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by

J.W. Cherwonogrodzky and V.L. Di Ninno

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ABSTRACT

Brief summaries are given on examples of bacteria (Bacillus anthracis, Francisella tularensis, Brucella spp., Coxiella burnetii), viruses (Venezuelan equine encephalitis, smallpox, Rift Valley fever), and toxins (botulinum, ricin, staphylococcal enterotoxin B) that are of medical importance. Descriptions, morbidity and mortality, vaccine availability, value of passive immune approaches, recommended therapy, suggested priority for further development and references are given for these examples.

RÉSUMÉ

Courts sommaires portant sur des exemples de bactéries (*Bacillus anthracis, Francisella tularensis, Brucella spp., Coxiella burnetii*), de virus (virus de l'encéphalite équine du Venezuela, de la variole, de la fièvre de la vallée du Rift) et de toxines (toxine botulinique, ricine, entérotoxine B staphylococcique) ayant une importance du point de vue médical. Pour ces exemples, on donne plusieurs types de renseignements : description, morbidité et mortalité, existence d'un vaccin, valeur de l'immunothérapie passive, thérapie recommandée, priorités proposées pour ce qui est des nouveaux développements et références.

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INTRODUCTION

During the Middle East Crisis, the Coalition Forces were faced with an opponent that was prepared to wage war with unconventional weapons. Chemical warfare agents were stockpiled, environmental terrorism had been initiated (e.g. massive oil spills in the Arabian Sea, detonation of several hundred oil wells in Kuwait), nuclear capability was researched, and a germ warfare program was considered to be in progress.

As effective antidotes were available for the chemical warfare agents while the need of "high-tec" for nuclear capability was temporarily restrictive for Iraq, biological warfare (BW) agents were potentially a greater threat than other weapons of war. For the use of BW agents:

- only "low-tec" capabilities are needed for their production
- pathogenic bacteria or viruses are easily obtained (several are known endemic to a given area)

BW AGENTS CAN BE ABOUT A MILLION-TOLD MORE EFFECTIVE THAN chemical agents in debilitating people (per unit)

- only small amounts are needed to contaminate an area (see Appendix A).

Also, unlike the other weapons, BW agents, such as some bacteria or viruses, can:

- infect an individual without their knowledge of such an occurrence
- grow within their hosts (instead of deteriorating or being inactivated with time, these may increase in potency)
- spread from soldier to soldier and be brought back to civilian populations,
- adapt and change their characteristics so as to overcome the defences of their host
- hide within tissues or cells of the host and cause relapses of disease over several years

Although BW agents are potentially a greater threat than other weapons of war, these are also the most readily countered with the appropriate vaccines, inactivating treatments, or antibiotics. Other consequences of having effective medical countermeasures are that:

- effective vaccines can protect a soldier against a BW agent for several years
- as several BW agents are also diseases endemic to some areas, a soldier protected against a BW agent may also be protected against a local disease (i.e. while he is on a peace-keeping duty).
- some medical countermeasures are broad spectrum in that a

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prophylactic measure or therapy against one disease may be effective against several others

- in a theatre of war, the victor may be the one, not with the most weapons, but the one with the only vaccine
- the disarmament and destruction of BW stockpiles in foreign countries will probably progress more readily when the weapons are rendered useless by medical defences.

With the recognition that diseases or toxins are serious threats to either military scenarios or peace-keeping roles, there have been several requests for brief summaries on the availability and efficacy of existing vaccines, passive immune approaches, chemotherapeutic and other treatments for some medically significant biological agents. The following review, therefore, has been written to answer some of these needs. However, it should be noted that as defensive measures do not exist at this time for some of these agents, some existing vaccines are only partially effective and then only against a low dose challenge, and some strains may be resistant to existing therapies, this paper should be viewed only as a first draft that will require subsequent updating, revisions and additions. For the time being, this paper does attempt to clarify what is presently available for each threat agent and, where none exists, to offer suggestions as to what may be possible.

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AGENT SPECIFIC INFORMATION

a) BACTERIA:

i) **Bacillus anthracis (anthrax)**: This large (1-20 um) gram-positive non-motile bacterium has a protective poly-glutamate capsule. It grows rapidly and in fulminative infections, at the later stage of disease, as much as 1/4 of the blood volume may be bacteria (personal communications, John Ezzel, USAMRIID). Toxins are then released and, after the blood cells are lyzed, the serum has a black (anthracite) appearance. At the end of a growth cycle, endospores form which are resistant to high temperatures and can persist in the environment for decades. These are susceptible to bleach or steam sterilization.

