0031-6997/96/4802-0289$03.00/0
PHARMACOLOGICAL REVIEWS

Vol. 48, No. 2
Printed in U.S.A.

Toxicology and Pharmacology of the Chemical Warfare Agent Sulfur Mustard[a]

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I. Introduction

Chemical warfare, which is cost effective, is present today. The efficacy of producing large number of casualties through use of weapons made from cheap and readily available chemicals has given rise to a new designation for chemical weapons: "the poor man's atomic bomb." All countries, regardless of size or economic condition, capable of producing petrochemicals, pesticides or detergents have the potential and capability of conversion to production of a wide variety of chemical warfare agents, of which mustard gas is the most accessible.

Chemical attacks, such as those in which mustard may have been used on Iranian soldiers and civilians during the Gulf War of 1984 to 1985 (Marshall, 1984; Dickman, 1988) and heavy use of chemical weapons in Afghanistan by the Soviet military, are recent innovations in military tactics that have been highly successful and may ensure further use of future military conflicts as a profitable adjunct to conventional military arms (Marshall, 1984; Segal, 1987). This weapon has been used recently by Iraq to attack its own Kurdish population in the Iranian-occupied village of Halbja in 1988, which resulted in many civilian casualties (Dickman, 1988; *Japan Times*, 1988; *New York Times*, 1988).

Today, many nations have the ability to produce and use chemical agents. Nations that do not have the expertise to build factories for production of these chemicals have been aided by European nations that are eager to share their own scientific expertise in these matters. The means of production, the means of delivery, and the stockpiling of such chemicals make their use against armies and civilian populations only a matter of time. Concurrently, in the United States (US)[b], recommendations are being made to provide safe measures and appropriate health standards for handling the national stockpiles of all chemical agents that are mandated for demilitarization. To resolve questions of public concern about possible exposure and potential health hazards resulting from destruction of these stockpiles of chemical agents, scientific data are being accumulated concerning potential adverse effects, including toxicology, carcinogenicity, mutagenicity, and teratogenicity. The "need to know," applicable to the scientific community as well as to an enlightened and educated public, implies that evidence be scientifically scrupulous and verifiable.

Although this review is exclusively on sulfur mustard, on occasion, references and illustrations will be made to the nitrogen mustards because of the similarity of structure and mode of action. This is done to maintain a clarity and continuity in our survey where evidence is

[a] The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other official documentation.

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[b] Abbreviations: US, United States; HD, sulfur mustard [bis(2-chloroethyl)sulfide]; H, Levinstein mustard; T, bis[2(2-chloroethylthio)ethyl]ether; bw, body weight; HT, a mixture of 60% HD and 40% T; LD_{50}, lethal dose for 50% of subjects (median lethal dose); HN-1, HN-2, nitrogen mustard; Ct, concentration; DNA, deoxyribonucleic acid; NAD^+, oxidized form of nicotinamide adenine dinucleotide; CNS, central nervous system; BAL, British anti-Lewisite (dimercaprol); LDH, lactate dehydrogenase; S-330 or M-5 (Army designation), 7,8-diphenyl-1,3,4,6-tetrachloro-2,5-diaminoglycoluril; merophan, o-di-2-chloroethylamino-DL-phenylalanine); RNA, ribonucleic acid; *N. crassa*, *Neurospora crassa*; poly U, polyuridylic acid; *E. coli*, *Escherichia coli*.

sparse or is lacking regarding the involvement of sulfur mustard in specific instances. Mustard gas and sulfur mustard are used interchangeably and refer to the same chemical compound, viz., 1,1′-thiobis(2-chloro)ethane and more commonly called ββ′-dichlorodiethylsulfide.

II. Chemistry of Mustard Gas

Although there are presently more highly effective chemical warfare agents that are more toxic, mustard gas has not lost its military usefulness because of its special characteristics: it is very toxic and difficult to treat, versatile, persistent, cheap, easy to produce industrially, and difficult to protect against. Moreover, mustard gas is toxic as droplets, liquid, vapor, and, most of all, as "a poisonous cloud" in the form of an aerosol (Gates and Moore, 1946).

A. Physical Properties

Pure sulfur mustard is a transparent liquid with a slight odor of castor oil, whereas technical sulfur mustard is a dark liquid with an unmistakable odor of mustard or garlic. It is barely soluble in water (0.07% at 10°C) and very soluble in organic solvents, fuels, and lubricants (Aleksandrov, 1969).

It is easily absorbed by many foodstuffs, porous materials, paint and varnish coatings, and rubber articles, all of which will remain contaminated for long intervals (Aleksandrov, 1969; Rosenblatt et al., 1975).

Terrain and all objects present will become contaminated by sulfur mustard for very long periods of time because of its stability and persistence. Norwegian samples of snow analyzed for the presence of sulfur mustard attested to its persistence 2 weeks after its initial presence, but was not detectable 4 weeks later (Johnsen and Blanch, 1984).

B. Chemical Properties

Mustard gas, because of its alkyl properties, displays nucleophilic substitution of its chlorine atoms by hydroxyl groups on interaction with water and alkalies. This hydrolysis proceeds in two stages, with gradual substitution of the chlorine atoms by hydroxyl groups, and is reversible; the second hydrolytic reaction is more rapid than the first hydrolysis (fig. 1).

For further information about the solubility and hydrolysis of mustard gas, see tables 1 and 2 (Dacre and Burrows, 1988).

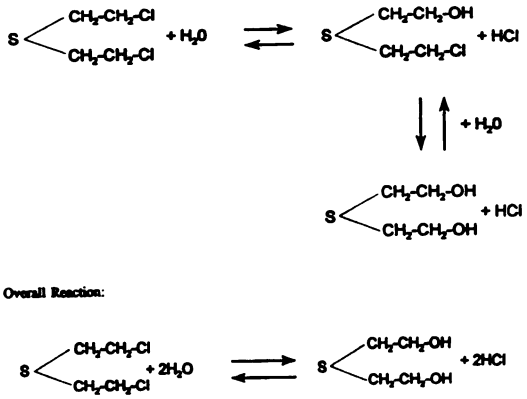

FIG. 1. Hydrolysis of mustard gas: two stages.

TABLE 1
Chemical identity of sulfur mustard

Chemical abstract name
Ethane, 1,1′-thiobis (2-chloro) (after 1971)
Sulfide, bis (2-chloroethyl) (before 1971)
Other names:
Bis (2-chloroethyl) sulfide; 1-chloro-2-(2-chloroethyl-thio) ethane; 2,2′-dichlorodiethyl sulfide; di-2-chloroethyl sulfide; 2,2-dichloroethyl sulfide; Schwefel-Lost; 5-lost; 5-mustard; sulfur mustard; mustard; mustard gas; Levinstein mustard; Yellow cross mustard; Yperite
Other data
Chemical Abstracts Registration No. (CAS No.) 505-60-2
Defense Department Symbols: H, HD
Edgewood Arsenol No. EA 229
Registry of Toxic Effects of Chemical Substances (TECS) No. WQ 0900000 (1983–1984 Supplement)

No., number.

TABLE 2
Physiochemical constants of sulfur mustard

Property	Value
Melting point, °C	14.4
Boiling point, °C	215–217 at 760 mm Hg 108 at 14 mm Hg
Flash point, °C	105
Vapor pressure (20°C), mm Hg	0.72
Heat of vaporization Kcal/mol	15.0
Heat of fusion, Kcal/mol	4.3
Heat of combustion, Kcal/mol	708
Heat of formation, Kcal/mol	32
Viscosity (20°C), poise	0.046
Liquid density d^{20}_4	1.274
Specific heat, liquid, cal/g°C	0.330
Refractive index n^{20}_D	1.531

C. Preparation of Mustard Gas

Mustard gas was first synthesized in 1822 by Despretz. Since then, it has been produced by several different methods:

- the Victor Meyer (1887) process, in which 2-chloroethanol reacts with solium sulfide to produce thiodiglycol, which then will react with hydrogen chloride;
- the Levinstein process, in which ethylene reacts with sulfur chloride;
- the American process, in which ethylene oxide reacts with hydrogen sulfide to yield thiodiglycol, which will react with hydrogen chloride (International Agency for Research on Cancer, 1975; Rosenblatt et al., 1975). A US Department of the Army

Military Specification (1969) for Mustard Gas [sulfur mustard (HD)] has been approved by the US Army. The specification sets out assay limits and the specific assay method based on freezing point determination on the compound.

III. General Toxicity

A. Humans

Sulfur mustard was first used in World War I as an offensive weapon by the Germans during an attack against the British at Ypres, Belgium, in July 1917 (Jackson, 1936). The British called it mustard gas because of its peculiar odor, the French called it Yperite because it was first used at Ypres, the Germans called it Lost, an acronym of the first letters of the names of two German chemists (Lommel and Steinkopf) who were associated with the pioneer industrial development of the agents, and everyone alluded to it as "yellow cross" because the German shells containing the agent were marked with a yellow cross (Vedder, 1925; Aleksandrov, 1969). The code designation *H. S.* is generally believed to stand for HunStoffe (Vedder, 1925). In the US, mustard gas is now commonly called sulfur mustard and is designated HD. Indeed, the national stockpiles of mustard actually consist of HD, Levinstein mustard (H), bis[2(2-chloroethylthio)ethyl]ether (T), and a mixture of 60% HD and 40% T (HT). It was a devastating weapon in World War I and is still regarded as a very dangerous chemical warfare agent (Haber, 1986; Medema, 1986).

Mustard is a poisonous chemical agent that exerts a local action on the eyes, skin, and respiratory tissues, with subsequent systemic action on the nervous, cardiac, and digestive systems in humans and laboratory animals. It causes lacrimation, malaise, anorexia, salivation, respiratory distress, vomiting, hyperexcitability, and cardiac distress (Lynch et al., 1918; Marshall and Williams, 1920; Vedder, 1925; Smith, 1943; Anslow and Houck, 1946; Gates and Moore, 1946; Anslow et al., 1948). Under extreme circumstances, dependent upon the dose and length of exposure to the agent, necrosis of the skin and mucous membranes of the respiratory system, bronchitis, bronchopneumonia, intestinal lesions, hemoconcentration leucopenia, convulsions with systemic distress, and death occur (Lynch et al., 1918; Warthin and Weller, 1918, 1919a; Smith, 1943; Morgenstern et al., 1947; US Department of the Army, 1974; Lohs, 1975). Severe mustard poisoning in humans is associated with systemic injury that is manifested as headache, epigastric distress, anorexia, diarrhea, and cachexia (Marshall, 1919; US Department of the Army, 1974; Lohs, 1975). Damage to hematopoietic tissues with progressive leucopenia occurs with mustard doses of 1000 mg-min/m^3 (Krumbhaar, 1919; Krumbhaar and Krumbhaar, 1919; Bodansky, 1945; Philips, 1950).

1. Mustard intoxication studies. Four human subjects who were injected intravenously with 0.1 mg tris(2-chloroethyl)amine developed headaches and nausea within 9 hours. These symptoms persisted for some time; although only two of four subjects displayed dizziness and weakness, all four developed thrombophlebitis. Whereas exposure to nitrogen-mustard vapors caused nausea, vomiting and headaches, neither vomiting nor anorexia were observed after i.v. injection. Although lymphocytopenia developed, the normal count fell from 2500 cells/mm^3 to 700 cells/mm^3 on the third to sixth days and the lymphocyte counts returned to normal by the fourteenth to twentieth days (Anslow and Houck, 1946).

Oral ingestion of 2 to 6 mg tris(2-chloroethyl)amine dissolved in tap water caused anorexia, recurrent nausea, vomiting, fullness and tenseness in the epigastric region with lassitude, depression, occasionally diarrhea and gaseous eructations. These symptoms were associated with 2-mg doses, pronounced with 4- to 6-g doses, and absent with 1 mg dose after ingestion. Repeated oral ingestion, three times per day with 1 mg of the nitrogen mustard for 5 days, resulted in moderate symptoms of discomfort with prompt recovery once oral intake ceased. The ingestion of a total of 15 to 18 mg caused a moderate leucopenia in five of seven cases within 7 to 9 days that was persistent in all instances for 7 to 25 days and continued for as long as 7 weeks. Consumption of a total of 7 to 9 mg in those individuals on a 1-mg dose schedule caused a definite leucopenia. All subjects were asymptomatic 18 months later (Anslow and Houck, 1946; Jager, 1946). When solutions of tris(2-chloroethyl)-amine were hydrolyzed and the dominant product, bis-β-hydroxyethyl-ethylenimonium chloride, was given, only those subjects who had ingested 24 mg displayed nausea and vomiting but no alterations in leukocyte count (Anslow and Houck, 1946).

2. The Bari incident. Destruction of 16 cargo vessels carrying several thousand tons of high explosives and 100 tons of mustard gas by German bombers on December 3, 1943, in the harbor of Bari, Italy, led to the formation of a giant oil slick mixed with mustard gas that coated the surface of the water for several days. While one thousand men were killed or missing and 800 men were hospitalized, 617 of the latter suffered from exposure to mustard. Indeed, 83 deaths occurred that were attributable to mustard contamination. Some of the casualties of mustard contamination displayed first- and second-degree burns, in some cases involving up to 90% of the body surface. Those casualties who had floundered in the oil slick for many hours before rescue and who had endured additional time waiting for medical aid while wrapped in blankets displayed shock and clinical symptoms consequent to systemic intoxication by mustard. In addition, low arterial pressure and hemoconcentration that resisted treatment was associated with depression and apathy that persisted from 18 hours to 3 days and was followed either by slow recovery or death. By the second day, the few men who removed the blackened oil from their bodies appeared well, while those

covered with the black fuel and mustard gas mixture were dying. Severe conjunctivitis and generalized brawny edema of the skin and subcutaneous tissues were observed in the majority of the rescued men. Additional deaths occurred and were ascribed to the decreasing blood leukocyte counts, which reached levels as low as 50 to 100 cells per cm^3. In such cases, in which there was a failure in the granulocyte response, death occurred (Rich and Ginzler, 1944; Alexander, 1947; Rhoads, 1947; Philips, 1950).

In this incidence, the lungs, kidneys, and skin were the major tissues sustaining the most damage, probably as a consequence of the inhalation of mustard vapor, aspiration of foreign material and mustard-in-oil into the lungs and stomach, blast injury, and subsequent infection. The clinical features associated with the casualties of this disaster were a reflection of systemic intoxication by mustard absorbed through the skin from the oil-mustard gas-water mix on the surface of the harbor water (Rich and Ginzler, 1944; Alexander, 1947; Rhoads, 1947; Philips, 1950).

Colonel Stewart F. Alexander, a medical officer who had been trained by the US Chemical Warfare Service, was stationed in North Africa; and on being informed of the Bari tragedy, he confirmed the findings and immediately realized that the casualties were dying from mustard gas poisoning and not from blast or immersion shock (Alexander, 1947). He was able to obtain some tissue specimens from the casualties that were sent to the Medical Research Laboratories of the US Chemical Warfare Service at Edgewood Arsenal. The specimens were reviewed, and his diagnosis was confirmed (Rhoads, 1947). Interestingly, skin burns caused by mustard exposure do not have the same toxic properties as do thermal burns (Sollmann, 1957).

3. An early incident. There have been a number of incidents in which ignorance of the hazards of mustard gas have led people to suffer injury and death from mustard gas intoxication. In 1919, at Salaise, France, a large can of alcohol that had been used to clean out Yperite from the apparatus of a nearby shell filling station was stolen and kept in a room for several days. Because the can leaked, the alcohol-contaminated Yperite saturated the floor of the room and subsequently dripped down below into a room occupied by a family consisting of a man, his wife, and two children, all of whom died of Yperite poisoning. Apparently, the house was heated, and rapid evaporation of the Yperite was effected (Zanetti, 1919).

4. Recent incidents. More recent reports state that 197 people were contaminated with mustard gas from handling 50,000- to 150,000-lb bombs that had been netted by fishermen off the coast of Borholm, Denmark. These bombs had been sunk previously in the Baltic Sea in 1946 to 1947. Approximately 90% of the hospitalized people were fishermen who had entangled the bombs in their nets and had become contaminated with mustard from the corroding steel casings. Twenty-seven men showed skin lesions, erythema, vesication, necrosis, and eye lesions; there were two deaths (Aasted et al., 1985; Jorgensen et al., 1985).

On March 2, 1984, 15 Iranians were brought from Teheran to Vienna, Austria, suffering from burn injuries caused by a gas attack in the Iraqi-Iranian War. Clinical examination of mustard gas in the urinary specimens of two of the patients by the University of Vienna provided evidence that the injuries were a consequence of a mustard gas attack. This was confirmed by Heyndrickx's (Heyndrickx and Heyndrickx, 1984) laboratory in Ghent, Belgium, which found mustard gas present in urine, feces, and blood (Mandl and Freilinger, 1984; Pauser et al., 1984; Vycudilik, 1987), urinary excretion of thiodiglycol (Wils et al., 1988), and inhibition of serum cholinesterase (Vojvodic et al., 1985). Indeed, careful scrutiny of the chemical records of 65 Iranian patients who were casualties of the Iraqi-Iranian War affirmed the conclusion that these patients had been poisoned by sulfur mustard (Willems, 1989a, b).

B. Animals

Single intravenous injection of 0.5 mg/kg of sulfur mustard in young male rats caused widespread degenerative damage to all hemopoietic tissues such as spleen, thymus, and bone marrow and resembled the hematological condition that prevails after X-ray irradiation (Kindred, 1947). This was also observed by (Graef et al., 1948) in rats, mice, rabbits, and dogs: the most dramatic and pervasive feature of mustard poisoning was the marked effect on the hemopoietic system. Within 12 hours after subcutaneous injection of 3 mg/kg of sulfur mustard, granulocytosis was observed, quickly followed by leukopenia, with bone marrow depletion 24 to 48 hours later. Damage to the spleen, thymus, small intestine, and injury to the testes with inhibition of spermatogenesis were also observed. Associated with these destructive changes was a widespread and prevalent systemic poisoning of the gastrointestinal, pulmonary, and nervous systems (Kindred, 1949a, b). There was a loss in control of body temperature, a fall in respiration, diarrhea, muscular weakness with tremors, and convulsions (Anslow and Houck, 1946). Although most animals recovered from such intoxication, some were not able to sustain the sequence of damaging events and shock, and succumbed, particularly, if great fluid loss and anoxia had been incurred (Graef et al., 1945, 1948) (tables 3 and 4).

Subcutaneous injection of 0.625 mg/kg of mustard gas in young male rats (50% of the LD_{50}) caused injury to the thymus gland, with inhibition of cell division on the fifth day; recovery was slow (Cataline et al., 1971). Friedberg et al. (1983) injected 15 mg of mustard gas/kg i.p., which depressed the activity of bone marrow cells of the femur 3 to 8 days after administration of the agent. The depression persisted for 3 days, after which recovery was

TABLE 3
LD_{50} values (mg/kg) for sulfur mustard in various animal species

Animal Species	Route of Administration		
	Subcutaneous	Intravenous	Dermal
Rat	1.5, 2.0	0.7, 3.3	9
Mouse	26	8.6	92
Dog	–	0.2	20
Rabbit	–	Ca 1.1, 2.7	Ca 100
Guinea pig	–	–	20
Goat	40	–	50

TABLE 4
LCT_{50} values for sulfur mustard in various animal species

Animal Species	LCT_{50} mg/min/m^3	Time Range (min)
Mouse	860–4140	2–360
Rat	840–1512	2–360
Rat	420	2
Guinea pig	1700	10
Rabbit	900	10
Cat	700	10
Dog	600	10
Goat	1900	10
Monkey	800	10
Human	1500	1

observed (8 days later). In dogs, nausea, vomiting, and anorexia were observed a few hours after mustard intoxication, with diarrhea on the second and third day. Weakness with diminution in body temperature followed so that the extremities were cold and the animals sank into coma and ultimately died from respiratory failure (Anslow and Houck, 1946).

