SKIN SENSITIZATION TO VESICANT AGENTS OF CHEMICAL WARFARE

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2 The statements in this article are the private ones of the authors and are not to be construed as official or reflecting the views of the government agencies which sponsored or assisted in the execution of the work herein reported.

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DEFINITIONS

The following definitions apply to the immunologic terms employed in this report:

Allergic Sensitization: The state of being, or the process of becoming, sensitive or more sensitive than before, as the result of a specifically acquired alteration in the capacity to react.

Desensitization: The process of becoming or the process of making, an individual or tissue insensitive or less sensitive than before.

Drop Test: A test of the skin consisting of the application of a small drop of the proper concentration and form of test material to the surface of a grossly normal skin area. No covering is applied.

Eczematogenic: Having the capacity to produce eczematous reactions.

Flareup: A reaction, i.e. exacerbation or recurrence at the site, either of an active or quiescent clinical lesion or of an active or quiescent site of test or of other application.

Hypersensitive: More sensitive than is normal.

Incubation Period: The period of time elapsing between the exposure of living tissue to an allergenic agent and the acquisition of the degree of sensitivity required for the production of characteristic allergic reaction on exposure to the agent in question.

Normally Sensitive: As sensitive to a particular agent as is common to individuals in the same age group in the particular community at the particular time and under equivalent conditions.

Patch Test: A test of the skin consisting of the application of the proper form and concentration of test material to the surface of a grossly normal skin area. The excitant or a piece of material impregnated with the excitant is placed on the skin, covered with a piece of occlusive material which in turn is covered by a larger patch of adhesive tape or other material. The test keeps the excitant in contact with the skin for a period of from twenty-four to forty-eight hours.

Primary Sensitivity: The capacity to react or respond upon first exposure to an agent, i.e. without preceding specific sensitizing exposure.

Reaction Time: The time elapsing between the moment of exposure of sensitive
tissue to the specific agent and the moment of appearance of the first characteristic gross change.

Sensitive: Capable of reacting or responding to an agent.

Sensitization: The process of becoming, or the process of making, an individual or tissue sensitive or more sensitive than before.

HISTORICAL

The study of skin sensitizations produced by vesicant agents of chemical warfare presents a combination of unusual and interesting circumstances. In contrast to most other substances which have been studied in relation to the production of allergic reactions of the skin, the vesicant agents are intrinsically, i.e. primarily, damaging to all skins and regularly and inevitably produce changes. This “primary” damage may have some features in common with the changes seen in eczematous allergic reactions. Therefore, when cutaneous sensitization to these vesicant agents takes place, the skin reaction which results from subsequent exposure to the agent generally consists of a summation or combination of the primary damage and of the allergic response of hypersensitivity. Moreover, since both the level of susceptibility to primary skin damage and the level of acquired allergic sensitivity vary from individual to individual, the resultant reactions can take the form of a wide variety of combinations of relative degrees of primary damage and of allergic reaction. Only when the exposure is so reduced (by dilution, by short exposure, etc.) as to eliminate all primary damage, can the degree of purely allergic response be accurately assessed. However, both the appearance and course of the allergic reactions are inclined to differ somewhat from those of the primary damage; and the experienced observer can often recognize and distinguish these two components of the skin lesions produced in sensitized individuals.

As early as 1886 Victor Meyer (1) noted that persons differ greatly in their skin sensitivity to the effects of mustard gas. However, not until World War I and the exposure of large numbers of individuals to mustard gas, was it observed that the same individual could at different times show varying degrees of skin response to approximately equal exposures. In general it was found that the skin of many individuals who once had sustained a mustard gas burn reacted much more violently to subsequent exposure to the agent. Moreover in some instances, old sites which had apparently partially or entirely healed became active, i.e. were seen to “flare up” when weeks, months, or years later the same individuals were exposed to mustard gas at other, distant, skin sites (fig. 1).

Subsequent to World War I vesicant agents other than mustard gas, such as lewisite, adamsite, phenyldichlorarsine, were also observed to produce the immunologic phenomena of acquired increased sensitivity and of “flareups.”

These observations all indicate that in cutaneous sensitivity to vesicant gases two main factors may be concerned:

1. differences in primary sensitivity of the skin of different individuals, i.e. differences in the capacity to react to the inherent irritant or damaging action of the vesicants.
b. differences in sensitivity of the skin of different individuals due to sensitization, i.e. due to immunologic changes which were acquired subsequent to—and as the result of—exposure to the vesicant agents.

In addition to the observations on cutaneous sensitization, a number of reports have appeared which deal with non-cutaneous immunologic changes brought on by vesicant agents. Among these are studies on the effect of mustard gas on antibody formation (Hektoen and Corper (2)), and others (3), its effect in active anaphylaxis (Corper, Rensch, Black and Moore (4)), its effects on the immunologic properties of proteins (Berenblum and Wormall (5)), and attempts to produce anaphylaxis with mustard gas protein conjugates (Moore (6)). These non-cutaneous immunologic changes are not the subject of the present report, which is confined to a discussion of the status of investigative work on skin sensitization to vesicant agents. The data presented here are based on both the available literature and on the authors' own studies and observations.
I. Mustard Gas (Dichlorodiethylsulfide)

A. Tests for Primary Sensitivity to Mustard Gas in Human Volunteers. As stated in the historical introduction, the very earliest observations showed that individuals differ markedly in their inherent susceptibility to cutaneous damage from mustard gas. That is, the primary sensitivity, the degree of response to the "obligate", inherent damaging action, varies considerably from person to person. A number of investigators have attempted to establish objectively the normal range of this primary sensitivity. The usual procedure has been to apply standard amounts or standard dilutions of mustard gas to the skin of groups of individuals and to observe the degrees of response elicited. These studies to ascertain the "normal" levels of skin sensitivity in both human beings and in animals are of course a prerequisite in all attempts to demonstrate and investigate acquired increases in sensitivity to mustard gas.

Marshall, Lynch and Smith (7) employed 2 test methods. The first—the saturated vapor method—consisted of exposing skin to mustard gas vapor from a test tube under constant conditions and ascertaining the minimum time of exposure which produced a visible reaction within the next twenty-four hours. The second method—"drop tests"—consisted of dropping onto the skin a standard quantity of solutions containing 1 per cent, 0.1 per cent and 0.01 per cent mustard gas in paraffin oil. Similar standard solutions of mustard gas in linseed oil, cottonseed oil and kerosene were "less reactive", while solutions in alcohol were "more reactive." Among white volunteers, 2—3 per cent reacted to the 0.01 per cent (1:10,000) solution, 10 per cent reacted to the 0.1 per cent (1:1000) solution, and 60—80 per cent reacted to the 1 per cent (1:100) solution.

Kimball, Tarr and Hanzlik (8) stated that the lowest effective vesicant concentration of mustard gas in absolute alcohol is a 0.2 per cent (1:500) solution, and that the skin of 71 per cent to 85 per cent of subjects vesicates after exposure to a 0.3 per cent (1:333) solution.

Vedder (9) applied drop tests using 0.01, 0.1 and 1 per cent solutions of mustard gas in absolute alcohol. He stated that in the normal individual the 0.01 per cent (1:10,000) solution should cause no inflammation and a 0.1 per cent (1:1000) solution should produce a definite erythema. If neither of these solutions caused redness, the subject was tested with a 1 per cent (1:100) solution; if this failed to redder the skin the subject was considered very resistant.

Kellinger and Hanzlik (10), employing drop tests with dilutions of mustard gas for establishing the primary irritant capacities of two different samples of mustard gas, found that benzene dilutions of mustard gas are much less irritating than alcoholic dilutions of corresponding strength.

Ferri (11) carried out drop tests on a group of soldiers with 0.05 per cent (1:2000), 0.1 per cent (1:1000), 0.2 per cent (1:500) and 0.5 per cent (1:200) solutions of mustard gas in chloroform. He considered the 0.5 per cent solution optimal for testing. Of 300 (sic) volunteers tested, 119 gave a slight reaction, 98 an intermediate reaction and 81 a strong reaction to this dilution.

