS12 AA 001.
As of June 1988, supplies of purified botulinum neurotoxins were as follows: Serotype A (36mg purified; 3mg used, 33mg on hand); Serotype B (112mg purified; 21mg used, 91mg on hand); Serotype C (41mg purified, 7mg used, 34mg on hand).633

Botulinum Immune Plasma (Equine and Human)

In FY1981, the army-owned horse, “First Flight” was hyperimmunized by the use of botulinal toxoids to all common types (ABCDEFG) and subsequently with the homologous botulinal toxins. He was transported and housed and the University of Minnesota. From this horse, over 200 liters of Heptavalent Botulism Immune Plasma (equine) were collected. From this 350 ml of immunoglobulin of IV quality was prepared with substantial neutralizing activity for all seven botulinal toxin types and became available for emergency use.634

Another project was underway as of FY1980 to collect human botulism immune plasma. As of FY1980, this program had yielded over 1200 liters of Botulism Immune Plasma (Human) (IND #1332).635 By FY1981, 2000 liters of plasma (human) was collected.636

633 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1988 (Frederick, Maryland: USAMRIID), Work Unit BS12 AA 001.
634 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1981 (Frederick, Maryland: USAMRIID), Work Unit A871 BA 150.
635 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1980 (Frederick, Maryland: USAMRIID), Work Unit A841 00 001.
636 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1981 (Frederick, Maryland: USAMRIID), Work Unit A871 BA 150.
In FY1982, 500 liters of human-derived botulism immune plasma were converted to botulism immune globulin and methods to fractionate and despeciate heptavalent (ABCDEFG) Botulism Immune Plasma (Equine) were evaluated.\textsuperscript{637}

Immunology and Therapy

Efforts to treat SEB toxemia involved the use of hemoperfusion of activated charcoal, bicarbonate-induced alkalosis or tannic acid. Neither tannic acid (5mg/kh) nor bicarbonate-induced alkalosis (blood pH 7.5-7.6) provided any protection against SEB in Dutch rabbits.\textsuperscript{638} However, 3,4-Diaminopyridine prolonged survival of mice poisoned with a lethal dose of type A botulinum toxin.\textsuperscript{639} (Because equine antitoxins neutralize free toxin, but are unable to reverse the toxin-induced blockage of acetylcholine release that occurs within poisoned nerves, the aminopyridines were studied because they can enhance acetylcholine release from botulinum-poisoned nerve terminals.)\textsuperscript{640} In FY1981, 4-aminopyridine was also found to be effective in antagonizing the blockage of transmitter release resulting from botulinal poisoning.\textsuperscript{641}

By FY1982, the compound 3,4-Diaminopyridine (3,4-DAP) was undergoing the pilot drug stage of development as part of the broader effort to develop a

\textsuperscript{637} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit A871 BA 150.

\textsuperscript{638} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1980} (Frederick, Maryland: USAMRIID), Work Unit A841 00 030.

\textsuperscript{639} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1980} (Frederick, Maryland: USAMRIID), Work Unit A841 00 071.

\textsuperscript{640} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1980} (Frederick, Maryland: USAMRIID), Work Unit A841 00 071.

\textsuperscript{641} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1981} (Frederick, Maryland: USAMRIID), Work Unit A841 BA 150.
chemotherapeutic approach to the treatment of botulism.\textsuperscript{642} In FY1985, studies showed that 3,4-DAP significantly prolonged the survival of mice poisoned with type A toxin, but was not effective in the treatment of mice poisoned with type B, E, or F toxin. Also, while survival was prolonged for type A toxin, it did not alter the final outcome: once treatment was stopped, the mice still died from the intoxication.\textsuperscript{643}

In FY1981, two efforts were underway to understand the structure and mechanism of action of toxins with proven BW potential. One employed the diphtheria toxin model.\textsuperscript{644} The other involved in vivo studies with botulinum toxin made possible by the development of two new cell lines (NG 108-15 and PC-12) that synthesize acetylcholine and release the neurotransmitter in response to pharmacologic or electrical stimuli.\textsuperscript{645}

In FY1985, studies were underway to develop synthetic peptide vaccines. Peptides were synthesized from a known amino acid sequence unique to type A, B, or E neurotoxin, coupled to carrier molecules and used to immunize rabbits and the sera then evaluated.\textsuperscript{646} While the animals produced high antibody titers, the antibody

\textsuperscript{642} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit A871 BA 150.

\textsuperscript{643} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1985} (Frederick, Maryland: USAMRIID), Work Unit A871 AB 150.

\textsuperscript{644} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1981} (Frederick, Maryland: USAMRIID), Work Unit BS10 AN 200.

\textsuperscript{645} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1981} (Frederick, Maryland: USAMRIID), Work Unit BS10 AN 200.

\textsuperscript{646} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1985} (Frederick, Maryland: USAMRIID), Work Unit A871 AB 150.
neutralized only a small amount of toxins. Therefore, those particular peptides had limited potential as candidate vaccines and therefore others were being evaluated.\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1986} (Frederick, Maryland: USAMRIID), Work Unit BS12 AA 001.}

In FY1990, the efficacy of anti-BOT IgG and anti-BOT Fab fragments were tested for their ability to protect non-human primates from botulinum toxin given by aerosol.\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1986} (Frederick, Maryland: USAMRIID), Work Unit BS12 AA 001.}

Detection

In FY1980, a rapid technique for the detection of type A toxin in various fluids was refined.\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1980} (Frederick, Maryland: USAMRIID), Work Unit A841 00 071.} However, while the mouse bioassay was extremely sensitive, a new method of detecting and assaying nanogram quantities of botulinal toxin was desired because that assay was both time consuming and involved cumbersome procedures.\footnote{US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit A871 BA 150.}

\textbf{T-2Mycotoxins}

USAMRIID’s interest in T-2 mycotoxins began after allegations of “yellow rain” incidents in Laos (1975-1981), Kampuchea (1979-1981), and Afghanistan (1979-1981), that resulted in 6,300 deaths, 1000 deaths, and 3,042 deaths in each respective case. Confirmation of the attacks has been difficult and controversial. There is no specific antidote and no specific immunotherapy or chemotherapy. The
only defense is to prevent exposure by wearing a protective mask and clothing during an attack. Simply washing within an hour of exposure may entirely prevent toxicity. The T-2 mycotoxins are small-molecular-weight compounds, extremely stable in the environment, and dermally active.

Work at on the mycotoxins began at USAMRIID in FY1981. A threat analysis study by the U.S. Army Medical Intelligence Agency determined that certain of the small nonprotein toxins were potential BW agents. To help in the development of medical defense against these toxins, it was necessary to develop a program for safe handling and decontamination; rapid detection and identification; determination of the molecular mechanism of action; elucidation of the pathogenesis and physiological aberrations; development of methods of prevention, diagnosis and therapy; and evaluation of potential aerosol threat from these small nonprotein toxins of BW importance. A dozen or more small nonprotein biological toxins were potential BW agents. However, because of limitations in personnel and resources, it was decided to carry out initial studies on T-2 mycotoxin and two marine toxins - saxitoxin and tetrodotoxin.

In FY1987, a decision was made to phase down work on trichothecene mycotoxins; the technology developed was transferred for studies of other small-sized toxins that were potential biowarfare agents, marine toxins (saxitoxin and

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652 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1981* (Frederick, Maryland: USAMRIID), Work Unit BS10 AQ 197.
brevetoxin); blue-green algal toxins microcystin and anatoxin-a; and coral toxins (palytoxin).653

Pathogenesis

The first experiments involved the systematic study of the measure of toxicity of T-2 toxin by various routes of administration.654 A year later, studies suggested a plasma membrane receptor or transport system for T-2.655

By FY1982, a researcher at USAMRIID concluded that, contrary to the literature, T-2 does not directly inhibit DNA synthesis. Also, cross-reaction studies concluded that the development of agents that would interfere with the binding step would be attractive since protection from several of the trichothecenes should result.656

By FY1982, a SOP for liquid and powder samples of T-2 was established. In general, it was found that trichothecenes are stable compounds that can be kept at room temperature in the dry state for a long time. T-2 toxin is not inactivated by autoclaving, but completely neutralized in temperatures in excess of 500 degrees F.657

Mice died between 12-24 hours after a subcutaneous injection of T-2 toxin of one LD50 (2.01 mg/kg body weight). T-2 caused marked destruction and depletion of rapidly dividing cells of the host, coagulation abnormalities, alterations in many

653 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1987 (Frederick, Maryland: USAMRIID), Work Unit A871 AF 135.