The LD_{50} for mustard, whether administered topically, subcutaneously or intravenously, was rather high in mice and rabbits compared with that observed in rats, which appeared to be the most sensitive species examined (Anslow et al., 1948). The LD_{50} values also varied widely and may have been influenced by the solvent used for dissolving the mustard; in rats, the intravenously injected "neat" mustard LD_{50} was about five times lower when dissolved in propylene glycol. In fact, "neat" mustard caused severe pulmonary necrotic lesions with associated damage to neighboring tissues, whereas mustard dissolved in propylene glycol, resulting in diffuse pulmonary congestion (Anslow et al., 1948).

Soon after introduction into body tissues, hydrolysis of mustard occurs to form thiodiglycol and semimustard, which are relatively nontoxic, having no effect on heart rate, blood pressure, or vagus nerve irritability in dogs or rabbits. However, 2-chloroethyl-2[bis(2-hydroxyethyl)-sulfonium]ethyl sulfide chloride was toxic and caused enteritis, necrosis of the liver, damage to lymphoid tissues, and adrenal congestion, but no bone marrow injury in mice and rats; in rats, large doses of this hydrolytic product, as the picryl sulfonate, produced diarrhea and reduction in body weight (bw) in addition to the previous noted effects. Both the picryl sulfonate and chloride given i.v. caused a slight leucopenia. Despite the toxicity of this compound, it was not considered likely to be contributing to the overall toxicity of mustard because thiodiglycol was necessary for its formation. However, the concentration (Ct) of thiodiglycol that would actually be present after i.v. injection of mustard gas would be inadequate for transformation of mustard into the bimolecular sulfonium salt. Moreover, the Ct of a radiolabeled sulfonium derivative actually extracted from pig skin, after injection of labeled mustard, was very small: 2.5% of the radioactive material present (Anslow and Houck, 1946; Anslow et al., 1948). In addition, incubation of labeled 0.0008 M mustard with blood plasma for 30 minutes at 37°C resulted in 2.4% of labeled mustard being converted into the labeled sulfonium derivative (Anslow and Houck, 1946). The investigators concluded that the active toxicant after mustard gas administration was mustard gas itself or its cyclical form, β-chloroethylethylenesulfonium chloride, which was the first reaction product in the hydrolysis of mustard gas (Anslow et al., 1948).

Injection of a lethal dose of mustard intravenously in dogs elicited the following course of action within 10 to 20 minutes: salivation that increased in flow followed by a diarrhea that persisted until death of the animal. Rapid respiration, muscle spasms, tetanic muscular contractions, extension of the hind legs, arching of the neck and back with subsequent convulsions were also observed. The pulse became irregular, the blood pressure fell slowly, the cardiac rhythm became anomalous with more auricular beats than ventricular (3:1); also, the vagus became unresponsive. The convulsions ceased eventually, and the animals died in coma less than 24 hours after injection of the mustard. Autopsy revealed an intense congestion of the intestinal mucosa from pylorus to anus with hemorrhaging into the lumen (Lynch et al., 1918; Warthin and Weller, 1919b).

C. Human Health Criteria

Recently, drinking-water criteria have been derived for the chemical agent sulfur mustard, for the protection of human health and specifically for the health of the soldier in the field. Criteria have been calculated for a daily drinking water intake of both 5 L and 15 L over a maximum period of 7 days, using the basic formula proposed by the US Environmental Protection Agency. The recommended drinking-water criteria, based on an uncertainty factor of 100, are 28 μg/L (5 L/day) and 9.3 μg/L (15 L/day intake) (Dacre and Burrows, 1988). A no-observable-effect-level of 0.1 mg/kg/day was determined from the available toxicology data-base from a 90-day study in the rat given the compound by oral administration (Sasser et al., 1988, 1989c, 1995).

IV. Respiratory System

A. Animals

Next to eye lesions, the greatest discomfort produced by exposure to mustard gas is that resulting from irritation and injury of the respiratory system (Giraud, 1917; Mandel and Gibson, 1917; Warthin and Weller, 1919a). Indeed, Victor Meyer, who synthesized mustard gas in 1887, showed experimentally that irritation and inflammation of the upper respiratory tract resulted from exposure to the agent and terminated eventually in pneumonia.

When rabbits were gassed in special gassing chambers, symptoms of nasal irritation were observed immediately, such as rubbing of the nose and turning about of the animals to present their backs to the in-flowing gas. Photophobia and lacrimation together with increased nasal secretion and inflammation of respiratory membranes appeared 2 to 3 hours after exposure. Rabbits exposed to low Cts of mustard gas such as dilutions of 1:100,000 for 10 to 15 minutes or to higher Ct for 1 to several minutes displayed some degeneration and necrosis of the epithelium of the mucous membrane, with congestion, edema, and mucus secretions. The lesions were mild and limited to the anterior nares, pharynx, larynx, and upper portions of the trachea; however, some pulmonary congestion and edema with increased bronchial secretions were often noted. Recovery without any secondary infection usually followed (Warthin and Weller, 1919a). However, rabbits exposed to higher Cts of mustard gas for short periods of time, such as 1 to several minutes, or to prolonged exposures, showed the usual respiratory clinical symptoms; somewhat later, respiration became difficult and coughing, rales, exudation, and death ensued. The clinical symptoms were associated with laryngeal and tracheal edema attended with congestion and marked necroses of the mucosa of the respiratory tract which generated the formation of diphtheritic membranes (easily detached) in the anterior nares, neopharynx, larynx, trachea, and bronchi as a consequence of the fibrous inflammatory exudation. Secondary infection with *Staphylococci* followed in severe exposures and led to purulency of the lesions in the larynx, trachea, and bronchi within 4 to 6 days. The lungs displayed congestion, edema, hydropic, and mucoid degeneration of the epithelia in mild cases of exposure. Necrosis of the epithelia that extended into the smaller bronchioles, accompanied by secondary infection, was observed in severe cases of exposure; edematous and hemorrhagic atelectatic areas caused by plugging of the bronchioles with exudates alternated with emphysematous areas and resulted in hemorrhagic bronchopneumonia. In most cases, death was caused by an infective purulent bronchopneumonia that was secondary to the gassing; however, in severe cases of exposure, death occurred quickly because of diphtheritic or purulent laryngitis in absence of pneumonia. Under some circumstances in which bronchopneumonia was not extensive, recovery ensued when the localized diphtheritic patches in the nose, mouth, larynx and trachea healed, and cicatrization with resulting cicatricial contraction in the trachea and larynx occurred (Warthin and Weller, 1919a).

No permanent effects could be demonstrated in dogs intoxicated by a single i.v. injection of 1 mg/kg of methylbis(β-chloroethyl)amine or tris(β-chloroethyl)amine, although some dogs did die of anoxia of the respiratory centers because of peripheral circulatory failure promoted by a reduction in blood volume (Houck et al., 1947). Exposure to mustard gas at a Ct of either 0.001 mg/m^3 for 16, 32, and 52 weeks or 0.02 mg/m^3 for 4, 8, 16, and 32 weeks did not alter the normal respiratory rate or values in dogs (McNamara et al., 1975).

B. Humans

1. War. Clinical descriptions of men exposed to mustard gas on the battle field during World War I emphasized the initial effects of mustard on the mucous membranes of the respiratory system (Sollmann, 1957; Blewett, 1986; Haber, 1986). Early symptoms included sneezing and coughing, with increasing irritation of the nose and throat and a discharge of mucus from the nose with loss of the sensations of smell and taste within 12 hours. Longer periods of exposure to mustard gas caused laryngitis, aphonia, incapacitation, bronchitis, and pneumonia within 36 to 48 hours. Dysphagia or difficulty in swallowing appeared on the second or third day, lasting from 4 to 6 days or longer and was associated with local white diphtheritic necroses in the mucosa of the pharynx that covered a great part of the oropharynx and laryngopharanyx. As a result, tickling, dryness, burning sensations were experienced in the larynx and in the sternum for several days or even weeks, depending on the extent of the lesion present in the mucosa. There was constant coughing that was painful, dry, paroxysmal, and troublesome, particularly at night; occasionally, a bloody and even purulent expectoration was also present. Aphonia was sometimes present, but, more usually, the voice was merely rough or husky and was associated with changes in the mucosa of the larynx. The larynx displayed a marked hyperemia, swelling, and local necroses that developed into whitish or grayish eschars that tended to form pseudomembranes. The most frequent lesions were the isolated eschars associated with the arytenoids, epiglottis, and vocal cords, all of which healed very slowly, taking several weeks. Tracheal lesions were similar to those described for the larynx and were associated with subjective symptoms of bronchitis. Inflammation of the airways of the pharynx, larynx, trachea, and bronchi led to bronchitis, bronchopneumonia, and death (Warthin and Weller, 1919a; Buscher, 1944; Sollmann, 1957; Blewett, 1986).

Lesions of the respiratory tract in humans caused by mustard gas exposure did not appear to be different

from those observed experimentally in laboratory animals. Hyperemia and a slight necrosis led to inflammation of the nose, laryngeal huskiness, cough, and sore throat, all of which were commonly observed in human beings who had been exposed to either light or moderate exposures of mustard gas; although recovery was rapid, some irritation, cough, and huskiness might persist for some time. Severe exposure caused more degeneration, necrosis, and exudation in the epithelia of the respiratory tract, resulting in eschars on the plate, back of the tongue, pharynx, and larynx associated with tracheal inflammation, bronchitis, pulmonary congestion, and edema. Indeed, severe exposure as may be encountered on the battle field may produce diphtheritic lesions of the larynx, trachea, and bronchi, leading to bronchopneumonia (Warthin and Weller, 1919a; Sollman, 1957; Haber, 1986).

2. Chemical Factories. People employed in factories manufacturing mustard gas can become partially or totally disabled because of injury to the mucosa of the respiratory system after protracted exposure to small quantities of mustard gas vapor. Typically, such workers develop an aggregate of several or many symptoms:

- eye problems, such as "red" eyes, photophobia, lacrimation, impaired vision, and blepharospasm;
- respiratory problems, such as loss of sensation of taste and smell, bleeding from the nose, sore throat, hoarseness, difficulty in swallowing, chest pain, wheezing, and dyspnea;
- gastrointestinal problems such as anorexia, vomiting, weight loss;
- and nervous problems, such as insomnia and irritability.

Treatment of these sick people is difficult and takes a very extensive course of time; invariably, the exposure results in total or partial disability (Morgenstern et al., 1947).

In another study, Brown (1949) reported that a large number of the employees (20 to 60 years of age) who were working at the Huntsville Arsenal in Alabama and were exposed to continuous Cts of mustard gas vapor for long periods of time displayed coughing, chest pain, shortness of breath, and fatigue. Eventually, the coughing contained foul sputum, and the workers developed bronchiectasis with progressive emphysema and narrow attenuated bronchioles. Of 224 workers at the plant who were considered to have some degree of disability as a consequence of mustard gas exposure, 80% displayed disabilities that were rated from 25 to 100%; 25% of those with disabilities were considered to be 100% disabled.

Weiss (1958) has reported such delayed effects as changes in the mucous membrane that led to swelling and formation of polyps in the paranasal sinuses. Lohs (1975) has provided a summary of such slowly evolving effects.

V. Skin

A. Humans

Ordinarily, the skin serves as a bulwark, a vital and important defense system against penetration of potentially toxic and even life-threatening chemical agents. However, on exposure to mustard gas, the skin itself becomes a target that is vulnerable and defenseless against the rapid penetration and fixation of sulfur mustard. This rapidly leads to a sequence of reactions that result in severe damage to the basal cells, which are vital for the replacement of epidermal tissue, thereby attenuating the healing process so that lesions take several months to heal. In general, 80% of the mustard making contact with the skin will evaporate, and the 20% remaining will penetrate the skin: however, only 2% of the mustard becomes fixed, so that 18% is absorbed into the circulation to cause systemic intoxication (Pruit, 1987).

Humans exposed to mustard gas vapors display simultaneous irritation to the eyes, skin, and respiratory system evidenced by lacrimation, acute conjunctivitis, sneezing, running nose, burning in the throat, coughing, hoarseness, and erythema of the skin (Marshall and Williams, 1920; Smith, 1943; Morgenstern et al., 1947). Soldiers exposed to mustard gas vapors or mists complained mostly about injury to the eyes, respiratory tract, scrotum, face, and anus as body areas that were most vulnerable (Blewett, 1986). Such exposure is usually not associated with any immediate discomfort or pain because of mustard's long latency period, which thereby adds to its insidious nature; later discomfort and pain are caused by the lesions themselves and not by the causative agent itself (Warthin and Weller, 1919a; Daily et al., 1944). Lewisite is a vesicant that can provoke the immediate initiation of protective measures because it gives adequate warning through its acute irritation to the eyes and respiratory passages (Daily et al., 1944), but severity of skin lesions as a consequence of exposure to mustard is dependent upon the dose of the agent, the humidity, and the length of exposure (Renshaw, 1946; McNamara et al., 1975). Tremendous differences in skin sensitivity have also been reported (Marshall et al., 1918). Under hot and humid conditions, mustard gas contamination may cause maceration and desquamation in certain dry and most moist areas of the body without previous development of vesicles, particularly in the skin of the scrotum and penis and in the axillae (Renshaw, 1946). Indeed, mustard contamination may cause an erythema resembling sunburn or widespread vesications (McNamara, 1960).

Mustard gas applied to the forearms of humans, as droplets by capillary pipet (0.002 ml), spread rapidly to occupy an area 3 to 4 mm in diameter, with erythema and edema appearing 2 to 3 or even 8 to 12 hours later (Warthin and Weller, 1918). On the other hand, the erythema after Lewisite contamination appeared rap-

idly, spread immediately over a wide area, and diminished just as quickly (Daily et al., 1944). Then, 16 to 24 hours after contact with mustard, a vesicle formed that increased in height to 4 mm, measured 25 mm in diameter, and was filled with a clear, pale yellow fluid; it was at this time that some pain was first perceived. Absorption of the fluid within the vesicle caused folding and wrinkling of the epidermal covering of the vesicle 46 hours after application of the mustard. Vesicle collapse occurred within 72 hours and the delicate wrinkled epidermis rubbed off within 4 days. The brown crust at the base of the vesicle became loose and detached, and was accompanied by itching during the following 10 days. The excavated area began to fill in with a yellow brown crust and came off as a scab during the ensuing 10 to 18 days. Healing was completed with formation of a scar 50 days later (Warthin and Weller, 1918, 1919b). A characteristic brown pigmentation may be observed during the healing and may persist for a very long time (Chiesman, 1944).

Vesicles may even appear on different parts of the body as late as 7 to 12 days after exposure. Australian studies held in the tropics have reported that most men were incapacitated 10 to 12 days after exposure to mustard rather than at either earlier or later times. Men wearing tropical Australian battle-dress together with respirators were subjected to mustard gas vapor for several successive days and rated on their ability to carry out standard physical performance tests. Exposure to high doses of mustard led to an inability to perform these tests because of the prevalence of a combination of systemic intoxification, surgical shock, and severe generalized burns. Moderate doses of mustard resulted in the incapacity to perform these tests because of severe skin burns on the genitalia, axillae, and buttocks, all of which became severe, with maximal disability 11 days after exposure (Chiesman, 1944; Sinclair, 1950; Stockholm International Peace Research Institute, 1973). Although the skin of the genitalia and buttocks required effective protection against exposure to moderate doses of mustard, the rest of the body did not appear to be as sensitive under these conditions when ordinary tropical Australian battle-dress was worn (Nagy et al., 1946; Cullumbine, 1947; Sinclair, 1950; Medema, 1986). The severity of skin lesions consequent to mustard contamination was influenced not only by the degree of exposure but also by the prevailing weather conditions: hot, humid weather intensified the severity of the lesion, so that the healing process took longer (McNamara, 1960; Stockholm International Peace Research Institute, 1973). Indeed, certain regions of the skin were more sensitive to the action of vesicants, so that hot, sweaty skin (Smith et al., 1919) accelerated the fixation of mustard in skin (Cullumbine, 1947; Sollmann, 1957).

Two days after exposure, the severity of injuries produced by mustard gas or nitrogen mustard (HN-1) vapors in contact with the skin of forearms of human volunteers was markedly increased in skin that had a thin continuous layer of water. Evidently, hot sweaty human skin incurred greater damage from these agents, because its sensitivity to the injurious vapors were enhanced by a film of moisture on the skin surface (Renshaw, 1945).

Fairley (1943) has reported the occurrence of prolonged vomiting that was frequent and violent in four men who wore respirators but whose skin was exposed to a Ct of 660 mg/min/m^3 under tropical conditions; no leucopenia was observed in these men.

1. Vesicant action of mustard and Lewisite. An extensive comparison of the modes of action of Lewisite and sulfur mustard applied to human forearm skin has been reported by Buscher (1944). One to two large drops of each of the compounds were applied to the skin approximately 15 to 20 cm apart. Detailed comparisons were made immediately and during the following times after exposure: 5, 14, 30, 50 minutes; approximately hourly up to 5 hours; intermittently up to 24 hours; daily up to 15 days; and, finally, at 20 days. The reader is referred to Buscher (1944) pp. 93–115 for the summarized results of these comparative experiments.

In general, the lesions caused by sulfur mustard were more severe than those due to Lewisite, and the resulting third-degree burns not only exposed, but also damaged the corium layer of the skin (Buscher, 1944). A study by Daily et al. (1944) confirmed these observations. Nagy et al. (1946) has estimated that as little as 6 μg/cm^2 of liquid sulfur mustard will cause lesions in human skin in most exposed sites. The results of studies of the effects of Lewisite alone on the skin of men and other mammalian species have been reviewed by Goldman and Dacre (1989).

2. Models for studying vesication. A recent study using electron microscopy to investigate the effects of mustard gas on human skin grafts, borne by athymic nude mice, did not reveal any new information: results were the same as those reported using light microscopy (Papirmeister et al., 1984a). Electron microscopy reemphasized the finding that mustard gas poisoning of human skin—using either moderate doses of mustard, such as 60 μg/cm^2 or higher doses, such as 635 μg/cm^2—was initiated at the basal cell layer of the epidermis and that injury to these cells led to separation of the epidermis from dermis with the formation of a subepidermal microblister. Although the collagen fiber bundles remained intact, they separated from one another; however, the fibroblasts and endothelial cells in contact with the dead basal cells were damaged (Papirmeister et al., 1984b).