Keller (12) performed drop tests on volunteers with 0.01 per cent (1:10,000), 0.05 per cent (1:2,000) and 0.1 per cent (1:1000) solutions of mustard gas in benzene. 26 of the volunteers showed damage from the 0.01 per cent solution, 84 from the 0.05 per cent solution, and 96 from the 0.1 per cent solution. He classified 2 per cent of his volunteers as hypersensitive, 52 per cent as normally sensitive, 25 per cent as resistant, and 21 per cent as very resistant.

British investigators (13) used the skin reaction to a drop test with a 1:10,000 w/v. dilution of mustard gas in benzene as an arbitrary standard. In a series of 6370 volunteers they found that only 0.5 per cent of not previously exposed individuals reacted to this dilution.
Barre (14) conducted drop tests on 2728 men with alcoholic solutions of mustard gas. He classified 4.9 per cent of his subjects as hypersensitive (reaction to 0.01 per cent (1:10,000) solution); 47 per cent as normally sensitive (reaction to 0.1 per cent (1:1000) solution); 41.9 per cent as resistant (reaction to 1 per cent (1:100), but not to 0.1 per cent solution) and 0.2 per cent as very resistant (no reaction to 1 per cent solution).

Sulzberger and Baer (15) found that among not previously exposed volunteers only 3—4 per cent reacted to drop tests with dilutions of 1:10,000 and 1:100,000 mustard gas in benzene; 61.4 per cent reacted to 1:1000 and 86.7 per cent to the 1:500 dilution.

As compared to the many studies employing dilutions, skin testing with the quantitative application of varying amounts of undiluted mustard gas has been but rarely employed. This is probably due to the great technical difficulties of the latter method. William Bloom and co-workers (16) have apparently overcome the major technical difficulties and have been able to test large series of human subjects with doses of undiluted mustard gas ranging from 2.5 gamma to 70 gamma. The results of 5,828 such measured applications showed that: 2.5 gamma produced erythemas in 37 per cent and vesication in 1 per cent of the subjects; 5 gamma produced erythemas in 70 per cent and vesication in 3 per cent; while doses of from 30 gamma to 70 gamma produced erythemas in 100 per cent of the subjects and vesication in from 52 per cent to 81 per cent.

In the most exact quantitative studies to date, Nagy, Golumbic, Stein, Fruton and Bergmann (17) found that there was only a small difference between the dose of mustard gas necessary to produce erythema and that necessary to produce vesication. With mustard gas vapor at 22°C. no vesicles appeared at twelve sites after 4 gamma had penetrated at each site, while vesication appeared at 7 of 9 sites after 8 gamma had penetrated. Furthermore they were able to demonstrate that at 21–23°C. the mean rate of penetration was about 1.4 gamma per minute on an exposed area of skin cm² while at approximately the same humidity but at a temperature of 30–31°C. the mean rate of penetration was 2.7 gamma per cm² per minute. There was little difference in penetration between symmetrical sites in each individual and between different subjects. Biological variations appeared to play a negligible role in the penetration of vesicant vapors into human skin.

**COMMENT**

There is no absolute agreement among investigators as to the “normal” level of sensitivity to mustard gas. The discrepancies in the findings can be explained by many factors, the most important of which are the differences in the purity of the mustard gas; in the techniques and diluents used; in the temperatures and humidities at the time of the tests; and perhaps also the differences in the groups of test subjects. All investigators agree, however, that different individuals differ greatly in their primary sensitivity, i.e. in their level of response to the obligate damaging effect of mustard gas; and that every large group of human volunteers can be shown to include representative groups of very sensitive, normally sensitive, resistant, and very resistant individuals. From an analysis of the figures given, it can be concluded that many human beings with a “normal” degree of mustard gas sensitivity begin to react to a droplet of the 1:5,000 dilution in benzene and almost all “normals” react to dilutions of 1:500. **Strong reactions to dilutions of greater than 1:5,000 therefore indicate a very sensitive skin; and lack of reaction or weak reactions at 1:500 indicate a very resistant skin.**

**B. Non-Specific Factors in Primary Mustard Gas Sensitivity.** 1. **General tendency to skin damage by vesicants.** Not much attention has been paid to the question of whether some human beings have an abnormally high or low degree
of primary sensitivity to the damaging action of various, not necessarily related, vesicant compounds, i.e. have a high or low general level of susceptibility to vesicant damage. Keller (12) saw in general no parallelism between primary sensitivity to mustard gas and that to lewisite. An analysis of the results of tests of the present authors (18) in which mustard gas and lewisite were applied simultaneously permits the conclusion that there is no regular parallelism between the degrees of primary sensitivity to mustard gas and primary sensitivity to lewisite. However, occasional instances were observed in which the skin of a subject was unusually sensitive or unusually resistant to both mustard gas and lewisite.

2. The effects of temperature, humidity and exercise. It has long been noted that damage tends to be greater in environments with high temperatures and high humidity. Breazeale and Hunt (19) attempted to analyze and separate the effects of temperature from those of relative humidity. They stated that an increase either in temperature or in relative humidity intensified the vesicant action of mustard gas. In Gorrill’s experiments (20) an increase in outside temperature was followed by an increase in damage produced by mustard gas, while the degree of humidity seemed to be unimportant in determining the degree of damage. Exercise, according to Marshall (21) and Gorrill (22), also increased the damage produced by mustard gas. Nagy et al. (17) investigated whether the generally observed greater damaging properties of mustard gas at higher temperatures are due to the increased volatility or to the enhanced sensitivity of the skin at higher temperatures. They showed that the skin of human forearms is not markedly more sensitive to mustard gas at 30°C than it is at 22°C, but that the greater damage is due to the greater volatility of the mustard gas at higher temperatures which leads to availability of a larger quantity of vesicant agent per unit of time and skin surface for penetration into the skin; and consequently to greater skin damage. Renshaw (23) recently showed experimentally that the presence of water on and in the superficial layers of the skin increases the damaging effect of mustard gas, thus supporting the statements of Smith, Clowes and Marshall (24) and of Sollman (25).

3. Racial differences in susceptibility. All investigators agree that the skin of negroes as a group is much less sensitive to mustard gas than the skin of whites. Marshall, Smith and Williams (26) reported that about 78 per cent of negroes are “resistant” as compared with 20–40 per cent among the whites. We also have been impressed with the relatively low primary sensitivity to mustard gas of negro volunteers. Nagy et al. (17) did not observe significant differences in the penetration rate of mustard gas into the skin of a series of white subjects and of 2 negro subjects. There was also no difference in the clinical response of these 2 negroes and the white subjects. They postulate that this lack of difference in clinical response may have been due to the fact that the exposure period used for the negro subjects was more than four times that required to produce 50 per cent vesication in the white subjects, a technic which may have prevented detection of differences which were less than three or fourfold in extent. Ferri (11) found that white soldiers are more sensitive than colored (Ethiopian) soldiers. He also stated that blonde individuals are more sensitive to mustard
Experiments by the present authors did not indicate that there are marked differences between blondes and brunettes, and Ferri's statement in this regard is not supported by evidence in the experimental literature.

On the other hand Carlisle (27), Bloom (28) and Sulzberger, Baer and Kanof (18) could demonstrate no significant difference in the primary mustard gas sensitivity of white and nisei (Japanese-American) soldiers.

4. Influence of thickness of the skin (horny layer?). The “thickness of the skin” (a vague designation which presumably often refers to the thickness of the horny layer of the skin) is said to have a marked influence on primary sensitivity to mustard gas: the thicker the horny layer, the greater the resistance of the particular individual or skin area. The relatively high resistance of the palms and soles against mustard gas has long been observed, and is noted, for example, by Marshall, Lynch and Smith (7) and Cullumbine (29). The relatively thicker horny layer of the negroes' skin may be one of the factors which contributes to their relative resistance.