654 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1981 (Frederick, Maryland: USAMRIID), Work Unit BS10 AQ 197.

655 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1982 (Frederick, Maryland: USAMRIID), Work Unit BS10 AN 200.

656 Ibid.

657 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1982 (Frederick, Maryland: USAMRIID), Work Unit BS10 AQ 197.
metabolic pathways, and decreased protein synthesis in cellular proliferation. These effects could lead to a shock-like syndrome and might be the eventual cause of death. However, in FY1985, in vitro studies on inhibition of protein synthesis did not correlate with data from mouse lethality studies, raising doubts as to whether protein synthesis is the major mechanism of action.

Other dose-response studies showed that higher doses of T-2 resulted in an increased efficiency of penetration and that the administration route could markedly influence the toxicity in the mouse lethality assay.

In FY1985, studies of the molecular events leading to T-2 toxicity in cell cultures was successful in identifying the transport system as the major factor determining a tricothecene’s potency. Researchers quantified many aspects of T-2 binding to isolated ribosomes and found that virtually all tricothecenes bind to the same site on the ribosome as T-2 does. Because of this, protection at this stage of action would be expected to cross-protect from a large number of tricothecene toxins.

In FY1987, it was found that the macrocycline trichothecenes were much more slowly metabolized and excreted than the T-2 toxins. A new class of such toxins (mycotoxin B) was found to be 100 times more toxic than T-2 in the mouse bioassay and twenty times more potent as a skin irritant.

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658 Ibid.
659 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1985 (Frederick, Maryland: USAMRIID), Work Unit BS12 AD 004.
660 Ibid.
661 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1985 (Frederick, Maryland: USAMRIID), Work Unit A91C 00 136.
662 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1987 (Frederick, Maryland: USAMRIID), Work Unit A871 AF 135.
Challenge Tests

In FY1982, propylene glycol was used as a solvent for T-2 toxin, allowing ready aerosolization with an average particle size of three microns. In rats exposed to these T-2 aerosols, inhalation was not a markedly more lethal means of delivering T-2 than systemic exposure.663

In FY1985, aerosol challenge tests were done with Diacetoxyscirpenol (DAS), a mycotoxin found in close association with T-2 toxin. Like T-2, it was only slightly soluble in water, but soluble in ethanol (ETOH) and dimethylsulfoxide (DMSO). When aerosolized, both solutions produced droplets with an average size of less than a microgram. When rats were exposed to aerosols of DAS-ETOH, all succumbed, while 50% died when exposed to DAS-DMSO.664

In FY1986, aerosol tests were completed in mice and rats exposed for ten minutes to an aerosol of various concentrations of T-2 mycotoxin. T-2 was ten- to fifty-times more potent when inhaled as compared to systemic exposure. Researchers concluded that the aerosol toxicity of T-2 mycotoxin is equivalent to that for nerve agents and a log more toxic than aerosolized mustard.665 When these tests were extended to the guinea pig model, T-2 was found to be twice as toxic when inhaled as compared to the intravenous route.666

663 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1982 (Frederick, Maryland: USAMRIID), Work Unit BS10 AQ 197.

664 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1985 (Frederick, Maryland: USAMRIID), Work Unit A871 AJ 138.

665 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1986 (Frederick, Maryland: USAMRIID), Work Unit A871 AF 135.

666 US Army Medical Research Institute of Infectious Diseases, Annual Progress Report: FY 1987 (Frederick, Maryland: USAMRIID), Work Unit A871 AF 135.
Prophylaxis and Therapy

In FY1982, two radioprotective compounds, glutathione and cysteamine, were screened for protective effect, but increased rather than decreased the toxicity of T-2 toxin in mice.\textsuperscript{667} Several classes of drugs seemed to be effective in reducing the lethality of the toxin and attempts were underway to generate a vaccine by FY1985.\textsuperscript{668}

In FY1985, charcoal, dexamethasone, and glutathione prodrugs, as well as soap and water decontamination were shown to be successful for combating tricothecene exposure. In addition, anti-T-2 antibodies were shown to be effective in vivo, even post-exposure.\textsuperscript{669} When T-2 was given orally to animals, followed immediately with activated charcoal, dexamethasone was 100% effective in preventing T-2 intoxication.\textsuperscript{670}

In FY1988, Emetine was found to block the binding of T-2 to target cells and its molecular mechanism of action defined.\textsuperscript{671}

Detection

In FY1985, an ELISA was developed that could detect T-2 or its metabolite in organs of exposed animals.\textsuperscript{672} Reagents were being generated for production of test

\textsuperscript{667} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1982} (Frederick, Maryland: USAMRIID), Work Unit BS10 AQ 197.

\textsuperscript{668} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1985} (Frederick, Maryland: USAMRIID), Work Unit A871 AJ 138.

\textsuperscript{669} Ibid.

\textsuperscript{670} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1986} (Frederick, Maryland: USAMRIID), Work Unit D807 AK 022.

\textsuperscript{671} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1988} (Frederick, Maryland: USAMRIID), Work Unit BS12 AA 001.
kits. As of FY1987, the methodology was developed for the immunodetection of the major urinary metabolites of T-2 (H-2 and tetraol). These products were detected in rats and monkeys after IV and oral exposure using urine samples obtained within two to three days of exposure. High-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) were both used to trace the metabolism of T-2 toxin in monkeys and allowed detection of T-2 at 100 picograms.

*Staphylococcal Enterotoxin B (SEB)*

SEB is one of seven antigenically distinct enterotoxin produced by the coagulase- positive *S. aureus*. SEB is intoxicating by the aerosol route after a 3-12 hour latency period. Exposure to high levels of SEB can result in toxic shock and death. Because of its ability to incapacitate large numbers of people for 1-2 weeks, it was stockpiled by the US BW program. When ingested or swallowed, SEB provokes profound gastrointestinal symptoms. There is no human vaccine available to prevent SEB intoxication. Immunity acquired through natural exposure does not provide complete protection from an aerosol challenge. Work on SEB at USAMRIID

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672 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1985* (Frederick, Maryland: USAMRIID), Work Unit D807 AK 022.


674 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1987* (Frederick, Maryland: USAMRIID), Work Unit D807 AK 022.

675 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1987* (Frederick, Maryland: USAMRIID), Work Unit A871 AA 130.

encompassed several work units each year from FY1969-FY1982, after which research on SEB diminished significantly.

Toxoid Studies

An SEB toxoid in use in FY1969 retained immunogenicity for at least 18 months when stored at 4C; vaccinated monkeys showed no significant decrease in circulating antibody or in protection against challenge throughout the course of one year.\(^677\) Rhesus monkeys given the toxoid were partially protected against illness and lethal effects produced by SEB toxin given by the aerosol and IV routes. It had a safety margin at least ten times the proposed vaccination dose.\(^678\) Five production lots of SEB toxoid was produced by Pfizer for USAMRIID, but three were found unsatisfactory. From the remaining batches, the optimizing immunization schedule was found to be two doses of 50 micrograms antibody N 28 days apart.\(^679\)

Work on a polyvalent toxoid that included \textit{S. aureus} enterotoxins A, B, C, and D and other exoproteins was underway in FY1970. Cultural and toxoiding conditions were determined for organism 10-275, a potent producer of alpha hemolysin and B toxin, grown in a 10-liter fermenter.\(^680\) A polyvalent toxoid was prepared in FY1970

\(^{677}\) US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1969} (Frederick, Maryland: USAMRIID), Work Unit 096 03 800.

\(^{678}\) US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1969} (Frederick, Maryland: USAMRIID), Work Unit 096 01 801.

\(^{679}\) US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1970} (Frederick, Maryland: USAMRIID), Work Unit 096 02 007.

\(^{680}\) US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1970} (Frederick, Maryland: USAMRIID), Work Unit 096 02 800.
utilizing purified enterotoxins A, B, and C protected monkeys 33-100% after oral challenge with 40-400 micrograms/kg of purified exotoxins.  

Toxin Preparation

In FY1972, gram amounts of enterotoxins SEA, SEB, and SEC were prepared. The SEA previously used had an oral median illness dose (ID-50) of 40 micrograms/kg. A procedure developed by Schantz resulted in a highly purified enterotoxin A with an oral ID-50 of 4 micrograms/kg. A SED toxin was prepared and purified, too.  