An in vitro model has been developed recently for investigating the degree of injury and the role of the various components participating in the early phase of the inflammatory response provoked by addition of different Cts of sulfur mustard to the skin (Dannenberg, 1988). This model, called the paranuclear vacuolization test, consists of full-thickness 1.0 cm^2 human skin

pieces, to which are added topically various dilutions of sulfur mustard, and incubated in organ cultures for 24 hours at 37°C. The explants are examined histologically to determine the number of paranuclear vacuoles and the organ culture fluids assayed for various lysosomal enzymes and other factors released by the epidermis exposed to sulfur mustard. The usual markers of cell death and early inflammatory events such as lysosomal enzymes, trypsin, and chymotrypsin-like proteases, angiotensin-coverting enzyme are assayed from the culture fluid. Dannenberg (1988) has reported that the culture fluids of both normal and sulfur mustard-treated explants displayed no significant difference in any of the enzyme Cts except for one mediator of early inflammation, the plasminogen-activating processes that were significantly increased in the organ culture fluid of sulfur mustard-treated human skin explants. The plasminogen-activating proteases are released by the basal epidermal layer of human skin (Justus et al., 1987) and may play an important role in blister formation, because they have also been associated with activity of alkylating agents (Brdar, 1986).

Another recent model for studying sulfur mustard toxicity of skin is the use of human lymphocytes. Human lymphocytes are incubated with either 1×10^{-3} M sulfur mustard and 1×10^{-3} M niacinamide or with sulfur mustard alone for 24 hours at 37°C. Light microscopy revealed a loss of viability and various phases of cytotoxicity such as fragmented cells and ghosts of necrotic cells present among surviving cells (30%) that had been treated with mustard alone. The surviving cells showed condensed nuclear chromatin and pyknosis, with paranuclear vacuolation surrounding the nucleus, whereas cells treated with both mustard and niacinamide had a viability of 87% with normal cytology. Although scanning microscopy showed the normal surface features of numerous microvilli and an intact plasma membrane in those lymphocytes treated with both mustard and niacinamide, lymphocytes incubated with mustard took on a rounded shape, because they had lost their microvilli and revealed numerous perforations of the plasma membrane. Finally, transmission electron microscopy showed that those lymphocytes that survived treatment with mustard had nuclear pyknosis, condensation of nuclear chromatin with loss of euchromatin, paranuclear vacuolation, cytotoxic organelles, extensive blebbing of the nuclear envelope with perforation and fragmentation of the plasma membrane; but simultaneous treatment with mustard and niacinamide resulted in the absence of any cytotoxic effects and presence of an essentially normal ultrastructure. The pathology observed in lymphocytes treated with mustard is very similar to that which has been found occurring in epidermal basal cells of human skin grafted onto athymic nude mice and which leads to cytotoxic effects and ultimately cell death. This suggests that human lymphocytes may be used as a model for studying mustard toxicity (Petrali et al., 1988).

B. Animals

The application of 25 to 250 $\mu g/cm^2$ mustard gas to rabbit or guinea pig skin provoked an erythema within 30 to 60 minutes. This spread over the ensuing 4 hours so that in 5 to 8 hours, the erythema had progressed beyond the site of application. Edema, necrosis, and an exudate were present during the subsequent 48 to 72 hours (Vogt et al., 1984). After 3 to 5 days, attenuation of the erythema and edema occurred, with formation of a scab that sloughed off and was replaced with a new epidermis within 10 to 14 days. The developing inflammatory response was evident, as noted by activation of the lysosomal enzymes in the basal epidermal cells at early times with increased activity of mesenchymal elements sometime later (Dannenberg et al., 1987; Higuchi et al., 1988). The reaction of the skin to the mustard insult revealed a biphasic response (Dannenberg and Vogt, 1981; Vogt et al., 1984), characterized by an early phase in which damage to the basal epidermal cells, superficial small capillaries, and venules resulted in limited vascular leakage. This was followed by a massive infiltration of granulocytes that were dominated by basophils into the area of injury; this was termed the intermediate phase. The delayed phase occurred 8 hours later and was characterized by the death of the epidermal cells, increased lysosomal enzyme activity, muscular leakage, and edema. Massive infiltration of heterophils (equivalent to neutrophils in humans) with an ensuing reduction of basophils into the area below the dying epidermal cells occurred over the next 24 hours, with consequent ulceration. The inflammatory response achieved its maximal activity between 27 to 72 hours and was followed by the formation of a crust over the ulcerated skin with repair and healing. Subsequent shedding of the scab exhibited the presence of a new skin some 10 days later. The above investigation was an extremely thorough undertaking in which light and electron microscopy, histochemistry, and injections of Evans blue dye were used to study the pathogeneses of skin lesions caused by sulfur mustard. Intramuscular injection of 25 mg cortisone acetate/kg alone, or together with one topical application of 1.0% hydrocortisone, to the multiple sites where lesions were developing as a consequence of the application of 100 $\mu g/cm^2$ sulfur mustard to rabbit skin, reduced the degree of edema developing during the immediate phase but had no influence on the rate of healing (Vogt et al., 1984).

1. Penetration of the skin by mustard gas. The mustards are oily lipophilic liquids that easily penetrate skin and mucosal surfaces within 3 to 5 minutes after contact to provoke local discomfort both to the respiratory tract and to the skin. The injury resulting may be transitory or might lead to permanent damage to the more sensitive proliferating tissues such as bone marrow, the re-

ticuloendothelial system, and the intestinal tract (Anslow and Houck, 1946; Cullumbine, 1947; Rudin, 1953).

The initial step in the development of injury to the skin appears to be the fixation of mustard molecules at the site of contact where they react with the proteins of the skin. No free mustard can be detected within the skin, even when the skin has been massively contaminated with the agent, and the mustard that has become fixed cannot be physically removed without causing further injury (Renshaw, 1946). A large portion of the mustard that penetrates the skin is carried away by the circulation to produce systemic injury (Anslow and Houck, 1946). Although the reactions between the mustard and the systemic tissues are completed within a few minutes (Rudin, 1953), there is a latency period of many hours before the appearance of overt cellular damage as a consequence of the disruptions of nucleic acid and protein metabolism at the cellular level (Cullumbine, 1947).

Thirty minutes after topical administration of 5 mg of radioactive mustard to the skin of a rat, 0.17 mg of the compound was found distributed throughout the animal. The Cts of labeled ^{35}S reached maximal activity 2 to 6 hours after application and attained a steady activity between 24 and 72 hours. Although the activity was about the same in all tissues, kidney tissue activity was consistently higher. The radioactivity in the blood increased during the first 6 hours, fell, and then rose again as a consequence of hemoconcentration at 48 and 72 hours. The plasma radioactivity was higher than that in the cells during the first 6 hours, but 12 hours later, the cells showed a higher activity than the plasma. Fifty percent of the radioactivity applied topically was excreted into the urine by 72 hours, with the greatest fraction being excreted during the first 24 hours (Anslow and Houck, 1946).

In human skin, the penetration rate of saturated vapor or liquid mustard is 1 to 4 $\mu g/cm^2$/min (Dutra et al., 1944; Cullumbine, 1947; Rudin, 1953). Using mustard gas labeled with radioactive sulfur, it was found that penetration of the skin occurred within 10 minutes, and 12% of the labeled mustard gas became fixed (Dutra et al., 1944; Cullumbine, 1947). The unfixed fraction of labeled mustard (88%) remaining in the skin during the first 10 minutes disappeared rapidly, being carried away by the circulation.

In humans and pigs, there appears to be a correlation between the amount of labeled mustard fixed per unit area of skin and the severity of the injury. Fixed mustard disappears from the skin slowly, remaining constant for approximately 1 week, then disappearing slowly at a rate that parallels the rate of healing and the rate at which the epidermis desquamates; apparently, the body is not able to metabolize the fixed mustard. In pig skin exposed to liquid mustard in vivo, 80% of the fixed sulfur is present in the epidermis and approximately 70% of that is in the cornified layer, whereas only 20% is present in the dermis. The presence of approximately 0.25 μg of mustard/cm^2 of skin surface that has been fixed in the Malpighian layer is associated with exposures that will produce vesication in humans (Dutra et al., 1944; Renshaw, 1946).

Apparently, mustard vapor is more toxic than are mustard aerosols in mice. Approximately 50% or more of ^{35}S-labeled mustard vapor was fixed to the skin of mice than was ^{35}S-labeled mustard as an aerosol. Moreover, less of the labeled mustard as an aerosol was retained in the respiratory tract or blood, because it was not transported very far into the respiratory tract (Southern Research Institute, 1953).

2. Alkylation of deoxyribonucleic acid and inhibition of glcolysis. Addition of mustard either to minced tumor tissues or rabbit brain tissue depressed glycolysis markedly, whereas thiodiglycol addition was without effects (Berenblum et al., 1936). Marked reduction in glycolysis of rat skin by mustard was also demonstrated by Barron et al. (1948). It is now known that mustard inactivates those enzymes, the phosphokinases, such as hexokinase and adenosine triphosphatase, which are involved in the transfer of phosphate groups (Dixon and Needham, 1946). As a matter of fact, both oxygen uptake by pyruvate oxidase and anaerobic oxidation of glucose are inhibited, in particular hexokinase phosphorylation of glucose (Dixon and Needham, 1946). Moreover, poisoning of hexokinase plays an important role in vesication, and the features associated with the vesicant's action on skin appear to be correlated with the inactivation of the glycolytic system. Indeed, there appeared to be a correlation between the ability to inactivate hexokinase and manifest vesicant properties that certain chemical compounds such as mustard gas displayed (Dixon and Needham, 1946). Accordingly, Wheeler (1962) concluded that there was a correlation between the ability of an alkylating agent to inhibit glycolysis and its vesicant, antineoplastic, mutagenic, and carcinogenic activities.

Moreover, the mechanism of action of mustard on enzyme activity unlike that of Lewisite (Peters et al., 1946; Johnstone, 1963) was not through combination with sulfhydryl groups essential for enzymic activity (Dixon and Needham, 1946; Peters, 1947). An important objection to the "enzyme inactivation" hypothesis of vesication by mustard was that although many enzymes, particularly hexokinase, are inactivated immediately by mustard in vitro, there is a delay time of several hours before hexokinase activity in contaminated skin begins to diminish. By that time, there is usually adequate evidence of tissue damage, so that the delay in inactivation of hexokinase in vivo may be secondary to cell injury (Cullumbine, 1947; Peters, 1947). In addition, very large doses of mustard that are lethal within 2 to 3 hours do not appear to inhibit carbohydrate metabolism, because neither glycogenolysis, gluconeogenesis, nor glycolysis display any alteration until the animal is moribund

(Black and Thomson, 1947). Such evidence does not appear to support the position that mustard directly inactivates hexokinase or other phosphokinases.

3. Deoxyribonucleic acid-alkylation-damage hypotheses of Papirmeister. A recent hypothesis of Papirmeister et al. (1985) attempts to elucidate the phenomenon of vesication in human skin in biochemical terms, emphasizing the damage incurred because of alkylation of deoxyribonucleic acid (DNA) and inhibition of glycolysis. This causes an alteration in metabolism that results in a sequence of pathological events that terminates in vesication.

Fixation of mustard in human skin occurs rapidly by alkylation of DNA in the keratinocytes of the basal layer in the epidermis (Renshaw, 1946; Papirmeister et al., 1984a, b). This results in monofunctional adducts at N-7 in guanine (60%), N-3 in adenine (16%), and bifunctional adducts at N-7 in two guanines (16%) adjacent to one another on the same strand, or in two guanines of opposite strands of DNA, with trace amounts of monofuctional adducts on other DNA purines and pyrimidines (Brookes and Lawley, 1961). The resulant apurinic sites are rapidly cleaved by apurinic endonucleases; the N-3 alkyladinine adducts are the most sensitive to these enzymes, with further degradion of the DNA generated by exonucleases. The DNA breaks activate the chromosomal enzyme poly(adenosine diphosphate-ribose) polymerase (Berger et al., 1979), which uses the oxidized form of nicotinamide adenine dinucleotide (NAD^+) as its substrate. As a consequence, the levels of coenzyme are depleted below a critical Cts (Roitt, 1956), which depresses glycolysis in the skin (Dixon and Needham, 1946; Peters, 1947; Renshaw, 1946; Wheeler, 1962). The levels of alkylation by ^{14}C-labeled sulfur mustard and the reduction in Cts of NAD^+ (Rankin et al., 1980) are correlated with the severity of lesions in human skin implants in athymic nude mice 4 hours after exposure to the agent (Gross et al., 1985). Indeed, fixation of 0.1 to 1.0 μg mustard/cm^2, of 1.0 to 2.5 μg mustard/cm^2, and of 2.5 μg mustard/cm^2 caused mild erythema, vesication, and necrosis, respectively, and correlated well with proportionate reductions in levels of NAD^+ in human skin implants of mice 4 hours after exposure. The resultant reductions in NAD^+ levels (*a*) in mildly injured skin are reversible, (*b*) in vesicant skin are partially reversible, and (*c*) in necrotic skin are irreversible. Moreover, 3-aminobenzamide, which is an inhibitor of poly(ADP-ribose)polymerase (Purnell and Whish, 1980), increased the 4-hour NAD^+ levels from 45 to 58% in mildly injured skin, from 37 to 68% in vesicant skin, and from 23 to 50% in necrotic skin after mustard exposure.

The diminution of NAD^+ levels in the basal epidermal keratinocytes inhibits glycolysis and causes an accumulation of various intermediate metabolites such as glucose-6-phosphate, which stimulates the activation of the $NADP^+$-dependent hexosemonophosphate shunt. Additionally, DNA damage also causes the release of several proteases that may be responsible for the sequence of pathological events that result in vesication in human skin. The reduction in NAD^+ levels is associated with the presence of pyknotic nuclei in basal cells; this is first seen 6 hours after exposure to mustard. The number of basal cells showing pyknosis increases by 12 hours and becomes maximal 18 to 24 hours later (Papirmeister et al., 1984a, b). The epidermis separates from the basement membrane at the epidermal-dermal junction, forming small clefts, which fill with debris and cells accompanied by the formation of extracellular vacuoles below the moribund basal cell layer. The vacuoles increase in number, fill with fluid and debris, coalesce, and cause widening of the clefts to form subepidermal blisters consisting of necrotic basal cell tissue to cause vesication (Papirmeister et al., 1984a, b, 1985).

4. Protein thiol depletion hypothesis of Orrenius. A variety of cellular defense mechanisms exist for the detoxification of many different toxic metabolites produced intracellularly, once cells are penetrated by toxic molecules (Orrenius, 1985; Orrenius and Nicotera, 1987). One such cellular mechanism is the glutathione and protein thiol system, which plays a critical role in preventing an increase in intracellular oxygen radicals such as hydrogen peroxide and organic hydroperoxides produced by redox active substances such as quinones, paraquat, etc. (Jones et al., 1981; Eklow et al., 1984; Orrenius and Nicotera, 1987). If these systems are overwhelmed and the pools of glutathione and protein thiols exhaused in coping with an influx of toxic molecules, disruption in Ca^{2+} homeostasis occurs, leading to a continual rise in cytosolic Ca^{2+} that results in plasma membrane blebbing in isolated hepatocytes, with subsequent cell killing as a result of the oxidative stress (Schanne et al., 1979; Jewell et al., 1982; Thor et al., 1982; DiMonte et al., 1984; Thor et al., 1985). Surface blebbing of membranes has also been induced by the presence of calcium ionophores such as A23187 and is preceded by changes in intracellular thiol levels (Jewell et al., 1982; DiMonte et al., 1984). Ordinarily, a rise in cytosolic Ca^{2+} is prevented by intracellular compartmentalization of Ca^{2+} by mitochondrial and microsomal Ca^{2+} pumps and binding to proteins such as calmodulin. Active transport of Ca^{2+} through the endoplasmic reticular and plasma membranes is mediated by a Ca^{2+}-stimulated, Mg^{2+}-dependent adenosine triphosphatase, which in turn is dependent on the presence of free thiol groups (Moore et al., 1975; Bellomo et al., 1983; Thor et al., 1985). Thus, an oxidative stress leading to depletion of glutathione and protein thiol levels disrupt those mechanisms regulating Ca^{2+} sequestration and results in blebbing of the plasma membranes of hepatocytes with subsequent cytotoxicity (Orrenius, 1985; Orrenius and Nicotera, 1987). Exposure of isolated hepatocytes to reducing agents such as dithiotreitol or precursors for glutathione synthesis to prevent thiol depletion results in protection against cytotoxicity (Moore et al., 1975). Thus, calcium

homeostasis appears to be involved in the mechanism of cytotoxicity (Orrenius, 1985; Orrenius and Nicotera, 1987).

It has been reported recently that glutathione concentrations in mouse skin are 0.75 and 0.32 μmoles/g in epidermis and dermin, respectively (Wheeler et al., 1986), which were about one-tenth of the levels found in mouse liver, 6.74 μmoles/g (Burton and Aherne, 1986). If it turns out that human skin also has very low glutathione and protein thiol levels, this would explain why skin is so very sensitive to alkylating agents such as sulfur mustard.

The two hypotheses discussed here offer some glimmer of hope for understanding the complicated pathological phonomenon of vesication by putting in some order a multitude of different effects that occur on first contact with a vesicant such as sulfur mustard and at later stages so that the data takes on some coherent perspective for researchers. Neither hypothesis is contradictory nor exclusionary, and each may be operating in some sequence not yet known or, perhaps, simultaneously, that is yet to be discovered; each has been further discussed (Medical Chemical Defense, 1989, 1991).

VI. Eyes

A. Humans

Of the three immediate target tissues of chemical warfare agents, namely, skin, eyes and respiratory system, the one that is immediately susceptible to the toxic action of mustard are the eyes (Mandel and Gibson, 1917; Warthin et al., 1918; Pickard, 1919; McNamara, 1960). Because mustard gas penetrates the cornea more rapidly than it does the skin, the eyes must be cleaned rapidly, within 2 to 3 minutes after contact, to minimize damage (Friedenwald and Woods, 1948).

The acute effects of mustard incurred by the eyes of humans with different exposures were as follows: (*a*) at 12 mg-min/m^3, mild reddening with no effect at lower doses; (*b*) at 50 mg-min/m^3, mild reddening with no injury but some erythema; (*c*) at 100 mg-min/m^3, onset of eye effects such as grittiness, photophobia, lacrimation, discharge, and staining initiated 6 to 24 hours after contact and persisting for 1 to 3 days; and (*d*) at 200 mg-min/m^3, temporary blindness caused by blepharospasm (spasmodic winking) beginning 3 to 12 hours after contact and persisting for 2 to 7 days (Mandel and Gibson, 1917; Warthin et al., 1918; McNamara et al., 1975). The last condition can become severe and lead to conjunctivitis, corneal opacity, and blindness (Morgenstern et al., 1947; Sollmann, 1957; McNamara et al., 1975; Geeraets et al., 1977; Dahl et al., 1985).

When men were subjected to mustard gas in chambers where the concentration of the vapor was 0.0005 mg/L of air for less than 1 hour, 8 of the 13 men displayed definite conjunctivitis, while 3 of 13 men also suffered from erythema, which persisted for several days (Reed, 1920).

Contact with mustard gas vapor caused lacrimation, pain, temporary blindess caused by blepharospasm, photophobia, edema, and a discharge within 3 to 12 hours that persisted for 2 to 7 days. This might be followed later by conjunctivitis, opacity, and ultimately blindness (Morgenstern et al., 1947; McNamara et al., 1975).

Approximately 60 to 90% of the workers in a plant producing mustard gas at the Edgewood Arsenal who had been on the job for 15 months or longer displayed a range of corneal effects such as conjunctival changes, reduced corneal sensitivity, superficial punctate staining of the corneal epithelium, and pigmentation of the corneal epithelium (Laughlin, 1944a). In another study performed at the Huntsville Arsenal in Alabama, men who had been exposed to long-term low level mustard gas vapors displayed acute but not serious eye conditions that abated when they absented themselves from the workplace, even for a short time (Brown, 1949).

