STUDIES ON THE SENSITIZATION OF ANIMALS WITH SIMPLE CHEMICAL COMPOUNDS

VIII. Sensitization to Picric Acid; Subsidiary Agents and Mode of Sensitization

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In sensitization experiments with a fair number (17) of compounds all similar in chemical constitution, namely Cl and NO_2 substitution products of benzene, a definite correlation has been found (1, 2) between the capacity to sensitize guinea pigs under given conditions and the ease with which the substituents are replaceable. For example, the labile 2,4-dinitrochlorobenzene proved to be a powerful sensitizing agent whilst the compounds 1,2,4- and 1,4,2-dichloronitrobenzene resistant to treatment with alkali or aniline were ineffective. The demonstration of a correlation between chemical properties and allergic activity led to the detection of new groups of reactive chemicals endowed with sensitizing capacity, namely acyl chlorides and benzyl chlorides. Analogous observations on acid anhydrides have recently been made by Jacobs (3) and his colleagues, and other reactive compounds as diazomethane and mustard oil have been added to the list of experimental sensitizers (4). Moreover, the above results were corroborated in human beings by Sulzberger and Baer (5). Here, likewise, 2,4-dinitrochlorobenzene sensitized a considerable percentage of individuals (*cf.* Haxthausen (6)) and again 1,2,4- and 1,4,2-dichloronitrobenzene failed to do so, with a possible exception in one person.

Whereas the connection of sensitizing power and the reactivity of substances under conditions as they may exist in the animal body is well established, there remains the fact, as mentioned previously (2), that compounds which from their chemical character would not appear to enter readily into firm combination with constituents of the animal body, still engender sensitivity in human beings even though not as frequently as certain typical incitants, *e.g.* dinitrochlorobenzene or urushiol (poison ivy). Indeed, with the latter substances experimental sensitization succeeds almost regularly, a single dose often sufficing to induce hypersensitiveness in man as well as in guinea pigs when applied on the intact skin and,

broadly speaking, all such substances of the reactive sort are presumptive allergens.

Some attempts made in this laboratory to repeat recently described experiments on sensitization of guinea pigs to phenolphthalein and pyramidon (7, 8),—which cannot be considered as reactive in the above sense—were unsuccessful.

Experiments with Picric Acid

From the point of view outlined, allergy to picric acid is of decided interest. The substance is chemically similar to the nitro compounds previously tested but the NO_2 groups are not readily replaced. It is decomposed by boiling in alkaline solution but is rather stable under milder conditions. Still picric acid now and then produces skin sensitivity in human beings (see (9, 10)).[1] Hence one must assume that its sensitizing capacity is due to some mechanism other than the formation of conjugates by elimination of NO_2, as is very probably the case with 1,2,4-trinitrobenzene (1).

On boiling picric acid with an excess of aqueous alkali, HCN, NH_3, and HNO_2 were found to be formed (11); heating with aromatic bases at high temperatures yielded dyes (12). On treating picric acid with 3 moles of aniline in alcoholic solution for 15 hours as previously described (1, page 630), the solution became dark brown. After removal of aniline, crude picric acid amounting to 97 per cent of the quantity used was recovered by extraction with toluene from acid solution. Upon recrystallization the melting point corresponded to that of picric acid, with no depression on mixture with a known sample. Furthermore, after refluxing with 2.2 moles of sodium methylate in methanol for 1 hour picric acid could be recovered in good yield with very little decomposition.

In a number of cases treatment of burns with butesin picrate,[2] as it is often applied, has been followed by allergic reactions. Thus Sulzberger and Wise (13) remark "that the application to a burned area (injured area) may constitute a situation peculiarly favoring sensitization." With this in view and on account of previous negative results with picric acid (1) upon intracutaneous injection in guinea pigs, butesin picrate was applied superficially to skin areas inflamed following application of cantharidin which produces lesions similar to burns. Other clinical experience, too, indicates that irritation of the skin aids the development of allergic eczematous affections due to various substances (14, 15), and similar effects were noted in animals ((16), (4, page 506)). It was seen, however, in the course of the

[1] Over twenty cases of sensitivity to picric acid or butesin picrate are reported in the medical literature.

[2] Butesin picrate is a compound containing one molecule of picric acid and two molecules of butesin (*n*-butyl ester of *p*-aminobenzoic acid).

work that superficial (skin) treatment with butesin picrate or with picric acid alone yielded positive results but less regularly and on the whole with a lower degree of allergic reactions.

In the experiments with cantharidin lesions 2 drops of a 0.1 per cent solution of cantharidin in methyl cellosolve (ethylene glycol monomethyl ether) were put on one side of the posterior half of the back of male albino guinea pigs and spread gently with a glass rod, and this was repeated the next day; sometimes 1 drop of the solution was applied some days afterwards. Cardboard collars (17) were fastened to the animals in order to protect the muzzles from contact with the cantharidin. 1 or 2 days after the first application 5 drops of a solution of 0.4 per cent of picric acid, or 1 per cent butesin picrate, in olive oil were spread around the burn daily for 5 days; in the remaining 7 treatments because of the skin alteration 8 drops were needed to cover the whole area. Other experiments were conducted in a like fashion except that no cantharidin was employed. Usually about a week from the beginning of the administration of butesin picrate or picric acid the treated site in a number of animals would show conspicuous changes (not due to cantharidin itself) consisting in redness of the skin, thickening, and scaling, often with a sharp line of demarcation. In all cases the areas of the skin to be treated (or tested) were clipped and whenever indicated scales were removed (soaking with olive oil, washing with alcohol).