Morbidity and mortality: Transmission is made through scratches or abrasions of the skin, wounds and inhalation of spores. The infectious dose is about 1300 spores by inhalation (LCDC Material Safety Data Sheet). Incubation is 1-7 days but may be less than 1 day for pulmonary cases. Mortality for untreated cutaneous anthrax is up to 25%, for pulmonary cases it is almost 100%, and for rare intestinal cases it is fatal (1).

Vaccine availability: Protection is not significant if vaccination is with killed cells, but vaccination is effective if Protective Antigen (PA), a toxin found in culture supernatants,

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is used with an adjuvant. A vaccine made by absorbing the culture supernatant to alum is available through the Biologic Products Program, Michigan Department of Public Health, East Lansing, Michigan (2). During the Middle East Crises, supply was a key limitation. Priority should be given to stockpiling the vaccine, determining its limitations and having quality assurance.

Value of passive immune approaches: Recovery from an anthrax infection does result in a high titre of serum antibodies to the whole cell (3). However, as protective antigen (PA) is the key component for making the edema factor (EF) and lethal factor (LF) active (4), antibodies to PA would probably be effective in prophylaxis against disease. Since the vegetative cells of B. anthracis grow rapidly in the blood and have a protective capsule, antibiotic prophylaxis may limit the disease.

Recommended therapy: The vegetative cells are usually sensitive to several antibiotics. If penicillin-sensitive, give 2 million units/i.v/every 2 hours, if penicillin-resistant give either ciprofloxacin at 1000 mg p.o., then 750 mg/p.o./12 hours or doxycycline at 200 mg/i.v. then 100 mg/i.v./12 hours (5).

Priorities for further development: Animal studies should be done on the efficacy and storage limitations of the current and newly developed vaccines. As PA is a key element to protection,

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research should be done to maximize production and purity of PA (see above, vaccine availability), determine if adjuvants (other than alum) better enhance immunity, and if PA "inactive" mutants produce a component which can be given directly as an immunogen. Studies of the live attenuated vaccines, such as B. subtilis containing PA, "Sterne-like" strains, or the newly developed recombinant vaccinia expressing PA should continue (4).

Prophylaxis with antibiotics, especially within release-delayed forms of liposomes or microspheres to extend their persistence in the body, should be investigated.

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ii) **Francisella tularensis (tularemia)**: This small (0.2-0.3 µm) gram-negative pleomorphic bacterium is a fatal disease of wild rodents. The organism is viable for weeks in water, soil and hides, and for years in frozen rabbit meat. It is very sensitive to high temperatures (40°C) and common disinfectants.

Morbidity and mortality: Transmission is made by infection through the skin, eyes or lungs, or by being bitten by infected flies and ticks. The infectious dose is about 10 organisms (LCDC Material Safety Data Sheet). For people accidentally infected, there is a sudden onset of chills, fever, incapacitation and pneumonic complications. The chronic form may cause enlargement of the lymph glands or typhoid-like symptoms. Clinical signs of disease are either pleuropulmonary or ulceroglandular (headache, fevers, ulcers at site of entry). Strains can be categorized as being either type A (North America, highly virulent, 10% mortality in people without treatment) or type B (Europe, Asia, less virulent, 1% mortality rate).

Vaccine availability: A live attenuated vaccine strain (F. tularensis LVS) is available from the CDC in Atlanta, Georgia (1). A retrospective analysis of researchers vaccinated and then exposed to the bacterium in the laboratory, showed that the incidence of typhoidal tularemia fell about 20-fold while the incidence of ulceroglandular tularemia remained unchanged (2).

Another study (3) showed that although it did proliferate in monkeys at the site of intratradermal or aerogenic inoculation, the infection was benign and temporary. It has been suggested that the vaccine has its limitations and may offer inadequate protection by the current schedule of immunization (4, 5).

Value of passive immune approaches: Protection appears to be given by cell-mediated responses following vaccination. However, the protection by antibodies (monoclonal vs polyclonal) should be investigated.

Recommended therapy: F. tularensis is usually sensitive to streptomycin. For the treatment of infections, either 15-20 mg streptomycin/kg/day for 14 days or 3-5 mg gentamicin/kg/day for 14 days should be given. For possible aerosol infection, either 2 gm tetracycline/day for 14 days or 100 mg. doxycycline b.i.d. for 14 days should be given (6).

