STUDIES ON THE SENSITIZATION OF ANIMALS WITH SIMPLE CHEMICAL COMPOUNDS*

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PLATE 30

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Not a few investigations have been carried out on the sensitization of animals to simple compounds known to produce hypersensitiveness in man, but the progress of this subject has been hindered to a certain extent by difficulties encountered in reproducing the experiments.

Sensitization with salvarsan has been reported by Swift (1), and with neoarsphenamine by Frei (2), and Sulzberger (3). Swift recorded general symptoms,[1] whereas in the experiments of the latter two authors the effects consisted in the development of skin lesions in the treated animals. Similarly, cutaneous hypersensitiveness was induced with phenylhydrazine by Jadassohn (4) and with *p*-phenylenediamine by Mayer (5) and Dienes (6). In the case of *p*-phenylenediamine the mechanism of sensitization can be more easily understood than in other instances because this compound, after oxidation, is apt to enter into a firm chemical union with proteins, and, in fact, *p*-phenylenediamine is used extensively as a fur dye. This interpretation is evident for the experiments of Klopstock and Selter (7) with diazonium solutions and actually their treatment produces a state of typical anaphylaxis which would seem to set this case apart from the common instances of drug and occupational allergy.

Apparently the most striking and consistent results have been obtained by Bloch and Steiner-Wourlisch (8) with primulin, in animals as well as human beings, and more recently by Rackemann and Simon (9) with poison ivy in guinea pigs. The latter experiments were performed with extracts, whereas in the former a pure substance of simple composition ($C_{14}H_{18}O_3$ or $C_{14}H_{17}O_2OH$) was used which, however, has the drawback of not being easily accessible.

On attempting to reproduce, in New York, the results he had obtained in Europe with neoarsphenamine, Sulzberger (10) found that the experiments were

* The experiments have in part been briefly reported in *Proc. Soc. Exp. Biol. and Med.*, 1934, **31**, 790, 1079.

[1] Other experiments along this line are critically reviewed by Frei (2).

no longer distinctly positive.[2] He summarizes his findings by saying that guinea pigs reacted differently when sensitizations with the same brand of neoarsphenamine were attempted in various places. In Breslau 98 per cent of the animals became sensitized, but none in New York; Zurich animals showed a state of susceptibility placing them between those of New York and Breslau in this respect. From a study with Mayer (11) of the factors involved, he concluded that the diet of the animals was of decided importance; green fodder inhibiting, dry fodder favoring sensitization.

The observations of Walthard (12) on the sensitization of guinea pigs with nickel salts[3] could not be duplicated by Coca and Milford (13). Experiments by Mu (14) on neosalvarsan were definite but variable in guinea pigs, and in rabbits positive in some batches, negative in others. Frei (2) was unable to obtain clear-cut results with the latter species.

The passive transfer of human idiosyncrasy to iodoform and iodine, described by Bruck (15) and Klausner (16), has, so far, not been corroborated; and likewise Meyer (17), Bock (18), and Mayer (19) were unsuccessful in attempts to produce active anaphylaxis to *p*-phenylenediamine in guinea pigs as reported by Curschmann (20), Gerdon (21), and Mehl (22). The first two authors and Mayer (23) also reported positive transfer to guinea pigs with the serum of human beings hypersensitive to ursol, but the latter was not able to transfer hypersensitiveness from guinea pig to guinea pig.

It is apparent from a survey of the work bearing on the subject that the conditions which influence the experiments are not yet fully understood and so far, in most cases, the lesions obtained in animals have not been equal in intensity to those occurring in human beings.

Other pertinent questions also are still unsolved. It is not known whether the capacity to induce sensitivity is connected with certain peculiarities in chemical constitution, and with a number of substances to which some human individuals are hypersensitive it has not been possible, as yet, to induce such a state in animals. Moreover, the mechanism underlying the sensitization effects is uncertain, as is their relationship to the condition of anaphylaxis as produced by typical antigens.

[2] We also had irregular results with neoarsphenamine, but the results were markedly improved by using arsphenamine without neutralization. According to a personal communication by Sulzberger, he encountered difficulty in repeating the experiments by Jadassohn on phenylhydrazine, also, whereas in a small series of animals we found definite evidence of sensitization.

[3] An experiment of our own with intracutaneous injections of small amounts in a few animals was negative.