In FY 1975, the conditions for large scale production of _S. aureus_ exfoliative toxin was developed. This included optimal fermentor conditions for growth in a 50-liter vessel, centrifugation, and concentration, purification, and lyophilization methods. In addition, a procedure was developed for enhancing enterotoxin B production of _S. aureus_: by this method one strain increased its enterotoxin production 100-fold. This method of isolating carbohydrate-negative mutants from selected membrane mutants was believed to be a general method for enhancing enterotoxin production starting with any wild type of _S. auereus_.

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681 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1971* (Frederick, Maryland: USAMRIID), Work Unit 096 02 800.  
682 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1972* (Frederick, Maryland: USAMRIID), Work Unit 096 02 800.  
683 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1975* (Frederick, Maryland: USAMRIID), Work Unit 096 01 802.  
684 US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1975* (Frederick, Maryland: USAMRIID), Work Unit 096 01 804.
Pathogenesis

In FY1969 and FY1970, studies were still underway to understand the mechanism of action of the toxin. At high concentrations, it seemed that SEB toxin acted directly on macrophages, but at lower doses, it inhibited macrophage migration, due in part to SEB stimulation of lymphocytes to produce migration inhibition factor.\(^6\)^8\(^5\)

In FY1978, monkeys were given a lethal dose of SEB (50 or 100 micrograms/kg), with hemoperfusion started at 15 or 60 minutes after SEB inoculation and continued for six hours. (25 micrograms/kg IV is usually sufficient to cause death). Monkeys receiving the lower dose survived, but those receiving the higher dose died. The results suggested that once SEB is introduced into the circulatory system, it is difficult to restrict the deteriorating processes which ensue. The only way to diminish SEB toxicity is to remove the toxin from circulation.\(^6\)^8\(^6\)

Detection

A solid phase RIA system was developed in FY1971 to assay SEB in body fluids, broths, and purified forms. The method was satisfactory for detecting as little as 0.01 micrograms per milliliter of enterotoxins A, B, and C.\(^6\)^8\(^7\)

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\(^{685}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1969* (Frederick, Maryland: USAMRIID), Work Unit 096 03 006 and US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1970* (Frederick, Maryland: USAMRIID), Work Unit 096 03 006.

\(^{686}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1978* (Frederick, Maryland: USAMRIID), Work Unit A841 00 030.

\(^{687}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 19871* (Frederick, Maryland: USAMRIID), Work Unit 096 03 801.
Basic Research

In FY1976, it was found – as had been reported for endotoxins – that total-body irradiation prolonged survival and increased percentage survival in animals inoculated with lethal doses of SEB.\(^{688}\) The mechanism of protection was hypothesized to involve the reduction of circulating leukocytes as an effect of x-irradiation: since SEB molecules bind to leukocytes, a reduction of circulating leukocytes results in less SEB transported to the lungs where it would otherwise cause pulmonary capillary damage and eventual edema.\(^{689}\)

In other studies, the role of myocardial depressant factor (MDF) was studied to understand its role in the pathogenesis of SEB shock and sever infections.\(^{690}\)

*Ricin*

Work on ricin toxin was initiated in FY1989 at USAMRIID. Ricin is a lethal toxin when inhaled. There is no vaccine or prophylactic antitoxin currently available. Ricin was used in the assassination of the Georgii Markov in 1978 and several other attempts were been made to use ricin powder (as recently as 2004). It is considered a terrorist threat because it is relatively easy to produce and use, but such large

\(^{688}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1976* (Frederick, Maryland: USAMRIID), Work Unit 096 01 010.

\(^{689}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1980* (Frederick, Maryland: USAMRIID), Work Unit A841 00 030.

\(^{690}\) US Army Medical Research Institute of Infectious Diseases, *Annual Progress Report: FY 1976* (Frederick, Maryland: USAMRIID), Work Unit 096 01 015 and 096 01 112.
quantities (tons) would be required to cover a battlefield, that it is not considered a likely weapon for large-scale use.\textsuperscript{691}

In FY1989, two lots of ricin from different suppliers were evaluated in in vivo and in vitro models. The LD50 for mice was approximated at 3-4 micrograms/kg and the 50\% inhibition of protein synthesis in Vero cells was approximately 100 pg/ml for both lots. The biological activity of the isolated A and B chains of ricin were evaluated in an attempt to find toxoid-vaccine candidates.\textsuperscript{692} Also in that year, an ELISA for ricin was developed.\textsuperscript{693}

In FY1990, active and passive immunization protected mice from the lethal effects of aerosolized ricin, but pulmonary lesions were still seen.\textsuperscript{694} To protect against the lethal effects of an inhaled pathogen or toxin, attempts were made in FY1990 to protect the respiratory mucosa through the secretion of antigen-specific immunoglobulins (Ig) of the IgA isotype. IgA binds to the inhaled pathogen or toxin and may be able to neutralize any direct effect (inflammatory and/or necrotizing) on the mucosal tissue or impair transport of the antigen across the mucosal surfaces to the systemic circulation.\textsuperscript{695} When inhaled, ricin produces a severe diffuse necrosis of respiratory epithelium: this effect alone can cause death by asphyxiation from


\textsuperscript{692} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit A871 AF 135.

\textsuperscript{693} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1989} (Frederick, Maryland: USAMRIID), Work Unit D807 AK 022.

\textsuperscript{694} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1990} (Frederick, Maryland: USAMRIID), Work Unit A871 AF 135.

\textsuperscript{695} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1990} (Frederick, Maryland: USAMRIID), Work Unit BS12 AA 001.
impaired gas exchange. Therefore, although a humoral immune response (IgG) was elicited in mice responding to systemic ricin, it was not sufficient because it did not protect against the necrotizing effects.\textsuperscript{696}

Mice vaccinated with ricin in Freund’s adjuvant or treated with goat antibody survived a lethal challenge and there was ricin-specific IgA in bronchial lavage from immunized mice before and after aerosol ricin challenge. The ricin toxoid was not toxic at 1,000 times the lethal ricin dose and vaccine studies showed it to be an effective immunogen without adjuvant. A bivalent vaccine was developed consisting of whole ricin and saxitoxin that reacted with anti-saxitoxin and anti-ricin antibodies.\textsuperscript{697}

In chemotherapeutic studies, no drugs were found by FY1990 that provided protection against ricin.

\textsuperscript{696} Ibid.

\textsuperscript{697} US Army Medical Research Institute of Infectious Diseases, \textit{Annual Progress Report: FY 1990} (Frederick, Maryland: USAMRIID), Work Unit D807 AK 022.
### Appendix B: USAMRIID FY1969-FY1990 Pathogenesis Studies