In addition to the usual reports of eye lesions and blindness incurred during World War I exposure to mustard gas, Weiss (1958), quoted in *Delayed Toxic Effects of Chemical Warfare Agents* (Lohs, 1975), has reported studies of people employed in plants producing mustard gas during World War II. These people developed delayed effects, such as chronic conjunctivitis and other severe eye diseases, many years later. Although most eye damage incurred by soldiers exposed to mustard gas during World War I were not permanent, keratitis or delayed keratopathy, which is associated with corneal ulceration and gradual erosion of the cornea, eventually led to blindness or vision impairment. This condition can have a latency of 8 to 40 years and has been noted in several cases (Berens and Hartmann, 1943; Mann, 1944; Atkinson, 1948; Geeraets et al., 1977; Dahl et al., 1985; Grant, 1986).

On exposure to fairly high concentrations of mustard gas vapor, the eyes began to water immediately, the conjunctiva became erythematous, and the sensation that a foreign body was present in the eyes produced great discomfort. There were also complaints of pressure exerted on the eyelids so that eyelids felt too heavy to open, and the eyes could not endure bright light for many week thereafter. Inflammation progressed very rapidly and within a few hours, very severe swelling in the lids was present, and the eyes became completely closed. Nevertheless, the prognosis was often good, without much permanent damage, even if severe keratitis with some opacity was initially present; however, severe ulceration of the cornea leads to blindness (Mann, 1944; Atkinson, 1948).

1. Mustard gas dump at Breloh. When empty mustard gas shells were burned at a gas dump in Breloh, Germany after World War I to destroy any residue of the agent so that the casings could be used for scrap metal, a great many workers incurred eye damage from mus-

tard vapor, ranging form slight irritation to very severe injuries to the cornea. Eyes that were severely inflamed and caused severe headaches that were localized in the forehead seemed to indicate that the supraorbital nerves were affected. Such patients groaned and complained of pain; their eyes were swollen shut, their faces swollen beyond recognition, their coughing hoarse, their voice toneless, and the eyes could not be opened for several days. The possibility of secondary infection was always imminent, especially when there was continual itching and scratching. Usually, after a few days, the inflammation of the conjunctiva and swelling of the eyelids receded sufficiently so that the patient could see. Although the corneal injuries might improve with time, scar formation became a complicating factor. The symptoms of pain and pressure in the frontal region occasionally continued for several weeks (Buscher, 1944).

When the inflammation caused by mustard vapor from the shell casings was treated early, corneal changes often disappeared in 3 to 4 weeks, and even coreal opacities cleared up. Although the physicians attending these patients injured at the gas dump at Breloh did not know the concentrations of vapor that the patient had been exposed to, eye damage caused by mustard vapors frequently cleared up, whereas contact with liquid mustard caused destruction of the eye with subsequent blindness (Buscher, 1944).

B. Animals

Exposure to mustard gas vapors causes discomfort, pain, redness, and a mild edema in laboratory animals (Warthin et al., 1918; Anslow et al., 1948), followed by lacrimation, photophobia, conjunctivitis, blepharospasm, and edema with a discharge observed 24 to 48 hours after exposure. Whether or not there was complete destruction of the eye and subsequent blindness depended upon the severity of exposure (Mann and Pullinger, 1942; McNamara et al., 1975).

A single drop of mustard gas, 0.0004 mL, applied with a fine pipet to the center of the cornea of the eye of a rabbit caused the animal to blink and rub its eye with its fore paws. After 1 to 2 minutes exposure, irritation of the conjunctiva with lacrimation was observed, followed within 30 minutes by hyperemia and by edema after 60 minutes. The edema progressed for approximately 12 hours, at which time chemosis was definitely present. These effects are identical to those obtained by a 15-minute exposure to mustard vapor at a concentration of 1:20,000; consequently, the drop method appeared to be much more convenient and safer to handle experimentally. In animals, the edema developed first and most markedly in the palpebral conjunctiva when a drop of liquid mustard was applied, in contrast to the vapor, which produced edema first in the bulbar conjunctiva. Within 15 minutes after eye exposure, a milky white discharge began to flow from the nose, and the eye became swollen and completely shut. The edema became severe and obscured the eye so that when the eyelids were first opened, a profuse exudation was seen with badly swollen tissues around the eye. While the edema persists for several weeks in animals, it is more irregular and less severe in humans, in whom the hyperemia is more marked. Necrosis of the cornea was manifested by a cloudiness that developed 5 to 6 hours later; by the eighth hour, the cornea had acquired a porcelain-like appearance with a bluish-white opalescence. In mild lesions, only a slight cloudiness was observed. Frequently, an opaque band or line was observed running horizontally across the cornea, immediately below its transverse diameter. At this time, the eyelids contained a seropurulent exudate that increased in flow and sealed the eyelids 10 hours later; the eyelids remained closed until the inflammatory process diminished 3 weeks later. By the fifth or sixth day, when a diminution of the edema has occurred, a kinking or ruffling of the upper eyelid appeared at the same time that the lower lid displayed some aversion. Both facial hair near the eye and eyelid hair were lacking at this time. During the second week, changes in corneal curvature were observed, with clouding of the anterior chamber. After the third week, the lesions began to undergo repair very slowly, and progressive vascularization of the cornea was observed, with vessels reaching the center of the vertex by the end of the sixth week. Consequently, corneal cicatrization (that is, fibrous tissue that forms scars as it contracts) was noted at this time, accompanied by marked impairment of vision and thickening of the eyelids and nictitating membrane. Even in mild cases of exposure in humans, the edema and hyperemia of the conjunctiva showed a chronic progression toward visual disturbances and reduced vision. In animals, whose eyes were treated medically, purulent panophthalmitis did not develop; however, when larger doses of mustard were given than could be delivered with the drop method or with vapor concentrations of 1:20,000 and no medical treatment, suppurative panophthalmitis occurred and terminated in the complete destruction of the eyeball (Warthin et al., 1918; Buscher, 1944; McNamara et al., 1975).

When rats, rabbits, guinea pigs, and dogs were subjected to mustard gas in a chamber, either to a concentration of 0.001 mg/m^3 or 0.1 mg/m^3 for 52 weeks, only the dogs receiving the mustard gas vapors at the higher concentration, 0.1 mg/m^3, displayed corneal opacity, keratitis, vascularization, pigmentation, and granulation after 16 weeks of exposure; these manifestations persisted until termination of the project (McNamara et al., 1975).

Laughlin (1944b) has developed a bioassay for mustard in which the eyes of a rabbit are exposed to mustard vapor for 30 to 60 minutes and observations made for 24 hours. He found that the same dose of mustard was less toxic when given over a longer period of time such that a Ct delivered in 2 minutes of exposure produced more

severe eye effects than the same Ct delivered in 30 to 60 minutes.

VII. Gastrointestinal System

A. Humans

Mandel and Gibson (1917) reported that nausea, vomiting, and epigastric distress were frequent symptoms displayed 4 to 16 hours after mustard gassing. Canelli (1918) reported that the stomach was distended with gas, had a cloudy mucosa with many minute ulcerations, and a thickened congested mucosa and a colon that was necrotic and covered with a purulent exudate at autopsy. The duodenum also displayed a cloudy and congested mucosa covered with minute ulcers, while the jejunum and ileum were anemic with a desquamative epithelium. Canelli (1918) (*a*) diagnosed the findings as acute gastroduodenitis with hemorrhagic erosions, acute desquamative enteritis, and severe hemorrhagic necrotic colitis and (*b*) called attention to the selective action of mustard gas on the gastrointestial tract.

There is a report of violent, frequent, and prolonged vomiting in four cases of experimental mustard burns in which the skin had been exposed only to the agent. These men, wearing respirators, were subjected to a Ct of 660 mg/min/m^3 under tropical conditions; no leucopenia was observed (Fairley, 1943).

A young man who was drunk drank five mL of mustard gas in an attempt to commit suicide; he vomited, collapsed, and became unconscious within 8 minutes; this was also associated with urination and defecation. On recovering from unconsciousness, his stomach was lavaged and twice rinsed with permanganate, but he died 5 hours later when his heart ceased beating. Although no mustard was chemically demonstrable in the blood or gastric contents, gauze strips soaked with either his blood or gastric contents when applied to the skin of rabbits caused hyperemia with subsequent development of a crust. Autopsy revealed the presence of hyperemia of the oral, laryngeal, tracheal, and pharyngeal mucosa, but only slight inflammation of the intestial mucosa, swollen kidney tubules, and a fatty liver. The brain, spinal cord, meninges, and sympathetic nervous system showed congestion, degeneration, and loss of cells with pyknotic nuclei. Sympathetic ganglia of the neck and chest, Purkinje cells of the cerebellum and olivary nucleus also showed pathological changes (Jankovich, 1938).

B. Animals

Rabbits and guinea pigs were given capsules containing 0.06 to 0.24 mL of mustard gas in olive oil, butter, and lard; other capsules containing mustard were mixed with meat and given to dogs. Vomiting, irritation of the mucous membranes of the mouth, profuse salivation, foul discharges from nose and mouth, diarrhea, tarry stools, depression, and refusal of food and water occurred within 1 to 12 hours. Anorexia and weakness persisted so that 6 days later, the animals were weak and prostrate. Death followed 12 days later from ingestion of 0.03 to 0.06 mL doses and 3 to 5 days after a 0.24-mL dose for dogs; animals such as guinea pigs and rabbits died sooner (Warthin and Weller, 1919a).

At autopsy, those animals that had died of mustard given orally showed the following symptoms:

- emaciation, congested liver, dilation of the gall bladder, and anemic spleen;
- dilation of the right side of the heart with clots in the right ventricle;
- congestion of the lungs but no pneumonia;
- and congestion of the kidneys.

Very prominent lesions were observed in the gastrointestinal tract, as was distention of the stomach and duodenum, which were filled with a green or black fluid. Necrosis of the mucous membrane of the stomach with occasional congestion and thin necrotic areas were present, but very little hemorrhaging was observed. Peritonitis occasionally was noted, and ulcers were observed later, 6 to 12 days after ingestion of the mustard. However, stomach lesions did not display the characteristic edema that one associates with mustard contamination of the skin and conjunctiva nor hemorrhagic lesions. The intestines showed patches of necroses and congestion, with slight hemorrhages and some inflammation (Warthin and Weller, 1919a; Young, 1947). Air, saliva or secretions from the upper portion of the respiratory system, if contaminated with mustard, might, upon swallowing, cause some irritation and necrosis of the alimentary mucosa, varying from inflammation to eschar formation; these can develop into gastric ulcers and eventually cause perforation (Warthin and Weller, 1919b).

Extensive intestinal injury was incurred by rats and rabbits that had reeived one to two LD_{50} doses of either sulfur mustard or any of the nitrogen mustards. The intestinal lesions involved the entire intestine, starting just below the pylous and being most severe in the regions of the ileum; however, the stomach itself was unaffected when the mustards were given by i.c., i.v., i.m., or i.p. routes. The mucosa showed degeneration and inflammation, with marked erosion and injury to the epithelium with edematous villi within 24 hours of injection. The most severe lesions were found 72 to 96 hours after dosing when the glandular tissue appeared cyst-like with denuded mucosal surfaces; the epithelial tissue displayed hypertrophy and hyperplasia; maximal sloughing, fluid distention, and mucous diarrhea were also observed (Mayer et al., 1920; Smith, 1943; Graef et al., 1948).

Histological examination of the small intestine, liver, kidneys, and spleen of rats 1, 3, and 5 days after i. v. injection of ½ LD_{50} of sulfur mustard revealed that the most severe necrotic damage was observed on day 3.

Tissue renewal was found on day 5 in the glandular epithelium of the small intestine, liver, and kidneys and in the histiocytic reaction of the stroma in the myocardium, but no recovery was seen in the lymphatic follicles of the spleen. Rats that had been injected previously with sulfur mustard were injected with 140 μCi/animal of sulfur mustard labeled with ^{35}S. Historadiographic examination of the small intestine, liver, kidneys, myocardium, and spleen showed that the distribution of the labeled sulfur was concentrated in fragments of intact tissue but was irregular in necrotic tissues (Andrzejewski and Scianowski, 1978).

VIII. Nervous System

A. Humans

In humans, large doses of mustard gas (2 mg/kg) injected i.p. that affect the neuromuscular system provoked convulsions simulating an epileptic crisis. Whereas weak doses of mustard gas caused stupor, large doses, in addition to inciting convulsions, also resulted in a fall in body temperature to 35°C at the time of death (Mayer et al., 1920).

In 1961, Spiegelberg reported on the psychopathological and neurological lesions incurred by workers in German plants producing war gases during World War II; delayed effects were noted following an earlier association with mustard gas. Likewise, U. Hellmann (1970a) and also Lohs (1975) reported on the development of delayed lesions in these workers, previously examined by Spiegelberg (1961) in 1958 to 1960, such as debility, loss of vitality, impaired concentration, sensory hypersensitivity, diminished libido, weakened potency, neuralgic complaints, and disorders in autonomic regulation of the heart. Delayed effects on impaired regulation of the heart by the autonomic system in human beings have been reported by Weiss (1958).

Vomiting follows exposure to even mild doses of mustard and might reflect cholinergic activity or excitation of the vomiting center in the central nervous system (CNS) as a consequence of nasopharyngeal irritation (Buscher, 1944). This is followed by less rapid gastrointestinal disturbances such as anorexia, epigastric pain, constipation, and diarrhea, all of which may persist for some time (Warthin and Weller, 1919b; Velden, 1921; Buscher, 1944). The return of one's appetite was considered as one of the most desirous of diagnostic signs (Velden, 1921; Buscher, 1944).

The literature on the effects of mustard on the nervous and neuromuscular system is sparse. Although some behavioral changes are occasionally mentioned, no concerted effort has been expended on investigating behavioral modifications associated with mustard insults.

B. Animals

Injection of mustard gas into dogs resulted in hyperexcitability followed by unsteadiness of gait, muscular weakness, and defecation (Lynch et al., 1918). Indication of central nervous stimulation was observed in mice 20 minutes after injection of 500 mg/kg i.p., when convulsions, seizures, hind feet and leg flexion, Straub-tail, progressive weakness, respiratory distress, and death ensued (Philips and Thiersch, 1950). Rats given 15 mg/kg of nitrogen mustard displayed weakness, ataxia within 60 minutes, and diminution in righting reflexes. Marked activation of both cholinergic and sympathetic activities were observed in cats and dogs (Philips and Thiersch, 1950).

Smith (1943) found that the cells of the cerebral cortex, basal ganglia, pons, and the medulla had degenerated, and the Purkinje cells of the cerebellum were reduced in number in a cat that had received a total of ten drops of mustard gas over a period of 5 months; it was suggested that the dose had selectively exerted a destructive action on ganglia cells of the CNS and may have been mediated through skin absorption. The salivation, vomiting, defecation, and diarrhea after intoxication with mustard might be a reflection of parasympathetic stimulation (Smith, 1943).

Rabbits, guinea pigs, and dogs display a marked depression and refusal to eat after exposure to mustard gas contained in capsules given orally (Warthin and Weller, 1919a).

1. Cardiovascular system. Although a lethal dose of mustard gas (2 mg/kg) injected i.p. in rabbits caused a significant fall in blood pressure within 5 to 10 minutes (Mayer et al., 1920), mustard gas (1 mg/kg) injected i.p., i.v. or given percutaneously showed no effect on the activity of the heart, blood pressure, or vagal activity in dogs or rabbits (Cordier and Cordier, 1950).

2. Immune system. Because of the special affinity of the sulfur and nitrogen mustards for injuring hematopoietic tissues, it is not surprising to find that mustard gas and bis(2-chloroethyl)methyl amine suppress and retard antibody production (Philips, 1950). This action of the mustards was put to use as an experimental tool in studying tissue hypersensitivity (Berenbaum, 1962).

An attempt was made to sensitize rabbits by applying different concentrations of liquid mustard to the right eye at a rate of 0.1 mL, 3 days/week for 3 weeks. Concentrations of 0.2% caused redness, chemosis, and coreal opacity, 0.02% caused redness and a discharge, whereas 0.002% caused redness in only one rabbit after the first application. A challenging dose of 0.002% was then applied to the other eye (left) 2 weeks after the last sensitizing dose. Because the challenging dose was without effect, another challenging dose at the came concentration was given but without effect (McNamara et al., 1975).

When the eyes of the rabbits were exposed to a mustard Ct of 400 mg/min/m^3 for a second time, 2 weeks later, the eyes displayed a more severe reaction as noted by an increased conjunctival reaction and discharge (Laughlin, 1944b). There have also been a few reports of

sensitization to mustard in people who worked in mustard gas filling plants (Marshall, 1919; Morgenstern et al., 1947).

McMaster and Hogeboom (1945) have reported that human skin contaminated with mustard became very sensitive to repeated treatment with British anti-Lewisite (dimercaprol) (BAL) (63% of volunteers) and that the rate of sensitization was many times greater in mustard contaminated skin than that observed when BAL was repeatedly applied to normal skin or skin contaminated by Lewisite or by arsenical compounds.

IX. Endocrine Gland

A. Adrenals

Male rats injected i.v. with several different agents such as 0.4 mg/kg bis(β-chloroethyl)sulfide, 0.04 mg/kg ethyl bis(β-chloroethyl)amine, 1.2 mg/kg methyl-bis(choroethyl)amine, and 0.6 mg/kg tris(β-chloroethyl)-amine showed marked hypertrophy of the adrenal glands (Chanutin, 1944; Ludewig and Chanutin, 1946; Chanutin and Ludewig, 1947). Total lipid and ester cholesterol were decreased in the adrenal glands and replaced by water. The investigators interpreted these findings as indicating a conversion of adrenal cholesterol into adrenal cortical hormones such as corticosterone and other adrenocorticosteroids and pointed out the parallelism between the alteration of adrenal lipid and cholesterol and the alarm reaction of Selye, which is a response to stress (Ludewig and Chanutin, 1946; Chanutin and Ludewig, 1947). Indeed, Anslow et al. (1948) reported that mice and rabbits given mustard showed some adrenal congestion, which was more marked in mice. Male dogs injected i.v. with several different nitrogen mustards also showed evidence of damage to the nuclei of cells located in the adrenal cortex, whereas the nuclei of cells in the adrenal medulla were not affected. The fasiculata and reticularis zones of the cortex displayed characteristic necroses and damage, implying reduced production of cortical hormones (Kindred, 1949b). Intravenous injection of 100 mg/kg of mustard gas in rats resulted in the death of all animals within 2 to 3 hours, including intact and adrenalectomized rats receiving cortin as replacement therapy. Those adrenalectomized rats unsupplemented with cortin survived for only about half that time (Black and Thomson, 1947). Kindred (1947) likewise reported that adrenalectomized rats that received several different doses of tris(2-chloroethyl)amine i.v. that were not lethal suffered damage to the lymphoid tissues. Consequently, it was suggested that mustards appeared to exert a direct action on lymphoid tissues, although part of this action might be mediated through the adrenal cortex (Kindred, 1947; Karnofsky, 1948b; Philips, 1950).