Priorities for further development: antibiotics: In mice, liposome-encapsulation of ciprofloxacin greatly enhanced its effectiveness. A single intravenous dose offered either 2-3 days protection against acquiring the disease (10 LD_{50}), or effective treatment 3-7 days after infection (7). Greater effectiveness was obtained for intranasal delivery. Histopathologic studies showed that, even if the bacterium was given to mice by different routes

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(i.v., i.p., i.n.), the most affected organ was the lung (8). Further work is needed on liposome formulation, aerosol delivery mechanisms and challenge limits.

Vaccines: We are investigating the concept of one O-polysaccharide vaccine to protect against several BW agents (see brucellosis). Also, although other researchers have found no evidence of toxins nor virulence factors associated with F. tularensis, it is likely that some do exist that account for differences in virulence with similar appearing strains and for the reports of necrosis at the sight of infection. We have observed that growing the bacterium in synthetic medium derepresses capsule formation (9) and will investigate these culture supernantants for the expression of other virulence factors of vaccine potential.

Passive immunity: Protection by monoclonal vs polyclonal antibodies to this bacterium should be investigated.

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iii) **Brucella spp. (brucellosis)**: This gram-negative facultative parasitic bacterium thrives in white blood cells, resides quiescently in tissues and bone-marrow, and is seldom eliminated. At low temperatures it can survive in soil for 10 weeks and in liquid manure for 2.5 years. Contaminated materials are easily sterilized or disinfected by common methods.

Morbidity and mortality: Transmission to humans is usually by the ingestion of contaminated dairy products or meat, although laboratory workers are usually infected by the aerosol route. The infectious dose is unknown, but it is probably about 10 organisms. Symptoms are "undulant" fever, headaches, arthritis, depression and nauseau. Some strains are more pathogenic than others with B. melitensis ≥ B. suis > B. abortus (still very pathogenic) > B. canis. Brucella ovis and B. neotomae are not known to be pathogenic. Incubation before disease is from 6-60 days or more (the average is 14 days). The susceptibility of humans to B. abortus is about 50% and of these the mortality is 2-3%. The susceptibility of humans to B. melitensis or B. suis is about 75-80% and mortality is 3-6% (1).

Vaccine availability: There is no human vaccine available for Canadians. The Russians have used B. abortus strain 19, an attenuated strain that is used to immunize cattle, but the resulting illnesses showed little advantage. The Chinese have

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developed the vaccine strain B. suis biotype 1 strain 2 that is given orally, and they recently view this vaccine as "Top Secret" (though a stock is available from the Ministry of Agriculture, Weybridge, England). There is a phenol insoluble (PI) crude residue fraction (Brucellin-INRA) developed in France which appears to be protective but does give a high incidence of reactogenicity (?).

Value of passive immune approaches: In the mouse model, monoclonal antibodies against the O-polysaccharide (OPS) of Brucella have provided some protection. However, despite bacterial numbers being reduced about 1000-fold, infections persisted and increased after treatment stopped (3). The Russians have studied a combination of therapies in the mouse model; notably, immunization with lipopolysaccharide (LPS) from Brucella followed by passive immunity.

Recommended therapy: Most clinicians prefer doxycycline (100 mg daily) rather than oral tetracycline (500 mg/4 times daily) for 4-6 weeks. For more seriously ill patients, streptomycin (1 g/d intramuscular, 2-3 weeks) is combined with doxycycline or tetracycline therapy (4). Colleagues that have had brucellosis, found the treatment effective but only after the symptoms were expressed, and infection was still life-long.

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Priorities for further development: vaccines: i) We have had success in liposome-encapsulated LPS and OPS as effective vaccines in the mouse model (5). Large animal studies, possibly in Venezuela (pigs), Peru (goats), or Colombia (cattle) are required.

ii) DRES has a contract with the United Nations University (Tokyo) for the development of novel vaccines (an LPS-free 14 Kd protein, Chile; an 18 and 28 Kd protein clone, Argentina), and the testing of strain RB51 (in guinea pigs, Colombia; pregnant cattle, Ecuador). Within a Mexican laboratory investigating Brucella, of the 10 researchers that received the French vaccine, none acquired the disease although their sera has shown sero-conversion or exposure to the organism; of the 3 workers that refused the vaccine, all came down with the disease. Comparisons should be done on the efficacy of this vaccine.

iii) As B. abortus, B. melitensis, B. suis, Vibrio cholerae, Salmonella landau and godesburg, Yersinia enterocolitica O:9, and possibly F. tularensis and Y. pestis, have derivatives of the same highly immunogenic O-polysaccharide sugar on their cell walls (6), we have initiated a study (7) with the goal of developing 1 vaccine to protect against 9 diseases.

