STUDIES ON THE ECZEMATOUS SENSITIZATION

IV. Sensitization to Metadinitrobenzene and Its Relation to 2:4 Dinitrochlorobenzene*

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In the study of sensitizations to simple chemicals, there are two somewhat related chemical problems. One is the nature of the union between the sensitizing chemical and the tissues of the host in order to engender the eczematous sensitization, and the other is in what chemical respects can a substance that is capable of evoking a reaction in the already sensitized animal deviate from the parent sensitizing compound? It has been reasonably well shown, originally by Landsteiner (1) and subsequently confirmed by others, that simple chemical compounds which are capable of inducing the eczematous variety of the delayed type of allergic sensitization enter into some sort of union with the tissues of the host. The simplest hypothesis is that a protein conjugate is made. There seems to be direct chemical evidence for this type of union with certain classes of compounds, such as substituted nitro- and halo-benzenes, especially the dinitrophenyls. Such a union may not be true for all substances which yield an eczematous sensitization, e.g., nickel, for, as Gell (2) states:

"Many chemical compounds which act as sensitizers have one general feature in common: they are capable of reacting with a group or groups in the molecule of a protein to form a conjugate. A second type of sensitizer is not able itself to combine with protein, but may be metabolized in the body to a derivative possessing such powers of combination. There may be a third category of sensitizers which do not react chemically but form strong adsorption complexes with protein. In any case the conjugate or adsorption complex so formed will become antigenically distinct from the parent protein, and if the sensitizer gains access to the tissues of an animal the proteins of the latter may be rendered antigenic."

Much less work has been done to determine how far and in what respect one can deviate chemically from the compound to which the host was originally sensitized and still obtain a reaction. Rostenberg and Kanof (3) studied this problem in persons who had been sensitized to 2:4 dinitrochlorobenzene and came to the conclusion that "in the case of the substituted halo-benzenes, for the body to react to a compound other than the one to which it is sensitized, it appears that two factors must be present: 1) a geometric resemblance of the original to the new compound; and 2) the new compound must be capable of forming conjugates of a kind similar to the original."

Subsequently Eisen and colleagues (4) in several studies have furnished rather detailed knowledge concerning the nature of the union and the chemical dif-

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ferences between elicitors (that is, compounds capable of producing a reaction in the eczematously sensitized individual) and non-elicitors (that is, compounds not capable of producing the eczematous reaction). Eisen et al state: "It is concluded that a necessary condition for the elicitation of delayed allergic skin reactions by haptens is the combination of the latter with skin protein through the formation of bonds of the covalent type. No choice is yet possible amongst the several possible explanations which are advanced to account for the obligatory character of protein combination." While possibly not chemically elegant terminology, it might be well to think of compounds such as 2:4 dinitrochlorobenzene (2:4 D) as consisting of two parts: 1) the "active" or conjugating portion of the molecule, which is the means by which it attaches to the protein. This may be termed the molecular hook or linker, and 2) the "passive" portion of the molecule, which may be thought of as the configurational residue. In other words, for compound B to yield a reaction in an animal eczematously sensitive to compound A, two factors must be present: 1) compound B must have a group (a hook) by which it can attach to protein, possibly a particular kind of union is required, 2) having attached, the configuration of the residue insinuated onto the surface of the protein molecule must be geometrically (?) close to the configuration introduced by A. In the case of sensitization with 2:4 D, the chlorine atom is the hook by which the compound attaches to the protein, and the metadinitrophenyl residue is the configurational structure introduced onto the protein. As examples of the need for both an appropriate "hook" and a proper configurational residue, the results of testing individuals sensitized to 2:4 D with various structurally similar compounds will be cited. Animals or persons sensitized to 2:4 D react just about as well 2:4 dinitrobromobenzene or 2:4 dinitroiodobenzene because in both of these compounds an adequate hook exists, namely, the halogen atom and the configuration introduced onto the protein is the same as that introduced by 2:4 D. A compound such as 2:4 dinitroaminobenzene has the appropriate configuration but lacks the ability to attach, for Rostenberg and Kanof, as well as Eisen and colleagues, found that individuals sensitive to 2:4 D failed to react to this compound. On the other hand, 3:4 dichloronitrobenzene probably attaches adequately to the protein but the configuration introduced is apparently not sufficiently akin to the metadinitrophenyl structure to elicit reactions in an individual sensitive to 2:4 D (Rostenberg and Kanof).

Several investigators (Landsteiner, Haxthausen (5), Rostenberg) have shown that both guinea pigs and persons sensitive to 2:4 D fail to react to m-dinitrobenzene (mD), either by application or by intradermal injection. Now, as mD has the configuration of the groups introduced by 2:4 D, the failure of the sensitized animal to react implies that mD is incapable of entering into the requisite union with body protein to form the complete antigen, or at least is not able to form these conjugates sufficiently rapidly to yield an adequate local concentration. This last point is considered in more detail later.

In the course of some studies on the general problem of "desensitizing" the animal made eczematously sensitive to a simple chemical it was thought that

mD might inhibit reactions to 2:4 D in animals sensitized to this compound. Landsteiner (6) showed that certain simple chemical haptens inhibit precipitation reactions to the complete antigen. As is well known, he demonstrated that if a precipitating serum was first exposed to suitable concentrations of the simple chemical hapten and then the conjugated antigen (a protein united with the simple chemical hapten) introduced precipitation failed to occur; the theory being that the hapten was capable of uniting sufficiently with the antibody so as to utilize its combining sites, so that when antibody so treated was exposed to the complete antigen (the conjugated protein) there was no means by which a union between the two could occur; but the hapten-antibody combination did not produce molecular aggregates sufficiently large to cause precipitation. In a somewhat analogous fashion it was thought that mD might inhibit reactions to 2:4 D.