5. Rate of destruction of mustard gas by the tissues. Ungar (30) on the basis of experiments on men, horses, guinea pigs, and rabbits came to the conclusion that there is a relationship between degree of sensitivity to mustard gas and the rate at which the vesicant is destroyed, i.e. “the more a species is sensitive to mustard gas, the slower is the destruction of this substance in their skin.”

C. Tests for Primary Sensitivity to Mustard Gas in Laboratory Animals. 1. Tests in guinea pigs. A good deal of work has been done on the primary sensitivity of the guinea pig's skin to mustard gas. The results of this work are of particular interest, as the guinea pig has been found to be the most suitable experimental animal for sensitization experiments with mustard gas.

Marshall (21) tested guinea pigs with 0.01 per cent (1:10,000), 0.1 per cent (1:1,000) and 1 per cent (1:100) mustard gas in paraffin oil. 33 per cent reacted to the 1 per cent and none to the 0.1 per cent and 0.01 per cent solutions. Kidd and Landsteiner (31) reported that guinea pigs did not react to drop tests with 1/40 per cent (1:1,000) and 1/2 per cent (1:500) solutions of mustard gas in castor oil. Moore (6) reported that “normal” guinea pigs reacted to an “average maximum dilution” of 1:1,600 in benzene and that no reactions occurred at dilutions of 1:4,000 and higher. Holiday (32) produced skin reactions in normal guinea pigs with a droplet of benzene solution containing 42 gamma mustard gas, while 22 gamma of mustard gas produced no reaction. David P. Barr and Sulzberger (33) found that normal guinea pigs gave skin reactions to a dilution of 1:100 mustard gas in olive oil, but did not react to dilutions of 1:1,000.

2. Tests in rabbits. There is little information on the primary sensitivity of rabbits' skin to mustard gas. Marshall (21) reported that the 2 rabbits which he tested reacted to a 1 per cent (1:100) solution in paraffin oil and did not react

*According to the recent studies by Herrmann and coworkers at the New York Skin and Cancer Unit this resistance of palms and soles may perhaps be attributed in great measure to absence of hair follicles.*
to 0.1 per cent (1:1,000) and 0.01 per cent (1:10,000) dilutions. The present authors also tested 2 rabbits and found that a 1:1,000 dilution in olive oil failed to elicit a skin reaction.

D. Tests for Sensitization to Mustard Gas in Human Volunteers. In September 1918 Boycott (34) stated that "it is well known, or at any rate commonly understood, that those who have been burned with mustard gas or who have come into contact with it in any way are liable to become much more sensitive to its action." This same investigator carried out what is to our knowledge the first series of tests demonstrating a significant difference in the level of mustard gas sensitivty of previously exposed and of not previously exposed individuals. Boycott employed drop tests with 0.01 per cent (1:5000), 0.1 per cent (1:1000) and 0.5 per cent (1:200) solutions of mustard gas in benzene and was able to show:

1. that of 16 not previously exposed control subjects, 14 reacted to the 1:200 solution, while none reacted to the 1:1000 and 1:5000 solutions.
2. of 20 previously exposed subjects all reacted to the 1:200 solution, 13 reacted to the 1:1000 solution and 8 to the 1:5000 solution.

These results indicate a sensitization incidence of 13/20, i.e. approximately 65 per cent among the previously exposed individuals. In general, among subjects who had had severe mustard gas burns there appeared to be a higher incidence of sensitization and a tendency to develop higher degrees of sensitization than among subjects with mild mustard gas exposures.

Gimingham (35) applied drop tests in 6 subjects with a 1:1000 dilution and in 1 subject with a 1:2500 dilution of mustard gas in alcohol and remarked about the "increased sensitiveness to mustard gas which is sometimes seen after a burn by that substance." One of his subjects had been tested one month previously and had not reacted to a 1:1000 solution and only slightly to a 1:500 solution; at that time the subject was then tested with a 1:100 solution which caused a marked burn. One month later tests on this subject caused "marked effects" from the 1:1000 solutions.

Marshall, Lynch and Smith (7) tested a series of workers exposed over a period of four months to mustard gas, using their two previously described testing methods (page 369). In contrast to the other studies cited, these authors could find no experimental evidence to support the "general impression that workers became more susceptible from continued exposure." However, they described the case of a subject who had been exposed to small doses of mustard gas for ten months, after which period a two minute standard vapor exposure was necessary to elicit a skin response. Five months later the subject received a more severe experimental burn with liquid mustard gas. Subsequent to this burn, retesting with the standard vapor exposure showed that the minimum exposure time for eliciting a skin response had been reduced to five seconds.

British investigators (36) reported on drop tests with a 1:10,000 dilution of mustard gas in benzene. Of 53 previously exposed subjects, 24 (i.e. 45 per cent) gave a positive reaction, but only one reaction occurred among 249 not previously
exposed subjects. There was some indication that frequent small burns induce sensitization more readily than does a single large burn. The 4 most sensitive subjects were then tested with higher dilutions of mustard gas in benzene and it could be demonstrated that 3 of these subjects reacted to a 1:500,000 dilution, while the 4th subject reacted to a 1:100,000 dilution. In the group of sensitized individuals, the "most sensitive" subjects were about 1000 times as sensitive as the "normal" subjects and the "least sensitive" were about 20 times as sensitive as the "normal" subjects. This demonstrates that even among sensitized individuals there was a 50-fold difference in the degree of acquired sensitivity to mustard gas.

A subsequent British report (13) presents a series of 6,370 not previously exposed subjects, 0.5 per cent of whom gave a positive and 99.5 per cent of whom gave a negative reaction to drop tests with a 1:10,000 benzene solution of mustard gas. Subsequent to exposure (accidental or experimental) 63 of these subjects were retested with the same solution: 30 per cent then gave a positive reaction and 70 per cent a negative reaction.

Fairley (37) suggested that drop tests be used as confirmatory evidence to help identify workers who had developed such a high degree of mustard gas hypersensitivity that continued employment in the manufacture of the gas became uneconomical or that health was likely to be impaired. He considered a positive reaction to a 1:100,000 dilution of mustard gas in benzene as sufficient evidence to discharge a worker because of extreme hypersensitivity.

Sulzberger and Baer (15) performed drop tests with 1:500, 1:1,000, 1:10,000 and 1:100,000 dilutions of mustard gas in benzene on a series of 27 previously exposed and burned subjects and 68 not previously exposed subjects. A high percentage (about 60 per cent) of the previously burned individuals could be shown to have acquired a skin hypersensitivity to mustard gas. However, in contrast to the findings of British investigators the hypersensitivity among these subjects was apparent not so much in an increased number of positive and/or stronger reactions to the two higher dilutions, but rather in an increased number of stronger reactions to the two lower dilutions (i.e. higher concentrations). In another series of tests (Pillsbury, Talbot, Karnofsky, Sulzberger and Lowenberg (38)) on 135 previously exposed subjects and 48 with little or no previous exposure, more than 25 per cent of the former could be shown to have acquired skin hypersensitivity. Among the exposed group there were 2 subjects who had evidenced great clinical hypersensitivity by reacting violently to very small exposures of mustard gas vapor. These 2 subjects were the only ones who reacted strongly to drop tests with dilutions as high as 1:100,000.

The studies of Nagy et al. (17) tend to show that hypersensitivity to mustard gas is not due to an increased capacity of the skin to permit penetration of the vesicant agent but is due to an increased capacity of the skin to respond to a given (penetrated) quantity of the agent.

E. Clinical Features of Sensitization to Mustard Gas. 1. Incubation period of sensitization to mustard gas. In one of the studies (15) described above we found that a group of subjects tested eight days after first exposure already
showed some increase of sensitivity as compared with a non-exposed control group. But a group of subjects who were tested four weeks or more after first exposure, had become considerably more hypersensitive than the eight day group. This suggests that at eight days after the first “adequate” exposure to mustard gas, hypersensitivity was already present but that it had not yet reached its maximum.