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Dr. Om Surijbahli of Animal Diseases Research Institute (Nepean, Ontario, Canada) has cloned the B. abortus O-chain expression into E. coli. Dr. Tom Dees of Texas A&M University is comparing the efficacy of B. neotomae and the Chinese B. suis strain 2 live vaccines.

antibiotics: We have found that liposome encapsulation improves the efficacy of ciprofloxacin both in the prophylaxis and treatment of Brucella. Unfortunately, it also appears that the formulation of these liposome (i.e. "designer" liposomes) is crucial for effective treatment. We are about to begin the study of this treatment in clearing brucellosis infections from goats in Peru.

passive immunity: It is unknown whether the limits to passive immunity can be overcome by using monoclonal antibodies with greater affinities and wider specificities, or if polyvalent antibodies (which can coat the bacterium more effectively and hence initiate the complement cascade) are more effective.

In the model of mice receiving anti-'flu antibodies encapsulated in liposomes, protection and treatment was greatly improved (8). Potentially this technology can be applied also to the treatment of brucellosis. Dr. Thomas Ewart, Managing Director of TherImmune Scientific Ltd., 110 McClure Dr., King City, Ontario, L0G 1K0 (416-979-5395) voiced the potential for cost effective human monoclonals (these would have a longer half-life in the body and would provoke fewer allergic responses).

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iv) **Coxiella burnetii (Q fever): This small, obligately parasitic** gram-variable bacterium grows profusely in the yolk sac of embryonated hen's eggs (1) and matures from a pleomorphic cell to a very resistant body. It can probably persist on surfaces from 5-60 days, is resistant to 40°C and 0.5% phenol, but is susceptible to 0.5% formalin (2).

Morbidity and Mortality: This bacterium is unique among the rickettsial diseases in that it does not require transmission by an insect bite but can infect by the aerosol route. Clinical symptoms are severe fever, sweats, pneumonitis, pericarditis, hepatitis, and generalized infections. The incubation period is from 14-26 days with a mean of 19 days (2). Only 10 bacteria are needed to cause infection, and the fatality rate is usually less than 1% (LCDC Material Safety Data Sheet) although it can be as high as 4% during an epidemic.

Vaccine availability: An investigational inactivated vaccine is available through USAMRIID, Fort Detrick, Frederick, MD. Phase I cells are purified, killed with formaldehyde, lyophilized, extracted with chloroform:methanol at 4:1 (the solvent removes a toxic component), and the residue (suspended in saline) has been shown to be effective in giving protection to mice (3).

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Value of passive immune approaches: The disease is self-limiting and in the patient antibodies to Q-fever occur throughout a chronic infection. As the parasite resides within host cells, it is sequestered from the effects of humoral antibodies. It also immunosuppresses lymphocytes (3) and this may be an evolutionary means of the parasite controlling the host to ensure its survival. If antibodies are encapsulated within liposomes, these might reach the parasite within the host cell and limit disease.

Recommended therapy: Antibiotic prophylaxis has not been shown to be effective (2), possibly because the bacterium sequesters within host cells where it is protected from circulating antibiotics. For treatment, tetracycline is the antibiotic of choice, with chloramphenicol an alternative, over 2 weeks (4).

Priorities for further development: Efficacy of the noted vaccine has not been determined in humans. The use of liposomes to enhance the prophylaxis and treatment with antibiotics or antibodies (see F. tularensis) should be investigated.

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b) VIRUSES

) **Venezuelan equine encephalitis (VEE)**: This alphavirus that belongs to the family Togaviridae, is an enveloped single-stranded RNA virus that is about 70 nm in diameter (1). Although it is resistant to low temperatures and phenol, it is susceptible to heat (78^{o}C/30 min) and formalin (2).

