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From the Editor…….

My apologies to those who read and subscribe to The Chemist since it has taken much too long to get this issue out. This issue includes papers from the laboratory of Dr. Margot Hall and her students on a variety of interesting topics. There is a modest contribution on Ion Mobility Spectrometry and its application in a variety of fields. Finally, we have a book review to round out this issue. The next issue scheduled for later this fall will include book reviews, meeting reports, contributed papers and general information for the membership. As with all vehicles such as this we continue to solicit more manuscripts since they are the life blood of The Chemist. Should you wish to discuss a submission, please feel free to call me at 717-534-5145 or contact me via email through the AIC Office.

Best wishes for the fall.

W.Jeffrey Hurst, PhD, FAIC
CoEditor, The Chemist

The History and Science of CBRNE Agents, Part I

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Abstract

Various unconventional forms of warfare have existed throughout history and include intentional contamination, poisoning, and delivery of a variety of weapons—virulent microorganisms, deadly toxins, and high-yield explosives, including atomic weapons. Such weapons have been studied and utilized on the battlefield, in political struggles, and in terrorist activities for centuries. Chemical, biological, radiological, nuclear, and explosive (CBRNE) substances assumed a new prominence in the public consciousness following recent terror attacks. These agents pose a public health risk; thus, scientific professionals, including biochemists, should understand the history of CBRNE agents, their potential for harm, and the technologies—both common and advanced—used when handling a suspected CBRNE incident.

Introduction

The acronym *CBRNE* (pronounced SEA-BURN-EE), meaning Chemical-Biological-Radiological-Nuclear-Explosive, has replaced the passé acronyms NBC (Nuclear, Biological, Chemical) and ABC (Atomic, Biological, Chemical), employed to describe agents used by some party to intentionally inflict harm on another party. The official definition from 18 U.S.C. Section 2332a is:

> Any explosive, incendiary, poison gas, bomb, grenade, or rocket having a propellant charge of more than four ounces [113 g], missile having an explosive or incendiary charge of more than one-quarter ounce [7 g], or mine or device similar to the above. (2) Poison gas. (3) Any weapon involving a disease organism. (4) Any weapon that is designed to release radiation at a level dangerous to human life. [1]

The separate words that comprise the acronym CBRNE describe each agent that could be employed malevolently (e.g., *Biological* describes microbial agents such as *Bacillus anthracis*). Another analogous term currently en-vogue and heard often in media reports and political circles is *weapons-of-mass-destruction* (WMD). Historically, the planning and use of such agents was reserved for warfare between opposing state forces, but recent events have shown a new, frightening utility for CBRNE among terrorist groups (both domestically and abroad), who have shown an interest and willingness to use the agents to spread fear and chaos among civilian populations for political and ideological reasons.

Scientific professionals will be among the first to respond to the aftereffects of any CBRNE incident. Imperatively, biochemists must understand: the history of CBRNE to grasp the injurious potential of past usage; agent biochemistry (modes of action, infection, etc.); and the usefulness of traditional and new laboratory methods for diagnosis.

The History of CBRNE

The malicious use of chemical and biological agents is not a recent phenomenon. Evidence exists that prehistoric humans used arrowheads and spears dipped in feces. [2] As early as 1000 B.C. during the late Bronze Age [3], the Chinese recorded hundreds of recipes for compounds that were mixed with gunpowder in hopes of producing a toxic smoke to incapacitate their enemies; mixtures such as “soul-hunting fog” (containing arsenic) and “five-league fog” (enriched with wolf excrement) were described in detail and still used over ten centuries later.

The first suspected use of a biological agent occurred circa 500 B.C. during the Classical Age [3], when it is believed the Assyrians poisoned their enemy’s water supply using rye ergot, a poisonous mycotoxin obtained from diseased

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rye. [4] Even the Spartans utilized an early form of chemical warfare, when they harnessed toxic smoke from tar-and-sulfur-soaked burning wood and used it against the Athenians during the Peloponnesian War. Later, hellebore roots were used by Solon of Athens to taint a tributary used by Cirrhaeans as a water source. [5]

In 1155 A.D., Barbarossa contaminated his enemy's wells with the bodies of his dead troops during the Battle of Tortona. [6] During the Hundred Years War at the siege of Thun l'Eveque in 1340 A.D. (as the Plague began rampaging in one of its many historical epidemics), the practice of propelling animal carcasses at enemy fortifications was grasped more as a harassment technique versus an intentional means of spreading disease. Jean Froissart, a contemporary chronicler, stated the besiegers "...cast in deed horses, and beestes stynking..." and that "...the ayre was hote as in the myddes of somer: the stynke and ayre was so abominable." Such was the misery of the defending contingent that they surrendered, though no record of consequential illness exists.[5]

In thirteen years, the dreaded Plague had traveled from Asia to the Middle East, arriving in 1346 A.D. Once it manifested at the walled port city of Kaffa in present-day Feodosiya, Ukraine, inventive, retreating Turkish besiegers decided to catapult their own plague-ridden dead over the walls of the city. [7] Thus was born the first confirmed use of a microbial agent to inflict harm, though no one believes these warriors understood the exact etiology of the disease; the use of cadavers as a source of contagion, or "bad air", obviously was understood. [5] The supposition that warriors believed decaying matter tainted the air and caused disease was definitely confirmed in 1422 A.D.; Corbut launched dead soldiers and loads of feces amidst his enemies at Carolstein, such that "a great number of the defenders fell victim to the fever which resulted from the stench." [6] Spanish troops attempted to induce disease in French soldiers by using wine tainted with the blood of lepers in 1495 A.D., but this effort was unsuccessful. [6]

Around 1500 A.D., the use of fomites as agent vectors became a new strategy of biological warfare. Pizarro used clothing contaminated with smallpox as "gifts" for the natives of South America. The British would resurrect Pizzaro's fomite vector tactic again in 1754 A.D. during the French-Indian War; blankets from a smallpox hospital were presented to Native Americans camped around Fort Pitt, causing disease that quickly spread throughout the tribes. However, it was not confirmed that the fomites were the direct cause of the disease, as it was already endemic in Native American tribes at that juncture. [6]

The American Civil War marked an era of advancing military technology, though several archaic methods of biological warfare were revisited. In 1863, Dr. Luke Blackburn, a Confederate physician, was incarcerated for importing clothing from yellow fever and smallpox patients and selling them to Union troops; only one Union officer was purportedly killed by this scheme. During the summer of that same year, General W.T. Sherman wrote that, as the Union retreated from Vicksburg, Confederate troops deliberately shot farm animals and deposited them in ponds to slow the Union withdrawal. [6]

The truly modern era of biological and chemical warfare commenced during World War I. The early Twentieth Century was a milestone in microbiology, with new erudition on the causative agents of disease and culturing techniques. Germany developed the first-known comprehensive biological warfare program; their plan was to use *Bacillus anthracis* (anthrax) and *Burkholderia mallei* (Glanders) to infect Allied livestock, but success cannot be certain, as these are naturally occurring diseases in certain farm animals. A German physician, Anton Dilger, suffered a nervous breakdown and was discharged from the German army in 1915; subsequently, he traveled to the United States to live and rest with his parents in Virginia at a time when America was still neutral in the conflict. Dilger brought with him cultures of *B. anthracis* and *B. mallei*, which he gave to a German operative, Captain Frederick Hinsch, who inoculated horses bound for Allied troops in Europe. German agents would use this tactic of infecting horses and livestock extensively during the period between 1915 and 1917. [6]

The chemists were not to be outdone by the biologists in the First World War. The Second Battle of Ypres on April 22, 1915 marked the very first widespread usage of synthesized chemical agents in full-scale war, when the Germans attacked French and Algerian forces with chlorine gas. A total 50,965 tons of pulmonary, lacrimatory, and vesicant agents, including numerous variants of chlorine (Cl_2), phosgene, and mustard gas, were utilized by both the Central and Allied powers, causing

176,500 non-fatal casualties and 85,000 fatalities directly. [8]

The horrors of World War I led many statesmen to question the justness and humaneness of biological and chemical agents. The *1925 Geneva Protocol for the Prohibition of the Use in War of Asphyxiation, Poisonous, or Other Gases, and of Bacteriologic Methods of Warfare* was the first worldwide attempt to halt proliferation of these agents, but effectively became a "no-first-use" policy when the French, Soviets, and British proclaimed they would use the agents if first attacked with them. Possession of the agents was not forbidden under the Geneva Protocol; therefore, proliferation was paradoxically assured. Although a signatory, the United States Senate did not ratify the Geneva Protocol until 1975. [6] In 1918, the Japanese formed a special military division to investigate the practicality of biological weapons; the Japanese would later take up their newfound knowledge when occupying China during World War II. [2 8]

During World War II, Germany further expanded on the embryonic chemical agent technology of World War I through its discovery of the nerve agents tabun (Figure 1), sarin (Figure 2), and soman (Figure 3). Although the Nazis developed and manufactured several chemical agents during this period, neither the Axis nor the Allied Powers used them in the European theater. Documents recovered in Germany after the war showed that Germany believed the Allies also had access to nerve agents, and so fear of retaliation is believed to have discouraged their use by the Nazis. However, the Axis Powers did not completely ignore these agents. Japan used mustard gas (Figure 4) and another recently developed blister agent Lewisite (Figure 5) against Chinese troops during the Japanese occupation. The Japanese also experimented with biological warfare agents, intentionally testing the agents of cholera, dysentery, typhoid, plague, and anthrax on enemy human subjects during their occupation of Manchuria from 1931 until their eventual surrender in 1945. [8 9] Ultimately, the United States would overlook the atrocities of the implicated Japanese scientists during the war crimes tribunals following the war. Intelligence had long indicated a robust Japanese bioweapons program, including purported plans to attack North America using paper balloons containing bioagents. [9] Given U.S. isolationism and reluctance to enter World War II, American biological and chemical weapons research had lagged behind other world powers for decades. [9]

A senior Palestinian Islamic religious authority, Haj Amin el-Husseini (a close ally of Hitler), spearheaded a chemical attack on Jews in Palestine that was ineffective. Agents carrying canisters of German "fine white powder" were instructed to empty the canisters at strategic points in the Tel Aviv water system. Each of the five canisters was described as containing enough chemical agent to kill 25,000. [8] With Adolf Eichmann's effort to address "The Final Solution of the Jewish Question," the Nazis utilized the insecticide Zyklon B, containing hydrogen cyanide (HCN) gas, to murder hundreds of thousands of unsuspecting Jews and other "undesirable" victims in the "showers" of the concentration camps during the Holocaust. [8 10]

A new, terrifying weapon ended the struggle of the Japanese in August 1945, when the first atomic weapons were employed by the United States against the Japanese mainland in the cities of Hiroshima and, later, Nagasaki. For the first time ever, entire cities could be leveled in mere seconds, with thousands of instantaneous fatalities and the subsequent casualties resulting from radiation toxicity and contamination. Paralleling the mid-war U.S. rush to acquire biological and chemical weapons technology, the United States heavily invested resources in the race to be the first nuclear power ahead of Germany and Japan, who were also working towards nuclear weapons programs at the time. [11 12] Debates still rage about whether the U.S. should have used this new, powerful weapon to end the war, but there is no doubt that the 1945 employment let the nuclear "cat out of the bag" and helped trigger arms races that have not yet resolved.