For the reasons stated it seemed desirable to search further for sensitization effects which could be obtained regularly with easily available simple substances of known chemical composition.

EXPERIMENTAL

Technique—Guinea pigs were used as experimental animals and for the sensitization various procedures proved effective. In a large part of the experiments the method of repeated injection of small quantities was adopted, but other methods were used besides; *i.e.*, single injections, or applications of an olive oil solution on the skin, or salve. A more extensive study of various methods of administering the substances will be necessary finally to evaluate their comparative merits.

Injections were made, after clipping the hair with an electric hair clipper, with a No. 26 short bevel needle into the skin of the back, near to the surface, without abrading the skin. The volume of the injected fluid was always 0.1 cc. Olive oil solutions (1 per cent) were administered by gentle spreading with a glass rod. White guinea pigs 350–450 gm. in weight were used throughout.

For treatment and tests the substances were weighed, dissolved in a small amount of 1 per cent saline, or alcohol if they were too insoluble in water, and made up to the required dilution with saline. Usually a stock 0.3 per cent solution in alcohol was kept and the required saline dilutions made up before injections. In the case of relatively unstable substances, as neosalvarsan, *p*-aminophenol, and *p*-phenylenediamine, the dry substance was dissolved in saline before injection.

The substances used were commercial preparations, recrystallized when necessary, excepting several substituted (Cl, NO_2) benzenes (prepared in this laboratory, or obtained from the chemical laboratory of the University of Amsterdam[4]), *m*-chloroacetylaminophenol and chloroacetyl-*p*-chloroaniline,[5] dextran,[6] and carbohydrate preparations (made by the authors).

Reactions were read on the day following the test injections, which were made on the flanks of the guinea pigs. The hair was clipped with an electric clipper and the tests read mostly after application of a depilatory. Intracutaneous injections were made as described above.

In the tables figures give diameters of the lesions in millimeters, the shade of which is described as colorless (c.), almost colorless (a.c.), faintly pink (f.p.), pale pink (p.p.), and pink (p.). Other designations are negative (neg.), almost negative (a.neg.), flat (fl.), faintly elevated (f.el.), slightly elevated (sl.el.), elevated (el.), and markedly elevated (m.el.). Insignificant reactions, namely small elevations at the place of puncture, frequently present in non-sensitized animals,

[4] To Professors Wibaut and Holleman we are greatly indebted for furnishing us with these preparations.

[5] Available through the kindness of Dr. Walter Jacobs.

[6] Kindly furnished by Dr. Hibbert of Montreal.

are designated as nodules (nod.). Livid center (liv.c.) or necrotic center (necr.c.) is used to describe stages of necrosis. Oil contact tests are rated as negative, faint, weak, strong.

p-Nitrosodimethylaniline  .—Distinctly positive results were arrived at with *p*-nitrosodimethylaniline. The use of repeated injections of small quantities was adopted following the recommendation of Kolle (24), who, in this way, in experiments on salvarsan induced a condition in guinea pigs in which they succumbed after administration of quantities otherwise well tolerated. The fact that nitroso-

TABLE I

Animals Tested by Intracutaneous Injection of 1/30 Mg. Nitrosodimethylaniline

Treated		Controls	
Guinea pig No.	Lesions	Guinea pig No.	Lesions
1	9 x 7, p., sl.el.	7	c., nod.
2	11 x 9, p.p., fl.	8	4, p.p., nod.
3	9 x 6, p., sl.el.	9	c., nod.
4	14 x 13, p.p., m. el.	10	f.p., nod.
5	9, p., el.	11	neg.
6	9, p., el.	12	neg.

dimethylaniline is capable of producing hypersensitiveness in human beings was brought to our knowledge by van der Scheer, who had observed a striking instance of such an affection. A batch of animals were given repeated intracutaneous injections of 1/30 mg. each in 0.1 cc. saline solution on the back, two a week for 10 weeks, and tested on the side with the same dose 3 weeks after the last treatment (Table I). Beginning with the 2nd week the injections were followed in all the animals by reactions, which gradually became stronger; not seldom a decrease was noticed later in the course. The lesions, which were pinkish to pink and elevated, were fully developed after 1 day. In normal control guinea pigs but slight effects were produced by injections of the substance into the skin.