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<td>A841-00-069</td>
<td>Growth hormone and infection</td>
<td>Dec-79</td>
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<td>Bunner, DL</td>
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<td>BS03-00-15</td>
<td>Effects of infection/intoxication upon structure and function of cellular membrane</td>
<td>Oct-76</td>
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<td>Little, JS</td>
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<td>BS12-AF-005</td>
<td>Mamalian Peptides</td>
<td>Oct-84</td>
<td>Oct-85</td>
<td>Saviolakis, GA</td>
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<td>A871-AP-142</td>
<td>Defense.</td>
<td>Apr-85</td>
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<td>Higbee, GA</td>
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<td>BACTERIA</td>
<td>Subcellular biological effects of militarily important microbial diseases and toxins</td>
<td>Jul-60</td>
<td>Oct-77</td>
<td>Canonico, PJ</td>
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<td>096-01-803/BS03-00-10</td>
<td>Capillary Ultrastructure in Bacterial Infections of Military Medical Importance</td>
<td>Dec-70</td>
<td>July-73</td>
<td>Hudson, TH</td>
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<td>096-01-105</td>
<td>Effect of infectious disease on cellular respiration</td>
<td>Aug-72</td>
<td>July-74</td>
<td>Neufeld, HA</td>
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<td>096-01-108</td>
<td>Nucleic acid metabolism in infectious bacterial disease of military medical importance</td>
<td>Nov-71</td>
<td>July-74</td>
<td>Earp, HS</td>
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<td>096-02-104</td>
<td>Mechanisms of complement activation in infectious diseases</td>
<td>Oct-72</td>
<td>Jul-75</td>
<td>Fine, DP</td>
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<td>096-02-108</td>
<td>Characterization of host protease inhibitor responses in infectious disease.</td>
<td>Mar-74</td>
<td>Jul-75</td>
<td>Beringer, RW</td>
<td>$126,000</td>
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<td>096-01-108</td>
<td>Bactericidal ability of phagocytes in nonspecific host resistance.</td>
<td>Oct-72</td>
<td>July-74</td>
<td>Janssen, WA</td>
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<td>A91C-00-144</td>
<td>Mechanisms and determinants of microbial pathogenicity</td>
<td>Oct-77</td>
<td>Oct-80</td>
<td>Canonico, PG</td>
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<td>BS10-AO-199</td>
<td>Bacterial and Rickettsial Diseases of Potential BW Importance</td>
<td>Oct-80</td>
<td>Oct-82</td>
<td>Hedlund, KW</td>
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<td>A91C-00-133</td>
<td>Role of Anthrax Toxin Components in Virulence of B. anthracis</td>
<td>Jun-81</td>
<td>Oct-82</td>
<td>Leppla, SH</td>
<td>$353,000</td>
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<td>A871-AC-132</td>
<td>Studies to characterize recycling and modulation of the macropage anthrax toxin receptor; dlpmnt of a novel propionic acid technique.</td>
<td>Oct-83</td>
<td>Oct-85</td>
<td>Leppla, SH</td>
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<td>A91C-00-132</td>
<td>Genetic analysis of factors associated with virulence and vaccine resistance of Bacillus anthracis.</td>
<td>Oct-89</td>
<td>Oct-90</td>
<td>Novak, JM</td>
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<td>A91C-LA-133</td>
<td>Pathogenesis of hemorrhagic vascular lesions induced by nonindigenous rickettsiae /</td>
<td>Jan-90</td>
<td>Oct-90</td>
<td>Welkos, SL</td>
<td>$93,000</td>
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<td>096-01-300/</td>
<td>Pathogenesis of the vascular lesions of RMSF in the rhesus monkey</td>
<td>Mar-76</td>
<td>Jul-79</td>
<td>Moe, JB; Hall, WC; Ruch, GL;</td>
<td>$296,000</td>
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<td>A841-00-049</td>
<td>Histopathology of RMSF in the rhesus monkey</td>
<td>Oct-72</td>
<td>Jul-76</td>
<td>Sammons, LS</td>
<td>$491,600</td>
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<td>096-01-301</td>
<td>Rickettsial Diseases of Potential Biological Warfare Importance</td>
<td>Nov-72</td>
<td>Jul-74</td>
<td>Moe, JB</td>
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<td>A871-AN-143</td>
<td>Warroad and other bacterial and rickettsial diseases</td>
<td>Oct-84</td>
<td>Oct-85</td>
<td>Stephenson, E</td>
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<td>Arboviruses</td>
<td>Induced metabolic sequelae as diagnostic, prognostic and therapeutic indices of infections of unique concern to military medicine / Effect of bacterial and viral infections on host cell biosynthetic mechanisms.</td>
<td>Oct-62</td>
<td>Jul-77</td>
<td>Powanda, MC</td>
<td>$765,700</td>
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<td>096-01-401/</td>
<td>Host-parasite relationships in arbovirus infections</td>
<td>Jan-61</td>
<td>July-74</td>
<td>Spertzel, RO</td>
<td>$172,600</td>
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<td>BS03-00-009</td>
<td>Effect of ionizing radiation on progression and immune defenses against infectious disease of potential BW importance.</td>
<td>Jan-67</td>
<td>Oct-78</td>
<td>Spertzel, RO</td>
<td>$898,400</td>
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<td>A841-00-008</td>
<td>Mouse Brain Ultrastructure in Viral Infections of Military Medical Significance.</td>
<td>Dec-70</td>
<td>July-75</td>
<td>Evans, H; Gorelkin, L.</td>
<td>$238,600</td>
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<td>096-01-404</td>
<td>Lymphoid tissue ultrastructure in viral infections of military importance.</td>
<td>Jul-71</td>
<td>July-73</td>
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<td>096-01-406/</td>
<td>Analysis of subcellular structures in microbial infections of potential BW importance</td>
<td>Jul-72</td>
<td>Jul-80</td>
<td>White, JD</td>
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<td>A841-00-051</td>
<td>Pathogenesis of fetal deaths in viral infection</td>
<td>Oct-72</td>
<td>Jul-73</td>
<td>Terrell, TG</td>
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<td>096-01-409</td>
<td>Physiologic mechanisms of phagocytosis in host resistance</td>
<td>Jul-72</td>
<td>July-74</td>
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<td>096-01-106</td>
<td>VEE immunopathology in mice</td>
<td>Sep-73</td>
<td>Jul-74</td>
<td>Woodman, DR</td>
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<td>A841-00-016</td>
<td>Interaction of viruses with peripheral host leukocytes as an index of immunity against infections of BW importance.</td>
<td>Aug-76</td>
<td>Oct-79</td>
<td>Levitt, NH</td>
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<td>A841-00-019</td>
<td>Differentiation and specification of virulence of the North and South American encephalitic alphaviruses.</td>
<td>Dec-76</td>
<td>Oct-77</td>
<td>Walder, Raul</td>
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<td>A91C-00-131</td>
<td>Rift Valley fever virus infection: genetic and cellular aspects</td>
<td>Aug-78</td>
<td>Oct-80</td>
<td>Peters, CJ</td>
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<td>A91C-00-143</td>
<td>Antigenic Analysis of Phlebotomus Fever Group Virus Components</td>
<td>Jan-81</td>
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<td>Dalrymple, JM</td>
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<td>A870-BA-070</td>
<td>Risk Assessment and Evaluation of Viral Agents and Their Vectors that Pose a Potential BW Threat</td>
<td>Oct-80</td>
<td>Oct-82</td>
<td>Bailey, CL</td>
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<td>A871-AM-141</td>
<td>Laboratory Conditions Biology of Viral Agents of Potential BW Importance</td>
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<td>Bailey, CL</td>
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<td>BS10-AP-198</td>
<td>FY85: Biology of Viral Agents of Potential BW Importance / FY86: Basic Studies Seeking Generic Medical Countermeasures Against Agents of Biological Origin</td>
<td>Oct-80</td>
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<td>Dalrymple, JM</td>
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<td>BS12-AC-003</td>
<td>Exploratory Dvlpmnt Studies of Conventional Agents of Biological Origin for Dvlpmnt of Medical Defensive Countermeasures</td>
<td>Oct-84</td>
<td>Oct-90</td>
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<td>A91C-LA-141</td>
<td>FY85: Biology of Viral Agents of Potential BW Importance FY86: Basic Studies Seeking Generic Medical Countermeasures Against Agents of Biological Origin</td>
<td>Apr-85</td>
<td>Oct-87</td>
<td>Dalrymple, JM</td>
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<td>A91C-LA-138</td>
<td>Development of attention and subunit Phlebovirus vaccines based on the function and immunogenicity of the non-structural viral proteins.</td>
<td>Jan-90</td>
<td>Oct-90</td>
<td>Smith, JF</td>
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<td>A841-00-018</td>
<td>Arenavirus defective-interfering particles and their characterization as potential vaccine candidates.</td>
<td>Dec-76</td>
<td>Oct-77</td>
<td>Luscri, BJ</td>
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<td>BS03-00-034</td>
<td>Mechanism of viral binding to its cell surface receptor and internalization.</td>
<td>Jul-80</td>
<td>Oct-80</td>
<td>Huggins, JW</td>
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<td>A91C-00-131</td>
<td>Role of Macrophage Proliferation and Activation in the Control of Viral Infections</td>
<td>May-81</td>
<td>Oct-82</td>
<td>Friedlander, A</td>
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<td>A91C-00-132</td>
<td>Role of T-Cells in Pathogenesis of Argentine Hemorrhagic Fever</td>
<td>Jun-81</td>
<td>Oct-82</td>
<td>Kenyon, RH</td>
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<td>A91C-00-133</td>
<td>Immunopathogenesis of Junin Virus infection in Guinea Pigs and Humans</td>
<td>Apr-82</td>
<td>Oct-82</td>
<td>McKee, Jr, KT</td>
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<td>A91C-00-134</td>
<td>Other Bunyaviridae (HF Viruses)</td>
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<td>A871-AL-140</td>
<td>Exploratory Research for Protection Against Hantaan Virus</td>
<td>Oct-83</td>
<td>Oct-85</td>
<td>Dalrymple, JM</td>
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<td>A91C-00-132</td>
<td>Medical Defensive Studies on Crimean-Congo Hemorrhagic Fever Virus</td>
<td>Dec-85</td>
<td>Oct-87</td>
<td>Watts, DM</td>
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<td>A91C-00-139</td>
<td>In vitro effects of Hemorrhagic Fever Viruses on Endothelial cells</td>
<td>Dec-84</td>
<td>Oct-90</td>
<td>Lewis, RM, Schmaljohn, C</td>
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<td>A841-00-068</td>
<td>Ebola virus infection</td>
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<td>A871-AE-134</td>
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<td>Johnson, ED</td>
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<td>096-01-012</td>
<td>Biophysical studies of bacterial toxins and other inert molecules</td>
<td>Jul-61</td>
<td>July-74</td>
<td>Buzzell, A, Rhoda, DA, Denniston, J, Kastello, MD, Elwell, MR</td>
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<td>096-01-800</td>
<td>Biological Effects of Microbial Toxins</td>
<td>Oct-66</td>
<td>July-75</td>
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<td>$430,000</td>
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<td>096-01-801/</td>
<td>Radioimmunoassay techniques as diagnostic and prognostic indicators and their use during therapy of infectious diseases.</td>
<td>Jan-73</td>
<td>Oct-77</td>
<td>Auerbach, D, Jung, AC,</td>
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<td>A841-00-022</td>
<td>Cellular membrane alterations: staphylococcal enterotoxin production</td>
<td>July-74</td>
<td>Oct-78</td>
<td>Altenbern, RA</td>
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<td>096-01-805/</td>
<td>Effect of potential BW agents on the Central Nervous System</td>
<td>Jul-74-Oct-77</td>
<td>Bailey, PT</td>
<td>$100,000</td>
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<td>096-01-806/</td>
<td>Role of bacterial exotoxins in disease pathogenesis</td>
<td>Jul-75-Oct-80</td>
<td>Leppla, SH</td>
<td>$540,100</td>
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<td>096-01-807/</td>
<td>Mechanism of action of bacterial exotoxins</td>
<td>Jul-75-Oct-80</td>
<td>Middlebrook</td>
<td>$791,200</td>
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<td>096-01-112/</td>
<td>Effect of potential BW agents on availability of host energy substrates</td>
<td>Dec-75-Oct-77</td>
<td>Yamada, T</td>
<td>$165,000</td>
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<td>841-00-024</td>
<td>Prevention of Pseudomonas aeruginosa infection and exotoxin</td>
<td>Oct-76-Oct-77</td>
<td>Petrella, VJ</td>
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<td>841-00-048</td>
<td>Role of the kallikrein-kinin system in infectious diseases of potential BW</td>
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<td>841-00-039</td>
<td>Mechanism of action of bacterial exotoxins</td>
<td>Oct-76-Oct-77</td>
<td>Taxy, JB</td>
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<td>841-00-021</td>
<td>Prevention of Pseudomonas aeruginosa infection and exotoxin</td>
<td>Apr-77-Oct-80</td>
<td>Thompson, W</td>
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<td>841-00-022</td>
<td>Cellular internalization of bacterial exotoxins</td>
<td>Aug-77-Oct-80</td>
<td>Cades, JS</td>
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<td>Characterization of Microbial Toxins</td>
<td>Nov-78-Oct-80</td>
<td>Dorland, RB</td>
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<td>10-AN-200</td>
<td>Characterization of Microbial Toxins</td>
<td>Oct-80-Oct-82</td>
<td>Middlebrook</td>
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<td>12-AA-001</td>
<td>Prevention and Treatment of Small Molecular Weight Toxins / Expl Studies for the Dvlpmnt of Medical Defensive Countermeasures to Toxins of Biological Origin</td>
<td>Oct-84-Oct-90</td>
<td>Bunner, DL/ Friedlander, AM</td>
<td>$6,997,000</td>
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<td>71-130</td>
<td>Molecular mechanism of action of T-2 mycotoxin</td>
<td>Oct-83-Oct-90</td>
<td>Middlebrook</td>
<td>$2,581,000</td>
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<td>91C-136</td>
<td>Cloning of Snake Toxin Genes for Novel Vaccine</td>
<td>Apr-83-Oct-85</td>
<td>Middlebrook</td>
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<td>91C-LA-140</td>
<td>Development</td>
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<td>91C-LA-134</td>
<td>Immunological protection against membrane-damaging protein toxins</td>
<td>Jan-90-Oct-90</td>
<td>Brown, JE</td>
<td>$122,000</td>
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<td>91C-LA-135</td>
<td>Purification of high-affinity polyclonal antibody directly from the blood of immunized animals w/ ligand-specific detoxification and specific-antibody hemoperfusion columns.</td>
<td>Jan-90-Oct-90</td>
<td>Hewetson, JF</td>
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<td>A91C-LA-131</td>
<td>Protection of respiratory mucosa from inhaled ricin aerosol by induction of ricin-specific secretory IgA antibodies</td>
<td>Jan-90 Oct-90</td>
<td>Creasia, DA</td>
<td>$72,000</td>
<td>0.5</td>
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<td>A91C-LA-137</td>
<td>Use of the isolated perfused rat lung model to study the pulmonary effects of ricin</td>
<td>Jan-90 Oct-90</td>
<td>Rivera, ER</td>
<td>$136,000</td>
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<td>A841-00-023</td>
<td>Effects of aerosol-disseminated respiratory infection in monkey models</td>
<td>Aug-76 Oct-77</td>
<td>Knutsen, GL</td>
<td>$68,000</td>
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<td>A841-00-041</td>
<td>Management of infections acquired via aerosol dissemination</td>
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<td>Kastello, MD</td>
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<td>A841-00-066</td>
<td>Characteristics of aerosol-induced Rift Valley fever infections</td>
<td>Dec-78 Oct-80</td>
<td>Brown, JL</td>
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<td><strong>TOTALS</strong></td>
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## Appendix C: USAMRIID FY69-FY90 Vaccine & Therapy Studies