Although corticosteroids did not influence the healing of lesions provoked by sulfur mustard application of rabbit skin, they did appear to have a general effectiveness in reducing the degree of edema and so may influence some important phase in vesication. Vogt et al. (1984) and Vojvodic et al. (1985) have suggested that corticosteroids used alone or together with other drugs be used in treatment of the local and systemic effects induced by mustards.

B. Gonads

1. Testes. Intravenous injection of ether nitrogen or sulfur mustard in male mice results in damage to the testes, with inhibition of spermatogenesis (Graef et al., 1948). Indeed, it has been reported that the administration of any one of nine different nitrogen mustards injected into young mice caused testicular damage (Landing et al., 1949). A study was performed in which three different nitrogen mustards were injected intraperitoneally: 4.0 mg/kg of bis(2-chloroethyl)methyl amine, 1.5 or 2.8 mg/kg tris(2-chloroethyl)amine, 150 mg/kg 2-chloroethyl morpholine as single injections and 0.6 mg/kg bis(2-chloroethyl)methyl amine for seven injections. Histological examination 24 hours after the last injection revealed that disruption of spermatogenesis had occurred with many mitotic figures, showing abnormal metaphases or anaphase divisions, dark swollen chromosomes and pyknotic nuclei with cell degeneration, and lysis. At 48 and 72 hours, there was a marked disorganization of the layers of spermatogenic cells, damaged tubules filled with abnormal-appearing cells, and fusion of spermatids into multinucleate giant cells; marked acellularity with disorganization and fragmentation of spermatogenic tissue and loss of sloughed cells through the epididymis were also prominent (Landing et al., 1949). Repeated injections of small doses of bis(2-chloroethyl)methyl amine caused more severe damage than did a single injection of the same total dose. Nevertheless, the damage was usually transient, because testicular recovery was observed at 2 weeks, with the formation of mature sperm 4 weeks after exposure (Landing and Eisenberg, 1949; Landing et al., 1949).

Female rats never exposed to mustard gas were mated with male rats that had been exposed to either 0.001 mg/m^3 or 0.1 mg/m^3 mustard gas continuously for 52 weeks. At the end of the gestation period, the pregnant rats were sacrificed, but no evidence of fetal abnormality was found. In addition, pregnant rats exposed to either 0.001 mg/m^3 or 0.2 mg/m^3 mustard gas during the first, second, or third week of gestation (or throughout the gestation period as well) did not display any fetal abnormalities (McNamara et al., 1975).

2. Ovaries. Administration of nitrogen or sulfur mustard did not affect the reproductive potential of female mice, because the fertility of the mice was not altered, and no injurious effects were observed in the ovaries (Graef et al., 1948). Pregnant rats subjected to low levels of exposure to sulfur mustard vapors revealed no increase in fetal mortality or fetal abnormalities (McNamara et al., 1975).

Recently, a two-generation reproductive study was completed by Battelle Pacific Northwest Laboratories. Male and female Sprague-Dawley rats of each generation were distributed into several groups and gavaged with either 0, 0.03, 0.1, or 0.4 mg/kg sulfur mustard for 13 weeks before mating, and continued throughout gestation, parturition, and lactation for 42 weeks. The treatment did not affect the course of reproduction nor that of pregnancy in any of the generations. The highest dose did depress growth of adult male and female rats of the F_1 generation, and growth of the F_1 and F_2 offspring during lactation. The presence of a gavage-related hyperplasia was noted in both sexes in the forestomach and was dose-related (Sasser et al., 1989a, 1996).

C. Other Effects

An interesting but rather peculiar phenomenon of mustard gas is its ability to induce acetylcholinesterase activity and axonal growth in mouse neuroblastoma cells; however, the mechanism involved is still obscure (Turnbull et al., 1973; Lanks et al., 1975). The addition of 0.5 μg/mL of mustard gas to the culture medium provoked a five-fold increase in enzyme activity 4 days after exposure to the agent, with a 25-fold increase in the rate of reappearance of acetylcholinesterase activity after an essentially irreversible inhibition, which implied the induction of new enzyme. The very low concentrations required to induce axonation and acetylcholinesterase activity in neuroblastoma cells suggested to the investigators that the site of action might be DNA (Turnbull et al., 1973). Because a monofunctional mustard such as semimustard required much larger concentrations to produce the same effects as the difunctional mustard gas, it was concluded that cross-links were necessary to produce these two remarkable activities (Turnbull et al., 1973).

Gille (1969) reported that mustard inhibited cholinesterase activity. More recently, it has been reported that cholinesterase activity determined, 4 days after s.c. injection of lethal doses of sulfur and nitrogen mustards (three LD_{50} doses), showed a profound depression in several tissues such as diaphragm, intercostal muscle, liver and serum, but no inhibition was observed in either brain or erythrocyte cholinesterase; the cholinesterase activity was 36%, 43%, 44%, and 52%, respectively, in the depressed tissues and 96% in brain, 100% in erythrocytes and controls (Vojvodic et al., 1985).

After exposure to mustard given intravenously, dogs showed a moderate hyperglycemia for 2 days, succeeded by a reduction to normal or below normal levels by the fifth or sixth day (Dziemian, 1945). Although Chanutin (1944) showed a progressive hyperglycemia after i.v. administration of mustard in dogs and rats, Ball (1944) was not able to find any alteration after i.v. injection of mustard in rabbits. Indeed, Dziemian (1945) was not able to report any significant or consistent alteration in blood glucose levels in dogs after i.v. injection of mustard and concluded that the action of mustard on carbohydrate metabolism, incurred by the two different routes of delivery (i.v. or whole-body gassing) led to different effects; this was noted by the dissimilar influences on certain parameters of carbohydrate metabolism, such as the glucose tolerance test, gastrointestinal motility, and blood glucose concentrations. Black and Thomson (1947) suggested that the alterations in carbohydrate metabolism were a reflection of the anoxic conditions that developed during mustard intoxication.

Sulfur mustard administered i.v. to adult male Wistar rats at ½ LD_{50} resulted in the depression, for several days, of lactate dehydrogenase (LDH) total activity in homogenates of heart and skeletal muscle, liver, spleen, kidney cortex, and ileum. Serum LDH activity rose on day 1, fell on day 3, and returned to normal levels on day 5 after administration of the agent. The investigator suggested that sulfur mustard caused a temporary inhibition of LDH synthesis that was reflected as a fall in LDH activity and that the LDH increase in activity in the serum was a consequence of increased membrane permeability, with leakage of enzymes from the cytosol (Andrzejewski and Scianowski, 1975; Scianowski, 1977). These findings appeared to be contrary to the findings of Needham et al. (1947), who reported that sulfur mustard inhibited only the phosphokinases and several proteinases.

When tissues were exposed to X-ray irradiation, lysosomal damage resulted in the release of hydrolytic enzymes, which play an important role in tissue injury (Watkins, 1975). Although sulfur mustard and 2-chloroethyl ethyl sulfide did result in a slight release of three lysosomal enzymes (aryl sulfates, β-glucuronidase, and acid phosphatase), their release occurred only at very high concentrations of the agents; therefore, lysosomal release of enzymes that could play a role in the cytotoxic activity of sulfur mustards does not appear to be relevant (Gross et al., 1981).

The destruction of cells of bean root plants, *Vicia faba*, occurred after 10 minutes of immersion in liquid mustard gas; however, the destruction of such cells occurred more rapidly and was more severe if the roots were immersed in Lewisite (Milovidov, 1949).

X. Metabolism of Mustard

Intravenous injection of sublethal doses of 5 mg kg ^{35}S-labeled mustard gas into rabbits resulted in a rapid diffusion of most of the radioactivity from the circulation to the urine and bile within 20 minutes after injection, while the remainder of the ^{35}S was distributed to most of the tissues, including the liver, kidneys and lungs, where it became firmly fixed or bound to protein complexes (Boursnell et al., 1946b). Injection of ^{35}S-labeled oxidation products of mustard gas, bis-β-chloroethyl sulfoxide and bis-β-chloroethyl sulfone, i.v. into rabbits resulted in the distribution of the ^{35}S in the urine and bile in the same manner as ^{35}S-labeled mustard (Boursnell et al., 1946a, c).

A major portion of the radioactivity of ^{35}S-labeled mustard gas injected intravenously into mice was excreted within 24 hours, with an additional 10% on the following day, while a considerable amount of radioactivity was once again found in the bile. There was no evidence of any intact mustard being present because of its rapid hydrolysis, and only small amounts had been oxidized completely to sulfate. Although 25% of the urinary substances excreted were the hydrolytic product of mustard, thiodiglycol, the major portion of the remaining radioactivity was associated with an unidentified anionic component (Davison et al., 1957).

In cancer patients, i.v. injection of labeled ^{35}S-mustard gas resulted in the disappearance of 90% of the radioactivity, almost as soon as the injection ceased. The portion that remained in the plasma turned over very slowly and remained constant for the entire period of observation. The radioactivity was excreted to the extent of 25% in 48 hours; however, the greater fraction of that (⅔) was excreted during the first 12 hours (Davison et al., 1957).

In another study of the fate of ^{35}S-labeled mustard gas, 1–5 mg/kg of the agent was injected into mice, rats, and humans. A major fraction of the radioactivity was excreted in the urine within the first 24 hours in rodents, whereas a considerable amount of radioactivity was retained for very long periods of time in humans (Davison et al., 1961). The metabolites excreted in the urine after injection were present mainly as conjugates, expecially conjugates of glutathione. This suggested that these products of metabolism were probably the result of alkylation reactions rather than a consequence of enzymatic alterations (Davison et al., 1961). Among the major products excreted in the urine were thiodiglycol, conjugates of thiodiglycol with glutathione, and other substances; bis-β-chloroethyl sulfone was present also as conjugates with either glutathione or cysteine (Davison et al., 1961). In terminal patients, the labeled urinary metabolites isolated after injection of ^{35}S-labeled mustard gas were the same as those found in the urine of mice and rats (Davison et al., 1961).

A more recent study of the toxicokinetics and disposition of ^{14}C-sulfur mustard in the rat by Maisonneuve et al. (1993) describes similar results, but in more detail than given in the above-mentioned studies by Davison et al. (1957, 1961). A similar biological fate study using ^{35}C-sulfur mustard vapor was reported by Hambrook et al. (1993). The intake, distribution, and retention of ^{35}S in the skin and blood of rats after cutaneous application is described and compared with in vitro studies with both human and rat blood.

The i.p. injection of 1 mg/kg ^{35}S-labeled mustard gas into rats resulted in the urinary excretion of labeled bis-cysteinylethylsulfone and small amounts of thiodiglycol metabolites (Roberts and Warwick, 1963b). Some reactions of bifunctional mustards with cysteine or glutathione have also been reported (Roberts and Warwick, 1963a).

In the mouse and rat, almost all of the ^{35}S of the labeled mustard gas injected was excreted within 3 days: approximately 50% during the first 6 hours and 90% for the first day, whereas only 50% was excreted in humans within 2 days. Chromatographic analysis revealed the presence of six substances in the urine, all of which were either neutral or anionic; 20% of these urinary products was associated with the neutral peak. The same pattern was observed in mouse, rat, and humans. Isotope dilution showed that 10% of the neutral peak was identifiable as thiodiglycol. Traces of two sulfone acids were also found: isethionic and sulfoacetic acids. The suggestion was made that thiodiglycolic acid could be a logical intermediate between thioglycol and sulfoacetic acid and thereby account for about 1.5% of the radioactivity in the urine (Smith et al., 1958). There was no evidence that sulfur mustard was excreted as a conjugate of taurine or taurocholate (Rozman et al., 1957; Smith et al., 1958).

Whole-body autoradiography of mice given ^{35}S-labeled mustard gas by i.v. or percutaneous injection showed that the highest levels of radioactivity were mainly located in the nasal region, kidneys, and liver, with some activity in the CNS and in fetuses of pregnant animals (Clemedson et al., 1963).

Perfusion of isolated lungs of dogs with ^{35}S-labeled mustard revealed that equilibrium between the blood and tissues occurred within 5 minutes. Moreover, 14% of the radioactivity was fixed to the lung tissue, while 74% of the labeled sulfur remained in the perfusate. The remaining radioactivity, which could not be accounted for, was assumed to have been thiodiglycol that had left the lungs when it had been washed free of blood and had been distributed into the intracellular fluid. Other than the radioactivity fixed to lung tissue, negligible amounts of radioactivity were found in lymph, blood, spleen, liver, brain, thoracic duct, and kidney tissue (Pierpont and Davison, 1962).

XI. Decontamination and Antidotes

A. Decontamination of Mustard

1. Skin. The high toxicity of sulfur mustard requires rapid and very effective removal or decontamination to prevent cutaneous absorption with consequent vesication (Jelenko, 1974; Trapp, 1985). Liquid mustard must be removed from the skin in less than 3 to 5 minutes, that is, as rapidly as possible, but the efficacy of this is also affected by weather conditions, because hot, humid weather aggravates the effects of mustard (Stockholm International Peace Research Institute, 1973. An additional consideration is quick and effective removal, without spreading of the vesicant. This may be performed in a number of different ways such as prolonged washing with soap, frequent changes of water, and chemical neutralization to minimize penetration and absorption to preclude local and systemic intoxication (Chiesman, 1944; US Department of the Army, 1974; Lindsten and

Schmitt, 1975; van Hooidonk et al., 1983).

There is no universal skin decontamination procedure that is effective and safe. During World War I, a bleach cream was used that had to be removed by washing because it was a severe skin irritant. This was followed by application of antigas ointments that could be used readily in the field; antigas ointment No. 1 (obsolete, no longer used) was an excellent antidote against mustard but needed to be washed off because of its skin irritancy; this was replaced by antigas ointment No. 2 (composition not given), which was very effective but became an irritant when covered by bandages and clothes. Hair contaminated with mustard was also treated with a bleach cream and washed out or rubbed off with antigas ointment or removed by kerosene (Chiesman, 1944).

An effective decontaminant that neutralized free mustard on skin during the initial critical exposure interval was 7,8-diphenyl-1,3,4,6-tetrachloro-2,5-diaminoglycoluril (S-330 or M-5 Army designation). If used during the critical period, it could reduce the harsh effects of large liquid splashes of mustard to a mild erythema (Rudin, 1953). The US Army also used M-5 as a protective ointment after removal of the mustard or soap and water to wash the area. However, decontamination is of little value against vapor exposure (McNamara, 1960). Washing the area with oil, kerosene, or gasoline followed by copious washing with soap and water has also been recommended (Jelenko, 1974), as well as using neutral hypochlorite (1% chlorine) (Chiesman, 1944).

The system used in the US is the Personal Decontamination Kit, M258A1; this is used against nerve agents and blister agents but consists of chemicals that are both irritating to the skin and eyes, as has been confirmed in rabbit eye tests (Hayes et al., 1984; Harrington, 1987). This system has been recently replaced by resins, such as Ambergard XE-555 and Ambergard XE-556, which readily absorb and detoxify the agent without themselves being toxic or irritating (Harrington, 1987).

Effective methods of decontamination used by the military are not available to the civilian population. A recent report suggests that the use of common household products by ordinary people during a mustard gas attack might be a very effective means of decontamination. Household products such as flour, talcum powder, salad oil, Dutch powder, dry tissue paper, and wet tissue paper removed more than 97% of the sulfur mustard from guinea pig skin contaminated with the agent. Thus, simple household means are available and can be used by civilian populations to protect themselves. Any excess powder may be removed from the skin by copious amounts of water. Indeed, more important than the type of decontaminant used is the time delay between contamination and initiation of the decontamination process (van Hooidonk et al., 1983).

Aspiration of blisters with subsequent cleaning with mild soap and water or a bland antiseptic, such as 8% Dithol, followed by washing with saline, has also been advocated (Chiesman, 1944). Because there is a question as to the toxicity of the fluid within mustard-induced vesicles, it has been suggested that blisters of bulla larger than 2 cm be debrided or opened while being lavaged and treated topically with sulfamylon cream (mafenide acetate) or silvadene cream (silver sulfadiazine) for their antibacterial activity (Pruit, 1987).

Repeated application of ¼ to ½ mL of a 5% BAL and petrolatum ointment on gauge dressings for 48 hours after contact with 2.0 mg liquid mustard gas, or its saturated vapor ½ hour after contamination, or after the appearance of erythema, 2 to 2½ hours later, was very effective in inhibiting vesiculation. Although BAL ointment does not accelerate healing, pain and tenderness are reduced during the first week. Both BAL and chlorinating decontaminants may be used simultaneously without interference of each other action. There are a number of disadvantages that outweigh the efficacy of BAL treatment of mustard gas lesions: BAL ointment tends to trap any residual mustard still on the skin surface and, in so doing, prevents further evaporation, thus causing worse lesions. In addition, skin contaminated with mustard displays a higher degree of sensitization to repeated treatment with BAL than does normal skin or skin contaminated with Lewisite or other arsenical compounds (McMaster and Hogeboom, 1945).

The Federal Republic of Germany and the Union of Soviet Socialist Republics have included sodium thiosulfate and unitiol (2,3-dimercapto-propane-1-sulfonate) for detoxification of mustard in pretreatment and or early treatment of skin and systemic mustard exposures. However, recently, these have been found ineffective in reducing the severity of cutaneous mustard incurred injuries (Papirmeister, 1987).

2. Eyes. Contamination of the eyes by liquid mustard requires immediate decontamination by the individual who has been splashed (Chiesman, 1944; Stockholm International Peace Research Institute, 1973). BAL ointment which is rubbed gently into the eye followed by irrigation with copious volumes of water has been used; this treatment may be effective within seconds of contamination but is of little aid after 2 minutes of contact (McNamara, 1960). Ainsworth (1945) reported that the BAL ointment was very effective in removing surface mustard from the eyes of rabbits, if applied within 1 minute of contact with the agent; however, the application of an irritant ointment, containing carbon disulfide, to the eyes of rabbits contaminated with a drop of mustard was equally effective. Since administration of anesthetic to reduce pain also inhibited the normal blink reflex that would ordinarily retard the rate of absorption of mustard by the eye, the implication was that the effective agent was not the BAL ointment but the involuntary opening and shutting of the eye caused by any substance in contact with the eye. Indeed, BAL ointment will reduce the severity of lesions caused by mustard contamination of the eyes and allow recovery, because

the ointment will remove surface mustard by taking it up into the greasy base of the ointment and thereby stimulate the blink reflex; lacrimation will then expel most of the resulting fluid solution. The use of BAL in such a procedure is problematical and might be eliminated in the future (McNamara, 1960). It has been suggested that, because ointments contain fatty substances, they should be avoided because of mustards' solubility in lipids; hence, the eyes should instead be irrigated immediately with large volumes of water or with 1.5% sodium bicarbonate solution within 15 minutes, before most of the mustard is absorbed (Berens and Hartmann, 1943).

Eyes may also be washed with alkaline solutions such as 2 to 5% $NaHCO_3$ or with neutralizing solutions such as 0.5% dichloramine-T dissolved in chlorinated paraffin or chlorinated diphenylether with previous installation of a local anesthetic (Hughes, 1942). Flushing the eye with dichloramine-T for the first 15 minutes after contact with mustard gas may prevent eye damage (Berens and Hartmann, 1943).

Hypertonic solutions have also been recommended for inducing drainage of eyes that have been contaminated by mustard by using saturated solutions of sodium sulfate or magnesium sulfate in water containing syrup termed Boonefon solution (Bonnefon, 1939). Zinc or boric acid instilled during convalescence may also be useful (Berens and Hartmann, 1943).