Captured German and Japanese technology (and scientists) fueled the arms races of the succeeding Cold War. The United States and the Soviets both recovered German artillery shells containing nerve agents and used them to expand their own chemical weapons arsenals. [8] German rocket technology was the basis of the U.S.-U.S.S.R. space race and complex CBRNE missile delivery systems, which would relegate atomic bomb technology to obsolescence.

Since World War II and the conclusion of the Cold War, several nations acquired or are believed to possess nuclear weapons, such as the United States, former Soviet Republics (e.g., Russia), France, the United Kingdom, Pakistan, India, and Israel. The nuclear stockpiles of the world are believed adequate to destroy the world

many times over. [13] Nuclear proliferation by unstable Third World countries (such as Iran and North Korea) is a present, vehement focus in U.S. national security and foreign policy circles.
In 1969, President Richard Nixon signed an executive order that renounced U.S. preparations for biological war; the U.S. limited its research efforts to vaccines and defensive measures. Between 1971 and 1973, offensive stockpiles of biological weapons were destroyed at Ft. Detrick, Pine Bluff Arsenal, and Rocky Mountain Arsenal. A small portion of biological agents was retained at the newly established United States Army Medical Research Institute for Infectious Diseases (USAMRIID) for defensive studies. [6]

Early in the Iraq-Iran War that started in 1980, Iraq began to employ mustard gas (Figure 4) and tabun (Figure 1) against Iranian forces, causing 5% of all Iranian casualties. Iran was also alleged to have used chemical weapons manufactured by Iraq and the United States, but this was never confirmed. After the war ended in 1988, the Iraqi Kurdish village of Halabja was exposed to mustard, sarin (Figure 2), tabun, and VX (Figure 6) by Saddam Hussein's regime, killing about one-tenth of the town's 50,000 residents. [8]

However, accidents, both related to CBRNE proliferation and benign civilian purposes, managed to exemplify for civilian populations on both sides of the Cold War just how deadly CBRNE agents are. The Three-Mile Island incident was a near disaster for the U.S., with widespread nuclear contamination only narrowly avoided. Chernobyl was the nuclear power disaster that Three-Mile Island could have been, with vast, detectable radiological contamination "…subsequent[ly] transport[ed] across Asia to Japan, the North Pacific, and the west coast of North America." Neither disaster, however, equaled the levels of radiation released in the atmosphere from Cold War nuclear weapons tests. [14] There were also accidents involving bioweapons or potential bioweapons in the Soviet Union and United States. In April 1979, the Soviet city of Sverdlosk experienced an accidental release of weaponized anthrax spores from a military research facility, which caused a small-scale epidemic. [6] In 1989, a research facility in Reston, Virginia had a scare when a certain strain of *Ebola* virus (typically a highly virulent, usually fatal hemorrhagic Filovirus) was discovered in a group of imported research monkeys. There have been subsequent outbreaks of this strain, *Ebola-Reston*, among research primates imported from the Philippines, with human seroconversion but fortunately no illness. [15]

The United States experienced its first case of confirmed bioterrorism in 1984. Followers of the Indian cult leader named Bhagwan Shree Rajneesh deliberately contaminated several restaurant salad bars with *Salmonella typhimurium* causing 751 cases of gastroenteritis. [6]

Japan experienced chemical terrorism in 1995 with a release of sarin nerve gas into the Tokyo subway system by members of the Aum Shinrikyo cult, which resulted in 12 fatalities and over 3,000 injuries. The cult had previously attempted bioterror attacks with several potential bioweapons, including botulinum toxin. [6]
About one month after the September 11, 2001 terror attacks in Washington, D.C. and New York City, several cases of cutaneous and inhalational anthrax appeared among employees of media outlets and the U.S. Postal Service. The source was determined to be letters sent through a post office in New Jersey, and additional letters bound for prominent U.S. politicians were intercepted. A massive epidemiological investigation ensued, but those responsible for this latest bioterror attack in the United States have not been apprehended. [6] The source of the weaponized anthrax (a product beyond the capabilities of the layman) was never definitively determined.

The history of CBRNE agents is long and sordid, and this brief, historical timeline is by no means complete. The past has shown the threat of these agents will never be eliminated, either in warfare or terrorism. The most recent events of history have shown a remarkable, rapid escalation in technology that has unfortunately included improvements in CBRNE technology.

CBRNE Agents

This two-part article will look at specific examples of probable CBRNE agents in each category—those chemical, biological, and radioactive compounds most likely to have the greatest deleterious effects with the least expense and difficulty for the perpetrators.

In attempting to evaluate and discuss agents that can be used as WMDs, the question, "What can cause a *maximum credible event*?" is hopefully answered. A *maximum credible event* is one that could cause a large loss of life in addition to disruption, panic, and an

> overwhelming use of civilian healthcare resources. For an agent to be considered capable of causing a maximum credible event, it should be highly lethal, inexpensively and easily produced in large quantities, stable in aerosol form, and have the ability to be dispersed (1-5 mm). The ideal agent also is communicable from person to person and has no treatment or vaccine.[16]

Scientists and medical professionals must be familiar with key CBRNE agents that could be encountered in the current uncertain sociopolitical climate, either via state-initiated warfare or through terrorist plots and actions. Radical idealists of every persuasion, though small in number, can cause exceptional harm and panic with considerably few resources.

Time and space do not permit an adequate treatment of every possible agent; indeed, many common household or agricultural products could be used as CBRNE agents. The 1995 bombing of the Federal Building in Oklahoma City is one such example, where fertilizer was used as a key ingredient in a high-yield explosive, which erased the front half of a large, multistory building. Even something as familiar as carbon monoxide (CO) could be employed as a CBRNE agent in the right circumstances. Therefore, it is important be aware of the plethora of agents, both known and unforeseen, to provide the best support to providers and investigators (i.e., FBI, CDC, epidemiologists, toxicologists, etc.) during any incident. In many situations, symptoms and mechanisms of action discussed in this paper are similar among many disparate agents; therefore, the professional should attempt to glean how routine laboratory results might apply to agents not discussed in this study (e.g., pulmonary agents would obviously cause hypoxia with a low PO_2).

In addition to examining the biochemical pathways and mechanisms of each agent type, this article will also examine probable clinical presentations (i.e., symptoms, routine test results, etc.) when known; possible means of delivery (i.e., vectors); and other investigative, confirmatory test methods employed in the laboratory to aid in mitigating the effects of a CBRNE incident.

Chemical Agents

In a sense, all CBRNE agents produce their insidious effects on living tissue at the molecular level. The mechanisms of biological agents, for example, are ultimately biochemical in nature, even if they do not involve the actions of synthetic chemical compounds. However, this section focuses solely on synthetic chemical compounds in four main groups: blood agents, mostly based on cyanide, which cause chemical asphyxiation at the cellular level; vesicants (the so-called blister agents), such as mustard gas, that cause blistering of the skin; pulmonary agents (or choking agents), such as chlorine, that suffocate by hindering the lungs; and, perhaps the most lethal, nerve agents, such as sarin and VX, that inhibit the breakdown of the neurotransmitter acetylcholine in nervous tissues. [8]

This section will not discuss incapacitating or lachrymatory chemical agents (such as tear gas or pepper spray), which are typically non-lethal compounds producing short, temporary physiological or mental effects. Such agents are used by law enforcement for crowd and riot control, offering limited utility for the terrorist or combatant at war.

Chemical Delivery Vectors

Salts and other solids have been used for years in various poisons, but solids offer limited utility in causing widespread damage. At first, tainting a water supply might seem a good means of inflicting harm, but water quickly dilutes any agent and mitigates its effects. Another possible delivery tactic is solid or vapor dispersal from low-flying aircraft, but weather, as with gaseous vapors from munitions, can confound the applicability of this strategy

In military applications, the most effective, proven means of delivery has been in the vaporized form via large munitions (e.g., bombs or missiles), as smaller munitions fail to provide adequate air volume saturation. Typically, liquid agents are volatile by nature or design; therefore, liquid agents that will rapidly vaporize are employed to cause the greatest damage with greatest ease of storage and maintenance.[17]

Whether vectored to target by munitions or aircraft, attackers must carefully plan for changes in weather (especially wind direction and speed) to achieve maximum effect with the least damage to one's own side. In terrorist incidents such as the Aum Shinrikyo subway attacks, a non-explosive, vaporizing mechanism proved useful at incapacitating large numbers of people in a relatively confined space; an

explosion preceding dispersal could alarm intended victims, speed their escape, and prevent the maximum effects of the agent.

Blood Agents

Cyanide, such as the Nazi's infamous Zyklon B, is a deadly agent that interferes with oxygen utilization at the cellular level. Typical volatile forms of this agent are seen in cyanogen chloride (ClCN) and hydrogen cyanide (HCN). The use of the term "blood agent" is actually a misnomer; it has no direct effect on erythrocytes or plasma. [18] Rather, cardiac and nervous tissue damage accounts for its lethal effects.

The active atom in any such compound, whether as a gas or salt, is the cyanide ion (CN^-), which is a metabolically aggressive species causing immediate injury to the optical and respiratory systems. Symptoms of exposure are lethargy or coma, dyspnea, tachypnea, tachycardia, and hypotension. Severe poisoning results in bradypnea, bradycardia, cardiovascular collapse, and ultimately death. [19] [20] Patients may report smelling bitter almonds. [17] Multiple clinical presentations of this type would constitute evidence as to its use.

Cyanide's toxic effects stem from its inhibition of electron transfer in the mitochondria along the electron transfer chain to oxygen during ATP synthesis. Cyanide binds to a crucial enzyme called cytochrome oxidase, which is utilized in the mitochondria for aerobic respiration. With the impairment of oxidative phosphorylation, ADP, H^+, Na^+ (sodium pump failure), and Mg^{2+} accumulate in the mitochondria and cytosol, and ATP is quickly depleted. [21] Lactic acid increases as anaerobic respiration attempts to fill the void left by the failure of aerobic respiration. [17] Therefore, major cellular respiration and energy production is rapidly hindered, and cell, tissue, and organ death ensues.