### Basic Research: Vaccines

<table>
<thead>
<tr>
<th>Project Title</th>
<th>Start Date</th>
<th>End Date</th>
<th>PI</th>
<th>Total funds spent</th>
<th>Total Prof'l Man Years</th>
<th>Total Publications</th>
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<tr>
<td>Determinants for virulence and attenuation of group A arborvirus vaccine candidates / Comparative pathogenesis of VEE virus for rodents in relation to host defense mechanisms</td>
<td>Aug-72</td>
<td>Jul-80</td>
<td>Jahrling, PB, Bartelloni, PJ, Hill Jr., JE, Edelman, R, Ascher, MS, Anderson, JH</td>
<td>$1,224,800</td>
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<td>Studies in Combined Antigens</td>
<td>Apr-63</td>
<td>Jul-73</td>
<td>H</td>
<td>$535,000</td>
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<td>Properties of Antibodies Evaluation of Humoral Factors Other Than Antibody in the Immune response</td>
<td>Oct-67</td>
<td>Jul-73</td>
<td>Wilke, MH</td>
<td>$201,000</td>
<td>5.0</td>
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<td>Role of Secretory Immunoglobulin in Host Immunity to Militarily Important Diseases</td>
<td>Aug-69</td>
<td>Jul-70</td>
<td>Kirkland, BB</td>
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<td>Lipid metabolism and mechanisms of host defense</td>
<td>Nov-69</td>
<td>Jul-73</td>
<td>Rabinowitz, SG</td>
<td>$289,000</td>
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<td>Characterization of the intestinal immune response</td>
<td>Jul-71</td>
<td>Jul-74</td>
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<td>Cellular responses in lymphatic tissues following immunization</td>
<td>Jan-73</td>
<td>Jul-75</td>
<td>Mathis, RK</td>
<td>$197,100</td>
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<td>Evaluation of Vitafiber® system for continuous cell culture during vaccine production</td>
<td>Dec-74</td>
<td>Oct-80</td>
<td>Anderson, AO</td>
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<td>Oct-77</td>
<td>Johnson, AD</td>
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<td>BS12-AB-002</td>
<td>Aerosol Exposure Technology (FY85) / Basic Studies on Conventional Agents of Biological Origin &amp; Dvlpmnt of Medical Defensive Countermeasures</td>
<td>Oct-85</td>
<td>Oct-90</td>
<td>Stephenson/ Dalrymple, JM</td>
<td>$11,760,000</td>
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<tr>
<td>A870-BD-147</td>
<td>Man for BW Defense Evaluation of Experimental Vaccines in</td>
<td>Oct-61</td>
<td>Oct-82</td>
<td>Brown, III J., Peters, CJ</td>
<td>$872,000</td>
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<td>D809-AN-002</td>
<td>Vaccine, Advanced Development</td>
<td>Oct-84</td>
<td>Oct-88</td>
<td>Cosgriff, TM</td>
<td>$848,000</td>
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<td>D809-AU-011</td>
<td>Vaccine, Advanced Development</td>
<td>Jul-89</td>
<td>Oct-90</td>
<td>Dalrymple, JW</td>
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<td>D847-AN-002</td>
<td>Vaccine, Advanced Development</td>
<td>Oct-84</td>
<td>Oct-89</td>
<td>Cosgriff, TM</td>
<td>$367,200</td>
<td>4.0</td>
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</table>