To relieve pain or to permit the separation of the eyelids for examination, 0.5% pontocaine may be installed into the eyes one or twice; however, cocaine and atropine are to be avoided (Berens and Hartmann, 1943). Additionally, mercurial salve has been applied to eyelids contaminated with mustard to prevent the formation of small tumors, styes, and multiple small abscesses of the eyelids (Pickard, 1919).

Rabbits were injected i.v. with 500 mg of ascorbic acid every day for 6 days. When the first injection was given 20 minutes before the application of a small drop of liquid mustard from a fine glass pipette into the eyes of rabbits, the spread of keratitis and eyelid inflammation were prevented. When 10% sodium thiosulfate was injected intravenously in 500 mg doses, in the same manner as with ascorbic acid, it was concluded that treatment with the thiosulfate was effective, but the results obtained were not as good as that found with ascorbic acid (Livingston and Walker, 1940). Mann and Pullinger (1940) reported that i.v. injected ascorbic acid had no effect on mustard gas lesions of the eyelids, cornea, and conjunctiva.

There does not appear to be any effective decontamination procedures for eyes contaminated by mustard vapor, because damage is initiated on contact, and it does take several hours for the final outcome to be determined. Treatment requires analgesics to ease pain and irritation, antiseptics to prevent infection, and sterile petrolatum to keep the eyelids patent. Secondary infections can increase scarring, but this can be prevented by the use of solutions of sodium sulamyd every 2 hours or penicillin solutions every 4 hours (McNamara, 1960; Rim and Bahn, 1987).

If sterile solutions or drops for cleansing eyes exposed to mustard gas vapors are not available, an absence of treatment is preferred to avoid incurring devastating secondary infections (Berens and Hartmann, 1943). Although eyes that have been exposed to mustard vapors might appear to be seriously damaged at first, recovery has occurred even without any treatment, if secondary infections can be avoided; thus, no treatment at all is preferred if sterile solutions are not available.

B. Systemic Intoxication: Antidotes

Systemic intoxication by mustard has been treated with (*a*) atropine to reduce gastrointestinal activity, (*b*) morphine or barbiturates to reduce discomfort and restlessness, and (*c*) whole blood, plasma, destrose, and saline to replace lost fluids and electrolytes and to maintain good nutritional status (McNamara, 1960).

1. Sodium thiosulfate. Sodium thiosulfate is relatively nontoxic and can be injected intravenously in large doses without incurring any morphological or chemical changes in the circulation (Schultz et al., 1962; Bonadonna and Karnofsky, 1965). When sodium thiosulfate is introduced into the circulation, it becomes rapidly distributed throughout the extracellular fluid, with little of the thiosulfate entering the cells because of its negative charge and the absence of a specific cell membrane transport system (Cardozo and Edelman, 1952); however, glomerular filtration clears the body of thiosulfate very rapidly (Gilman et al., 1942, 1946), even though a large concentration of thiosulfate is necessary for long periods of time (Gilman and Philips, 1946; Gilman et al., 1946; Cardozo and Edelman, 1952; Fasth and Sorbo, 1973).

Sodium thiosulfate reacts with mustards when these agents are in the cyclized form, but offers no protection against the systemic effects of mustard intoxication if thiosulfate is injected after previous injection of cyclized mustard because of the very rapid reactions of the cyclized species with the body cells (Litwins et al., 1943; Gilman, 1963; Connors et al., 1964; Connors, 1966). Pretreatment with sodium thiosulfate or simultaneous injections with mustard indicates that thiosulfate is a systemic antidote that neutralizes the mustard and so prevents or reduces systemic intoxication (Hatiboglu, 1960; Owens and Hatiboglu, 1961; Foster et al., 1962; Lawrence et al., 1964).

Thus, sodium thiosulfate is an effective antidote to systemic intoxication by mustard, if taken before mustard exposure and so is of practical significance only when there is advanced notice or warning of a pending chemical attack. Additionally, skin lesions produced by mustard cannot be medicated by thiosulfate present in the extracellular fluid (McKinley et al., 1982).

Injections of 300 mg/kg thiosulfate i.p. 10 minutes

before 6.27 mg/kg sulfur mustard offered protection to 80% of the rats treated, but no protection if the thiosulfate was infused s.c. over a 33-minute interval, immediately after injection of the mustard; oral administration of thiosulfate was not very effective (Callaway and Pearce, 1958).

It has been suggested that sodium thiosulfate may act as a "mustard scavenger" in the extracellular spaces to reduce the lethal effects of mustard (Callaway and Pearce, 1958). Apparently, a scavenger can be anything; an enzyme, antibody, or low-weight compound that specifically binds or chemically reacts with an agent to effectively lower the active toxicant levels in the body.

2. Sodium thiosulfate in combination with cysteine. Thiosulfate has been used in combination with several other drugs to increase the effectiveness of the antidote (McKinley et al., 1982). Thiosulfate in combination with cysteine or together with an additional drug such as methenamine (1 g/kg of each) decreased the mortality in mice from 100 to 10% when injected i.p. 30 to 40 minutes before receiving a lethal dose of 10 mg/kg nitrogen mustard (Scarborough and Thomas, 1962). Interperitoneal injection of 2 g/kg thiosulfate and 1 g/kg cysteine, together or separately, before i.p. injection of an LD_{50} dose of HN-2 and merophan (o-di-2-chloroethylamino-DL-phenylalanine) resulted in the following findings:

- cysteine offered protection against both nitrogen mustards;
- thiosulfate was effective only against HN-2, a SN_2 reactor (mustard that cyclize rapidly) but not against meropan, a SN_1 type of mustard (cyclizes slowly);
- in combination, they gave only slight protection against HN-2 and little protection against merophan.

The authors suggested that cysteine efficacy against both mustards was because of its entrance into cells to increase the thiol levels, while the presence of thiosulfate might prevent cysteine from entering into cells (Connors et al., 1964).

3. Sodium thiosulfate in combination with sodium citrate. The injection of 2750 mg/kg thiocit (sodium citrate) i.p. 10 minutes before or after injection of 6.75 mg/kg sulfur mustard provided protection for all rats subject to the agent. Moreover, i.v. injection of thiocit was more protective against sulfur mustard than even i.v. injections of thiosulfate, but oral administration of thiocit was not any more protective than thiosulfate (Callaway and Pearce, 1958).

4. Sodium thiosulfate in combination with other drugs. A comparative study of the effectiveness of several drugs on the degree of protection offered to rats acutely poisoned by either sulfur or nitrogen mustards (s.c. injections of $3LD_{50}$) revealed that, in addition to sodium thiosulfate (i.p.), (*a*) dexamethasone (i.m.), (*b*) promethazine (i.m.), (*c*) heparin (i.m.), (*d*) vitamin E (i.m.), and (*e*) atropine (i.m.), all injected 30 minutes after the mustards, gave good protection. The most effective protection (increased survival time and reduced lethality) against sulfur mustard was achieved by dexamethasone and vitamin E, and against nitrogen mustard by sodium thiosulfate and vitamin E, whereas atropine was the least effective of all the drugs tested. Simultaneous administration of thiosulfate with any of the other drugs further increased the protective activity, particularly in acute poisoning with nitrogen mustard; however, much better protection was offered when three of the drugs, sodium thiosulfate, dexamethasone, and promethazine, were simultaneously injected, protecting 90% of the rats compared with 50% when two drugs were combined (Vojvodic et al., 1985).

A steady loss in body weight (bw) was observed in all rats injected with the mustard, whether or not they had received a protective drug, and this loss became maximal 4 days after injection of the mustard. However, treament caused a steady bw increase from that point to the termination of the experiment 7 days later (Vojvodic et al., 1985).

Inhalation of dexamethasone immediately in large doses has been suggested in order to prevent lung edema after mustard contamination. Inhalation of dexamethasone every 10 minutes by taking five deep breaths of the drug for 1 day is the preferred therapy (Wegner, 1975a, b).

C. Recent Iranian Mustard Exposure Victims

The burn injuries of several Iranians were initially treated in several Vienna hospitals as second degree burns, and as such were covered with antibiotic ointment; the patients also were given calcium and antihistamines to reduce itching. On assuming that these burns had been incurred by mustard gas, further treatment was pursued with dimaval, a BAL derivative (Mandl and Freilinger, 1984). After confirmation (by laboratories both in Vienna and Ghent) that the burns had been induced by mustard gas, a collaborative effort resulted in the following schedule of therapy:

- skin decontamination by washing with 5% chloramine solution;
- 10 to 20 g/day animal charcoal, together with magnesium sulfate as a laxative and to accelerate urinary excretion: patients had to drink 2 to 3 L/day or receive these drugs by infusion;
- four infusions of 10 to 20 mL 0.5% cysteine solution to decontaminate the blood or hemoperfusion over charcoal in difficult cases;
- and vitamin K was given prophylactically for 3 days.

This treatment was successful in six of ten patients, three of whom had been considered terminal cases (Heyndrickx and Heyndrickx, 1984; Mandl and Freilinger, 1984; Pauser et al., 1984).

A pamphlet distributed at the Third International Symposium on Protection Against Chemical Warfare Agents, June 11 to 16, 1989, gave the latest treatment for poisoning with sulfur and nitrogen mustards; it advises immediate i.v. infusion of up to 500 mg sodium thiosulfate/kg bw (Kohler, 1989). Injection of 500 mg sodium thiosulfate/kg bw i.v. within the first 20 minutes after contamination with mustard will avoid systemic intoxication and even lethal effects (Wegner, 1975a, b).

XII. Mutagenicity of Sulfur Mustard

Sulfur mustard is a cell poison that causes disruption and impairment of a variety of cellular activities that are dependent upon a very specific integral relationship. These cytotoxic effects are manifested in widespread metabolic disturbances whose variable characteristics are observed in enzymatic deficiencies (Dixon and Needham, 1946; Cullumbine, 1947, 1954), vesicant action (Lynch et al., 1918), abnormal mitotic activity and cell division (Dustin, 1947; Darlington and Koller, 1947), bone marrow disruption and disturbance in hematopoietic activity (Krumbhaar, 1919; Krumbhaar and Krumbhaar, 1919; Pappenheimer and Vance, 1920), and systemic poisoning (Lynch et al., 1918; Warthin and Weller, 1919a; Anslow et al., 1948; Graef et al., 1948; Walpole, 1958; Hassett, 1963). Indeed, mustard gas readily combines with various components of the cell such as amino acids, amines, and proteins (Price, 1958; Wheeler, 1962; Lawley, 1966). In 1947, Berenblum and Schoental reported that crude extracts of rabbit skin shaken with mustard gas formed precipitates that contained not only sulfur but also phosphorous, and so appeared to indicate that the interacting material was not a protein but a nucleoprotein. This was confirmed when calf thymus nucleoprotein formed similar precipitates with mustard, whereas pure proteins such as serum globulin, albumin, fibrinogen, and gelatin did not. Also, Butler et al. (1950) reported that mustard caused depolarization of nucleoprotein and that the loss in structural viscosity of the nucleoprotein incurred by the action of mustard and X-ray irradiation were quite similar.

In 1946, Auerbach and Robson reported that mustard gas vapor was as effective as X-rays in producing chromosomal breaks and rearrangements. When *Drosophila melanogaster*, male fruit flies, were exposed to mustard gas, 95 sex-linked lethals of 1300 treated sex chromosomes were obtained, which represented a mutation rate of 7.3% compared with three sex-linked lethals observed in a similar number of untreated sex chromosomes, representing a mutation rate of 0.2%. Later tests reported much larger mutation rates of sex-linked lethals induced with mustard, such as 24% (Auerbach, 1947; Auerbach and Robson, 1947a; Auerbach et al., 1947). In addition to the potent tissue penetrability, vesicant action, and cytotoxic activities, mustard also appeared to be a mutagen. Dustin (1947) has alluded to those chemical substances such as mustard that affect chromosomes in similar fashion, as do the ionizing radiation of X-ray, as being radiomimetic.

When pollen grains of *Allium cepa* were exposed to the vapors of sulfur mustard, the mitotic and meiotic activities were impaired, with chromosome breakage observed similar to that after the ionizing radiation of X-rays (Darlington and Koller, 1947). When embryos of *Amblystoma punctatum*, at the tail-bud stage, were exposed to 0.001% bis(2-chloroethyl)methyl amine for 2 days, all mitotic activity was abolished (Bodenstein, 1947). Thus, evidence appeared to indicate that both X-ray irradiation and radiomimetic poisons acted on the level of the nucleus, specifically on the chromosomes (Darlington and Koller, 1947; Loveless and Revell, 1949).

Elmore et al. (1948) suggested that the cytotoxic effects of sulfur mustard might be a reflection of the interaction of mustard with nucleic acids through the cross-linking of mustard with certain groups of the polynucleotide chains. Bodenstein and Kondritzer (1948) reported that developing embryos of *Amblystoma punctatum* showed a steady increase in the nucleic acids, ribonucleic acid (RNA) and DNA, as the embryos passed through the various developmental stages. With increasing age, they continued to show a definite increase in RNA, whereas the DNA did not rise but remained at the same level as that earlier observed, if the embryos had been exposed to 0.001% bis(2-chloroethyl)-methyl amine for 45 minutes.

Male mice of an inbred wild-type strain were injected i.p. with 0.08 mg bis(2-chloroethyl)methyl amine; although most of the treated males died, one did survive and was mated. Of the 24 offspring, 16 were tested for the presence of recessive gene mutations by out-crossing each, and then back-crossing the offspring or mating them among themselves. Only one mutant was obtained, which was due to a single recessive gene giving normal rations with 100% penetrance. Phenotypically, they displayed folding ears, absence of hair on the tail or behind the ears, small kinks at the tip of the tail, and a short thin coat with hairs pointing toward the midline of the back; both sexes were fertile (Auerbach and Falconer, 1949). The investigators contended that spontaneous visible mutations in mice were quite rare, even in inbred strains, and that the mutation observed was induced by the action of the nitrogen mustard.

Asexual spores of wild-type 1A of *Neurospora crassa* (*N. crassa*) were exposed to two drops of mustard gas for 30 minutes and mated sexually with wild-type E5297a; single ascopores were isolated for germination on a complete medium (Horowitz et al., 1946). In the treated series, 760 spores germinated, of which 29 or 3.8% were mutants, compared with the untreated controls in which one doubtful mutant or 0.13% of 769 germinated spores was obtained. The mutants in the mustard-treated group consisted of 17 visible mutants (morphological and pigmentation) and 12 biochemical mutants, one of

which was a new type never encountered previously in that laboratory, termed "albino." Indeed, nutritionally deficient mutant strains of *N. crassa* were induced to revert to the nondeficient state, reverse mutation, by sulfur mustard and monochloromustards (Auerbach and Moser, 1950; Stevens and Mylroie, 1950).

The pioneering work of Avery et al. (1944) demonstrated that the transforming principle that was responsible for the conversion or transformation of a harmless strain of *Pneumococcus* into a virulent one was the nucleic acid, DNA, the genetic material of heredity. Evidence that the cytotoxic activity of mustard was exerted through an action on DNA was shown when the transforming principle from *Pneumococcus* was completely inactivated by a 2-hour exposure to sulfur mustard in concentrations as low as 6×10^{-5} M (Herriott, 1948) and that of *Haemophilus influenza* by 6 hours of exposure in nitrogen mustards such as 10^{-5} M bis(2-chloroethyl)-ethyl amine or 10^{-4} bis(2-chloroethyl)methyl amine (Zamenhof et al., 1956).

Crathorn and Roberts (1966) have shown that doses of mustard gas, at the mean lethal dose, inhibited the incorporation of thymidine into DNA but had no influence on the incorporation of uridine into RNA. Indeed, at a level of 0.5 to 1 mole ^{35}S-labeled mustard gas, it had no effect on the incorporation of amino acids into the polypeptides directed by polyuridylic acid in the Nirenberg-Matthaei cell-free system other than alkylation of the terminal 5′-phosphate group of polyuridylic acid (Abell et al., 1965). Thus, the primary action of a difunctional alkylating agent, such as sulfur mustard, was the inactivation of DNA through the formation of cross-linkages between DNA and mustard; this prevented the separation of the strands of DNA for replication, whereas RNA synthesis did not appear to be disturbed (Price, 1958; Kohn et al., 1965; Lawley and Brookes, 1965; Lawley, 1966; Lawley et al., 1969).

Mustard gas-induced mutations in specific regions of DNA such as those ribosomal RNA regions coding for the bb (bobbed) locus on RNA forming genes in *Drosophila* (Fahmy and Fahmy, 1971, 1972). Indeed, linear relationships were observed between the frequency of X-linked recessive lethals in *Drosophila* spermatozoa and molar doses of several different mustards (Fahmy and Fahmy, 1960), and for mustard gas-induced chromosome breaks and rearrangements in *Drosophila* (Nasrat, 1954; Nasrat et al., 1954).

Using murine leukemia L5178Y/As$^-$ cell suspensions, mustard gas induced chromosome mutations for asparagine dependence and reversed cells to asparagine independence with doses of 100 mg/kg (Capizzi et al.1973, 1974).

Mice bearing implants of 10^7 asparagine requiring L5178Y murine leukemia cells were exposed to 0.1 mg sulfur mustard vapor administered in a closed chamber for 6 hours a day for 5 days a week; however, the frequency of spontaneous reversion to asparagine independence was not statistically significant (Rozmiarek et al., 1973). In another experiment, the dominant lethal mutation procedure, which measures germ-cell mutations, was performed on adult male virgin rats that were exposed to sulfur mustard from 1 to 52 weeks, using two concentrations, 0.1 mg/m^3 and 0.001 mg/m^3. A significant difference in dominant lethality was observed only at the higher concentration, and the dominant lethal mutation rate was cumulative, reaching a maximum at 12 weeks of exposure. It was estimated that a total dose of 0.63 mg sulfur mustard/kg might have entered the lungs after 12 weeks of exposure at 0.1 mg/kg (Rozmiarek et al., 1973).

The mutagenic potential of sulfur mustard was evaluated in the standard plate incorporation version and preincubation modification of the Ames *Salmonella*/microsomal assay with tester strains TA97, TA98, TA100, and TA102, with and without S9 activation, using concentrations of 1, 10, 50, 100, and 500 μg/plate. Sulfur mustard induced point mutations in strain TA102 and frameshift mutations in TA97, but no mutagenicity was observed against strains TA98 and TA100; it was approximately four times more potent for the frameshift mutants (TA97) than for the substitution mutant (TA102). The mutagenic response caused by sulfur mustard was dose-dependent over the range of 1 to 50 μg plate and was independent of metabolic activation by Aroclor-induced rat liver microsomes (S9). Extensive sulfur mustard induced cell killing was seen with the excision repair deficient strains (TA100, TA98, and TA 97) but not with the wild-type for excision repair strain TA102 (Stewart, 1987; Stewart et al., 1989).