2. Local differences in sensitization to mustard gas. Another series of drop tests (18) were performed by Sulzberger and Baer on the backs and the buttocks of white and nisei volunteers who three weeks previously had been exposed to mustard gas in the vapor chamber and to minute quantities of liquid mustard gas applied as skin tests to the upper back and the forearms. The entire skin, except for the areas protected by the gas mask and the impregnated shorts, had been exposed to the vapor. None of these volunteers could be shown to have become sensitized to mustard gas. In about 25 per cent of the white volunteers and 50 per cent of the nisei volunteers, the not previously exposed buttock area was less sensitive than the previously exposed back area. These results are suggestive of local increased sensitivity following exposure to vapor. However, no definite conclusions can be drawn since no data are available on the relative mustard gas sensitivity of the back and buttocks of subjects who have not been exposed to mustard gas.

3. Eczematous form of sensitization to mustard gas. The skin test reactions produced in the hypersensitive individuals quite regularly presented an eczematous component, i.e. they were erythematous, papular and vesicular and suggested classical sensitization dermatitis. The reaction time was twenty-four to forty-eight hours, as is characteristic of the eczematous form of sensitization. This form of sensitization was regular in subjects exposed by the present authors, but unfortunately other investigators have not stated the exact type of sensitization reaction seen in their large series of volunteers. However, failing information to the contrary, the present authors believe it may be assumed that the *usual reaction of skin sensitization to mustard gas is eczematous* and thus analogous to the usual sensitization by well known eczematogenic allergens, such as poison ivy, dyes, anesthetics, etc.

4. Flare-up phenomenon of eczematous sensitization to mustard gas. The flare-up of old sites, another characteristic phenomenon of eczematous sensitization in human beings, is frequently observed also in mustard gas sensitization. At the time of re-exposure, the flare-up may manifest itself either as itching at old healed sites of mustard gas burns (Gimingham (35)) or as erythema and edema, and in some instances, as a recurrence of full vesication (McDermott and Armstrong (39)) (fig. 1).

5. Urticarial form of sensitization to mustard gas. In addition to these usual eczematous sensitizations, two cases of urticarial sensitization have been reported. Marshall et al. (7) described one of their subjects as having acquired an immediate wheal type of reaction which appeared six minutes after exposure to mustard gas. They wrote “this reaction appears to be of anaphylactic nature, a sensitization to some tissue decomposition product formed by the action of
mustard gas.” Their subject also developed what was presumably an eczematous sensitization to the vesicant agent.

The second case, reported by Talbot (40), was that of a subject who had been repeatedly exposed to mustard gas and had acquired a sensitization to the agent which manifested itself in an urticarial reaction within two hours after exposure followed by an eczematous reaction within twenty-four to forty-eight hours after exposure.

**COMMENT**

With the exception of Marshall et al. all the investigators cited above agree that sensitization of human skin to mustard gas occurs in a considerable percentage of subjects adequately exposed. Thus the objective evidence gained by quantitative skin tests is confirmatory of the common clinical impression that many individuals who have been exposed to mustard gas in the field, in the factory, or in experiments, tend to be much more sensitive to this agent than do not previously exposed individuals.

The incidence of hypersensitivity subsequent to exposure varies from about 30 per cent to about 65 per cent. It appears that the acquired hypersensitivity is demonstrable by skin tests in at least two ways.

a. by skin reactions of sensitized subjects to dilutions of mustard gas to which “normal” subjects do not react.

b. by the “stronger” reactions of sensitized subjects to dilutions to which “normal” subjects also react, but in lesser degree.

The stronger reactions in sensitized subjects are apparently the result of the summation of primary damage and allergic response at the same site (see historical introduction). This combination of reactions at the same site was observed by the present authors in many of their subjects.

**F. Tests for Sensitization to Mustard Gas in Laboratory Animals.** 1. Tests in guinea pigs. Guinea pigs gave promise of being particularly well suited for sensitization experiments with mustard gas since they had proved to be the only laboratory animals suitable for experiments in skin sensitization to other simple chemicals (W. Frei (41), Sulzberger (42), W. Jadassohn (43), and R. L. Mayer (44)).

The first experimental sensitizations of laboratory animals to mustard gas appear to be those performed by Kidd and Landsteiner (31) on guinea pigs. These investigators applied 8 drops of a 1/10 per cent or 1/50 per cent solution of mustard gas in ligroine 10 times over a period of three weeks onto the clipped skin of the back of 70 white albino guinea pigs. When skin was tested two to three weeks after the last “sensitizing” application, almost all the animals had become hypersensitive.

Sensitization was demonstrated by a positive reaction to a drop of 1/1000 per cent solution (1:1000) of mustard gas in castor oil, a solution which did not produce reactions in “normal”, not previously exposed, animals.

A smaller incidence of sensitization could be brought on by similar applica-
tions of a $\frac{1}{10}$ per cent solution, while a $\frac{1}{50}$ per cent solution "elicited practically no hypersensitivity." Guinea pigs burned with a single drop of undiluted mustard gas became hypersensitive, though much less so than animals treated repeatedly with $\frac{1}{10}$ per cent and $\frac{1}{50}$ per cent solutions. Repeated inhalations of mustard gas vapor elicited slight skin hypersensitivity in some animals but not in others.

Guinea pigs which had become hypersensitive to mustard gas appeared to develop burns from somewhat lower concentrations of mustard gas in ligroine than were required to produce similar burns in "normal" animals, but there was little or no difference in the healing of burns of comparable size in hypersensitive and normal animals.

Holiday (32) was able to show that hypersensitivity to mustard gas, demonstrable by drop tests with benzene solutions, could be produced in guinea pigs by the following methods:

1. Intraperitoneal injections of a suspension of formol-killed tubercle bacilli followed twenty-four hours later by an intraperitoneal injection of 0.4 mg. mustard gas in olive oil.

2. Scalding the skin sufficiently to produce edema without ulceration twenty-four hours preceding the intraperitoneal injection of 0.4 mg. mustard gas.

3. Repeated applications, to the same spot on the skin, of small doses of mustard gas in benzene. This last method was shown to produce generalized sensitivity of the skin, but a distinctly greater sensitivity at the site where the sensitizing doses were applied.

Sensitization appeared to be greater in animals which had received 11 applications of 4 gamma of mustard gas than in animals which had received 11 applications of 2 gamma or 5 applications of 8 gamma of mustard gas. Holiday observed that the degree of sensitivity in these sensitized guinea pigs was 4–8 times that of "normal" control animals. However, in a later report (45) Holiday gives figures which suggest that sensitized guinea pigs may be twenty times as sensitive to mustard gas as normal guinea pigs.

Further experiments on sensitization of guinea pigs to mustard gas were reported by David P. Barr and Sulzberger (33). Tests with a 1:1000 dilution of mustard gas in olive oil were carried out on a series of guinea pigs which had been exposed once previously to quantities of from 10 gamma to 7.5 mg. of mustard gas. A number of these animals had become hypersensitive; those which had not, either had had relatively small original exposure (10 gamma) or exposure of only the plantae, which are covered with a thick horny layer and have no follicular openings.

In another study Barr and Sulzberger (46) exposed 10 guinea pigs to 10 drops of 0.15 per cent mustard gas in ligroine, daily for ten days. These applications produced marked irritation consisting of erythema, scaling and crusting. Fifteen days after the last sensitizing application, drop tests with a 1:1000 dilution of mustard gas in olive oil showed evidence of sensitization in 8 animals and questionable reactions in 2.

Moore (6) applied 2 drops of a 1:1000 dilution of mustard gas in benzene
daily for ten days to the clipped skin of 46 guinea pigs. All animals became sensitized—38 reacting to a 1:32,000 dilution and 8 reacting to a 1:16,000 dilution (average dilution 1:29,000). In another series 2 drops of undiluted mustard gas were dropped on the clipped skin of each of 17 guinea pigs. All became sensitized—5 reacting to a 1:4,000 dilution and 12 reacting to a 1:2,000 dilution (average dilution 1:2,600). Injection of protein conjugates of mustard gas with pig, rabbit, guinea pig, or horse serum produced strong tuberculin-type reactions in sensitized animals but did not produce any reaction in "normal" guinea pigs.