Morbidity and mortality: Although its transmission to humans is usually by insect bites (e.g. mosquitoes), laboratory accidents suggest it can infect by the aerosol route (2). As little as 1 viral unit is needed for infection, and symptoms (after 2-6 days, though incubation can be as short as 1 day) range from 'flu-like symptoms (severe headache, chills, fever, myalgia, nausea, vomiting) to central nervous system involvement, disorientation, convulsions, paralysis, coma and death (1). Fatalities are rare (less than 1%).

Vaccine availability: A killed formalized vaccine C-84 does exist as does an investigational attenuated vaccine strain TC-83. Comparisons have been done on the protection of hamsters against aerosol or subcutaneous challenges (3) and although both vaccines were effective against subcutaneous inoculations, only the attenuated strain was effective against aerosol challenges. As

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formaldehyde will denature antigens (4), it is understandable why gamma- irradiation (which affects nucleic acid integrity rather than protein conformation) yields a better killed vaccine (5).

Value of passive immune approaches: There is some evidence, at least in animal studies, that antibodies (6) and immuno-modulators (7) may provide some protection against VEE.

Recommended therapy: Unlike Eastern Equine Encephalitis which has a mortality of 80% in patients, the mortality for those with VEE is less than 1% and most patients have mild symptoms (8, 1). No specific treatment is recommended for VEE (1).

Priority for further development: Although death seldom occurs with VEE, its highly infectious nature, short incubation time and its effect on orientation underline the need for protection. The efficacy of existing vaccines should be determined.

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ii) **Variola (smallpox)**: The double-stranded DNA poxviruses are the largest and most complex viruses of vertebrates, being brick-shaped with dimensions of 0.3 X 0.2 X 0.1 um. (1). The virus is viable for several years in water at 22-27^{o}C, and in the dry state it is more resistant than in the wet state. It is resistant to common disinfectants, but is susceptible to alcohol or acetone (22^{o}C/1 h) and to moist heat (78^{o}C/10 min.)(2).

Morbidity and mortality: Transmission is made by contact (skin, lesions, pus) with patients having the disease or with articles the patient has contaminated. Incubation is from 7-21 days, averaging 12 days (2). Symptoms are a high fever, generalized infection with pustular rash, toxemia and septic complications. Mortality is about 50% from the "major" form (1).

Vaccine availability: The disease has been eradicated due to the effectiveness of vaccinia, and indeed the word "vaccine" is derived from this treatment. As this agent had a long and varied passage history since Jenner's discovery of the efficacy of cowpox in 1798, different strains of vaccinia virus vary in their properties possibly because these were derived from either cowpox (1) or smallpox (3). As smallpox has been eradicated and since the vaccine may cause pustular lesions, encephalitis and fetal vaccinia, immunization against smallpox has been stopped. A vaccine is available but only in restricted stocks.

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Value of passive immune approaches: Gamma-globulin, prepared from the sera of persons who were recently vaccinated (4-6 weeks before bleeding) prevents smallpox in those recently exposed to infection (3). Passive immunity is feasible as a means of prophylaxis or treatment.

Recommended therapy: Without a vaccine, the drug thiosemicarbazone is 75-95% effective as a prophylactic measure to prevent disease. It's effectiveness is often diminished because it also induces severe vomiting (1).

Priority for further development: Vaccinia is often used as a vehicle for recombinanat vaccines. It is likely that these recombinants would protect also against smallpox.

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iii) **Rift Valley fever (RVF) : This 23-35 nm Phlebovirus has** 3 RNA segments, the smallest of which codes for the nucleocapsid (1). The virus is destroyed by 73°C/40 min.(2).

Morbidity and mortality: Transmission is usually by the bites of mosquitoes but incidences involving laboratory workers suggest infection by inhalation. It is highly infectious, incubation is from 1-4 days and both the onset of disease and recovery is rapid (2). Although clinical symptoms are often mild, when illness does occur the symptoms are pain in the extremities and joints, nauseau, violent headaches, high fever and malaise (1).

Vaccine availability: There have been several advances in the development of attenuated strains, killed preparations, deletion mutants and recombinant subunit vaccines (3). A formalin-killed vaccine does protect humans from RVF. A mutagenized strain and a vaccinia-vectored recombinant may be other alternatives.

Value of passive immune approaches: Recovery from RVF is usually followed by immunity and neutralizing antibody in the blood (5). Passive immunity has been observed in the hamster model (4).