Recently, an investigation has been concluded that sought to determine the dominant lethal effect in both male and female rats orally ingesting sulfur mustard. Male and female Sprague-Dawley rats 6 to 7 weeks of age were gavaged with one of several concentrations of sulfur mustard diluted with sesame oil to yield dosages of 0, 0.08, 0.20 or 0.50 mg/kg, for 5 days/week for 10 weeks. The dominant lethal effects were determined at the end of the gavaging period. To evaluate the female dominant lethal effect, females treated with mustard were mated to mustard-treated and nontreated males during a 3-week post-treatment mating period, and the fetuses were examined 14 days after copulation. Male dominant lethal effects were evaluated by mating of treated male rats with nontreated females. No significant female dominant lethality was observed at any of the sulfur mustard doses. However, significant male dominant lethal effects were seen in mustard-treated male rats that had been mated to untreated female rats 2 and 3 weeks after the 10-week exposure interval. Increased incidences of early fetal resorptions, preimplantation losses, and decreases in total live embryo implants were observed at the 0.50 mg/kg dose of mustard and frequently at the lower doses. Although no effects were observed on male reproductive organ weights, or on

sperm mortality, an increase in the percentage of abnormal sperm was found in those male rats treated with the 0.50 mg/kg dose of mustard. The investigators suggested that the dominant lethal effects observed were consistent with a reaction during the postmeiotic stages of spermatogenesis, involving the sensitive spermatids (Sasser et al., 1989b, 1993).

A doctoral dissertation by W. Hellmann (1970b) and a summary by Lohs (1975) citing the former investigator's work reported that dominant, sex-linked, lethal mutations were observed in the offspring of 134 former poison gas factory workers. This reproductive effect was seen as an increase in sex ratio, namely, the presence of more female births with, presumably, a very high mortality of male fetuses in offspring of fathers that had worked in a poison gas factory that produced both sulfur and nitrogen mustards during World War II. Abnormal spermatogenesis and damaged sperm were also found in these former poison gas factory employees. Although nitrogen mustard is a very effective mutagen, it is difficult to find sulfur mustard equally culpable under these conditions. There was a lack of information regarding the types of exposures the men were subjected to while employed in the factory. Epidemiological studies of germ cell mutations in human populations have been carried out on the children of retired Japanese workers who had been employed formerly at the Okuno-jima poison gas factory (Fujita et al., 1983; Neel et al., 1985; Yamakido et al., 1985a; Fujita, 1987). Examination of the blood proteins of these children by electrophoresis, and determination of enzyme activities using variant proteins as indicators of the genetic effects of sulfur mustard on the germ cells of the parents, did not reveal the presence of any variant proteins, which were a consequence of mutations of parental germ cells; also, no statistically significant difference in mutation rate from that of the spontaneous mutation rate was observed.

Fisherman trawling off the Danish island of Bornholm in the Baltic Sea have picked up in their nets containers and shells filled with mustard gas that had been dumped into the sea at the end of World War II. These shells were corroded and broke up easily to contaminate fishermen handling them. So far, 11 cases of acute intoxication have been observed in which the fishermen have displayed inflammation of the skin, axilla, and genitofemoral areas, with blisters on hands and feet, eye irritancy, and temporary blindness. Examination of their lymphocytes have revealed a statistically significant increase in sister-chromatid exchange, indicating mutagenicity (Wulf et al., 1985).

Considering the experimental evidence for the mutagenic action of the mustards in *Drosophila melanogaster* (Auerbach and Robson, 1947a, 1947b), *Aspergillus nidulans* (Hockenhull, 1948), *N. crassa* (Horowitz et al., 1946; Stevens and Mylroie, 1950), dormant barley and wheat seeds (MacKey, 1954), corn, *Zea mays* (Gibson et al., 1950), *Amblystoma* embryos (Bodenstein and Kondritzer, 1948), nucleoproteins (Berenblum and Schoental, 1947; Butler et al., 1950), ribosomal RNA regions in *Drosophila* (Fahmy and Fahmy, 1971), murine leukemia cell suspensions, and dominant lethal mutations in rats (Rozimarek et al., 1973), the inescapable conclusion was that the mutagenic action of mustards was on the hereditary maternal DNA (Papirmeister, 1961; Wheeler, 1962; Lawley, 1966; Hueper, 1971; Fox and Scott, 1980). Consequently, molecules such as the cytotoxic mustards were, essentially, "chemical bullets" of great reactivity that formed addition compounds with DNA to cause chemical alterations, mistakes or mutations (Wheeler, 1962; Hueper, 1971; Haddow, 1973; Fox and Scott 1980).

XIII. Alkylation

The special property that gives sulfur mustard its tremendous chemical reactivity is the presence of two chlorine atoms; hence, it is a strong bifunctional alkylating agent that can react with a wide variety of biological molecules. Under certain conditions, a cyclic ethylene sulfonium ion may form as a consequence of the ionization of one of its chlorine atoms. However, the reaction that is favored is the ionization of the chlorine atom with formation of a carbonium ion which then will react with a variety of nucleophilic centers in the cell, such as the guanine moieties of DNA (Loveless and Revell, 1949; Lawley, 1966; Van Duuren et al., 1974; Fox and Scott, 1980). This activity is enhanced by the presence of the two side chains in sulfur mustard, (C1-CH2-CH2-), so that the molecule acquires the capacity to insert alkyl groups into other molecules or form addition products, that is alkylation capability (Peters, 1947; Loveless, 1951; Auerbach, 1958; Wheeler, 1962; Lawley, 1966; Alexander, 1969; Hueper, 1971). Evidently, sulfur mustard reactions proceed much more rapidly with nucleophiles than do nitrogen mustards (Fox and Scott, 1980). Indeed, nucleic acids, such as DNA, contain nucleophilic sites in both strands of their polynucleotides that readily react with chloroalkyl groups to form ester linkages with their guanine residues (Haddow, 1959; Lawley, 1966; Van Duuren et al., 1974), resulting in cross-linkages within the same strand or between the two complementary strands (Elmore et al., 1948; Goldacre et al., 1949; Lawley, 1966; Fox and Scott, 1980).

Among alkylating agents, difunctional ones such as the mustard, bis(2-chloroethyl)sulfide, which has two chloroethyl groups, appears to exert a more potent cytotoxic activity than do monofunctional ones such as the hemisulfur mustard, 2-chloroethyl-2-hydroxyethylsulfide, which has merely one chloroethyl group (Loveless, 1959; Brookes and Lawley, 1961, 1963; Lawley and Brookes, 1965). Indeed, monofunctional mustards such as the hemisulfur, n-butyl-2-chloroethylsulfide and several monochloronitrogen mustards are effective in producing lethal mutations in *Drosophila melanogaster* (Auerbach and Moser, 1950) and many biochemical mu-

tations and reversions in *N. crassa* (Jensen et al., 1950; Stevens and Mylroie, 1950, 1952, 1953).

In 1959, Loveless reported that inactivation of bacteriophage T2 required the presence of two alkylating groups. Brookes and Lawley (1961) found that alkylation of nucleic acids occurred at the N-7 position of guanine and that hydrolysis of the alkylated products led to the formation of 7-alkylguanines by monofunctional agents and both 7-alkylguanines and diguanin-7-yl derivatives by the action of difunctional agents. These investigations suggested that difunctional alkylating agents such as mustard gas formed cross-linkages betwen the two strands of DNA to prevent strand separation and subsequent replication of DNA without any effect on RNA or protein synthesis or growth (Brookes and Lawley, 1961; Papirmeister, 1961; Brookes and Lawley, 1963; Lawley et al., 1969).

Although both resistant and sensitive strains of *Escherichia coli* do not show any difference in the extent of their initial alkylation by mustard gas, resistant strains continue to grow after alkylation when incubated in growth media, because they are able to excise the cross-linkages from their DNA (Papirmeister and Davison, 1964, 1965; Lawley and Brookes, 1965; Kohn et al., 1965; Venitt, 1968; Papirmeister et al., 1969). The resistant strains showed a preferential excision of the diguaninyl alkylation products from their DNA, but only partial excision of the monofunctionally alkylated guanine derivatives (Lawley and Brookes, 1965; Roberts et al., 1971). This difference between the resistant and sensitive strains in bacteria demonstrated that the significant lesion in DNA that prevents its replication is the difunctional alkylation. Thus, the cytotoxic action of mustard gas was caused by the formation of interstrand cross-linkages in DNA, and the excision of those cross-linked portions of the DNA strands restored the ability of DNA to act as a template for the synthesis of more DNA (Wheeler, 1962; Papirmeister and Davison, 1965; Hueper, 1971; Haddow, 1973; Fox and Scott, 1980). Thus, lesions caused by an alkylating agent such as sulfur mustard could be prevented, mitigated, or reversed by a variety of excision and repair mechanisms; this ability may account for the differential response of certain tissues to carcinogens. Such mechanisms appear to exist in *E. coli* (Kohn et al., 1965; Lawley and Brookes, 1965; Venitt, 1968), yeast cells (Kircher et al., 1979), HeLa cells (Crathorn and Roberts, 1966; Roberts et al., 1968; Reid and Walker, 1969; Roberts et al., 1971), mouse lymphoma cells (Crathorn and Roberts, 1966) and Yoshida lymphosarcoma cells (Scott et al., 1975; Scott, 1977). Moreover, the sensitivity of cells to the action of alkylating agents such as mustard gas varies at different stages of the cell cycle, so that mouse fibroblasts show the greatest resistance to mustard gas in the G_2 stage (Walker and Helleiner, 1963), whereas hamster cells exhibit resistance in the S period (Mauro and Elkind, 1967). Perhaps the sensitization, protection, and repair mechanisms involve enzymatically mediated elimination from the cell of some of the entering mustard (Kohn et al., 1965; Lawley and Brookes, 1965) through reaction with free thiol groups to reduce the extent of alkylation of DNA (Bacq and Alexander, 1964; Connors, 1966; Alexander, 1969).

XIV. Carcinogenicity of Mustard

A. Animals

Many chemical substances are mutagenic but not necessarily carcinogenic (Wheeler, 1962; Haddow, 1973; Fox and Scott, 1980). Now, although it has been demonstrated that the nitrogen mustards were among the most potent mutagens (Auerbach and Robson, 1946; Horowitz et al., 1946; Hockenhull, 1948; Stevens and Mylroie, 1950), the question of whether sulfur mustard is a potential nonhuman carcinogen has not been settled (Walpole, 1958; Haddow, 1959).

Using an inbread stain of mice, strain A, which had a known incidence of spontaneous development of pulmonary tumors, Heston (1950) injected i.v. 0.25 mL of a 1:10 dilution of a saturated solution of mustard gas (0.06 to 0.07%) for a total of four injections at 2-day intervals. Ten months later, 93% of the treated mice had developed pulmonary tumors, averaging 2.6 tumors per mouse, whereas 68% of the untreated mice had developed similar tumors, with an average of 0.93 tumors per mouse (Heston, 1950). In another experiment, strain A mice were injected i.v. with 0.1 mg of bis(2-chloroethyl)methylamine for a total of four injections at 2-day intervals. Indeed, 10 months later, 100% of the treated mice had developed multiple tumors, averaging 9.6 tumors per mouse, whereas 62.5% of the untreated mice had developed tumors with an average of 0.81 tumors per mouse, which was the usual incidence for that strain of mouse (Heston, 1950). This experiment confirmed the findings of an earlier preliminary investigation using bis(2-chloroethyl)methylamine (Heston, 1949) and led him to conclude that both nitrogen and sulfur mustards showed a positive correlation between their mutagenic and carcinogenic activities (Heston, 1950).

In another investigation, strain A mice, housed in cages, were placed in large desiccators that contained a piece of filter paper on which had been placed 0.01 mL of mustard gas. Vaporization occurred with the help of a small electric fan that also circulated the mustard gas vapors so that the mice received a 15-minute exposure to mustard gas daily. Ten months later, 49% of the treated mice had developed pulmonary tumors, averaging 0.66 tumors per mouse, whereas 27% of the untreated mice had developed tumors with an average of 0.31 tumors per mouse (Heston, 1953a). The difference in tumor incidence in treated and nontreated mice as well as the number of tumors per mouse were statistically significant, with no dimorphic differences being observed. Moreover, Heston (1953b) was also able to induce tumor

development in several different strains of mice in addition to strain A, such as C^3H and C^3Hf, using either sulfur or nitrogen mustards that were injected subcutaneously. Tumors developed, especially at the site of the injection, but in other regions that were remote from the injection site and included pulmonary and mammary tumors, hepatomas, and sarcomas 15 months after the last injection (Heston, 1953b).

Two groups of mice, 20 mice per group, received weekly s.c. injections of either 1.0 mg bis(2-chloroethyl)-methylamine or 1.0 mg tris(2-chloroethyl)amine/kg bw for 50 and 10 weeks respectively; 14 mice survived for more than 250 days and, of these, 10 had tumors that included eight lung tumors, two lymphosarcomas, a uterine fibroma, and, at the site of injection, a spindle-cell sarcoma (Boyland and Horning, 1949). Swiss mice and albino rats injected i.v., s.c. or i.p. for 40 weeks with either bis(2-chloroethyl)methylamine or tris(2-chloroethyl)amine and sacrificed 1 year later revealed a high incidence of tumors (Griffin et al., 1951). Tumors were first observed 6 to 7 months after the injections began, and s.c. injections appeared to be the most effective route of administration if one dose of the agent was given; however, all routes were effective if the agents were administered in multiple weekly injections (Griffin et al., 1951). No other details were given in the abstract. Haddow (1959) reported that aromatic nitrogen mustards such as N-phenyl nitrogen mustard, were also carcinogenic in the mouse, rat, and hamster when the agent was administered not only s.c. but also orally. Mustard gas injected s.c. was also carcinogenic in the rat (Haddow, 1959). A variety of alphatic and aromatic derivatives of nitrogen mustards such as uracil mustard, L-phenylamine mustard, and chlorambucil enhanced the development of pulmonary tumors in strain A mice (Shimkin, 1954; Shimkin et al., 1966).

Rats, mice, guinea pigs, rabbits, and dogs were maintained in closed chambers and exposed to mustard gas vapors at a concentration of either 1 or 100 $\mu g/m^3$, for 6 hours/day and 5 days/week, at Edgewood Arsenal. The duration of exposure to the agent varied from 1 to 52 weeks, depending on the different species, but was insufficient to derive any significant data for the guinea pigs, rabbits, and dogs. Chronic exposure to the higher concentrations of mustard gas vapor increased the frequency of skin tumors significantly in rats, but not in mice; the majority of tumors being squamous cell and basal cell carcinomas (McNamara et al., 1975).

In an earlier investigation, Fell and Allsop (1948a) reported that addition of low concentrations of mustard gas, 0.05 mg/mL, to tissue cultures containing small pieces of choroid and sclera obtained from 12-day-old chick embryos resulted in the appearance of multinucleate, hypertrophic, and other abnormal cells, resembling cells usually associated with malignant tumors. Consequently, these investigators applied small doses of mustard gas, 2.5 μg/mL to 12.5 μg/mL, to the skin of mice by means of a pipet, five times per week, for varying intervals, up to 278 days, with no evidence of any tumor formation (Fell and Allsop, 1948b). Although abnormal mitoses, multinucleate cells, and cystic hair follicles were observed in the epidermis, the dermis contained newly formed collagen fibers, and the treated areas of the skin appeared quite normal (Fell and Allsop, 1948b).

Recently, a treatment-related lesion associated with sulfur mustard gavage has been reported (Sasser et al., 1988, 1989c, 1995). Seventy-two Sprague-Dawley rats of each sex, 6 to 7 weeks of age, were distributed into 6 groups of 12 animals/group/sex and were gavaged with either 0, 0.0033, 0.011, 0.033, 0.1, 0.3 mg/kg of sulfur mustard in sesame oil 5 days/week for 13 weeks. A significant reduction in bw in both sexes was observed only in the group receiving the highest dose, and only 2 of 144 animals died during the 90-day study. Hematological and chemical determinations revealed no consistent alterations as a consequence of any of the doses used. The primary toxic effect observed was epithelial hyperplasia of the forestomach in both sexes receiving the 0.3 mg/kg dose and in males receiving the 0.1 mg/kg dose. These lesions were minimal and were characterized by increased mitotic activity of the basilar epithelial cells, with cellular disorganization and thickening of the epithelial layer. There was no evidence that the forestomach lesions observed were precancerous.

B. Humans

A comparative examination of the mortality records of 1267 British war pensioners, gassed with mustard gas in 1917 to 1918 during World War I, was made for the interval, January 1, 1930 to December 1, 1952 (Case and Lea, 1955). The study included two control groups consisting of men who had never been exposed to mustard gas and who comprised a group of 1421 war pensioners afflicted with chronic bronchitis and a group of 1114 war pensioners who were amputees. There were 29 deaths from lung and pleural cancer in the mustard gas exposure group, which was double the death rate expected for a normal population, and the same number of deaths from cancer in the chronic bronchitis group; for both groups, the incidence was 29 observed to 14 expected. However, there were only 13 deaths from lung cancer in the amputee control group; the incidence being 13 observed to 16 expected. It is particularly important to note that almost all of the pensioners in the mustard gas exposed group suffered from chronic bronchitis with a mortality estimate for that disease of 217 observed to 21 expected. The conclusion reached by the investigators was that the mustard gas had not acted as a direct carcinogen, but rather had increased the risk of lung cancer indirectly, by way of a variety of pulmonary disorders: most particularly, chronic bronchitis.

In another study (Beebe, 1960), the mortality records of World War I American veterans were examined who had been hospitalized after mustard gassing in 1918. All

of the veterans had been between 24 to 30 years of age in 1918, and comprised three groups: (*a*) 2718 men hospitalized after mustard gas exposure because of evidence of mustard gas injury to the respiratory tract, eyes, and skin; (*b*) 1855 men hospitalized for pneumonia during the influenza epidemic of 1918 who had no previous evidence of exposure to mustard gas; and (*c*) 2578 men hospitalized because of wounds to the extremities who had no previous contact with mustard gas. The interval between 1919 to 1955 was divided into calendar periods of approximately 10 years and revealed a difference in mortality only during the 1930 to 1939 decade, when the mustard gas-exposed group had the highest mortality, predominantly from pneumonia and tuberculosis. The number of deaths from lung cancer were 36 (1.3%), 14 (0.8%), and 126 (11%) in the mustard gas, pneumonia, and leg-wounded groups, respectively. However, chronic bronchitis was also prevalent: the incidence was 65% in the mustard gas group, 35% in the pneumonia group, and 20% in the leg-wounded group. When the incidence in mortality from lung, trachea, and bronchii were compared with the expected values based on US mortality rates, the following ratios of observed-to-expected were obtained: 39/26.6 (1.47); 15/18 (0.81); and 30/26.2 (1.15) in the mustard gas, pneumonia, and leg-wounded groups, respectively. The mustard gas group appeared to differ, but not always significantly, from the other groups in having higher mortalities from tuberculosis and pneumonia. Beebe's own statistical analyses of his data indicate, he stated, that the causal relation between mustard gas exposure and lung cancer in these circumstances was weak or equivocal. An additional examination of the same records perused by Beebe in 1960, but extended for an additional 10 years (from 1956 to 1966), included 2718 men who had been gassed with mustard gas; however, the original conclusions were not altered (Norman, 1975).

Of 511 people who had worked in a British factory producing mustard gas between 1939 and 1945 (World War II), 84% were traced up to the year 1974, and the mortality rate for cancer was determined for the 29-year interval. Although the number of deaths for all neoplastic diseases amounted to 37 men and eight women for a total of 45, these findings were only slightly higher than the expected mortality from the National Death Rate and were not significant statistically. However, seven deaths from carcinoma of the larynx was much more than the expected 0.75, and so the conclusion was that workers exposed to mustard gas over a long period of time have an increased risk of developing cancer of the larynx (Manning et al., 1981). Although mortalities from lung cancer, pancreatic cancer, pneumonia, and accidents were higher among those who had formerly been exposed to mustard gas, the findings were not statistically significant. Manning et al. (1981) reflected on the small scale of their study, the deficiencies in identifying the data recorded, the incompleteness of the records available, the inability to trace some of the former workers, and the lack of information about the personal habits of these people in regard to use of alcohol and smoking. Nevertheless, they concluded that the findings of their study provided evidence that exposure to mustard gas led to a significant risk of laryngeal cancer (Manning et al., 1981).