In the clinical laboratory, metabolic and lactic acidosis is seen, with an unexplained high anion gap ($[Na^+] - \{[Cl^-] + [HCO_3^-]\}$) and elevated lactate levels (if such testing is available). Blood gases show an elevated oxygen level, and all these presentations are due to the disruption of oxidative phosphorylation. [20] [22]

Confirmation would constitute a whole blood cyanide level greater than 0.05 µg/mL. [20] Methods are diverse and difficult, as cyanide is an elusive poison. The "gold standard" for all chemical toxins is the ubiquitous, time-consuming gas-chromatograph-mass-spectrometer (GC/MS). This technique involves first vaporizing a substance before injecting it into a GC column, which separates the substance into distinct compounds. The separated molecules immediately enter the MS, where they are ionized by a high-energy electron beam, transported and separated from uncharged species (based on mass-to-charge ratios), and detected. [23] Tung et al. introduced a more rapid method for determining blood cyanide levels by first binding cyanide to a sodium hydroxide trap [24]; with the addition of methemoglobin as the colorimetric indicator, cyanide levels can be determined spectrophotometrically, much like the traditional method for determining hemoglobin levels.

Blister Agents

As the name implies, these agents cause large, fluid-filled blisters to develop on exposed skin and other mucosal surfaces. Vesicants such as Lewisite (Figure 5) made their combat appearance during the First World War and caused more casualties than all other agents combined, including chlorine, phosgene, and cyanogen chloride. [25] The use of Lewisite was later abandoned when an effective antidote was synthesized to counteract the active arsenic component. [26]

Following Lewisite, mustard agents were introduced in two forms—complexed to sulfur (Figure 7) and soon after to nitrogen (Figure 4). The name *mustard* is derived from the characteristic color of the impure gas and the garlic or mustard plant odor often accompanying its release. [26] No effective antidote yet exists for this vesicant agent. [25] This paper will focus on the biochemistry of the vesicant sulfur mustard, as it has not been rendered obsolete. Nitrogen mustard has never been used; its effects are uncertain. [25]

A major complication of mustard use is its stealthy nature. Pain and blisters are major symptoms that do not manifest for hours after exposure, whereas Lewisite's effects are immediate. [25] Even if one survives exposure, which is likely given that mustard is not usually fatal [25], a strong correlation with lung cancers and mustard inhalation has been shown. [21] Sulfur mustard victims develop deep, itching or burning blisters where the agent contacts the skin; exposed eyes become sore and swollen, increasing the risk of conjunctivitis and blindness. Breathing high concentrations causes bleeding and blistering within the respiratory system, leading to pulmonary edema. The

greatest danger of fatality comes with extreme dosages (LD_{50} = 100 mg/kg). [25] [26]

The biochemistry of mustard is not clearly understood, which accounts for the lack of available antidotes. Mustards are strong alkylating agents; they act through cyclization with ethylene groups forming a strong sulfonium electrophilic center that reacts powerfully with any of the important macromolecular nucleophiles involved in a variety of metabolic processes, such as peptides and nucleic acids. In the case of nucleic acids, mustard is thought to cause breaks in DNA strands that increases the activity of the repair enzyme poly (ADP-ribose) polymerase, or PADPRP. The increased activity rapidly depletes stores of NAD^+, a crucial cofactor in glycolysis, causing a buildup of glucose-6-phosphate, and this buildup stimulates the hexose monophosphate shunt, which triggers cellular proteases. Proteases in basal epidermal cells are thought to cleave adherent fibrils connecting the basal epidermal cell layer to the basement membrane, resulting in the characteristic blisters. [25]

Another theory involves mustard's inactivation of the free radical scavenger glutathione; in such a situation, sulfhydryl groups are inactivated and a loss of free radical protection ensues. Calcium and magnesium adenosine triphosphotases are laden with sulfhydryl groups, so their quiescence would result in high calcium levels within the cell, triggering the activation of several cleavage enzymes, such as proteases and endonucleases. The final step in the hypothetical glutathione cascade is cell death. [25]
No specific test for mustard exposure exists. Standard laboratory panels would show leukocytosis, hyperglycemia, and hypercalcemia. Increased levels of thiodiglycol, a mustard metabolite, have been demonstrated in the urine of patients using GC/MS up to two weeks post-exposure. [25] [27]

Pulmonary Agents

As the name implies, pulmonary or choking agents interfere with breathing and cause suffocation, and the main agents include chlorine gas (Cl_2 – a yellow-green gas), chloropicrin (Figure 8), phosgene (Figure 9), and diphosgene (Figure 10), all containing chlorine in varying molecular configurations. [28] Other toxic inhalational agents exist and work by similar mechanisms, such as zinc oxides, nitrogen oxides, phosphorous smokes, and titanium tetrachloride [25]. For the purposes of this treatment, the focus will be on chlorine-containing gases, as history and science provide adequate data on these similar agents from which to draw conclusions.

The acute symptoms associated with pulmonary agents are all very similar; for example, chlorine and phosgene can cause skin irritation, ocular involvement, spasmodic coughing, a choking sensation, substernal tightness, aphonia, stridor, hemoptysis, dyspnea, tracheobronchitis, pneumonitis, and bronchopneumonia; peribronchial and perivascular fibrosis follow chronically in the case of chlorine. Phosgene causes acute pulmonary edema and bronchiolitis obliterans (as can lone chlorine in sufficiently high doses). Exertion, asthma, or other conditions that increase the respiration rate intensifies and hastens the effects of any respiratory agent. [21] [25] [29]

The biochemistry of chlorine gases is insidious. Soon after exposure, water in the lungs combines with the compound to form carbon dioxide, hypochlorous acid, and hydrochloric acid, the latter of which simply begin dissolving lung tissue. [30] The reaction can be summarized as follows:
$Cl_2 + H_2O$ -> HCl (hydrochloric acid) + HOCL (hypochlorous acid)

Segal discussed the somewhat controversial assertion that tissue damage is also caused by the generation of free oxygen radicals. [30] This once accepted, now debated method of generation is summarized in the following reaction:
$Cl_2 + H_2O$ -> 2 HCl + $[O^-]$ (nascent oxygen)
In addition, phosgene, a gas often emitting a smell of freshly cut grass, hay, or green corn, is an alkylating agent and carcinogen, as it interferes with DNA replication. [31]

Arterial blood gases (ABG) provide convincing clues as to the use of these agents. PO_2 levels provide nonspecific information as to the severity of the resulting hypoxia, as do increased CO_2 levels. ABG levels returning to normal within 4 to 6 hours post-exposure indicate a decreased risk of mortality. [25] One might also expect to see decreased pH and increased chloride values, depending on the level of absorption, but no concrete research exists describing the usefulness of these laboratory values [32].

Testing for these agents after exposure is not plausible, as they are quickly reduced into the acid compounds described previously. Instead, first-responders will need to collect anecdotal

evidence (i.e., witness accounts, circumstances at the scene, patient accounts, etc.) to determine whether these agents account for the symptoms and clinical presentation. Military and civilian first-responders (i.e., firefighters, FBI investigators, etc.) possess equipment and reagents to detect a variety of residual chemical agents at the incident scene.

Nerve Agents

Nerve agents are perhaps the most deleterious of all chemical agents. They occur in forms discussed previously, such as sarin, cyclosarin, soman, tabun, and VX, the latter of which gets its two-letter moniker from a United Nations military designation—the "V" stands for venomous. [25] All nerve agents belong to a class of compounds designated as *organophosphates*, resulting from the esters of phosphoric acid in various configurations. As the term implies, nerve agents affect elements of the nervous system by interrupting the breakdown of the neurotransmitters that signal muscle tissues to contract, which prevents them from relaxing. [33] [21] A lethal dose (LD_{50}) of VX, the most reactive, deadly nerve agent, is only a mere 10 mg/70 kg for cutaneous exposure, while the least reactive (but still quite deadly) is sarin (LD_{50} = 1.7 g/70 kg). Lethal doses by inhalation require far less. [25]

Initial symptoms of exposure include rhinorrhea, substernal tightness, and pupil dilation. Soon after, the victim experiences dyspnea, nausea, and salivation, followed by involuntary emesis, defecation, and urination. Muscle twitching progresses into convulsive involuntary spasms, and ultimately the victim becomes comatose and suffocates. [33] Effects on the parasympathetic autonomic nervous system result in bronchoconstriction, miosis, gastrointestinal symptoms, increased secretions, urination, and bradycardia; effects on the junctions between nerves and muscles result in tachycardia, hypertension, muscle fasciculation, tremors, weakness, and flaccid paralysis. [21] The effects of nerve agents are long lasting and cumulative with successive exposures; survivors of nerve agent almost invariably suffer from chronic neurological damage. [33] [21]

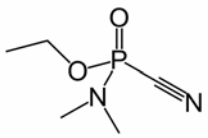
Nerve agents inhibit the key cholinergic enzyme, acetylcholinesterase (AChE). Esterases (as a class of enzymes) catalyze the hydrolysis of esters, and AChE has a high affinity for the esters of acetylcholine (ACh), a neurotransmitter of the autonomic nervous system. [25] Free, unbound ACh builds up at the endings of autonomic nerves due to the inhibition of AChE by the organophosphate agent, causing continuous electrical stimulation and the resulting physical symptoms. Nerve agents actually inhibit AChE by binding to a serine hydroxyl group at the enzyme's active site, forming a stable, phosphorylated, inactive enzyme. Dephosphorylation of the enzyme-agent complex is the rate-limiting step. [21]

Respiratory impairment involved in nerve agent intoxication produces expected abnormalities in arterial blood gas values, including a reduction in PO_2. Hypokalemia has been reported in sarin exposure, although the mechanism has not been ascertained. No standard laboratory tests exist to measure nerve agent levels directly; however, indirect evidence can be gathered. [34] One method of determining exposure via standard laboratory testing involves measuring erythrocytic cholinesterase (RBC ChE) and pseudocholinesterase (plasma butylcholinesterase or BuChE) levels, which are reduced 20-25% by the agent; however, baseline values are most useful when using suspected post-exposure enzyme levels for comparison. RBC ChE and BuChE levels that remain unchanged over time run counter to exposure, but conclusions and treatments should be based foremost on symptoms. [25] [34]

End of Part I

In this article, we examined the long history of CBRNE agents and introduced various chemical agents. In Part II, we will discuss radiological and biological agents, which also ultimately do their insidious work at the biochemical level.