**BACTERIA**

**Tularemia**

- 096-02-103/ A841-00-002 Enhancement of host resistance to facultative bacteria (Nov-72 - Oct-78) Eigelsbach, H, Hunter, DH, McGann, VG | $917,300 | 10 | 7 |
- 096-02-105 Immune responses of peripheral leukocytes (Mar-73 - Jul-75) McGann, VG | $280,700 | 4.0 | 0 |
- 096-02-106 Immunoprotective characterization of RNA-rich fractions (Jul-73 - Jul-75) Andron LA | $188,200 | 2.0 | 1 |

**BS03-00-001** Transfer mechanisms for cell-mediated immunity using a murine tularemia model (Oct-76 - Oct-80) Howell, HM | $280,700 | 4.0 | 0 |

**D809-AM-003** Vaccine, Tularemia (Oct-76 - Oct-78) Galloway, AK | $74,400 | 1.8 | 0 |

**Anthrax**

- A841-00-059 Pathogenesis of Anthrax (Oct-77 - Oct-80) Johnson, AD | $242,100 | 3.0 | 2 |
- D807-AA-011 Anthrax (Oct-83 - Oct-85) Leplla, SH | $68,000 | 0.7 | 2 |

**Plague**

- A91C-00-131 Isolation and Characterization of Immunogenic Components of Anthrax Toxin (Apr-85 - Oct-88) Welkos, SL | $328,000 | 4.5 | 8 |

**Rickettsia**

- 096-02-102 Development and Evaluation of an effective Vaccine Against Pneumonic Plague (Dec-62 - Jul-73) Marshall, JD, Cavanaugh, D | $375,000 | 14.0 | 21 |
- 096-01-303/ A841-00-007 Immunoprophylactic role of macrophages in airborne, rickettsial respiratory diseases (Role of macrophages in respiratory diseases) (Jul-75 - Oct-79) Kishimoto, RA | $487,500 | 4.1 | 10 |
<table>
<thead>
<tr>
<th>Project Number</th>
<th>Description</th>
<th>Start Date</th>
<th>End Date</th>
<th>Investigator(s)</th>
<th>Budget</th>
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<tr>
<td>A841-00-006</td>
<td>Immunochemical studies with the nonindigenous tick-borne rickettsiae.</td>
<td>Jul-67</td>
<td>Oct-79</td>
<td>Mieuse, JL, Robinson, DM, Kenyon, RH</td>
<td>$853,500</td>
<td>16.4</td>
<td>A841-00-027</td>
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<tr>
<td>A841-00-047</td>
<td>Physicochemical and biological characterization of components of Coxiella burnetii</td>
<td>Sep-72</td>
<td>Oct-80</td>
<td>Wachter, RF</td>
<td>$100,000</td>
<td>3.0</td>
<td>A841-00-047</td>
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<td>A841-00-047</td>
<td>Studies of Coxiella burnetii, Strain M44</td>
<td>Oct-72</td>
<td>Jul-75</td>
<td>Johnson, JW</td>
<td>$207,700</td>
<td>2.5</td>
<td>A841-00-047</td>
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<tr>
<td>A841-00-034</td>
<td>Immunopathogenesis of nonindigenous tick-borne rickettsial diseases.</td>
<td>Dec-74</td>
<td>Oct-77</td>
<td>Oster, CN</td>
<td>$260,000</td>
<td>3.0</td>
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<tr>
<td>A841-00-034</td>
<td>Characterization of nonindigenous tick-borne rickettsiae for vaccine development</td>
<td>Mar-75</td>
<td>Oct-80</td>
<td>Johnson, JW</td>
<td>$658,700</td>
<td>5.5</td>
<td>A841-00-034</td>
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<td>A841-00-035</td>
<td>Cellular immune response in rickettsial infections.</td>
<td>Jul-75</td>
<td>Jul-76</td>
<td>Rudzynski, A</td>
<td>$118,800</td>
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<td>A841-00-035</td>
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<tr>
<td>A841-00-036</td>
<td>Immunological studies of rickettsial proteins.</td>
<td>Feb-76</td>
<td>Jul-76</td>
<td>Pederson, C</td>
<td>$5,000</td>
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<td>A841-00-036</td>
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<tr>
<td>A841-00-047</td>
<td>Immunologic studies with typhus fever rickettsiae.</td>
<td>Jun-77</td>
<td>Oct-79</td>
<td>Kenyon, RH</td>
<td>$183,500</td>
<td>1.1</td>
<td>A841-00-047</td>
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<td>A870-BB-149</td>
<td>Prevention of Bacterial and Rickettsial Diseases of Potential BW Importance</td>
<td>Oct-80</td>
<td>Oct-82</td>
<td>Hedlund, KW</td>
<td>$1,433,000</td>
<td>13.7</td>
<td>A870-BB-149</td>
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<tr>
<td>D807-AB-012</td>
<td>Exploratory Development of Vaccines against Q fever.</td>
<td>Oct-83</td>
<td>Oct-90</td>
<td>Stephenson, Williams, JC</td>
<td>$2,511,000</td>
<td>7.5</td>
<td>D807-AB-012</td>
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<td>D809-AC-001</td>
<td>Vaccine, Advanced Development / Vaccine, Q fever.</td>
<td>Apr-84</td>
<td>Oct-90</td>
<td>Stephenson, Williams, JC</td>
<td>$1,704,000</td>
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<td>A91C-00-138</td>
<td>Application of recombinant DNA technology to develop new generation of Q Vaccines</td>
<td>Feb-84</td>
<td>Oct-87</td>
<td>Vodkin, MH, Williams, JC</td>
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<td>A91C-00-138</td>
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<td>VIRUSES</td>
<td>Influenza</td>
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<tr>
<td>096-02-0107</td>
<td>Cell-mediated immunity to pulmonary infection.</td>
<td>Sep-73</td>
<td>Jul-75</td>
<td>Hetsko, CM</td>
<td>$166,000</td>
<td>1.8</td>
<td>096-02-0107</td>
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<tr>
<td>096-02-0109</td>
<td>Evaluation of Efficacy of Combined Antigens in Laboratory Animals.</td>
<td>Oct-61</td>
<td>Jul-73</td>
<td>Spertzel, RO</td>
<td>$144,300</td>
<td>7.5</td>
<td>096-02-0109</td>
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<tr>
<td>A841-00-050</td>
<td>Therapy of respiratory bacterial infections transmitted via aerosols.</td>
<td>Feb-75</td>
<td>Oct-80</td>
<td>Berendt, RF</td>
<td>$1,063,200</td>
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<td>A841-00-050</td>
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<tr>
<td>ARBOVIRUSES (Alphaviruses, Flaviviruses)</td>
<td>Evaluation of Experimental Vaccines in Man.</td>
<td>Oct-61</td>
<td>Jul-73</td>
<td>Bartelloni, PJ</td>
<td>$382,000</td>
<td>8.0</td>
<td>096-02-008</td>
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<td>ARBOVIRUSES (Alphaviruses, Flaviviruses)</td>
<td>Evaluation of Efficacy of Combined Antigens in Man.</td>
<td>Feb-69</td>
<td>Jul-73</td>
<td>Hill, JE</td>
<td>$382,000</td>
<td>8.0</td>
<td>096-02-008</td>
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<td>ARBOVIRUSES (Alphaviruses, Flaviviruses)</td>
<td>Studies with Human Diploid Cell Cultures for Production of Military Vaccines</td>
<td>Nov-69</td>
<td>Jul-75</td>
<td>McManus, AT</td>
<td>$217,800</td>
<td>5.5</td>
<td>096-02-009</td>
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<tr>
<td>ARBOVIRUSES (Alphaviruses, Flaviviruses)</td>
<td>Comparative Studies of Various Routes of Immunization with Arbovirus Vaccines.</td>
<td>Aug-61</td>
<td>Jul-70</td>
<td>Kuehne, RW</td>
<td>$20,000</td>
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</table>
### Cross-Immunity Within the A Group of Arboviruses