EXPERIMENTAL

Guinea pigs which had been sensitized to 2:4 D were exposed to mD in three different ways: 1) by the intraperitoneal injections of a solution of mD, 2) by implanting crystals of mD under the skin of the guinea pigs, and 3) by injecting intradermally solutions of mD. In all cases the animals were subsequently tested by the cutaneous application of 2:4 D. In no case was there any effect upon the reactivity to 2:4 D. In other words, the animals treated with the mD reacted just as well as animals not so treated. In the case where the mD was injected intradermally the 2:4 D was subsequently applied to the very site into which the mD had been introduced, but as already stated, this did not appear to inhibit the reaction to the 2:4 D. It was also thought that mD might have an influence on the duration of a sensitivity to 2:4 D. Such did not appear to be the case. Animals eczematously sensitized to 2:4 D did not have the duration of their sensitivity altered by injections of mD, as compared to control animals treated with acetone.

In the course of these various experiments a group of guinea pigs *not previously exposed* to 2:4 D had been treated by placing mD crystals subcutaneously. This was done by cutting through the full thickness of the skin and with an instrument, such as a hemostat, bluntly separating the skin for a very short distance from the underlying fascia. A pinch of mD crystals was then placed in the pouch and the incision closed with one or two sutures. These animals when subsequently cutaneously tested with 2:4 D proved to be sensitive to the application of this material in dilutions of 1:1000 in acetone—in a few cases in higher dilution. This experiment has been repeated with the same results.*†

DISCUSSION

It thus appears that mD, a compound incapable of eliciting a reaction in persons or animals eczematously sensitive to 2:4 D, nevertheless can sensitize

* Very recently we have tried the effect of injecting intracutaneously 10 per cent acetone solutions of mD. So far we have failed to develop a sensitivity to 2:4 by this method.

† Distillation Products Industries, from whom we purchased the mD, kindly ran a test for chloro impurities and found none.

guinea pigs to this same compound. At first sight this would appear somewhat paradoxical, but it is believed that a reasonable explanation in line with accepted theories can be proffered.

As already stated, mD has the dinitrophenyl configuration of 2:4 D but fails to cause a reaction in the animal already sensitized to 2:4 D because it (mD) is incapable of conjugation. It would appear, however, that the phrase "incapable of conjugation" must be regarded in a relative fashion. It would now appear that mD is capable of conjugating with the same proteins that 2:4 D conjugates with, but the rate at which the conjugated protein forms with mD is below the rate at which the protein or the mD itself or both is carried away or degraded, or both; so that when a 2:4 D sensitive animal is tested with mD the concentration of conjugated protein existing at the test site at any one time is very small, presumably below the level of clinical detectability. However, when crystals of mD are introduced, over a period of time a sufficient number of appropriate protein conjugates are formed which will ultimately engender the enzymic adaptation in an adequate number of R.E. cells, so as to render the host generally eczematously sensitive to the material in question.* This finding is actually somewhat analogous to the situation that exists in anaphylactic sensitivities where it is reasonably well established that a much smaller amount of antigen is required to sensitize than to elicit the acute anaphylactic reaction in the already sensitized animal.

SUMMARY AND CONCLUSIONS

1. A review of the theory regarding the chemical characteristics of compounds that are able to elicit a reaction in an animal eczematously sensitized to some other compound has been given.

2. This theory has been explicitly discussed with respect to 2:4 dinitrochlorobenzene and metadinitrobenzene. It has been pointed out that metadinitrobenzene is incapable of causing a reaction in the eczematously sensitive animal, presumably, because it cannot form appropriate conjugates in sufficient concentration within a requisite time.

3. It has, however, been shown that metadinitrobenzene can engender a sensitivity to a metadinitrophenyl protein conjugate, so that when the animal is subsequently tested with 2:4 dinitrochlorobenzene the animal will be found to be sensitive.

4. Metadinitrobenzene was found to be incapable of inhibiting reactions to 2:4 dinitrochlorobenzene or to affect the duration of a sensitization to 2:4 dinitrochlorobenzene.

REFERENCES

1. Landsteiner, K., and Jacobs, J.: Studies on the sensitization of animals with simple chemical compounds II. J. Exper. Med., **64:** 625, 1936.

2. Gell, P. G. H., Harington, C. R. and Rivers, R. P.: The antigenic function of simple

* The theory of protein conjugates causing an enzymic adaptation as the basis of an eczematous sensitization is considered explicitly by me elsewhere (7).

chemical compounds: Production of precipitins in rabbits. Brit. J. Exper. Path., **27:** 267, 1946.

3. Rostenberg, A. and Kanof, N. M.: Studies on eczematous sensitizations: The specificity of the sensitization from the point of view of chemical configuration. J. Invest. Dermat., **6:** 201, 1945.
4. Eisen, H. N., Orris, L. and Belman, S.: Elicitation of delayed allergic skin reactions with haptens: The dependence of elicitation on hapten combination with protein. J. Exper. Med., **95:** 473, 1952.
5. Haxthausen, H.: Some problems concerning the pathogenesis of allergic eczemas, elucidated by experiments on sensitization with dinitrochlorobenzene. Acta dermat.-venereol., **20:** 257, 1939.
6. Landsteiner, K.: The Specificity of Serological Reactions, Harvard, 1945.
7. Rostenberg, A.: Concepts of allergic sensitizations: Their role in producing occupational dermatoses. Indust. Med., **23:** 1, 1954.