The first case of occupationally induced cancer caused by mustard gas that provoked an extensive and still ongoing series of investigations of former workers at a Japanese poison gas factory was reported in 1952, when a 30-year-old man died of bronchial cancer. He had been engaged in the production of mustard gas for 16 months during 1941 (Yamada et al., 1953; Wada and Maranishi, 1954). The poison gas factory was located in Okuno-jima, a small island in the Inland Sea of Japan, and produced several poisonous gases such as mustard gas, Lewisite, diphenylcyanarsine, hydrocyanic acid, chloracetophenone, and phosgene from 1929 to 1945, with intensive production between 1937 to 1944. The workers there were subjected to a working environment in which there were few health or safety precautions (Wada et al. 1963, 1968). The quantity of mustard gas produced on a monthly basis was almost three times the total quantity of all the other gases combined (Inada et al., 1978), resulting in a concentration of the gas in the work environment ranging from 0.05 to 0.07 mg/L (Nakamura, 1956). The protective clothing available to some of the workers did not prevent the gas from penetrating the clothing and caused acute symptoms of mustard gas exposure such as dermatitis, blistering, and skin lesions on the trunk and upper portions of the lower extremities, in addition to conjunctivitis, rhinitis, and bronchitis (Inada et al., 1978). More than half of the former workers previously engaged in the production of poison gas, particularly mustard gas, suffered from chronic bronchitis (Shigenobu, 1980) and also irreversible airway obstruction (Nishimoto et al., 1970).

Additional cases of carcinoma of the larynx and three of bronchial carcinoma were reported in workers 17 to 24 years after occupational exposure to mustard gas; the length of exposure had been from 5 to 13 years. Thus, a total of 32 deaths from cancer and 12 deaths from respiratory cancer during the period from 1946 to 1957 had been recorded (Yamada et al., 1957; Yamada, 1959). Another report found that 27.9% (48) of the deaths of former workers (172) at this poison gas factory were attributable to cancer and that 16.3% (28) had occurred in the respiratory system: particularly in the upper portion of the respiratory tract, where the inhaled mustard gas exposure was intense (Yamada et al., 1961; Yamada, 1963). According to Miyaji (1962), the primary sites of growth of pulmonary neoplasia were found in the hilar region and middle zone of the lung rather than in the peripheral regions, and their growths were predominantly adenocarcinomas. An additional study of deaths that had occurred between 1952 to 1967 in men who had

worked at the poison gas factory during 1929 to 1945 extended the number of deaths from neoplasms of the respiratory tract to 33 (Wada et al., 1968). Thus, there have been 33 deaths of former mustard gas workers since 1952, compared with 0.9 such deaths expected for males from 1952 to 1967, on the basis of the Japanese national mortality rates for males of the same age distribution as the gas workers (Wada et al., 1968). The association between mustard gas workers and the eventual development of respiratory neoplasms was significant.

Of 104 male former poison gas factory workers who had developed malignant tumors of the respiratory system in 1952, 93 had died by 1979 (Shigenobu, 1980). The means, respectively, for age of death, work period, and latent period for development of cancer of the lungs in these workers was 61 years, 5.6 years, and 32 years; for development of cancer of the upper respiratory tract, these means were 57 years, 7.4 years, and 25 years (Shigenobu, 1980). There appeared to be no significant difference in the rate of cancer of the respiratory system between those workers who smoked or did not smoke (Shigenobu, 1980). Observations of respiratory neoplasms in former poison gas workers from 1952 through 1981 revealed an increase in mortality of 102 cases up to that time: 79 cases of cancer of the lungs, 17 cases of cancer of the larynx, and six cases of cancer of the pharynx (Nishimoto et al., 1983; Yamakido et al., 1985b). Tokuoka et al. (1986) performed autopsies on former poison gas factory workers, 52 of whom had died of respiratory tract cancer, including 37 who had died of lung cancer. Histological examination of 19 lung cancer cases of former mustard gas workers confirmed the earlier findings of carcinogenicity (Yamada, 1963; Wada et al., 1968) and indicated an increase in frequency of moderate to severe bronchial lesions in former poison gas workers who had been diagnosed as not having lung cancer (Tokuoka et al., 1986). The majority of these former mustard gas workers were heavy cigarette smokers, and a correlation was found between smoking, mustard gas exposure, and pre- and early-cancerous changes in the lungs (Tokuoka et al., 1986).

Gastric cancer in former workers of the Okuno-jima poison gas factory has also been reported as a consequence of mustard gas involvement (Shimura et al., 1978). Also, one case of early gastric cancer with widespread metastases of the lymph nodes was reported in a man who had chronic bronchitis and had worked with mustard gas for 6 years (Hirono et al., 1984).

Lesions of the respiratory tract and skin were observed in former workers of the Okuno-jima poison gas factory as delayed effects of mustard gas exposure (Yamada, 1959, 1963; Wade et al., 1963; Inada et al., 1977, 1978). Inada and colleagues (1977, 1978) reported the presence of numerous pigmented and depigmented spots on the trunk and upper extremities as well as hyperkeratotic papular eruptions on the skin of five patients. All of these patients were former workers at the poison gas factory on Okuno-jima and had been engaged in the production of mustard gas. Examination of the skin lesions revealed the presence of Bowen's disease or intraepidermal squamous cell carcinoma, which suggested that multiple Bowen's disease had been caused by exposure to mustard gas (Inada et al., 1977, 1978). The average length of employment in the factory on mustard gas production was 9.3 years, and the interval from first contact with mustard gas to diagnosis of Bowen's disease was 39.2 years.

A close association of mortality from cancer and exposure to mustard gas in former German chemical warfare workers has been reported by Weiss (1958) and U. Hellmann (1970a). The average exposure of 4.6 years and an average interval from first contact with the agent and death from cancer was 18.5 years (Hellmann, 1970a).

The production, testing, and destruction of mustard gas and nitrogen mustard were performed from 1935 to 1945 in a German factory employing 878 workers, of whom only approximately half were directly engaged in handling the two chemical agents. During the interval between 1951 to 1972, 85 deaths occurred in the group of workers who had been exposed to nitrogen and sulfur mustard; work records available for these people suggested that 32 of these deaths were caused by cancer. When the incidence on mortality rates were compared with the mortality rates for Lower Saxony, the rates obtained exceeded the expected values; however, only the data on the mortality for bronchial carcinoma were significant: 11 obtained to 5 expected. The findings were confounded by the possibility of exposure to other dangerous chemicals such a phosgene, chloropicrine, bromoacetone, and several organic arsenicals produced at the factory (Weiss and Weiss, 1975).

Between 1945 and 1951, during the disbandment of a poison gas plant, Heerresmunitionsanstalt St. Georgen, in Germany, which had produced both sulfur and nitrogen mustards, approximately 400 people became contaminated through inhalation and handling of these agents because of inadequate precautions. Many of those people are only now, many years later, displaying multiple skin tumors such as basal cell carcinoma, Bowen's disease, Bowen's carcinoma, and skin spinocellulare, even in unexposed portions of the skin (Klehr, 1984).

The disparities in findings between the carcinogenicity of mustard gas in World War I and that observed in former workers in poison gas factories who had been exposed to mustard gas repeatedly over many years in the workplace would most certainly result in different effects from those contaminated by an acute poisoning incident on a battlefield (Norman, 1975; Manning et al., 1981).

A recent report provides strong evidence for a dependent relationship between mustard-induced cancer and former poison gas workers for both men and women who had been engaged in the production of mustard gas in

Cheshire, England during World War II. The work records of these former employees were examined and their mortality records followed up to the end of 1984; these individuals comprised a group of 3354 people. Comparison of National Death Rates from cancer of the upper respiratory tract showed very significant excesses in cancer of the larynx (11 observed vs 4.04 expected), cancer of the pharynx (15 observed vs 2.73 expected), and cancer of additional buccal and upper respiratory sites combined: i.e., lip, tongue, salivary glands, mouth, and nose (12 observed vs 4.29 expected). A more moderate, but still very significant, excess mortality was observed for lung cancer (20 observed vs 138.9 expected). Statistically, significant excesses in mortality from acute nonmalignant respiratory diseases were found: pneumonia and influenza (131 observed vs 9.87 expected) and chronic nonmalignant respiratory disease and chronic bronchitis (185 observed vs 116.31 expected) were also found. Significant excesses in portality for cancer of the esophagus (20 observed vs 10.72 expected) and stomach (70 observed vs 49.57 expected) were also obtained but displayed no consistency with regard to first time of exposure or duration of exposure and may have been confounded by chance, by other factors, or perhaps by a combination of these factors. The results, therefore, give strong evidence that mustard gas exposure causes cancer predominantly of the upper respiratory tract, cancer of the lungs, and nonmalignant respiratory disease; duration of employment (working with mustard gas) is very important (Easton et al., 1988).

Indeed, epidemiology, animal experimentation, and evidence obtained from accidental exposure to mustard gas supports the conclusion that mustard gas is a respiratory tract carcinogen in humans (International Agency for Research on Cancer, 1975, 1982; Cowles, 1983).

C. Therapeutic Uses

In 1929, Berenblum reported that the induction of warts in mice through the repeated application of tar to their skin could be prevented by the addition of 0.1% mustard gas to the tar; mustard gas apparently prevented the skin of the mouse from responding to the carcinogenicity of the tar. This antineoplastic effect observed by Berenblum (1929, 1935) was the rationale for using sulfur mustards and nitrogen mustards in the treatment of a variety of different tumors and neoplastic diseases (Berenblum, 1931; Karnofsky 1948a, 1948b, c; Burchenal and Riley, 1949; Burchenal et al., 1951). Mustard gas has also been used in the treatment of psoriasis in the form of an ointment that was applied one or twice daily from 2 weeks to 7 months (Illig, 1977). Mustard gas, in the treatment of this disease, was used not only as an ointment but also by inhalation, and was considered by the investigator as a comparatively weak carcinogen in humans (Illig, 1977) in contrast to what other investigators have concluded. When the bodies of patients were covered with radioactively labeled ^{35}S-mustard vaseline ointment (50 g) from 1 to 2 days, approximately 1 to 7% of the radioactivity was eliminated within 1 week, 0.5%/20 L of radioactivity was found in the breath, while the epidermis contained very little radioactivity; the ambient air contained 1.5 to 15.7 μCi/20 L of air (Illig, 1977).

In general, lymphomas and neoplasms of the hematopoietic tissues appeared to be the most susceptible to mustard therapy (Karnofsky, 1948a, b, c; Burchenal et al., 1951). Both nitrogen and sulfur mustards were once used extensively as theapeutic agents when radiation was ineffective, particularly in leukemia (Burchenal et al., 1948; Karnofsky, 1948a, b, c; Burchenal and Riley, 1949; Landing and Eisenberg, 1949). Indeed, these activities, particularly the inhibition of tumor growth, all pointed to the possibility that the mustards might be carcinogenic (Goldacre et al., 1949; Walpole, 1958; Haddow, 1973).

XV. Teratogenicity

During organogenesis, a single subcutaneous injection of 0.5 mg/kg or 1.0 mg/kg bis(2-chloroethyl)methylamine in Sprague-Dawley rats, given sometime between 12 and 15 days of gestation, resulted in abnormal development of fetuses. Reduction in fetal size and bw, receding lower jaws, cleft palate, deformed limbs, absence and fusion of digits, cranial defects, and short tails were observed, without any deleterious effect on the mother (Haskin, 1948). In the mouse, a single intra-abdominal injection of 1 μg bis(2-chloroethyl)methylamine/g bw, on any day between day 10 to 12 of gestation, resulted in a wide range of developmental anomalies and many dead embryos (Danforth and Center, 1954). Embryos are relatively resistant to the teratogenic influence of nitrogen mustards up to day 7 or 8 of gestation, because the critical period of gestation during which fetal development may be interrupted appears to be day 10 to 16 of gestation (Murphy and Karnofsky, 1956). Thus, a single dose of 0.3 to 0.5 mg bis(2-chloroethyl)methylamine/kg injected s.c. in pregnant Wistar rats between days 9 and 16 of gestation altered normal fetal development (Murphy and Karnofsky, 1956). Indeed, a single i.p. injection in pregnant Wistar rats on day 12 of gestation of any of several different polyfunctional alkylating agents, such as bis(2-chloroethyl)methylamine, triethylene meramine, and triethylenethiophosphoramide, chlorambucil, and busultan, caused fetal resorption with teratogenic effects in surviving fetuses (Murphy et al., 1958). These same agents injected into the yolk sac of 4-day-old chick embryos caused stunting, reduction in growth, retardation in development of the extremities, missing toes, and shortening of the lower beak (Murphy et al., 1958).

Exposure of pregnant mice to sulfur mustard vapors administered in a closed chamber, for 6 hours a day for 5 days a week, at a concentration of 0.1 mg/m^3, was not effective in inducing fetal toxicity or teratogenicity

(Rozmiarek et al., 1973). Male rats exposed to either 0.001 mg/m^3 or 0.1 mg/m^3 mustard gas vapor in closed chambers for different lengths of time varying from 1 to 52 weeks, when mated to female controls, displayed no reduction in fertility, and there was no evidence of any developmental abnormalities among the fetuses at the end of gestation (McNamara et al., 1975).

Recently completed teratology studies by Battelle Pacific Northwest Laboratories reported that neither rats that had received 0.5 to 2.0 mg/kg sulfur mustard between days 6 and 15 of gestation nor rabbits that had received 0.4 to 0.8 mg/kg mustard gas between days 6 to 19 of gestation, by gastric intubation and sacrificed on days 20 and 30 respectively, displayed any evidence of fetal abnormalities when maternal toxicity was not present. Because fetal defects were observed only at dose levels that caused maternal toxicity, the investigators suggested that mustard gas was not teratogenic in rats or rabbits (Rommereim and Hackett, 1986; Hackett et al., 1987).

Conclusions regarding the teratogenicity of mustard gas needs to be severely reassessed, particularly in light of the many perplexing factors might distort the presence of conclusive evidence: the use of many different routes of administration, the very high concentrations used, and absence of information regarding maternal toxicity (i.e., bw loss) incurred by the suspect teratogen. In a recent publication (Goldman and Dacre, 1989), we have questioned the use of parenteral routes for inducing teratogenicity and the absence of information of maternal toxicity in such studies. Maternal toxicity could be considered as an important diagnostic tool in the assessment of a suspected chemical as a possible teratogen.

XVI. Summary

There have been reports of chemical attacks in which sulfur mustard might have been used (*a*) on Iranian soldiers and civilians during the Gulf War in 1984 and 1985 and (*b*) in an Iraqi chemical attack on the Iranian-occupied village of Halbja in 1988, resulting in many civilian casualties. Heavy use of chemical warfare in Afghanistan by the Soviet military is a recent innovation in military tactics that has been highly successful and may ensure further use of chemical agents in future military conflicts and terrorist attacks as a profitable adjunct to conventional military arms.

Mustard is a poisonous chemical agent that exerts a local action on the eyes, skin, and respiratory tissue, with subsequent systemic action on the nervous, cardiac, and digestive systems in humans and laboratory animals, causing lacrimation, malaise, anorexia, salivation, respiratory distress, vomiting, hyperexcitability, and cardiac distress. Under extreme circumstances, dependent upon the dose and length of exposure to the agent, necrosis of the skin and mucous membranes of the respiratory system, bronchitis, bronchopneumonia, intestinal lesions, hemoconcentration, leucopenia, convulsions with systemic distress, and death occur. Severe mustard poisoning in humans is associated with systemic injury, which is manifested as headache, epigastric distresses, anorexia, diarrhea, and cachexia and is usually observed at mustard doses of 1000 mg/min/m^3 with damage to hematopoietic tissues and progressive leucopenia.

Sulfur mustard is a cell poison that causes disruption and impairment of a variety of cellular activities that are dependent upon a very specific integral relationship. These cytotoxic effects are manifested in widespread metabolic disturbances whose variable characteristics are observed in enzymatic deficiencies, vesicant action, abnormal mitotic activity and cell division, bone marrow disruption, disturbances in hematopoietic activity, and systemic poisoning. Indeed, mustard gas readily combines with various components of the cell such as amino acids, amines, and proteins.

Although evidence of an association between lung cancer and mustard gas encountered on the battlefields of World War I is at best suggestive if not problematical (Case and Lea, 1955; Beebe, 1960; Norman, 1975), the epidemiological data accumulated from the poison gas factories in Japan (Yamada et al., 1953; Wada et al., 1968; Inada et al., 1978; Shigenobu, 1980; Nishimoto et al., 1983; Hirono et al., 1984; Takuoka et al., 1986), in Germany (Weiss, 1958; Hellmann, 1970a; Weiss and Weiss, 1975; Klehr, 1984) and in England (Manning et al., 1981; Easton et al., 1988) are substantial (International Agency for Research on Cancer, 1975). Unfortunately, attempts to seek confirmatory and substantial evidence in laboratory animals such as mice (Boyland and Horning, 1949; Heston, 1950; Heston, 1953a; McNamara et al., 1975) and rats (Griffin et al., 1951; McNamara et al., 1975; Sasser et al., 1996) have not been consistent.

Sulfur mustard has been shown to be mutagenic in a variety of different species using many different laboratory techniques from fruit flies, microorganisms and mammalian cell cultures (Fox and Scott, 1980). Evidence is slowly accumulating from human data (Hellmann, 1970a; Lohs, 1975; Wulf et al., 1985).

Evidence for the teratogenicity of mustard has been negative in assessment of fetotoxicity and adverse effects of mustard on the reproductive potential of both human and animal studies. Indeed, investigations of women adversely affected by mustard are minimal because most of the studies have been performed on former men employees of poison gas factories and have been negative or questionable. We have recently emphasized the need to assess the affect of a suspected teratogen on maternal toxicity in laboratory animals before any conclusions can be made. Indeed, maternal toxicity should be considered as an important diagnostic tool in assessing whether a chemical is teratogenic. The significance

of parenteral routes for inducing teratogenicity is also a problematic one (Goldman and Dacre, 1989).

The special properties that give sulfur mustard its tremendous chemical reactivity are attributable to the presence of a sulfur atom with its unsaturated valence, which results in the formation of a cyclic ethylene sulfonium ion. However, the reaction that is favored is the ionization of the chlorine atom with the formation of a carbonium ion, which then reacts with a nucleophilic site such as the guanine moieties in DNA. This activity is enhanced by the presence of the two-side chains in sulfur mustard (C1-CH2-CH2-), so that the molecules acquire the capacity to insert groups into other molecules or form addition compounds, i.e., sulfur mustard is a strong alkylating agent. Evidently, sulfur mustard reactions proceed much more rapidly with nucleophiles than do reactions with the nitrogen mustards.

Acknowledgements. This work was performed under a US Army Summer Faculty Research and Engineering Program (Dr. Max Goldman) sponsored by the US Army Medical Research and Development Command, Fort Detrick, Frederick, MD, in collaboration with Dr. Jack Dacre, of the US Army Biomedical Research and Development Laboratory, Fort Detrick, MD

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