**096-02-403**
- Development of arbovirus vaccines for diseases of military importance
  - Role of Antibody in the Clinical Manifestations of VEE
  - Host-parasite relationships in virus immunization

**096-02-407/A841-00-011**
- Pathophysiology and Treatment of Yellow Fever
- Develop Serological Methods for Military Vaccine Evaluation

**096-02-413**
- Molecular structure of Group C, Bunyawera supergroup and ungrouped arboviruses.
- Evaluation of sequential immunization against selected attenuated group A arboviruses.
- Immunochemical studies of antigenic components of arboviruses.
- In vitro studies of human immune response to vaccines of military importance
- Defining vaccine efficacy by electron microscopy

### Rift Valley Fever Virus

**A841-00-015**
- Molecular structure and antigenic determinants of Rift Valley fever virus
- Exploratory Development of Vaccines Against Rift Valley Fever / Advanced Vaccine Development Studies of Viruses of Potential BW Threat

**D807-AC-013**
- Tick-borne encephalitis
- Exploratory Research for Protection Against Chikungunya
- Exploratory Development of Vaccines Against Rift Valley Fever / Advanced Vaccine Development Studies of Viruses of Potential BW Threat
<table>
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<tr>
<th>Date(s)</th>
<th>Authors</th>
<th>Funding</th>
<th>Duration</th>
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<tr>
<td>Oct-83-Oct-85</td>
<td>Dalrymple, JM</td>
<td>$4,400</td>
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<td>Exploratory Research for Protection Against RVFV</td>
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<td>Jan-90-Oct-90</td>
<td>Turrell, MJ, Terrell, TG, McLeod, C, Elwell, MR</td>
<td>$80,000</td>
<td>1.0</td>
<td>The use of mosquitoes as an in vivo bioassay for antiviral activity</td>
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<td>Oct-72-Oct-78</td>
<td>Gangemi, JD</td>
<td>$308,500</td>
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<td>Pathology of Bolivian hemorrhagic fever in the rhesus monkey</td>
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<tr>
<td>Mar-75-Oct-78</td>
<td>Rosato, RR</td>
<td>$195,900</td>
<td>2.1</td>
<td>Characterization of arenaviruses and their structural components for vaccine development</td>
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<td>Jun-71-Oct-80</td>
<td>Kuehne, RW, Eddy, GA</td>
<td>$1,841,200</td>
<td>17.3</td>
<td>Experimental Bolivian Hemorrhagic Fever: Pathogenesis and Vaccine Development</td>
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<td>Oct-83-Oct-90</td>
<td>Jahrling, PB</td>
<td>$8,917,000</td>
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<td>Exploratory Development of Vaccines Against Argentene Hemorrhagic Fever</td>
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<td>Oct-83-Oct-90</td>
<td>Lupton, HW</td>
<td>$958,000</td>
<td>4.0</td>
<td>Immunotheraphy of Hemorrhagic Fever Viruses / Adv Studies for the Dvlpmnt of Immunotherapy Against Viral Agents of BW</td>
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<td>Oct-83-Oct-90</td>
<td>Jahrling, PB</td>
<td>$1,112,000</td>
<td>8.4</td>
<td>Antibody, Lassa Fever</td>
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<td>Oct-76-Oct-80</td>
<td>French, GR, Rice RM</td>
<td>$734,100</td>
<td>4.5</td>
<td>Characterization and evaluation of selected hemorrhagic fever agents for vaccine development</td>
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<tr>
<td>May-80-Oct-80</td>
<td>Cosgriff, TM</td>
<td>$282,000</td>
<td>0.3</td>
<td>Evaluation of hemostatic derangement in infectious diseases o military importance</td>
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<td>Oct-80-Oct-82</td>
<td>Peters, CJ</td>
<td>$575,000</td>
<td>4.5</td>
<td>Advanced Studies for the Development of Therapeutics Against Primate Retroviruses</td>
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**Arenaviruses**

- **096-01-040/A841-00-044**
  - Description: Characterization of arenaviruses and their structural components for vaccine development
  - Principal Investigator: Terrell, TG, McLeod, C, Elwell, MR
  - Start Date: Oct-72
  - End Date: Oct-78
  - Funding: $308,500

- **834-02-425/BS03-00-004**
  - Description: Pathology of Bolivian hemorrhagic fever in the rhesus monkey
  - Principal Investigator: Gangemi, JD
  - Start Date: Mar-75
  - End Date: Oct-78
  - Funding: $308,800

- **834-02-426/A841-00-027**
  - Description: Characterization of arenaviruses and their structural components for vaccine development
  - Principal Investigator: Rosato, RR
  - Start Date: Jan-76
  - End Date: May-78
  - Funding: $195,900

- **096-03-045/A841-00-017**
  - Description: Experimental Bolivian Hemorrhagic Fever: Pathogenesis and Vaccine Development
  - Principal Investigator: Kuehne, RW, Eddy, GA
  - Start Date: Jun-71
  - End Date: Oct-80
  - Funding: $1,841,200

- **A871-AD-133**
  - Description: Exploratory Development of Vaccines Against Potential BW Threat
  - Principal Investigator: Jahrling, PB
  - Start Date: Oct-83
  - End Date: Oct-90
  - Funding: $8,917,000

- **D807-AF-016**
  - Description: Immunotheraphy of Hemorrhagic Fever Viruses / Adv Studies for the Dvlpmnt of Immunotherapy Against Viral Agents of BW
  - Principal Investigator: Jahrling, PB
  - Start Date: Oct-83
  - End Date: Oct-90
  - Funding: $958,000

- **D807-AG-019**
  - Description: Antibody, Lassa Fever
  - Principal Investigator: Jahrling, PB
  - Start Date: Oct-83
  - End Date: Oct-90
  - Funding: $1,112,000

**Hemorrhagic Fever Viruses (Other Bunyaviridae)**

- **A841-00-054**
  - Description: Characterization and evaluation of selected hemorrhagic fever agents for vaccine development
  - Principal Investigator: French, GR, Rice RM
  - Start Date: Oct-76
  - End Date: Oct-80
  - Funding: $734,100

- **A841-00-073**
  - Description: Prevention of Viral Diseases of Potential BW Importance
  - Principal Investigator: Cosgriff, TM
  - Start Date: May-80
  - End Date: Oct-80
  - Funding: $282,000

- **A870-BC-148**
  - Description: Advanced Studies for the Development of Therapeutics Against Primate Retroviruses
  - Principal Investigator: Jahrling, PB
  - Start Date: Oct-80
  - End Date: Oct-82
  - Funding: $5,235,000

- **H29-AE-035**
  - Description: Exploratory Research for Protection Against Arenaviruses / Exploratory Vaccine Dvlpmnt Studies on Conventional Agents of Potential BW Threat
  - Principal Investigator: Jahrling, PB
  - Start Date: Oct-83
  - End Date: Oct-90
  - Funding: $8,917,000

- **D807-AG-019**
  - Description: Antibody, Lassa Fever
  - Principal Investigator: Jahrling, PB
  - Start Date: Oct-83
  - End Date: Oct-90
  - Funding: $1,112,000

- **D807-AF-016**
  - Description: Immunotheraphy of Hemorrhagic Fever Viruses / Adv Studies for the Dvlpmnt of Immunotherapy Against Viral Agents of BW
  - Principal Investigator: Jahrling, PB
  - Start Date: Oct-83
  - End Date: Oct-90
  - Funding: $958,000

- **H29-AE-035**
  - Description: Exploratory Research for Protection Against Arenaviruses / Exploratory Vaccine Dvlpmnt Studies on Conventional Agents of Potential BW Threat
  - Principal Investigator: Jahrling, PB
  - Start Date: Oct-83
  - End Date: Oct-90
  - Funding: $8,917,000