2. Tests in rabbits. Pirie and Pullinger (47) found no evidence of skin sensitization in rabbits after subcutaneous or intraperitoneal implantation of "mustard gas collagen." Sulzberger and Baer applied mustard gas 1:1,000 in olive oil to the depilated skin of 16 rabbits which previously had been exposed to several milligrams of mustard gas. The results did not warrant a conclusion that the animals had become sensitized.

3. Test in rats. Maurice Sullivan (48) reported experiments suggesting the occurrence of a flare-up phenomenon in rats. When mustard gas was applied to sites previously injured by mustard gas and now healed, he noticed reactivation in healed scars at other sites. This phenomenon could not be elicited when mustard gas was applied to previously uninjured sites in these rats. The mustard gas dilution technique was not used to ascertain whether the animals presenting the reactivation phenomenon had developed a generalized skin hypersensitivity to mustard gas.

COMMENT

The fact that guinea pigs' skin can be deliberately sensitized to mustard gas is consonant with the observed sensitizations of human skin to this agent and with the successful experimental sensitizations of guinea pigs' skin with other simple chemicals. The experiments of Kidd and Landsteiner, of Holiday, of Moore, and of Barr and Sulzberger point to a definite quantitative factor both in regard to the amount required for the inauguration of hypersensitivity in the guinea pigs and to the degree of hypersensitivity produced in the animals. It appears that repeated small (but not too small) exposures to mustard gas lead to a higher incidence of sensitization than does a single large exposure to the vesicant. The level of hypersensitivity produced by the various methods described ranges from 4 to 20 times the normal degree of primary sensitivity (i.e. much lower than the more than 1000 fold increase of sensitivity which may be acquired by the human skin).

The question as to whether skin sensitization occurs after inhalation of mustard gas vapor needs further study. It is of great interest not only in connection with sensitization to mustard gas, but also in relation to the whole general problem of sensitization of skin after exposure to an agent through inhalation. Kidd and Landsteiner's experiments on skin sensitization after vapor inhalation did not answer this question, since their technique did not rule out the possibility that
the guinea pigs may have received sensitizing skin exposures to mustard gas during the inhalation exposure.

The experiments on guinea pigs suggest that sensitization to mustard gas is enhanced if the exposure takes place on skin which is already the site of an inflammatory process. This corresponds to the reported increase in sensitization produced by irritation and destruction of tissue as for example in butesin picrate sensitization (Sulzberger and Wise (49)), picric acid sensitization (Landsteiner and DiSOMMA (50)), and BAL sensitization (Sulzberger, Baer and Kanof (51)).

More work will be required to decide the question of whether rabbits' skin can be sensitized to mustard gas. However, it is to be noted that up to the present there have been no regularly successful sensitizations of rabbits' skin with any simple chemical compound (W. Frei (41)).

There is as yet no satisfactory evidence to prove a generalized cutaneous sensitization to mustard gas in rats.

G. The Duration of Acquired Skin Hypersensitivity to Mustard Gas. If mustard gas sensitizations are comparable to those produced by other contact-type eczematogenic allergens, it may be assumed that acquired hypersensitivity to mustard gas can persist for many years or for life, and all clinical experience in human beings appears to be in agreement with this assumption. Thus Fairley (37) stated that he had no evidence that the hypersensitivity was ever lost, but that it continued in undiminished degree many years after the last exposure.

A British report (13) presents data on the persistence of hypersensitivity to mustard gas in human beings. In 19 hypersensitive cases the original sensitizing exposure to mustard gas had occurred one to four years earlier.

Additional information was obtained in guinea pigs. Kidd and Landsteiner (31) stated that "the hypersensitivity to mustard gas in guinea pigs proved enduring." Retesting of groups of guinea pigs which had been rendered hypersensitive 22, 19, 14, 12, 10 and 6 months previously, showed that they all had retained the hypersensitive state, and that in most instances the degree of hypersensitivity had not changed during the 6 to 22 month period.

Moore (6) tested sensitized guinea pigs at three and six month intervals and found their state of sensitivity to mustard gas unchanged.

COMMENT

The acquired cutaneous hypersensitivity to mustard gas in human beings and in guinea pigs appears to persist for prolonged periods of time and perhaps for life, even without further exposure to the chemical allergen. This is in accordance with general experience in eczematous sensitizations to other agents.

H. Prevention of Sensitization to Mustard Gas. Although the concept is a logical one and has been envisaged as a possible prophylactic measure, there are no reported attempts at influencing susceptibility to mustard gas by immunologic measures in human beings.
There are, however, several reports on the prevention of sensitization in guinea pigs. Holiday (52) observed that previous immunization with diphtheria toxoid may abolish the capacity of these animals to become sensitized to mustard gas. Kidd and Landsteiner (31) found that when doses of mustard gas, which were usually effective in sensitizing normal guinea pigs' skin, were applied following repeated inhalations of mustard gas or following the cutaneous application of "sub-sensitizing" doses of dilute solutions of mustard gas in ligroine, a certain degree of resistance to sensitization had developed. Moore (6) applied undiluted mustard gas to guinea pigs' skin, producing a slight increase in sensitivity (reaction to an average maximum dilution of 1:2,600). He subsequently subjected these same animals to a series of applications of mustard gas 1:1,000 in benzene. Following this course of applications he found a further increase in sensitivity (reaction to an average maximum dilution of 1:9,250). However, not previously exposed control guinea pigs, which were given a similar series of applications of 1:1000 mustard gas in benzene, showed a much greater increase in sensitivity (reaction to an average maximum dilution of 1:29,000).

Jarman (53) repeatedly injected poison ivy extracts subcutaneously and intramuscularly into rabbits and failed to see any influence from this procedure on the size or course of subsequently produced mustard gas burns.

COMMENT

There is thus some evidence that the development of high degrees of skin sensitivity may be prevented in the guinea pig. This has been accomplished by prior immunization with diphtheria toxoid and by preceding the sensitizing exposure with the administration of mustard gas in such form or quantity as to produce either no sensitization whatsoever or only a slight degree. (In a manner similar to the last mentioned procedure, Sulzberger (54) was able to specifically inhibit skin sensitization of guinea pigs to nearsarsphenamine by the prior systemic administration (intracardial injection) of the drug. Recently Merrill W. Chase (55) has demonstrated that the preceding feeding of dinitrochlorobenzene to guinea pigs has a tendency to specifically reduce the animals' susceptibility to skin sensitization by external application of the compound).

It is conceivable that such an increase in resistance to mustard gas sensitization could also be achieved in human beings. The attempts at utilization of such subsensitizing exposures to inhibit sensitization to a number of eczematogenic (e.g. poison ivy) and other allergens are, of course, well known.

I. Desensitization or Hyposensitization to Mustard Gas. Kidd and Landsteiner (31) tried by various means to desensitize animals which had been previously sensitized to mustard gas. Repeated intraperitoneal injections of mustard gas in olive oil, repeated inhalations of mustard gas, applications of bis (β-chloroethyl) ether and bis (β-hydroxyethyl) sulphide to the skin, and repeated intraperitoneal injections of proteins modified by treatment with mustard gas, all failed to desensitize guinea pigs.

Moore (6) also was unsuccessful in his attempts to desensitize guinea pigs by
the following methods: 1) the production of severe mustard gas burns; 2) intraperitoneal injection of mustard gas in sesame oil; 3) injection of conjugates of mustard gas and guinea pig serum; 4) intraperitoneal injection of rabbit antiserum against a conjugate of mustard gas and pig serum globulin; 5) repeated application to the skin of mustard gas dilutions in benzene.