List of Figures



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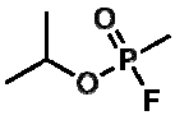

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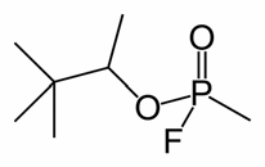

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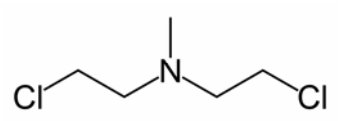

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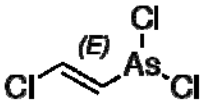

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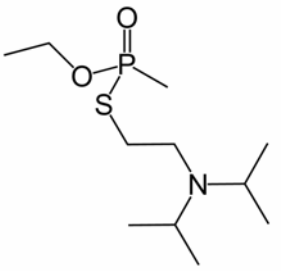

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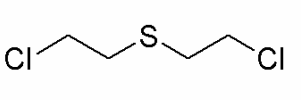

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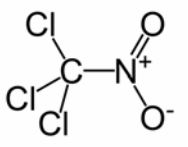

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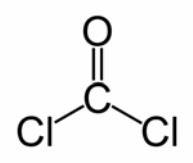

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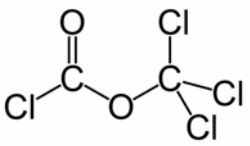

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The History and Science of CBRNE Agents, Part 2

Capt. G. Shane Hendricks[1] and Dr. Margot J. Hall[2]

Nuclear and Radiological Agents

Atomic apprehension

The atomic age was thrust upon the world when the United States military, backed by the highest levels of government and spurred on by Albert Einstein, assembled some of the best scientists in theoretical and atomic physics of the time to establish the heavily funded Manhattan Project in 1942. The team, led by U.S. Army General Leslie Groves and physicist Dr. Robert Oppenheimer, developed the first explosive, fissile nuclear device and detonated it at the remote Trinity test site in the New Mexico desert on July 16, 1945. Sand at the site was fused into glass from the heat, and the blast from the relatively small test device left a crater ten feet deep and 2,400 feet in diameter; the 100-foot tower supporting the device was nearly disintegrated, almost as if it had never existed. Light and heat from the blast were witnessed at up to 150 miles away. Later that year, the U.S. used the ensuing first atomic bombs, "Little Boy"—a bomb with a rifling mechanism containing uranium (U-235) dropped on Hiroshima, and "Fat Man"—a larger imploding type bomb containing plutonium that was dropped on Nagasaki. These bombs decisively ended the war with Japan and abruptly concluded World War II. Since that time, mankind has faced its own potential, self-induced annihilation from these weapons, powerful enough to destroy the earth's biomass many times over. [1 2 3]

The initial atomic weapons harnessed the tremendous heat and explosive energy of atomic fission, splitting atoms of unstable radioactive isotopes (radionuclides such as uranium or plutonium) that caused a cascading chain reaction. Neutrons released from splitting these atoms strike and split other atoms that release yet more neutrons (and other fission products such as new isotopes and various ionizing particles), and so on. [4] Later, hydrogen bombs were developed that inverted the process by fusing, instead of splitting, atoms, releasing even more explosive energy and radiation than fissile weapons. [5] A byproduct of such a high-energy release, whether fissile or fusional, is ionizing radiation (including that from radioactive fallout), which would contaminate the environment and induce occasionally mortal disease in survivors of an initial blast. For decades, the Soviet Union and U.S. faced off in the so-called Cold War that featured, at its core, a nuclear arms race coupled with the deterrence concept of mutual assured destruction (MAD). The anxiety over a contaminated, uninhabitable, and virtually destroyed planet instilled itself even among the warmongers of both powers, preventing nuclear weapons from being used again to the present day. [6]

The public was well aware of the danger; in the 1950s, a surge in the construction of family bomb shelters exemplified the fear associated with the potential of nuclear war. The government sought to mollify its citizenry in the face of total devastation by circulating contrived, worthless public service announcements that urged citizens to "duck and cover" during a blast. Fear of nuclear technology and radioactivity remains strongly entrenched today, especially after the nuclear power plant disasters discussed previously (see The History of CBRNE section). [7]

The public's apprehension with atomic energy is a bonanza for terrorists, whether through nuclear devices (fissile or fusional) or the far less deleterious radiological devices—the so-called 'dirty bombs' designed more to contaminate, and thus panic, rather than reap widespread death and destruction. Radioactive toxicity, by any means, is harmful to essential metabolic processes of living tissue.

Ionizing radiation

The term radiation refers to the ionizing energy of certain wavelengths of the electromagnetic

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spectrum (EMS) or from certain liberated subatomic particles.

Emitted neutrons are uncharged, but they can disrupt the nuclei of other atoms due to their relative mass and kinetic energy; they are considered more damaging than gamma radiation to living cells. [8]

Alpha particles are relatively large ionizing particles that are atomically equivalent to positively charged helium (He^{2+}) emitted typically from radioactive isotopes, such as radium, uranium, plutonium, americium-241 (^{241}Am) and polonium-210 (^{210}Po). They have limited ability to penetrate matter (a sheet of paper or skin will deflect them); their external effects are negligible, but if internally emitted (i.e., through ingestion), they can cause harm to adjacent tissues. [9 8 10]

Beta particles are ionizing particles that are either freed electrons (β-) or positrons (β+ or anti-matter), depending on the interconversion order of neutrons and protons within the split or decaying atom's nucleus; compared with alpha particles, they are smaller, at a higher energy, and typically produced from decaying isotopes, such as strontium-90 (^{90}Sr) or potassium-40 (^{40}K). In terms of a radiological incident, beta particles are found mostly in radioactive fallout and penetrate deeper layers of most matter compared with alpha particles. Epithelial injuries normally occur at the basal layer and resemble burns. Some forms of clothing and a mere aluminum plate will deflect beta particles. [9 8 11]

Gamma rays are a high frequency, short wavelength division of the electromagnetic radiation spectrum (EMS), which encompasses energy waveforms such as light, radio, and sound waves—each with varying wavelengths and frequencies. Gamma rays or waves occupy the highest reaches of the known EMS and result from the decay of radioactive nuclei, such as plutonium, radium, uranium, or cobalt-60 (^{60}Co), or from positron-electron annihilation (matter and anti-matter collision). Gamma rays, like other EMS radiation, exist as waves in one sense and particles called photons in another quantized sense (wave-particle duality); photons (literally meaning particles of light) are massless, energized, elementary particles (quantums) of the EMS capable of interacting with electrons and atomic nuclei and, therefore, ionizing molecules. Photons from gamma rays are especially penetrating and ionizing because of their high energy, which is derived proportionally from the gamma ray wave frequency (the higher the EMS wave frequency, the higher the energy of the associated photon). Gamma radiation passes easily through all matter and can damage all levels of tissue. [9 12 13 8]

Nuclear versus radiological exposure

Nuclear reactions release many random neutrons, new isotopes, alpha particles, beta particles, and photons as gamma rays. The energy of fission or fusion is greatly increased over the natural, spontaneous decay of radioactive material; this energy is in two forms: kinetic energy of the fission or fusion products and electromagnetic (EMS) radiation in the form of gamma rays. Only certain refined nuclear fuels (in a required amount—the critical mass) possess the properties that can sustain a nuclear chain reaction described earlier and release the enormous energy of a nuclear detonation. [4 14]

Certain natural or man-made isotopes (e.g., uranium or plutonium) decay or spontaneously emit radiation energy in the forms previously described, even without the amplified effects of fissional or fusional chain reactions. Radioactive elements of any type are dangerous in close proximity to living tissues and can cause varied deleterious effects depending on dosage. [8] Therefore, a simple, conventional explosive coupled with radioactive material—a 'dirty bomb'— could still contaminate a widespread area and cause localized illness and panic. [15] The effects would obviously be less dramatic and overt than a nuclear detonation, and the health risks associated with any such dispersal would be low. The economic and social impacts, however, would likely be high. [16] Achieving nuclear fission or fusion is beyond the technical and material means of most Third World nations, but an individual terrorist could possibly acquire radioactive material and build a dispersal device to achieve a desired outcome.

Symptomatology

Burns with redness, swelling, and itching can result from non-penetrating (beta particles) or penetrating (gamma rays) radiation, with the severest burns caused by gamma rays. Nausea, vomiting, watery diarrhea, cramping, bleeding, hair loss, prostration, confusion, seizures, cardiovascular collapse, and shock are all seen in exposure. Acute radiation exposure causes syndromes (i.e., hematopoietic, gastrointestinal,

and neurovascular) that manifest in four stages, depending on the radiation dose absorbed. The prodrome (from exposure up to 4 days) is characterized by a relatively rapid onset of nausea, vomiting, and malaise. The prodromal phase may progress directly into the manifest illness in high-level exposures. The latent period represents an interval of apparent wellbeing that lasts for 2-6 weeks; higher dosages shorten all stages of the illness. The manifest illness is characterized by the clinical symptoms associated with the affected organ system syndromes (e.g., the hematopoietic syndrome is characterized by a sharp decrease in circulating lymphocytes and bone marrow precursors of leukocytes and thrombocytes). Recovery (usually within 2 years of exposure) or death (usually less than 2 months following exposure) represents the last stage. [8] [17]

Clinical laboratory findings

Laboratory tests should include a baseline CBC. Lymphocyte counts fall rapidly after radiation exposure, and a 50% drop in lymphocyte count within 24 hours indicates significant radiation injury. [8] Other diagnostic data acquired during acute illness can be expected to describe the affected organ system (e.g., stool gross and occult blood due to gastrointestinal syndrome).

Biological Agents

Biological agents have garnered much of the attention of the CBRNE preparedness community following the anthrax mail attacks of 2001. Chemical, nuclear, and radiological agents require considerable expertise and abundant resources to acquire maximum benefit for nefarious perpetrators, while bioterrorists can often succeed with little training, equipment, or other resources, as the anthrax mail attacks demonstrated. [18]

This section begins with an overview of the modern molecular detection method known as real-time polymerase chain reaction (RT PCR); agent characteristics (i.e., where and how it occurs naturally, biochemical mechanisms of action, etc.); the resulting pathology (i.e., symptomatology, clinical picture, etc.); and traditional and biotechnological methods of identification (e.g., culture, RT PCR probes, etc.).

Real-time Polymerase Chain Reaction (RT PCR)

This article examines RT PCR (specifically the TaqMan assay) as the molecular method of choice for detecting bioweapons [19], due to its ubiquity, rapidity, and the author's experience. Other molecular methods are certainly acceptable with comparable advantages and disadvantages, but the complexity, expense, and skill involved with the plethora of available molecular techniques are beyond the means of many laboratories.