- **D807-AG-019**
  - Description: Antibody, Lassa Fever
  - Principal Investigator: Jahrling, PB
  - Start Date: Oct-83
  - End Date: Oct-90
  - Funding: $1,112,000

- **D807-AF-016**
  - Description: Immunotheraphy of Hemorrhagic Fever Viruses / Adv Studies for the Dvlpmnt of Immunotherapy Against Viral Agents of BW
  - Principal Investigator: Jahrling, PB
  - Start Date: Oct-83
  - End Date: Oct-90
  - Funding: $958,000

- **H29-AE-035**
  - Description: Exploratory Research for Protection Against Arenaviruses / Exploratory Vaccine Dvlpmnt Studies on Conventional Agents of Potential BW Threat
  - Principal Investigator: Jahrling, PB
  - Start Date: Oct-83
  - End Date: Oct-90
  - Funding: $8,917,000
### Toxin Studies

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<tr>
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<th>Title</th>
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<th>End Date</th>
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<td>Effects of Staphylococcal Enterotoxin B on Lymphoid cells in vitro in BW defense</td>
<td>Apr-70</td>
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<td>Metzger, JF; Adler, WH; DeRubertis, F; Zenser, TV</td>
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<td>Studies in immunization of the respiratory tract Mechanisms of immunoprophylaxis against aerosol-disseminated respiratory diseases</td>
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<td>096-01-023/ A841-00-042 Studies in immunization of the respiratory tract Mechanisms of immunoprophylaxis against aerosol-disseminated respiratory diseases Effects of respiratory infections on selected nonrespiratory function of the lung Assessment of Airborne Microbial Agents of Potential BW Threat Aerosol Studies on Agents of Biological Origin Exploratory Development of Vaccines, Therapeutic Agents and Immunomodulators Against Aerosols / Advanced Studies for the Development of Immunomodulators/Enhancers Against Infectious Agents of Potential BW Threat</td>
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<td>Therapeutic reversal of abnormal host amino acid, protein and RNA metabolism during infectious disease of unique military importance / Amino acid, protein changes, and RNA metabolism in blood in infectious disease</td>
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**OTHER THERAPIES**

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<td>Efficacy of zinc treatment in experimental endotoxemia and bacterial sepsis</td>
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## Appendix D: USAMRIID FY1969-FY1990 Detection Studies

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<td>Vollmer, RT, Rowberg, AH, Higbee, GA</td>
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<td>096-03-009</td>
<td>Application of electron spin resonance spectroscopy to infectious disease research</td>
<td>Nov-69</td>
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<td>096-03-403</td>
<td>Separation, Purification, and Concentration of Arbovirus Agents and Antigen-Antibody Complexes</td>
<td>Nov-61</td>
<td>Jul-76</td>
<td>Levitt, NH</td>
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<td>096-03-404</td>
<td>Use of antiglobulin for early detection of arbovirus antibody of diseases of military importance</td>
<td>Dec-70</td>
<td>Jul-75</td>
<td>McManus, AT</td>
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<td>096-03-406</td>
<td>Immunochemical studies of variants of arboviruses</td>
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<td>Jul-73</td>
<td>Pedersen, CE</td>
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<td>096-03-407</td>
<td>Morphogenesis and morphology of VEE virus</td>
<td>May-74</td>
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<td>Veltri, BJ</td>
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<td>096-03-408</td>
<td>Rapid diagnostic method based on singlet oxygen fluorescence</td>
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<td>A841-00-063</td>
<td>Rapid diagnosis of viral diseases of military importance</td>
<td>Jun-78</td>
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<td>BS03-00-25</td>
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<td>Role of coated vesicles in receptor-mediated endocytosis of biological substances</td>
<td>Jan-80</td>
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<td>A91C-00-143</td>
<td>Defects in cellular immunity after VEE vaccination and repair with transfer factor</td>
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<td>Oct-79</td>
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<td>Exploratory Development for Rapid Identification and Diagnosis / Advanced Studies for the Dvlpmnt of Rapid Diagnostic Procedures on Agents of Biological origin</td>
<td>Oct-83</td>
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<td>Bailey, CL, Linthicum, KJ</td>
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<td>Exploratory Dvlpmnt Studies Seeking Rapid Diagnostic Procedures to Detect Agents of Biological Origin in Clinical Specimens</td>
<td>Mar-84</td>
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<td>Early Immune Response in Infectious Disease and Toxemia</td>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>BDRP</td>
<td>Biological Defense Research Program</td>
</tr>
<tr>
<td>BFAC</td>
<td>BioForensics Analysis Center</td>
</tr>
<tr>
<td>BKC</td>
<td>Biodefense Knowledge Center</td>
</tr>
<tr>
<td>BTCC</td>
<td>Biological Threat Characterization Center</td>
</tr>
<tr>
<td>BW</td>
<td>Biological Weapons</td>
</tr>
<tr>
<td>BWC</td>
<td>Convention on the Prohibition of the Development, Production, and Stockpiling of Bacteriological (Biological) and Toxin Weapons, and on Their Destruction (or, the Biological and Toxin Weapons Convention)</td>
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<tr>
<td>CBW</td>
<td>Chemical and Biological Weapons</td>
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<tr>
<td>CDC</td>
<td>US Centers for Disease Control and Prevention</td>
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<tr>
<td>CWS</td>
<td>Chemical Warfare Service</td>
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<tr>
<td>DARPA</td>
<td>Defense Advanced Research Projects Agency</td>
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<tr>
<td>DHS</td>
<td>Department of Homeland Security</td>
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<tr>
<td>DOD</td>
<td>Department of Defense</td>
</tr>
<tr>
<td>DOE</td>
<td>Department of Energy</td>
</tr>
<tr>
<td>EF</td>
<td>Edema Factor</td>
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<tr>
<td>EID</td>
<td>Emerging Infectious Disease</td>
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<tr>
<td>EPA</td>
<td>Environmental Protection Agency</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>FEMA</td>
<td>Federal Emergency Management Agency</td>
</tr>
<tr>
<td>HEW</td>
<td>Department of Health, Education, and Welfare</td>
</tr>
<tr>
<td>HHS</td>
<td>Department of Health and Human Services</td>
</tr>
<tr>
<td>IC</td>
<td>Intelligence Community</td>
</tr>
<tr>
<td>IND</td>
<td>Investigational New Drug</td>
</tr>
<tr>
<td>IPMG</td>
<td>Interdepartmental Political-Military Group</td>
</tr>
<tr>
<td>JCS</td>
<td>Joint Chiefs of Staff</td>
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<tr>
<td>LF</td>
<td>Lethal Factor</td>
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<td>LVS</td>
<td>Tularemia Vaccine</td>
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<tr>
<td>NAS</td>
<td>National Academy of Sciences</td>
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<tr>
<td>NBACC</td>
<td>National Biodefense Analysis and Countermeasures Center</td>
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<tr>
<td>NIAID</td>
<td>National Institute for Allergy and Infectious Disease</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
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<tr>
<td>NRC</td>
<td>National Research Council</td>
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<tr>
<td>NSABB</td>
<td>National Science Advisory Board for Biosecurity</td>
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<tr>
<td>NSF</td>
<td>National Science Foundation</td>
</tr>
<tr>
<td>PA</td>
<td>Protective Antigen</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PHS</td>
<td>Public Health Service</td>
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<tr>
<td>PSAC</td>
<td>Presidential Scientific Advisory Committee</td>
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<tr>
<td>RMSF</td>
<td>Rocky Mountain Spotted Fever</td>
</tr>
<tr>
<td>RVF</td>
<td>Rift Valley Fever</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>SARS</td>
<td>Severe Acute Respiratory Syndrome</td>
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<tr>
<td>SEB</td>
<td>Staphylococcal Enterotoxin B</td>
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<tr>
<td>SGO</td>
<td>Army Surgeon General’s Office</td>
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<tr>
<td>SPA</td>
<td>Small Particle Aerosol</td>
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<tr>
<td>SPD</td>
<td>Special Projects Division</td>
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<tr>
<td>UNSCOM</td>
<td>United Nations Special Commission</td>
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<tr>
<td>USAMU</td>
<td>US Army Medical Unit</td>
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<td>USAMRIID</td>
<td>US Army Medical Research Institute of Infectious Diseases</td>
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<td>VEE</td>
<td>Venezuelan Equine Encephalitis</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<td>WMD</td>
<td>Weapons of Mass Destruction</td>
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<td>WRS</td>
<td>War Research Service</td>
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