Holiday's attempts (52) to desensitize small series of guinea pigs by feeding them either the powdered skin of mustard gas-sensitized guinea pigs, or mustard gas-keratin compound were unsuccessful. Oral ingestion of mustard gas in olive oil led to erratic results, one guinea pig becoming less sensitive, two guinea pigs becoming more sensitive, and a fourth guinea pig retaining its previous level of sensitivity.

COMMENT

From the above cited reports it is obvious that all attempts have failed to desensitize or hyposensitize guinea pigs once they have acquired a specific skin hypersensitivity to mustard gas. In human beings no such experimental attempts have been made, but it is quite unlikely that they would be any more successful than in guinea pigs, particularly since there are no accounts of spontaneous or clinical de- or hyposensitization of hypersensitive human subjects.

J. The Specificity of Sensitization to Mustard Gas. Boycott (34) investigated whether the acquired hypersensitivity to mustard gas in human subjects was specific or whether these subjects were also more sensitive to lewisite than "normal" subjects. Employing a quantitative method he concluded from his skin tests that persons who had become particularly susceptible to mustard gas were not abnormally susceptible to lewisite. Fairley and Mumford (56) reached the same conclusions.

Kidd and Landsteiner (31) showed the sharp specificity of the sensitization by demonstrating that several guinea pigs markedly hypersensitive to mustard gas failed to react to bis (β-chlorethyl) ether and to bis (β-hydroxyethyl) sulphide.

Holiday (45) carried out experiments on cross reactions to compounds related to mustard gas, in mustard gas hypersensitive guinea pigs. All the compounds tested had in common the group —S—CH₂—CH₂—Cl. The mustard gas hypersensitivity crossed over to propyl-chlorethyl-sulphide, N-heptyl-chlorethyl-sulphide and β-ethoxy-chlorethyl-sulphide but not to phenyl-chlorethyl-sulphide or mustard gas sulphone. Holiday concluded that cross reactions seem "to depend at most on the presence of the group —C₄H₉—S—C₅H₄Cl. A long chain attached to the S by covalent linkage does not abolish the cross reaction, whereas coordination of the S with oxygen abolishes it, as also a phenyl group attached to S."

In another experiment on guinea pigs hypersensitive to mustard gas, Holiday (45) found that there was no cross reaction with nitrogen mustard.

COMMENT

There is good evidence that sensitization to mustard gas is not associated with increased susceptibility to arsenical vesicants. The studies available on cross
reactions to compounds which are chemically related to mustard gas seem to indicate that cross reactions probably occur to compounds which have, attached to the sulphur, a \( \beta \)-chlorethyl group in which the chlorine possesses a reactivity similar to that of the chlorine groups of mustard gas. Insufficient data are available on possible cross reactions between mustard gas and nitrogen mustard gas compounds; but there are as yet no evidences of such crossing (see below).

II. Nitrogen Mustards (Chlorehylamine Compounds)

There is only one report on specifically acquired cutaneous hypersensitivity to nitrogen mustard:

Goldman and McNary (57) described a case in which they deliberately sensitized a subject to \( \beta,\beta'\beta'' \)-trichloroethylamine by applying \( \beta,\beta',\beta'' \)-trichloroethylamine hydrochloride suspended in 95 per cent alcohol (concentration not specified). The conclusion that the subject had become hypersensitive to the agent was arrived at from the violence and character of the reactions upon exposure to the free amine; no dilution tests were performed. The same subject had previously become hypersensitive to mustard gas but there was obviously no crossing over of the mustard gas sensitivity to the chlorinated ethylamine; the subject apparently gave a normal response to the first exposure to the hydrochloride of the chlorinated ethylamine at a time when he had already become hypersensitive to mustard gas. The fact that the subject became hypersensitive to both mustard gas and the chlorinated ethylamine must thus be attributed to a double sensitization, rather than to cross-sensitization.

Israel (58) reported a case of non-cutaneous allergy to ethylbis (\( \beta \) chloroethyl) amine. The chemical caused allergic conjunctivitis and acute asthmatoiph bronchitis. In this case there was good evidence that there was no cross-sensitization either to mustard gas or to lewisite.

At this time it is not evident whether the scarcity of reports on cutaneous sensitization to nitrogen mustards is due to the low sensitizing index (sensitizing potential) of these vesicants or whether it is due to the relatively small number of subjects who have been exposed, and particularly repeatedly exposed, to nitrogen mustards.

Nagy et al. (17) showed that although the penetration rate of ethyl-bis (\( \beta \) chloroethyl) amine into the skin of negroes was not different from that into the skin of whites, the clinical response of the skin of the negroes tested was strikingly less than that of the whites. The same authors carried out experiments on the influence of temperature on the damaging effects of several nitrogen mustards. They found that, as previously described for mustard gas, the greater volatility of the nitrogen mustards at higher temperatures is responsible for their greater damaging effects at these temperatures.

Renshaw (23) concluded from his experiments that the mere presence of water on and in the more superficial layers of the skin is to a considerable degree responsible for the increased susceptibility of hot sweating skin to the vapors of ethyl-bis (\( \beta \)-chloroethyl) amine.

No reports were found on experimental sensitization of animals with nitrogen mustards.
III. Lewisite (β-chlorovinyldichlorarsine)

A. Tests for Primary Sensitivity to Lewisite in Human Volunteers. Boycott (34) applied drop tests with 0.2 per cent (1:500), 1 per cent (1:100) and 2.5 per cent (1:40) solutions of lewisite in benzene to the skin of 36 not previously exposed volunteers. None of his volunteers reacted significantly to the 1:500 solution, while most of them reacted to the 1:100 and 1:40 dilutions. Boycott's findings of 1918 are fully confirmed by the results of extensive studies performed by the present authors in 1943 and 1944 (see below).

Keller (12) carried out drop tests on volunteers with 1.0 per cent (1:1,000), 0.5 per cent (1:200) and 1 per cent (1:100) dilutions of lewisite in benzene and alcohol. Eleven of the volunteers reacted to the 1:1,000 dilution, 42 to the 1:500 dilution and 61 to the 1:100 dilution. He classified 3 per cent of his volunteers as hypersensitive, 23 per cent as normally sensitive, 18 per cent as resistant and 56 per cent as very resistant (sic).

Sulzberger, Baer and Kanof (18, 59) performed skin tests on not previously exposed volunteers with dilutions of lewisite in mineral oil, in olive oil and in benzene. The mineral oil and olive oil dilutions were applied as patch tests; and the benzene dilutions as drop tests. It was observed that lewisite 1:100 and 1:250 in benzene, 1:500 and 1:1,000 in mineral oil, and 1:500 in olive oil were primary irritants regularly producing reaction in these not previously exposed subjects; while lewisite 1:500 and 1:1,000 in benzene and 1:1,000 in olive oil did not produce reactions in these volunteers.

In another series of skin tests with dilutions of lewisite in benzene (59) it was noted that some normal subjects did not react to lewisite 1:100 in benzene, and almost no normals reacted to the 1:250 dilution; while previously exposed subjects, who had become sensitized to lewisite, evidenced such sensitization in positive reactions to lewisite 1:250 and 1:500 in benzene.

This observation, that the levels at which normally sensitive and hypersensitive subjects react to lewisite dilutions vary from time to time is in agreement with the irregularity of damage which had been observed in the skin response to droplets of undiluted lewisite by Fairley, Hartley and Combe (60) and to dilutions of lewisite in benzene by Mumford (56).

B. Non-Specific Factors in Primary Lewisite Sensitivity. No reports have been found on the various non-specific factors which might influence primary skin sensitivity to lewisite. It appears that studies in this direction have been carried out only with mustard gas and nitrogen mustards. However, the experience of the present authors leads them to believe that, in general temperature, humidity, exercise, the thickness of horny layer and the race of subjects exert essentially analogous influences on the biologic effects of arsenical vesicants and of mustard gas. Thus, negroes were found to be significantly less susceptible to primary skin damage from lewisite than whites, and there was no difference in susceptibility to lewisite between white and nisei soldiers (Sulzberger, Baer and Kanof (18)). Likewise, the skin lesions produced during times of heat and high humidity tended to be greater than those produced by the same amounts
of arsenical vesicants applied during periods of lower temperature and lesser humidity.