The light-detecting thermocycler is an instrument that couples the principles of standard polymerase chain reaction (PCR) amplification with fluorescence detection via dye-labeled nucleic acid probes; this modified version of PCR is known as real-time polymerase chain reaction (RT PCR), and it provides a means of simultaneously amplifying, identifying, and semi-quantifying target sequences. Since known genomic or extragenomic sequences (DNA or RNA) are highly specific for particular phenotypic traits associated with an organism, and since RT PCR amplification is possible normally from start to finish within two to four hours of sample receipt (sample processing dependent), presumptive identification via this method is extremely reliable and expedient. However, PCR techniques currently require a high level of dexterity and laboratory skill on the part of the scientist to recover and identify target genetic material without exogenous contamination.

The basic principle of RT PCR identification involves multiple, sequential temperature-controlled steps. (Figure 1) Nucleic acids representing as little as one template molecule (1 organism) are extracted from the sample matrix and purified using a commercial extraction kit and bead beater mechanism. [20]

Genetic material (double-stranded) is heat-denatured (by heating to melting temperature or Tm = ≈94°C) into single-stranded deoxyribonucleic acid. (Figure 2) A heat stabile DNA polymerase obtained from the thermophilic bacterium *Thermus aquaticus* (TaqPol) is used in the reaction to prevent denaturing the catalytic protein at the high temperatures required in the thermocyclic reaction. TaqPol present in the TaqMan test system becomes chemically active during the initial cooling period of the cycle. As the sample continues quickly cooling, dual-dye-labeled oligonucleotide probes in the test system

first attach to their known complimentary target sequences, and primers then anneal to known complimentary bases on the forward and reverse strands (sometimes analogically called *bookending*). A probe's Tm must be higher than the primer's to ensure the probe anneals before the primer; otherwise, extension might occur without the bound probe—a critical step for detection. TaqPol then docks to the end of the primers (last three or four bases on the 3' end of the primers). (Figure 3) [21]

At ≈60ºC for 20 seconds, TaqPol polymerizes or extends complimentary bases to the forward and reverse strands in a 5' to 3' direction. If TaqPol encounters an attached probe, the probe's FAM fluorophore dye (5'-carboxyfluoroscein) will be cleaved off via 5' exonuclease activity of the TaqPol enzyme. Since FAM (i.e., donor or reporter molecule) and TAMRA (i.e., acceptor or quencher molecule—3'-carboxytetramethylrhodamine) are no longer in close proximity, FAM releases an electron as a photon of light (fluoresces at 522 nm λ) when first excited by light at 475 nm λ (i.e., TAMRA can not accept the electron or quench the energy from the FAM molecule as it does when they are in proximity). The fluorescence reading is taken at the end of 20 seconds, and the reverse strand (sans the probe) is used as template for subsequent PCR cycles. (Figure 4) The thermocycling and fluorescence detection is repeated 40-45 times and the total fluorescence corresponds to the presence of the target nucleobase sequence and thus indicates presence of the suspected agent. RT PCR showed comparable sensitivity and specificity among three different hardware testing platforms (assay limits defined as genomic concentrations producing positive results 97% of the time), when using standardized biothreat agent probes and primers for several different organisms.[22] [19] [21]

B. anthracis (anthrax)

Anthrax has received extraordinary attention as a bioweapon due to its use in the U.S. postal attacks following the renowned terrorist incidents of September 11, 2001. *B. anthracis* is found naturally in the soil, and anthrax is a zoonotic disease infecting agricultural livestock, certain wild animals, and humans. The disease occurs in three primary forms: inhalational or pulmonary (causing the highest mortality and thus the likely goal of bioterrorists); gastrointestinal; and cutaneous (the most common natural form). Unlike many other potential bioweapons, anthrax does not easily spread from person-to-person. [18] [23]

The symptoms and disease associated with anthrax depends on the form seen. The most common clinical manifestation of cutaneous anthrax is the black, painless lesion called an eschar from which anthrax gets its name (*anthrakis* is the Greek word for coal). Typically, cutaneous anthrax is self-limiting. [23] [22]

Gastrointestinal anthrax is believed to occur by ingesting vegetative cells, as spores likely could not germinate before passing through the digestive system. Gastrointestinal anthrax is more lethal than cutaneous anthrax, partly because of the difficulty in diagnosis. [18] [23]

The least common natural form of anthrax is the deadly inhalational form; this route of anthrax infection represents the greatest threat to public health. Inhalational anthrax appears flu-like initially with fever, malaise, myalgia, and fatigue after 1-6 days incubation, which confounds early diagnosis. After 2-3 days (and possibly some improvement), the patient's condition worsens drastically. Routine lab results observed in patients following the 2001 attacks included elevated white blood cell counts with neutrophilia, elevated alanine transaminase (ALT) and aspartate transaminase (AST), and hypoxia as indicated by arterial blood gases.[18] [23]

B. anthracis is a relatively large, gram-positive, spore-forming, nonmotile rod that grows well on sheep blood agar. The bacillus measures 1–1.5 µm x 3–10 µm, is nonhemolytic in aerobic conditions, and resembles bamboo shoots microscopically. The colonies the organism forms on solid media are large, rough, and grayish-white, with irregular, curving outgrowths from the margin. Both in vivo and in vitro in the presence of bicarbonate and carbon dioxide, the organism forms a prominent capsule, which is a factor related to its virulence. Traditional means of confirmation include lysis via specific bacteriophage, fluorescent antibody to the capsule, mortality in mice or guinea pigs, and demonstration of the protective antigen. [18] [23] Molecular methods of detection involve using RT PCR to confirm the presence of the organism's virulence proteins—protective antigen (PA), capsule (CAP), lethal factor (LF), and edema factor (EF). [19] The factors are coded on two plasmids—pX01 and pX02. pX01 is a 174-kb plasmid containing the toxin genes *pag*, *lef* and *cya* (coding for PA and LF), and the 95-kb plasmid pX02 contains the genes *capA*, *capB*

and *capC* involved in capsule formation (CAP). Both plasmids are necessary to confer full virulence. Ellerbrok et al. developed primers and probes for pX01 using *pag*, pX02 using *capC*, and *rpoB*, a chromosomal gene specific for *B. anthracis*; positive identification of spores using the RT PCR methodology was obtained in less than three hours. [22]

The disease-causing biochemical pathway of anthrax is complex and not completely understood. Protective antigen (PA), edema factor (EF), and lethal factor (LF) combine to form two toxins—edema toxin (PA + EF) and lethal toxin (PA + LF). PA, which as the name implies protects EF and LF from proteases, binds to an anthrax target receptor (ATR) on the cell membrane in groups of seven, forming a heptamer called the PA-ATR complex. EF and/or LF bind to the complex, which facilitates endocytosis of the complex and formation of an endosome around the ingested proteins. LF and EF are released from the endosome, free to do their intracellular damage. LF is believed to cleave certain key enzymes, such as mitogen-activated protein kinase kinase (MAPKK), which is part of the signal transduction pathway; LF also is believed to activate the Oxidative Burst Pathway. High mortality is linked to lethal toxin. EF is thought to disrupt water homeostasis (leading to edema) and impair neutrophil function. [23]

Yersinia pestis (plague)

Y. pestis is infamous in history as the causative agent of the Black Death, which eliminated approximately one-third of Europe's population during the Middle Ages; two other pandemics occurred before and after the Black Death, and *Y. pestis*, genetically similar to the organism of the last pandemic, still occurs sporadically even today. [24] [18] The highly communicable organism is most commonly transmitted from a host to human via the bite of an arthropod vector; however, close contact with infected tissue or body fluids or inhalation of the aerosolized bacterium will propagate the infection. More than 200 different rodents and other species can serve as hosts, such as domestic pets, squirrels, chipmunks, deer mice, rabbits, camels, and sheep. The natural vector is usually the rat flea, *Xenopsylla cheopis*, but thirty different flea species have been identified as carriers. Ticks and human lice can also carry the plague bacillus. An enzootic stage in resistant rodents guarantees survival of the bacillus, while an epizootic stage that kills infected animals spreads the organism to new hosts. The sylvatic stage occurs when humans are infected by animals. [25] Natural pneumonic plague (≈1% of cases) and meningeal plague (6 – 7% of cases) are rare; the occurrence of plague pneumonia in a large cohort would corroborate the employment of weaponized plague bacillus. [18]

Y. pestis is characterized by abrupt fever onset, chills, headache, diarrhea, localized lymphadenopathy, and *buboes* (i.e., inflamed swelling of one or more lymph nodes, usually in the groin, which may suppurate if untreated); the infection can rapidly progress to bacteremic and pneumonic stages (the highly lethal, least-common form). Untreated septicemic plague is fatal usually during the first day symptoms appear, but early treatment with antibiotics (usually streptomycin or gentamicin) can reduce mortality to ≈15%. The incubation period for pneumonic plague occurs between a few hours to up to four days and requires an inoculum of only 1-10 organisms. The initial symptoms of fever, headache, weakness, and coughing with hemoptysis make pneumonic plague indistinguishable from many respiratory illnesses, including influenza or even other respiratory CBRNE agents. Untreated pneumonic infection is fatal in one to six days with mortality as high as 95%. [18] [24] After infection, the plague bacilli multiply rapidly, evade cell-mediated immunity easily, and instigate an inflammatory response, which is accompanied by endothelial toxicity via the yersinial toxins. Later, necrosis causes vascular destruction and local hemorrhages that produce a darkened appearance under the skin and other tissues (hence the name 'Black Death'). These later presentations occur without further bacterial invasion of vascular structures. The bacillary toxin destroys phagocytic cells that manage to engulf the bacillus; some of the toxins cause peripheral vascular collapse and disseminated intravascular coagulation. [26]

Y. pestis, is a nonmotile, gram-negative bacillus measuring 0.5–0.8 x 1.5–2.0 μm that appears as a bipolar rod with safety-pin morphology in both Gram and Wright-Giemsa stains. The organism belongs to the Enterobacteriaciae family; is positive for catalase; and is negative for lactose fermentation, hydrogen sulfide, oxidase, indole, urease, sucrose, rhamnose, and melibiose. [18] It grows optimally at ≈28°C on blood agar (without hemolysis) or MacConkey agar, typically requiring 48 hours or more to form visible "beaten-copper" colonies measuring1-3 mm each—much smaller than other

Enterobacteriaciae. *Y. pestis* is homogenous, having only one serotype, one phage type, and three biovars. Research has shown the three biovars correspond genetically to the three historic pandemic strains isolated from remnant foci of ancient plague: Antiqua, Medievalis, and Orientalis biovars. Several new ribotypes of biovar Orientalis have appeared in the last century and have shown that chromosomal rearrangements coding for ribosomal RNA occur quickly, but no other significant genetic changes have been noted. [26] A direct fluorescent antibody (DFA) stain of a bubo aspirate, peripheral blood, and sputum for the presence of *Y. pestis* capsular antigen should be performed; a positive DFA is highly specific and represents a better preliminary identification than relying solely on safety-pin morphology, as other organisms such as *Pasteurella* sp., *Klebsiella* sp., and diplococci can closely mimic this microscopic characteristic. [18] Confirmatory testing should include traditional culturing, biochemical profiling, antimicrobial susceptibility testing, and identification of virulence factor genes by RT PCR, if available.