Guinea pigs treated in the manner described were tested 3 weeks after the last treatment by putting 1 drop of a 5 per cent solution of picric acid in equal parts of dibutyl phthalate and olive oil on the clipped skin of the thigh. Reactions began to be evident in these animals on the following or the 2nd day, mostly in the form of small or larger pinkish spots or papulae, increasing in color and size so that in the most pronounced cases by the 3rd day the test site was elevated and dark pink, and sometimes the treated site was surrounded by a wide zone of inflammation. Scaling developed and in the best reacting animals the area became covered with a thick adherent layer of scales which persisted for a week or longer. In normal guinea pigs used as controls the skin showed no changes at all. Histologically there was, in addition to leucocytic infiltration, marked thickening of the epithelium and formation of intraepithelial vesicles containing, in part, polymorphonuclear leucocytes.

Among various groups of six treated animals usually about half gave markedly positive tests and in addition some showed weaker reactions. To some extent, at least, the degree of sensitization seemed to be correlated with the intensity of the lesions observed during the period of sensitizing treatment. It must be noted that the results depend on the animal stock used and that with guinea pigs from a certain source only a few became hypersensitive although this same strain was well receptive in sensitization experiments with 2,4-dinitrochlorobenzene or picryl chloride. The exist-

ence of heredity differences in sensitization to the latter substance has been described lately (18).

In the slower development and longer persistence of the lesions in tests on sensitized animals, as well as in appearance, the reactions to picric acid differed from those seen in nearly all our previous experiments. Positive reactions recognizable by development of papulae and scaling were also seen in the sensitized animals when picramic acid (5 per cent) in a mixture of equal parts of "butyl carbitol"[3] and alcohol was put on the skin.

As already mentioned positive sensitization to picric acid was obtainable without the use of cantharidin or butesin. In order to compare the effects of the different procedures an experiment with four groups of guinea pigs was made, as given in Table I. It is seen from the tabulation that

TABLE I

Tests made by putting 1 drop of a 5 per cent solution of picric acid on the flank. The degree of reactions is indicated by plus signs, the figures giving the number of animals. The treatments and testing of the animals given in the first four lines were made simultaneously, the remaining set being done on a separate occasion.

	+++	++	+	±	−
Butesin picrate with cantharidin lesions	5	1	3	1	
Butesin picrate	2	3	2	1	
Picric acid with cantharidin lesions	2	3	2	1	
Picric acid	1	3	1		3
Picric acid	1	2		1	6

sensitization with picric acid alone gave poor results but they were improved by the employment of butesin or cantharidin lesions. This result is in accordance with the observations of Burckhardt on sensitization of guinea pigs to turpentine.

Other methods used were the repeated application (twelve times) of 2 drops of 5 per cent picric acid in a mixture of dibutyl phthalate and olive oil on the skin of the flank and daily intracutaneous injections of 0.1 cc. of 0.1 per cent solutions of picric acid in saline continued over a period of 2 weeks. The latter method did not produce clean-cut positive results; in the former two out of six animals developed marked skin changes—inflammation, scaling—during the treatment and on a test 3 weeks after the preparatory applications these two were found to be definitely allergic and two others weakly so.

[3] Diethylene glycol mono-butyl ether.

COMMENT

The above observations show the possibility of sensitizing animals with an aromatic compound—picric acid— which only occasionally causes allergy in human beings (see (19)). The lesions produced by application of oil solutions on the skin of sensitized animals were somewhat different from those seen in animals sensitive, for instance, to 2,4-dinitrochlorobenzene or poison ivy, and simulated human eczema. Sensitization could be achieved by treatment with picric acid alone but with low concentrations the effect was definitely greater when the substance was applied to inflamed skin areas and was enhanced by mixture with an aromatic amino compound (butesin), possibly because of a sensitizing activity of this substance.

Picric acid differs from such nitro substituted benzenes, as 1,2,4-trinitrobenzene, previously shown to be potent sensitizers (1, 5), in that its nitro groups are not readily detached and the formation of substitution compounds would not be expected. Therefore the mode of sensitization is in all probability different from that operative in the case of the previously examined NO_2 and Cl substituted benzenes where conjugates will be formed in the body by elimination of substituents. One may think that the formation of picrates is sufficient for inducing antigenic effects but at present evidence is lacking for this explanation.[4] It may be more likely that sensitization is brought about by reduction products of picric acid, since aromatic bases and allied compounds are well known to have sensitizing capacity (Mayer (20); Nitti *et al.* (21)), presumably by coupling with body constituents, after oxidation. Actually, conversion into picramic acid in the animal body has been demonstrated (22), and picric acid is reduced to azoxy compounds by glycyl-glycine anhydride in alkaline solution (23). Moreover, as mentioned, animals sensitized with picric acid were seen to be hypersensitive to picramic acid (although less than to picric acid), and picramic acid has itself sensitizing properties. In the case of sulfamidochrysoidine, Nitti and Bovet (24) were led to the conclusion that sensitization takes place after previous reductive cleavage of this azo compound.

SUMMARY

Sensitization of guinea pigs to picric acid was obtained by application of oil solutions to the skin, preferably on inflamed sites or by treatment with a compound of picric acid with *n*-butyl-*p*-aminobenzoate. The lesions

[4] Skin treatment with sulfosalicylic acid, like picric acid a protein precipitant, did not produce allergic reactions in a small group of animals.

obtained in sensitive animals on superficial administration bore resemblance to human eczema.

It seems probable that picric acid sensitization is an instance where a substance does not sensitize directly but after conversion into a more reactive compound, a principle which should be of wider application to instances where the original substance does not readily form conjugates.

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