COMMENT

There is no sharp line representing the level of skin sensitivity of "normal" subjects who have not previously been exposed to lewisite. The reasons for the variability and fluctuations in the primary irritancy of lewisite are not known. However, the authors of the present report feel that at any given time the level of lewisite sensitivity of a given group of subjects can easily be established by means of correct dilution skin tests (drop tests); and that in general a quite clear-cut differentiation can be made between subjects who are not sensitized and those who have become sensitized by exposures to lewisite.

C. Tests for Primary Sensitivity to Lewisite in Laboratory Animals. Barr and Sulzberger (61) have reported on primary sensitivity of animals to lewisite. These investigators tested guinea pigs and rabbits with dilutions of lewisite in olive oil. Guinea pigs which had not previously been exposed reacted to drop tests with 1:10, 1:100, and 1:1,000 dilutions, but not to dilutions of 1:2,000 and higher. Rabbits which had not previously been exposed reacted to the 1:10, 1:100, and 1:500 dilutions but not to dilutions of 1:1,000 and higher.

D. Tests for Sensitization to Lewisite in Human Volunteers. Sulzberger, Baer and Kanof (59) were apparently the first to describe and to prove by means of quantitative skin tests the occurrence of allergic skin sensitizations to lewisite. However, the literature shows that phenomena which could have been based on skin sensitization to lewisite had been observed many years previously. Thus Young (62) in September 1918 stated that in some cases (6 out of 40 examined) after the lesions produced with arsenical vesicant (related to lewisite) "had practically disappeared, a rash of small red pimples appeared extending round the former area and causing much itching. In two cases this effect has been very marked, the rash still being present after some weeks."

During the present war most experimental and even clinical contaminations with lewisite have occurred in individuals in whom BAL was used to treat all or some of the contaminated sites. Thus Barr and Sulzberger (60) reported a case of flareup dermatitis surrounding lewisite-contaminated areas which had been treated with BAL and considered the possibility that the dermatitis was a result of sensitization to BAL or to a BAL-lewisite complex. Subsequent observations indicated that such flareups, even though due to lewisite alone, can be confined to, or intensified at, BAL treated sites. These findings and their interpretations have been discussed in detail elsewhere (63).

Sparks and Levi (64) observed many cases of sensitization dermatitis among a group of volunteers whose lewisite lesions had been treated with BAL. Davis (65) reported an 18 per cent incidence of dermatitis when BAL was applied to one of several lewisite burns. These three authors apparently considered the observed dermatitis to be due to BAL or to a BAL-lewisite complex. However, it is noteworthy that in 8 of 11 volunteers in Davis' group, the flareup dermatitis
was not confined to the BAL treated sites but was present also at the other sites to which lewisite, but no BAL, had been applied.

Talbot (40) saw 3 cases of dermatitis which he ascribed to lewisite sensitization.

Mumford (56) wrote that the investigators attempted to test for hypersensitivity to lewisite but could not obtain a satisfactory "baseline" result for the reaction of normal persons, i.e. for the skins of not previously exposed subjects.

Sulzberger et al. (59) presented evidence that sensitization to lewisite occurred in 22 (63 per cent) of 35 volunteers previously exposed to 2.8 to 7.0 mg. of liquid lewisite. Hypersensitivity was demonstrated by skin reactions to drop tests.
with lewisite 1:500 and 1:1,000 in benzene, and patch tests with lewisite 1:1,000 in olive oil (fig. 2). Twelve of 13 volunteers who had had a local flareup dermatitis (fig. 3) eleven or more days after they were contaminated with lewisite

![Image of flare-up dermatitis]

**Fig. 3. Flare-up Dermatitis**

Proximal site on left arm was treated at 45 minutes with BAL ointment. Proximal and distal sites on right arm were treated with chlorinating ointments. Left distal site was the untreated control.

Note eczematous reaction of sensitization at and around each site with most intense reaction at the BAL treated site (left proximal). Skin tests showed that subject had become hypersensitive to lewisite while BAL skin tests gave only questionable reactions.

Photo 3 weeks after application of 1.4 mg. lewisite to each of 4 areas.

and treated with BAL, were shown to have an acquired general skin sensitization to lewisite, while only 3 of these 13 volunteers were shown to be hypersensitive to both lewisite and BAL (63).

That there is a definite quantitative relationship between the magnitude of the exposure to lewisite and the incidence of lewisite sensitization is indicated by the fact that in contrast to the 63 per cent sensitization which occurred following exposure to 2.8 to 7.0 mg. of lewisite, only one volunteer out of 79 became
sensitized when the original exposure was to approximately 0.2 mg. of lewisite (18).

**COMMENT**

In general the results of quantitative skin tests with dilutions of lewisite were successful in demonstrating skin hypersensitivity to this agent in those individuals who had evidenced a flareup dermatitis at the sites of previous lewisite exposures. The results therefore confirmed the clinical impression that the flareup dermatitis was in most instances due to sensitization to lewisite.

E. Clinical Features of Sensitization to Lewisite. Studies by the present authors indicated that clinically demonstrable hypersensitivity to lewisite develops rather slowly, increasing steadily between the 11th and 18th day after exposure.

Skin test reactions due to primary sensitivity, i.e. those which resulted from the application of primary irritant concentrations of lewisite, were pustular or superficially necrotic in appearance and could usually be distinguished quite clearly from the reactions produced in hypersensitive subjects by the higher non-primary-irritant dilutions. These “allergic” reactions were superficial, eczematous, papulo-vesicular and edematous and resembled closely the spontaneous flareup dermatitis appearing around areas damaged by lewisite. As previously stated (page 367), skin reactions in sensitized subjects can consist of two components: a primary damage due to the obligate, inherent effect of the vesicant, and a reaction based on acquired hypersensitivity. Thus, “mixed” primary-irritant and eczematous reactions could often be seen in hypersensitive subjects at those sites which had been tested with primary irritant concentrations of lewisite.

**COMMENT**

Although the occurrence of allergic skin sensitization to lewisite was not demonstrated until 1944, it is quite obvious from previous reports that many cases of cutaneous hypersensitivity to lewisite had occurred. These either were not recognized as manifestations of sensitization or were attributed to sensitization to other agents. With a properly adjusted test technique, skin sensitization to lewisite is demonstrable as reactions of the skin to dilutions to which nonsensitized subjects do not react.

The 63% incidence of sensitization to lewisite, demonstrated in our subjects after experimental exposures and burns, corresponds closely to the 60% incidence of skin sensitization after analogous experimental exposures to mustard gas.

Our observations, as well as the descriptions in the literature, indicate that the skin reactions of acquired lewisite hypersensitivity are characteristic for the allergic eczematous contact-type of hypersensitivity. The incubation period appears to be about 5 days to 4 weeks and the reaction time is 24 to 48 hours, both again corresponding to the characteristic periods in eczematous sensitization.
F. Tests for Sensitization to Lewisite in Animals. The only reported tests for skin sensitization in laboratory animals were carried out by Barr and Sulzberger (61) who could find no definite evidence of acquired hypersensitivity to lewisite in small series of previously exposed rabbits and guinea pigs. The solutions employed in these drop tests were lewisite 1:1000 in olive oil for rabbits and lewisite 1:2000 in olive oil for guinea pigs. These series are considered insufficient to permit the conclusion that the skins of laboratory animals cannot be sensitized to lewisite.

G. The Specificity of Skin Sensitization to Lewisite. While there are no data on the persistence of acquired hypersensitivity to lewisite and on possible prevention of sensitization or desensitization to lewisite, the specificity of lewisite sensitization has been discussed by several investigators. Those reports which deal with the negative findings on cross-sensitization between lewisite and mustard gas have been cited in the foregoing discussion on the specificity of sensitization to mustard gas (page 381).