Virulence of *Y. pestis* is provided via genes on three plasmids and on the chromosome. One plasmid encodes the low calcium response genes (LCR), which are active at 37°C in hypocalemic circumstances; these genes result in 12 proteins, including the secreted V antigen and Yops proteins (Yersinia outer proteins) that are both secreted and embedded in the outer membrane. Yop M binds human thrombin, Yop H provides antiphagocytic characteristics, and Yop E is a cytotoxin. Two other plasmids code for plasminogen activator, bacteriosin pestisin, murine toxin, and F1 capsule, which allows *Y. pestis* the ability to evade neutrophils and monocytes. *Y. pestis* can survive once engulfed by monocytes, but neutrophils are highly effective at killing the phagocytized bacillus; therefore, F1 capsule is essential for infection. [18] RT PCR primers and probes can be used to rapidly amplify and detect any of the virulence-associated genes in a manner similar to those seen previously in this paper [19], though, for reasons discussed previously, identifying the F1 capsule-encoding plasmid is necessary in any genetic study at a minimum. Researchers, using reverse transcription coupled RT PCR, have also studied the activation of certain host immune response genes (especially certain cytokines and other macrophage-related proteins) that quicken natural apoptosis of murine macrophages following infection by *Y. pestis.* Varying temperature gradients seem to alter the speed that macrophages expire; however, the reason temperature is important (increased cellular lifespan at 26° C versus shorter lifespan at 37° C) in delaying apoptosis has not been determined. [27]

Francisella tularensis (tularemia)

F. tularensis causes a naturally occurring, virulent, non-communicable zoonosis called tularemia (also commonly called “rabbit fever”), with fever, localized epithelial or mucous membrane ulceration, regional lymphadenopathy, and, sometimes, pneumonia. In 1911, the disease was discovered in Tulare County, California and was noted for causing an illness similar to plague in squirrels. G.W. McCoy, the microbiologist studying the disease, named it *Bacterium tularense*. The first human case was confirmed in 1914, and in 1921, Edward Francis described transmission of the bacterium via the deer fly vector and dubbed the condition tularemia. In 1926, researchers verified that transmission occurred among ticks via their reproductive system. The disease affects other reservoir hosts, such as deer and rabbits, while the natural vector appears to be arthropods, including ticks and deer flies. The genus was later changed to *Francisella* in honor of Edward Francis’ work with the organism. *F. tularensis* is believed to be a potential biothreat due to its high infectivity after aerosolization. Biovar tularensis (type A) produces acid from glycerol, demonstrates citrulline ureidase activity, and is the most common, virulent biovar isolated in North America, while biovar palaearctica (type B) is relatively avirulent, does not produce acid from glycerol, and does not demonstrate citrulline ureidase activity. [18 28 29] The largest clinical manifestation occurs as ulceroglandular tularemia after 3 to 6 days of incubation, with skin, eye, or other mucous membrane suppurative lesions and lymphadenopathy in 60% of cases. Lesions usually progress to necrotic granulomas. [29] Other symptoms include fever, chills, sweats, headache, cough, and myalgias, which complicates diagnosis by appearing flu-like and similar to many routine infections. Vomiting, diarrhea, dysuria, arthralgia, pharyngitis, pleuritis, anorexia, back pain, and neck pain are sometimes seen. The condition can also present as a typhoidal condition in a smaller cohort, without lesions seen on the skin or mucous membranes; patients with typhoidal tularemia often progress to atypical pneumonia. [18 29]

F. tularensis is a gram-negative, faintly staining, facultative, intracellular, pleomorphic coccobacillus, measuring 0.2µm x 0.2-0.7 µm. It does not form spores as seen with *B. anthracis* or exhibit bipolar "safety-pin" staining seen with *Y. pestis*. [30] [29] The organism is a highly fastidious, non-motile aerobe; it can be recovered (even after anti-microbial initiation) from blood, ulcers, conjunctival exudates, sputum, gastric washings, and pharyngeal exudates. Due to its parasitic nature, *F. tularensis* grows poorly on routine bacteriological media, such as blood, chocolate, and MacConkey's. Media containing cysteine or other sulfhydryl compounds (e.g., glucose cysteine blood agar or thioglycollate broth) are the best choices for suspected cases, but the bacillus has been recovered on charcoal yeast extract and Thayer-Martin agar. Colonies are small (1-2 mm), smooth, shiny, and opaque after 24 to 48 hours of incubation at 37°C. Growth characteristics and immunological techniques (i.e., DFA, bacteriological agglutination, or enzyme-linked immunosorbent assay) are proven methods of positive confirmation. Serologic diagnosis of tularemia must be considered with care, as antibody levels from previous infections can persist for many years. Attention is required to prevent confusion with *Brucella* sp., which is morphologically similar to *Francisella* sp. and can cross-react with some immunological assays. [30] [18] RT PCR can circumvent many of these difficulties and speed diagnosis via the rapid, specific detection of four genes. [19] Standard laboratory tests are usually unhelpful, as many analytes are normal or only mildly elevated, including major aspects of the complete blood count (CBC), liver and cardiac enzymes, and cerebrospinal fluid examinations. [18]

F. tularensis is introduced into the host via breaks in the skin (i.e., arthropod bites, cuts, etc.), or through the mucous membranes (i.e., eye, respiratory tract, or gastrointestinal tract). As few as ten organisms received through injection or inhalation can cause infection. Once inside a host, *F. tularensis* is phagocytized by macrophages and begins multiplying. The host attempts a defense through a variety of cell-mediated processes. Initially, through this major defensive strategy, the macrophage secretes tumor necrosis factor-alpha (TNF-α), inducing natural killer (NK) cells to produce interferon-gamma (IFN-γ) that stimulates the macrophages via a feedback pathway to destroy the ingested bacteria by producing nitric oxide. In another mechanism, macrophages present the antigen (in the context of the major histocompatibility complex—MHC) to the cluster of differentiation 4+ (CD4+) T lymphocytes, which then proliferate at the site and secrete TNF-α, interleukin-2 (IL-2), and IFN-γ. As before, the macrophages intercept these chemical messages to destroy the intracellular parasites with nitric oxide. Humoral and neutrophilic roles in defense are uncertain. [18]

Brucella sp. (brucellosis)

The genus *Brucella* causes a zoonosis in domestic and wild animals, and includes the species *abortus* (in cattle and bison), *suis* (in swine), *canis* (in dogs), *ovis* (in sheep), *neotomae* (in rodents), and *melitensis* (in sheep and goats). Infection in humans by *ovis* and *neotomae* has not been reported. Speciation is based on biovar designation, though there is disagreement on the existence of more than one species due to DNA homology among biovars. Humans become accidental hosts by consuming undercooked or unpasteurized animal products or inhaling infectious aerosols, usually through close contact with infected animals. The resulting non-communicable infection is known as brucellosis, undulant fever, Malta fever, or Crimean fever. The genus name *Brucella* is named after microbiologist David Bruce, who first isolated the etiologic agent in 1887 from the spleens of five fatal human cases on Malta; he initially placed it within the genus *Micrococcus*. [31] [18] The organisms can also gain entry into human hosts through breaks in the skin, mucous membranes, and conjunctiva. Percutaneous needle stick exposure, conjunctival exposure via eye splash, and inhalation are the most common means of infection in the United States. *B. melitensis* is the most pathogenic of the genus and is believed the most dangerous candidate for a biological weapon, though the United States actually developed munitions containing the less virulent *B. suis* in 1955. [31]

Symptoms and course of brucellosis are variable, which can confound diagnosis. Patients may present with an acute, systemic febrile illness; an insidious chronic infection; or a localized inflammatory process. The incubation period can range from three days to many weeks. Nonspecific symptoms include fever, cough, chest pain, dyspepsia, sweats, fatigue, anorexia, myalgias, bone pain, and arthralgia, which closely mirror symptoms seen in similar biothreat infections, such as tularemia. Genitourinary involvement may produce pain. Neurological and psychological symptoms are

frequently seen, with depression, headache, and irritability. Patient symptoms are indistinguishable based on routes of infection. Chronic infection produces symptoms lasting for 3 to 12 months or more, with hepatomegaly, splenomegaly, or lymphadenopathy occasionally seen. [18] [31]

Brucellae are small (0.5-0.7 × 0.6-1.5 μm), aerobic, non-motile, non-fermenting, non-sporulating, gram-negative, encapsulated coccobacilli that do not produce toxins. The fastidious, slow-growing organisms are catalase and oxidase positive, and produce urease and catalyze nitrite to nitrate. *Brucella* sp. produce a lipopolysaccharide coat with less pyrogenic properties than other gram-negative organisms; therefore, high fever in brucellosis is rare. [32] [31] [18] The organisms grow best on trypticase, soy-based, or similar enriched media, with binary fission requiring 2 hours. Carbon dioxide requirements; the ability to use glutamic acid, ornithine, lysine, and ribose; hydrogen sulfide production; growth in the presence of thionine or basic fuchsin dyes; agglutination by antisera directed against certain epitopes of the lipopolysaccharide coat; and by susceptibility to lysis by bacteriophage are all characteristics used to differentiate species phenotypically. [18] Verification of species-related Brucellae genes via RT PCR has been developed for *B. melitensis*, due to its reputation as a fearsome biothreat. [19] Debeaumont et al. evaluated an assay based on DNA amplification of a 169-bp portion of bcsp31, a gene found in all *Brucella* species and biovars. The RT PCR assay was evaluated using genomic DNA from 15 *Brucella* strains and 42 non-*Brucella* strains with 100% sensitivity and specificity. [33] Patients with the infection do not demonstrate leukocytosis with the CBC, and at times are neutropenic. Hepatitis and liver abscesses can occur, with mild elevations of serum lactate dehydrogenase and alkaline phosphatase. [18]

Both polymorphonuclear leukocytes and macrophages phagocytize brucellae organisms, but the bacterium resists attempts to kill it via prevention of phagosome to lysosome fusion; the organisms replicate in the phagosome and eventually destroy the phagocyte. [18] Brucellae are transported into the lymphatic system; they may reproduce in the lymph tissue, kidney, liver, spleen, breast, or joints. Granulomas occasionally accompany extracellular reproduction; this condition is usually observed in the liver and spleen. *B. abortus* receives its species name for its ability to propagate in fetal tissues, causing spontaneous abortion; however, this phenomenon is usually seen in cattle and only occasionally in humans. As in tularemia, cell-mediated immunity, rather than humoral, is the primary means of host defense. However, some immunoglobulins are produced during infection, but IgG titers are not elevated unless the infection is chronic or relapsing. Infectious exposure is between 10 - 100 organisms. [32] [31]

Variola virus (smallpox)

Until the late 20th Century, smallpox was a dreaded disease that had plagued mankind for centuries. By 1977, Somalia recorded the world's last naturally occurring case of smallpox; the World Heath Organization declared smallpox eradicated in 1980. Though it has not been seen naturally in 26 years, the utility of smallpox as a biothreat is debated because of the availability of the vaccine (from *Vaccinia* virus) that eliminated it. [18] Recently, the smallpox vaccine was being offered again in certain circumstances to certain groups of professionals, including military and medical personnel, due to the possibility of its employment as a bioweapon.