Recommended therapy: Current research has brought forth several means for therapy (ribavirin, ribamidine, liposomal-encapsulation of ribavirin, poly(ICLC), 9-beta-D-ribofuranosylpurine-6-carbox-

amides, immunomodulators such as glucan or interferon, lipoidal adjuvants and antibody treatment). Comparisons have yet to be done to determine which are the most effective.

Priority for further development: The efficacy of the various tried vaccines against those newly developed should be compared. Of the several means of treatment, comparisons should be made to determine which few are the most effective and if some are broad-spectrum for other viral diseases.

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c) **TOXINS:**

i) **Botulinum (Agent X):** Produced by the anaerobic bacterium Clostridium botulinum, this toxin is a high m.w. protein and 7 immunological types, A-G, have been found. It is stable for a week in non-aerated water, differs from other bacterial toxins in that it is not destroyed by gastro-intestinal secretions, but it is destroyed when boiled for 15 min. It should be noted that botulinum spores (which can germinate and produce the toxin under aerobic conditions) can survive boiling for 6 hours (1).

Morbidity and mortality: Transmission is usually through eating contaminated foods but the toxin may enter through breaks in the skin or by inhalation. The time before symptoms varies with the route of entry and the concentration of toxin (1). Symptoms are intoxication involving the nervous system and about 1/3 of the patients die within 3-7 days, usually to respiratory failure (2). The dose for an LD_{50} in humans is 0.00003 ug/kg (3).

Vaccine availability: An effective pentavalent (ABCDE) vaccine has been developed by USAMRIID. Previously, a type A and B toxoid gave suitable protection after multiple injections, and a booster inoculation at 1 year raised the titre 500-fold over minimal protective levels with high titres persisting for 2 years (4).

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Value of passive immune approaches: Anti-toxin antiserum, produced from horse serum, has been used as a treatment in the neutralization of the toxin. However, serum sickness and in severe cases, anaphylactic shock and glomerulonephritis, may result (5). There is probably more merit in the use of antibodies as prophylaxis, before the noted injury occurs to the neural tissue site.

Recommended therapy: Other than alleviating the symptoms and maintaining life-support, anti-toxin does reduce the side effects of botulism poisoning (6). The use of zinc (7), 4-aminopyridine and 3,4-diaminopyridine (8) appear to have a minor therapeutic value on some of the type toxins.

Priorities for further development: The efficacy of the vaccines is tested on laboratory animals, and yet animals appear to have different susceptibilities than humans. A better means of determining the effectiveness of existing vaccine or lots should therefore be considered (in vitro assays using human cell lines?). Work on novel vaccines (toxin subunits, de-activated derivatives, peptide fragment) should continue. Collaboration has begun between DRES (Dr. Les Nagata) and ADRI-Lethbridge (Dr. S. Masri) to develop genetically cloned human antibodies.

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ii) **Ricin (Agent W)**: Produced by the castor bean, Ricinus communis, ricin consists of two hemagglutinins (RCL I and II) and 2 toxins (RCL III or D and IV). The toxins are dimers (66,000 m.w.), the agglutinins are tetramers (130,000 m.w.) (1).

Morbidity and mortality: Symptoms depend on the dose and route of entry (gastro-intestinal hemorrhage if given orally, organ necrosis if given intramuscularly, nasal and lung necrosis if given as an aerosol (2). The LD_{50} is 0.02 ug/kg (3).

Vaccine availability: A ricin toxoid has been developed which when used as vaccine protects against toxin given by inhalation (2). Chemical modifications (4), reduction of the disulfide bridge (5), and deglycosylation (ricin enters the cell by a mannose receptor)(6) may be new tools for producing vaccines.

Value of passive immune approaches: Studies have shown that anti-ricin IgG protects animals, but its effectiveness is more pronounced against respiratory than intravenous exposures (2).

Recommended therapy: There is little literature on the treatment of ricin intoxication other than to maintain life support.

Priorities for further development: As ricin binds to galactose, if a polysaccharide of this sugar (agar is a polymer of galactose having sulfhydryl groups) can be introduced into the cell (i.e. liposome therapy?) it may have some therapeutic value as might inactivating monoclonal antibody that binds to ricin's galactose-binding domain (7). As there is little effective therapy once the damage has been done, the best option may be to protect troops with vaccines or protective human monoclonals.