Sulzberger et al. (59) were able to demonstrate that in 3 of 5 subjects who had been burned with lewisite and who had become demonstrably hypersensitive to that vesicant, a cross-sensitization to phenyldichlorarsine was present. No tests were performed for cross-sensitization to other organic or inorganic arsenicals.

IV. Phenyldichlorarsine

A. Tests for Primary Sensitivity and for Sensitization to Phenyldichlorarsine. Phenyldichlorarsine was not used in actual warfare in World War I and nothing was known of the sensitizing capacity of this vesicant agent until 1944 when Sulzberger, Baer and Kanof (59) observed flareup dermatitis in 10 of 11 volunteers whose arms had been contaminated with phenyldichlorarsine. This vesicant in benzene dilutions of 1:250 and higher did not elicit any skin response in normal subjects. In contrast, when drop tests were applied to the 11 subjects who had previously been exposed to phenyldichlorarsine, 9 gave positive responses to a 1:250 dilution, 7 reacted to a 1:500 dilution, and 5 to a 1:1,000 dilution. All the reactions to phenyldichlorarsine in the 9 sensitized subjects were eczematous in appearance and resembled the morphe of the flareup dermatitis previously seen.

The incidence of 9 sensitizations among 11 exposed subjects suggests that the capacity of phenyldichlorarsine as an eczematogenic sensitizing agent may approach that of the strongest previously known eczematogenic agents, e.g., the allergenic principle derived from primrose plants by Bloch and Karrer (66).

No experiments have been reported on skin sensitization of laboratory animals to phenyldichlorarsine.

B. The Specificity of Skin Sensitization to Phenyldichlorarsine. Sulzberger, Baer and Kanof (59) investigated the question whether sensitization to phenyldichlorarsine crosses over to lewisite. Drop tests with 1:100, 1:250, 1:500 and 1:1,000 dilutions of lewisite in benzene showed that 7 of 11 volunteers who had previously been exposed to phenyldichlorarsine had also developed an ec-
SKIN SENSITIZATION TO VESICANT AGENTS

Skin sensitization to lewisite. Although the number of test subjects was considered too small to draw definite conclusions, the results suggested that exposures to phenyldichlorarsine produced a higher incidence of skin hypersensitivity to lewisite than did exposures to lewisite itself.

![Image of dermatitis](image)

**Fig. 4. Flare-up Dermatitis**

Note the flare-up dermatitis particularly at the treated sites, left proximal and right proximal and distal. Skin tests showed that the subject had become hypersensitive to phenyldichlorarsine. Photo 16 days after the application of 2 mg. of phenyldichlorarsine to each of 4 areas.

V. Adamsite (diphenylamine chlorarsine)

A. Tests for Sensitization to Adamsite in Human Volunteers. In studying cases of dermatitis among workers exposed to adamsite, Longcope, Wintrobe, Luetscher and Jager (67) found that a history of recurrent attacks was not uncommon and investigated whether subjects with such recurrent attacks had acquired a sensitization to adamsite.

Patch tests were performed with adamsite as a dry powder on 25 subjects without previous exposure and on 25 subjects with previous exposure to adamsite. Three among the 25 not previously exposed subjects and 6 of the 25 subjects who had been exposed to adamsite gave positive reactions to the patch tests. Twelve of the 25 subjects with previous exposure to adamsite had no history of dermatitis; of these 12, 1 gave a positive patch test reaction. Thirteen of the 25 previously exposed subjects had a history of adamsite dermatitis; of these 13, 5 gave a positive patch test reaction. The investigators concluded that the results of their tests indicated "that some individuals who are exposed to adams-
site and are particularly prone to develop severe attacks of dermatitis, develop a sensitivity of the skin to this chemical.” No tests were carried out to determine the specificity of the sensitization to adamsite.

COMMENT

From clinical evidence and the results of patch tests with dry adamsite, it appears that adamsite may be capable of sensitizing the human skin. However, the results of the skin tests thus far performed appear to be suggestive—but not conclusive—of sensitization. It is possible that tests with graded dilutions of adamsite, analogous to the tests used for demonstration of sensitization to other vesicant agents, may show greater differences in the level of sensitivity of previously exposed and unexposed subjects, than did the employed method of patch testing with the dry powder.

B. Tests for Sensitization to Adamsite in Laboratory Animals. Lenton (68) attempted to sensitize guinea pigs to adamsite. In one series he applied to the skin one drop of a 1% solution of adamsite in equal parts of dibutyl phthalate and olive oil every third day for 21 days, and in another series a 2% solution of adamsite in cellosolve every third day for 21 days. A third series of guinea pigs received 8 intracutaneous injections of the 1% solution of adamsite in equal parts of dibutylphthalate and olive oil, one injection every 3 days. The absence of reactions to external application of the 1% adamsite solution and to intracutaneous injection of an adamsite-albumin complex demonstrated that all three methods of repeated exposure had failed to produce skin sensitization of the guinea pigs to adamsite.

VI. Diphenyldichlorarsine

Flury (69) stated that the intensity of the effects of diphenyldichlorarsine depended very much on the individual’s sensitivity. Relatively frequently, he observed an abnormal hypersensitivity which could be increased through repeated exposure to the vesicant agent. Some individuals who had undergone severe skin damage from diphenyldichlorarsine showed skin manifestations even from minimal, ordinarily clinically imperceptible, traces of this agent. Flury, however, did not report any attempts to prove skin hypersensitivity or specific sensitizations to diphenyldichlorarsine by means of skin tests.

CONCLUSIONS

In addition to their inherent or obligate damaging effects, certain vesicant agents of chemical warfare can produce specific skin sensitization in both guinea pigs and man. The susceptibility both to the inherent damaging action and to sensitization by these vesicants varies greatly from individual to individual. There is, however, no evident relationship between an individual’s susceptibility to primary damage and his susceptibility to sensitization. In addition to individual susceptibility, the quantity and manner of exposure play a considerable role in the production of the skin hypersensitivity to these vesicant agents.
Not only clinical and experimental observations in man and guinea pigs, but also quantitative skin tests (patch tests or drop tests) have proved that mustard gas, lewisite, and phenyldichlorarsine produce skin hypersensitivity. Di-phenylchlorarsine, adamsite, and agents of the nitrogen mustard type are in all probability also capable of sensitizing human skin.

After adequate exposures to mustard gas, lewisite, or phenyldichlorarsine, more than 60% of human subjects may acquire some degree of skin hypersensitivity. These vesicant agents are, therefore, of the same order of sensitizing potency as poison ivy extracts, primrose extracts, dinitrochlorobenzene, paranitrosodimethylaniline, etc., which are among the most powerful of the known eczematogenic sensitizers in man. The acquired skin hypersensitivity to these vesicant agents of chemical warfare evidences characteristics typical of the eczematous form of allergic hypersensitivity to simple chemicals, plants, etc.

Individuals vary considerably in their level of acquired hypersensitivity to mustard gas and to lewisite. In most instances the acquired hypersensitivity is of relatively low degree and probably plays little part in the causation of injuries. Moreover, the usual degrees of acquired hypersensitivity are of little significance in relation to the practical problems of military and civilian protection against the powerful primary vesicant action. However, for unknown reasons, a certain very small proportion of individuals acquires a much greater degree of skin hypersensitivity than the population in general. In these, the clinical damage produced by minute, ordinarily harmless exposures, and the difficulties of protecting against these minute exposures, may become practical military and medical problems. It is logical that whenever possible such excessively hypersensitive persons should be excluded from duties involving any exposures or hazards of exposure to the particular vesicants. Skin tests will often aid in the demonstration of these exceptional degrees of hypersensitivity—e.g., a reaction to a droplet of a dilution of 1:50,000 or 1:100,000 of mustard gas in benzene usually denotes a potentially dangerous degree of hypersensitivity.

Clinical observations and experimental results in analogous forms of human skin sensitization, as well as the results of animal experiments, appear to justify the continued search for immunologic measures capable of reducing, inhibiting or preventing skin sensitization of human beings to vesicant agents.

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