Variola is typically acquired by inhalation of infectious aerosols, inducing an asymptomatic viremia in as little as 72 hours after infection. Infection spreads from the lymph nodes to other organ systems quickly. Symptoms appear within 7-17 days of infection (average incubation is 12 days) and are, initially, very flu-like, with fever, myalgias, headache, chills (>50% of cases), vomiting (>50% of cases), delirium (15% of cases), and backache. Following the fever (48-72 hours later), a rash develops, predominantly on the face and extremities, that transitions (from macules to papules) into open, virus-filled pustular vesicles; the sores scab over within two weeks and begin healing. Patients are infectious until the scabs have fallen off. The most severe clinical manifestation of smallpox (seen in 2-5% of patients) is known as flat-type smallpox, with pronounced systemic toxicity and flat, soft lesions; mortality is as high as 66% in vaccinated patients and 95% in unvaccinated patients. Rarely, a hemorrhagic form of the disease occurs. [34] [18]

Variola is a highly contagious member of the family *Poxviridae* and the genus *Orthopoxvirus*, which also includes the viruses associated with cowpox, monkeypox, and molluscum contagiosum. *Variola* is known in two forms:

major (the predominant disease of Asia and Africa, with 30% mortality in unvaccinated victims) and *minor* (the less severe form of Africa, Europe, and South America, with 1% mortality). Poxviruses are large viruses with a double membrane layer enclosing a 200 kb segment of dsDNA. Some of the smallest bacteria are actually smaller than Poxviruses. They have a large genome consisting of a 200 kilobase (kb) double-stranded DNA segment enclosed in a double membrane layer, and these viruses, though requiring living cellular cytoplasm and organelles for reproduction, do not require the cell's nucleus to propagate. [35] [18]

The risk of misdiagnosing smallpox is high given that it can mimic other vesicular exanthematous conditions, such as chicken pox (*Varicella zoster*) and contact dermatitis; additionally, physicians have not seen the infection in over 26 years. Routine methods of laboratory confirmation have remained unchanged for decades; many of these methods (and the requisite skills and knowledge) are unavailable to most clinical laboratories. Demonstration of the characteristic virions from vesicular scrapings or drainage using an electron microscope is one method of identification, but the specificity is still limited to Poxviruses. Guarnieri bodies are B-type poxvirus cytoplasmic inclusions (therefore, non-specific) that stain reddish purple with Giemsa stain. The Guarnieri bodies can be enhanced for light microscopy using Gispen's modified silver stain, where the inclusions appear black. For non-molecular methods, growth on the chorioallantoic membrane (egg culture) demonstrates the more specific small, grayish-white pocks, which appear differently from the pocks seen with other Poxviruses. [18]

Genetic methods using RT PCR discussed previously may be the best diagnostic solution for clinical and local public health laboratories. Kulesh et al. developed and tested RT PCR assays 100% specific for *Variola* virus and other Poxviruses using the TaqMan methodology with thermocycling. [36] Target genes for *Variola* consisted of the hemagglutinin (HA) J7R, B9R, and B10R genes, and the HA and DNA polymerase-E9L genes were used as targets for the pan-orthopox viruses.

Ricin

Ricin is a potent biotoxin derived from the beans of the castor plant (*Ricinus communis*). In industry, the toxin is a byproduct of castor oil production; castor oil has been used both as a laxative and as a mechanical lubricant. In the 1800s, the word ricin was coined by Stillmark, who discovered the toxin within the castor bean; he noticed ricin's ability to agglutinate erythrocytes and precipitate serum proteins. Seventy-years later, Paul Erlich used ricin and another lectin, abrin, to induce murine immunity and thus helped to establish the modern field of immunology. In recent times, ricin has been examined for its potential as an oncological treatment, and it was developed as a bioweapon (with the moniker Compound W) by the United States at the end of World War I and into World War II but never used. [18]

The ricin toxin is easily and cheaply produced, has high toxicity, is stable in aerosolized form, and has no treatment or vaccine; however, a large volume of ricin is necessary to produce the desired effect of other CBRNE agents. To equal the lethality (LD_{50}) of 1 kg of *B. anthracis* dispersed over a 100-km² area, 4 metric tons of ricin is needed. For this reason, ricin makes a poor choice for the would-be bioterrorist, but the threat as a food and water contaminant in causing chaos cannot be denied. [37]

Ricin (weighing 66 kilodaltons and comprising up to 5% of the castor bean's mass) is a lectin with two polypeptide chains, the A-chain and B-chain, linked by a disulfide bond. (Figure 5) The plant protein belongs to a group of ribosome-inactivating proteins, which depurinate a single, specific adenosine nucleobase (A4323) in ribosomal ribonucleic acid (rRNA). The active A-chain catalytic site thus cleaves the 28S subunit of eukaryotic ribosomes (near the 3' end), effectively blocking protein synthesis. The B-chain binds to cell surface glycoproteins and allows transmembrane passage and endocytosis (forming an endosome) by some unknown mechanism. [18] Structurally, ricin closely resembles other biotoxins, including botulinum toxin. [37]

Animal studies have shown that dosage and route of exposure affects the symptoms seen. [18] Fever, sore throat, thirst, headache, nausea, pupil dilation, anuria, cramps, gastrointestinal hemorrhage, hematemesis, bloody diarrhea, melena, vascular collapse, and shock are seen via the ingestion route (the least toxic route due to poor absorption and the effects of digestive enzymes), and there may be necrosis of the kidneys, spleen, and liver. Inhalation leads to congestion, tight chest, wheezing, urticaria, pulmonary lesions, and respiratory distress with

hypoxia, cyanosis, labored breathing, tachypnea, tachycardia, and progressive respiratory failure. Injection of the toxin causes severe pain, nausea, muscle and lymph node necrosis, and moderate involvement of visceral organs at or near the region of injection. These route-specific properties are likely caused by ricin's tendency to rapidly bind cell-surface carbohydrate galactosides. [37] [18]

Leukocytosis (via CBC) is a common feature of ricin toxicity, and prothrombin time/international normalized ratio (PT/INR), activated partial thromboplastin time (APTT), and fibrinogen will be elevated with hemorrhaging. Important chemistries to perform include electrolytes, BUN, creatinine, glucose, liver enzymes, amylase, and lipase; increases in these analytes will depend on the organ systems affected. An arterial blood gas may reveal hypoxemia with respiratory exposure. [37]

Enzyme-linked immunosorbent assays (for blood or other body fluids) or immunohistochemical techniques (for analysis of tissues) can be used to confirm ricin intoxication. Ricin, however, is bound quickly and metabolizes before excretion, making identification in body fluids or tissues difficult by any method. [18]

DaSilva et al. studied the expression of 34 genes (from 1178 mRNA species) induced in pulmonary tissue following ricin inhalation using reverse-transcriptase polymerase chain reaction. The gene transcripts identified facilitate tissue healing (early growth response gene (egr)-1), regulate inflammation (interleukin (IL)-6, tristetraproline (ttp)), cell growth (c-myc, cytokine-inducible SH2-containing protein (cish)-3), apoptosis (T-cell death associated protein (tdag)51, pim-1) and DNA repair (ephrin type A receptor 2 (ephA2)). The hope is to use this information in designing treatment interventions in the event of a ricin inhalation incident. [38]

Other biothreats

A proper treatment of other potential biothreats facing public health officials, medical professionals, and scientists is not possible given the constraints of space and time, but there are many other possible threats, such as Glanders disease (*Burkholderia mallei*) and meliodosis (*Burkholderia pseudomallei*) [39]; Q Fever (*Coxiella burnetii*) [40]; Staphylococcal enterotoxin B (SEB) [41]; T-2 mycotoxin [42]; Viral encephalitides [18]; Hemorrhagic viruses (Ebola and Marburg) [43]; and *Clostridium botulinum* toxin [44] [18].

The modern age of genetics and biotechnology brings with it the possibility of engineered biothreats not yet anticipated. Scientists can purposefully delete and add certain genes to the bacterial chromosome to alter phenotype, or transform certain phenotypic characteristics of bacteria through plasmid vectors; viruses can also be manipulated. Many of these engineering feats have had benign, useful applications, but genetic engineering also allows for altering anti-microbial resistance genes and virulence factors, which can aid the bioterrorist. Time, knowledge, and technology advances make what was once the realm of the scientist increasingly available to the layman. These circumstances require clinical microbiologists to face the potential of organisms—once routine and easily treated—that were genetically manipulated to increase virulence for use as agents of diabolic mischief. State sponsors of genetically engineered, virulent bioweapons have existed since the middle to late 20th Century (see The History of CBRNE in Part I). The reality that a genetically-modified, weaponized bioagent could be used, either by a terrorist or a state sponsor of terror, seems to be more a question of when, and not if, it will happen.

Conclusion

CBRNE agents exist as numerous, frightening, and deadly foes of both their victims and various professionals. These usually fatal, panic-facilitating agents have existed and been used in various ways for centuries, but the amalgamation of modern technology and radical idealism gives the agents a new, menacing life in the present. Scientific professionals of various disciplines must be aware of CBRNE history and science to understand the present threat, using advancing technology to provide the most effective, appropriate response for patients, physicians, and investigators in the event of a CBRNE-related incident. The studious, well-trained professional will be a valuable, necessary commodity if the worst-case scenario is forced upon an unsuspecting public.