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iii) **Staphylococcal enterotoxin B (SEB):** The bacterium Staphylococcus aureus (a common skin contaminant) produces several enterotoxins (SEA-SEE) (1) of which SEB is usually produced in the greatest amounts and is one of the most potent. Unlike most bacterial exotoxins, SEB is stable in boiling water (resistant to boiling/30 min) and to potable quantities of chlorine (2).

Morbidity and mortality: Transmission is usually by the ingestion of contaminated foods, but death in animals does occur when it is given intravenously (toxic shock syndrome). Incubation is relatively short (30 min - 4h), and recovery usually occurs within 24 hours. Clinical symptoms are sudden and with violent onsets of severe nausea, vomiting, stomach cramps, severe nausea and incapacitation. Fatalities are rare but do occur (2). The dose for an ED_{50} (incapacitation) is 0.04 ug/kg (3).

Vaccine availability: A micro-encapsulated toxoid of SEB has been developed that produces an immune response in mice (4).

Value of passive immune approaches: To date passive immunity with antibodies raised to culture filtrates has not been successful, possibly due to the immune response to the other components, rather than to enterotoxin.

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Recommended therapy: In unsensitized monkeys, cimetidine and diatriazem completely prevented emesis and diarrhea upon gastric challenge with SEB. The former compound is a selective blocker of the H2 (histamine) receptor, the latter is a calcium channel blocker (1).

Priorities for further development: To develop a means of treatment or protection, the mechanism by which SEB induces its effects should be elucidated. Something as simple as diet, or the ingestion of an antagonist (4), may be all that is needed to prevent the symptoms of severe vomiting. If regions of SEB can be synthesized that convey antigenicity but not toxicity, novel vaccines could be developed (7). There is also considerable potential in the cloning of the SEB genome, or parts of the genome, into E. coli (1), and with this the expression of sufficient material for new toxoid vaccines.

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APPENDIX A:

1. Calculations for the relative effectiveness of a BW agent:

The chemical warfare agent, SOMAN, has an LD_{50} of 5×10^{1} ug/kg

The bacterial toxin, BOTULINUM TOXIN, has an LD_{50} of 3×10^{-5} ug/kg

The bacterium, Francisella tularensis, weighs about 1×10^{-6} ug. Assuming 10 organisms will causes infection in a 70 kg man, and without treatment there is a 10% chance of death then the LD_{10} is 1×10^{-5} ug/70 kg or about 1×10^{-7} ug/kg

2. Calculation for the theoretical contamination of an area 1 km in diameter and 0.5 km in height (a sphere is $4/3\ \pi r^3$)

Volume of air : $1/2 \times 4/3\ \pi r^3$

$= 1/2 \times 4/3 \times 3.14 \times (0.5\ km)^3$

$= 2.09 \times (5 \times 10^{4}\ cm)^3$

$= 2.09 \times 125 \times 10^{12} cm^3$

$= 2.09 \times 125 \times 10^{9}$ litres

$= 2.6 \times 10^{11}$ litres

If a soldier breathes 50 litres of air/minute and takes 10 seconds to don a gas-mask, he will have inhaled about 8 litres of air.

For an exposure to F. tularensis, assume 10 organisms are sufficient to cause an infection. Hence, the soldier may get infected if he breathes in 10 viable bacteria/8 litres of air (or 1.25 viable bacteria/litre).

The minimum theoretical amount of bacteria to contaminate the given area is therefore:

1.25 bacteria/litre $\times$ 2.6×10^{11} litres $= 3.2 \times 10^{11}$ bacteria

This organism weighs about 10^{-12} grams, so only 3.2×10^{-1} gram (about the weight of a paper-clip) of bacteria is needed to contaminate an area 1 km in diameter.

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Brief summaries are given on examples of bacteria (Bacillus arthracis, Francisella tularensis, Brucella spp., Coxiella burnetii), viruses (Venezuelan equine encephalitis, smallpox, Rift Valley fever), and toxins (botulinum, ricin, staphylococcal enterotoxin B) that are of medical importance. Descriptions, morbidity and mortality, vaccine availability, value of passive immune approaches, recommended therapy, suggested priority for further development and references are given for these examples.

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Bacillus anthracis	anthrax	vaccines
Francisella tularensis	tularemia	antibiotics
Brucella spp.	brucellosis	antibodies
Coxiella burnetii	Q-fever	passive immunity
Venezuelan equine encephalitis	VEE	disease
variola	smallpox	
Rift Valley fever	RVF	
botulinum toxin	bacteria	
ricin	viruses	
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