Figures

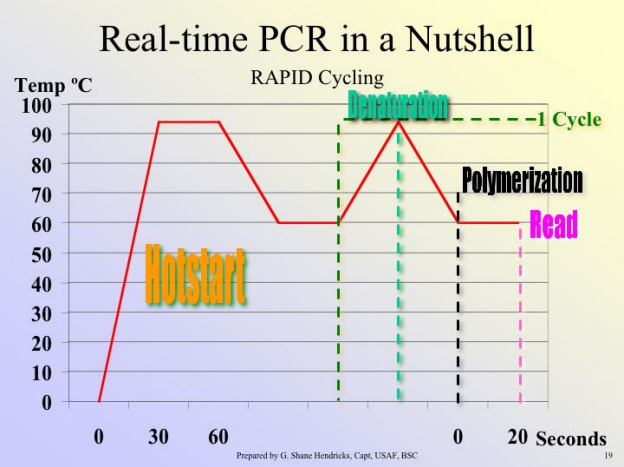

Figure 1. Temperature versus time cycle in RT PCR

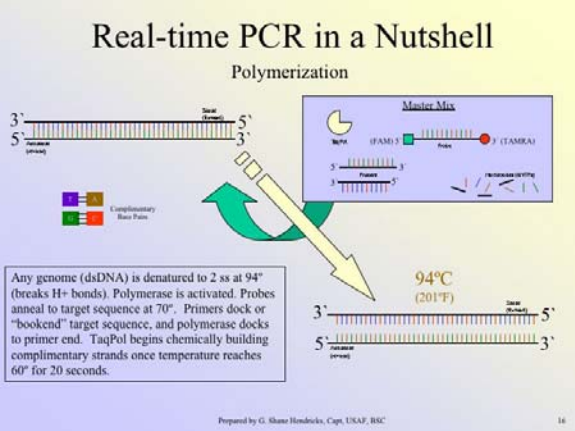

Figure 2. Initial steps of RT PCR

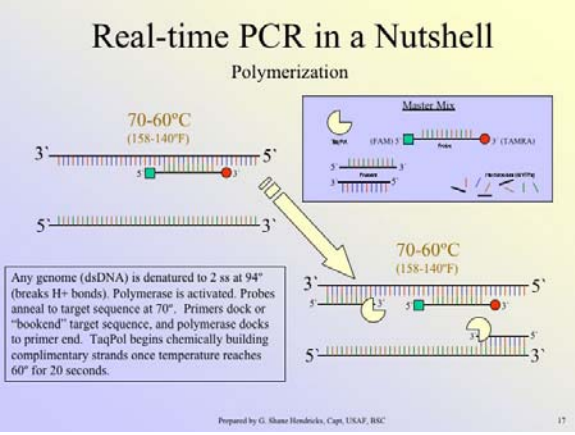

Figure 3. Intermediate steps of RT PCR

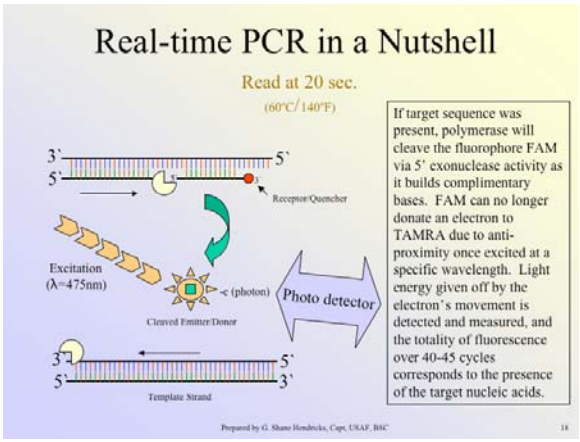

Figure 4. Final steps of RT PCR

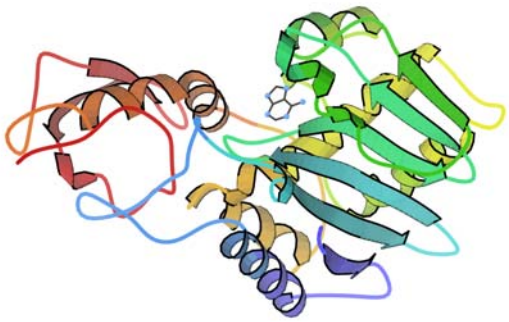

Figure 5. Ricin A-chain complexed with adenosine

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The Other Mass Spectrometry

W Jeffrey Hurst, PhD

Ion Mobility Spectrometry (IMS) is seen at many conferences in past years but people tend not to be familiar with all of its capabilities. I have titled this contribution the other mass spectrometry since I like many of you am extremely familiar with GC/MS, LC/MS, MALDI, TOF and a variety of other mass spectrometry techniques but not IMS. IMS responds to a variety of organic compounds with pg. to ng. levels of sensitivity. The applications of IMS have been focused primarily in the area of law enforcement with one of the largest applications and one that many readers might have seen in operation is in airports since IMS is used in many airports for explosive detection. It requires no large piece of equipment like other types of spectroscopy since there is a handheld instrument that can be seen in Figure 1.

Figure 1

Portable IMS

The instrumentation is used by airport security personnel who obviously would have only minimal training and no experience in IMS hence it has to be trouble and error free operate in a variety of environments with rapid analysis time. The technique has also been used successfully in the detection of chemical warfare agents. Finally, it has been used for identification of drugs and drug residues. Before I get much further, it is worthwhile to provide thumbnail sketch of the IMS and its operation.

In IMS samples after being vaporized are ionized using either a positive or negative soft ionization and travel down a drift tube where their mobility is measured having similarities to Time of Flight (TOF). This mobility is obviously influenced not only by the ion size but also its shape and this information can provide some information of the identity of the molecule. What results is not the classical spectrum many trained in classical mass spectrometry might be used to seeing but a fingerprint. Some molecules are easier to ionize than others are and developing this information is important to the appropriate use of IMS.

I believed that there are excellent potentially untapped applications in diverse market segments and to add some support to this premise, the DOE Idaho National Engineering and Environmental Laboratory (INEEL) has committed 1.7 million to establish a Center for Ion Mobility Spectrometry (IMS Center). The center plans to develop an affiliation with the Center for Process Analytical Chemistry (CPAC) at the University of Washington. For those unfamiliar with CPAC, it is s a consortium of national laboratories and

industrial sponsors who focus their efforts on problems emphasizing process analytical chemistry concerns. In my opinion, their focus is to bring the laboratory to plant or site.

In addition to the application base that has been previously mentioned, there are now activities by instrument vendors to broaden this base primarily with the emphasis in the pharmaceutical arena. Since many of the initial uses have been in the identification of drugs, this would seem to be a logical extension with one of the uses being the monitoring of line cleaning in cGMP environments. While Figure 1 provides an illustration of a handheld instrument there also are laboratory-based instruments that also take up very little laboratory "real estate". Three additional application areas for IMS could be food manufacturing operations and related industries for ingredient and product quality, environmental and clinical analysis. The rapid determination of pesticides and herbicides in soil, water and commodities would be an excellent fit since these compounds occur at low levels and the specificity and sensitivity of IMS would seem an excellent fit. These applications are not without problems related to sample integrity and homogeneity since some sample types will be more amenable to IMS than others. For example, soil and other environmental samples could present some challenges. Applying IMS to clinical samples would offer just in time (JIT) analysis and could adds another dimension to point of care testing (POCT).

The future for IMS is extremely bright since the application base in currently limited. The developments in miniaturization will likely further bring down the size of the instrumentation as will advances in the use of chemometrics since we will likely see the inclusion of neural networks and other techniques in the instrumentation and potentially the development of application specific instrumentation based on market requirements and demands.

About The Author

W. Jeffrey Hurst works for the Hershey Company as a Sr. Staff Scientist in the Analytical Research and Services Group and a member of the Hershey Center for Health and Nutrition. He also is Adjunct Professor of Comparative Medicine at the MS Hershey Medial Center, Penn State University College of Medicine.

BOOK REVIEWS

Introduction to Modern Inorganic Chemistry ; 6th edition.

Authors: K M Mackay; R A Mackay; W Henderson
Publisher: Nelson Thornes Ltd
ISBN: 0-7487-6420-8
Price: N/A

The 6th edition of Introduction to Modern Inorganic Chemistry published in 2002 is a thoroughly revised and updated version of earlier editions. The book is excellent and can be used by a freshman undergraduate chemistry student as a starting point. Attempts have been made by the authors to cover a detailed and explicit account of both basic theoretical principles and modern concepts of inorganic chemistry needed both at undergraduate and honor's levels. The book has twenty chapters. Each chapter begins with an introduction, is divided into a number of sub sections and contains many figures, diagrams, tables, toolboxes and margin notes. At the end of each chapter, a selection of cautiously chosen problems has been included to help the readers in learning the subject. Three appendixes and a subject index are given at the end of 610 pages book. Chapters from one to eight describe the principles of chemistry. Chapter nine and ten give the chemistry of hydrogen and 's' elements respectively. Chapters eleven and twelve cover the chemistry of scandium group , the lanthanides and the actinide elements respectively. Chapter thirteen gives the introduction of the transition metals, their general properties and complexes. Chapter fourteen describes the chemistry of the transition elements of the first series and chapter fifteen gives the chemistry of the second and third series of transition elements. Chapter sixteen outlines the selected topics on transition metals. Chapter seventeen describes the chemistry of the elements of the 'p' block and chapter eighteen gives the selected topics in main group chemistry and bonding. Chapter nineteen gives the description of general topics, namely, electron density determination, metal polychalcogenide compounds, fullerenes, nanotubes and carbon 'ONION' – a new form of elemental carbon and dendrimeric molecules. Chapter twenty describes biological, medicinal and environmental inorganic chemistry.

Appendix A lists a number of books, reviews and journals up to 2001, bibliographies for particular sections of the text and electronic access to chemical information. Appendix B gives a list of the name, formula and mode of coordination of some common polydentate ligands. Appendix C describes molecular symmetry and points. Relative atomic masses and a periodic table of the elements are also given at the end of the book. Overall, it is an excellent text book of inorganic chemistry for both the beginners and advanced levels students covering in depth knowledge of inorganic chemistry in a modern, readable and concise manner. It is a very popular and highly recommended text book for the inorganic chemistry courses.

Reviewed by : Gopendra Kumar, D Phil, FAIC

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Booth DE, Isenhour TL. The Chemist, 2000, 77(6), 7-14.

Books

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Turner GK in Bioluminescence and Chemiluminescence: Instruments and Applications, ed. Knox Van Dyke, CRC Press, Boca Raton, 1985, vol 1, ch. 3, pp 43-78.

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McCapra F, Tutt D, Topping RM, UK Patent Number 1 461 877, 1973.

Reports and bulletins, *etc.*

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Smith AB, Jones CD, “Environmental Impact Report for the US”, final report to the National Science Foundation on Grant AAA-999999, Any University, Philadelphia, PA, 2006.

Material presented at meetings

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Smith AB. Presented at the Pittsburgh Conference, Atlantic City, NJ, March 1983, paper 101.

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