A second experiment showed that much smaller doses may cause

sensitization. Thus it was seen that eighteen daily injections of 1/500 mg. produced unquestionably positive effects, and in another experiment, which would require repetition, even doses of 1/5000 mg. gave noticeable results. Sensitization effects were also brought about by rubbing guinea pigs with vaseline containing 5 per cent of the compound.

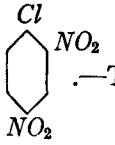
2:4 Dinitrochlorobenzene  .—The effects obtained with this substance were quite similar to those just described (Figs. 1 and 2) but in general still stronger. The fact that sensitization occurs in

TABLE II

Guinea Pigs Tested by Intracutaneous Injection of 1/100 Mg. of 2:4 Dinitrochlorobenzene

Treated		Controls	
Guinea pig No.	Lesions	Guinea pig No.	Lesions
13	7 x 5, p.p., el.	18	6, p.p., sl.el.
14	12, p.p., m.el., liv.c. 4½	19	4, f.p., f.el.
15	16 x 15, p., m.el., liv.c. 3	20	3, a.c., nod.
16	9, p.p., el.	21	p.p., nod.
17	17 x 15, p.p., el., liv.c. 3	22	5, f.p., sl.el.

human beings was called to our attention by Professor Zangger of Zurich. Reports on cases of human hypersensitiveness have been published by Wedroff (25, 26), who observed greatly increased hypersensitivity to the compound in many of the exposed factory workers.

One lot of guinea pigs, tested intracutaneously with 1/100 mg. dinitrochlorobenzene 2 weeks after a course of ten daily injections of 1/400 mg. on the back, showed strong reactions, three out of five with livid centers (indicative of necrosis) (Table II). Distinct reactions had been obtained in these animals a week earlier, following injections of 1/400 mg. A further experiment gave striking results when the animals were tested with 1/400 mg. intracutaneously 1 week after a course of two weekly injections of the same quantity over a period

of 10 weeks (Table III). The lesions, in four of the six animals, showed necrotic centers.

Again, as with nitrosodimethylaniline, reactions developed during the course of injections after the 1st week, later reaching a maximum.

In over twenty batches of guinea pigs treated with the substance at different times practically all animals responded with definitely increased hypersensitivity, although the effects were not equally good and not always so strong as those tabulated, and there were marked individual variations within each batch as is evident from a glance at the tables.

TABLE III

Guinea Pigs Tested by Intracutaneous Injection of 1/400 Mg. of 2:4 Dinitrochlorobenzene, and Application of a 1 Per Cent Solution in Olive Oil

Treated			Controls		
Guinea pig No.	Intracutaneous injection	Oil solution	Guinea pig No.	Intracutaneous injection	Oil solution
23	12, p.p., sw., necr.c. 5	Weak	29	a. neg.	Neg.
24	9, p., m.el.	Strong	30	4, f.p., sl.el.	Neg.
25	12, p.p., m.el., necr.c. 4	Strong	31	4, f.p., sl.el.	Neg.
26	17, p.p., el., necr.c.	Strong	32	a. neg.	Neg.
27	11, p., el., necr. c.	Strong	33	a. neg.	Faint
28	11 x 7, p., el.	Strong			

Apparently, a still better way than intracutaneous injection to demonstrate the increased sensitivity of treated animals was to spread a drop of a 1 per cent solution of dinitrochlorobenzene in olive oil on the skin with a glass rod. In sensitive animals the areas in contact with the substance showed a faint pinkish color after a few hours which increased to a distinct pink or red on the following day, and were, in many cases, elevated or actually swollen, in striking contrast to the negative controls (Table III). The same method proved to be applicable in other cases; *e.g.*, with nitrosodimethylaniline.

Also, by treating the skin with oil solutions of dinitrochlorobenzene, it was possible to sensitize guinea pigs.

In other tests alcoholic solutions were used as done by Wedroff (26), who described reactions after the application of alcoholic solutions of

dinitrochlorobenzene in sensitive patients. With this method well sensitized guinea pigs showed reactions following the application of 1 drop of a 1:100 or 1:1000 solution of dinitrochlorobenzene which compared to the degree of sensitivity of a large part of Wedroff's patients although a number of them reacted to dilutions much higher still.

In the preceding experiments repeated injections were administered, but it was found that positive results can be obtained with but few applications. Even after the first injection there was in a batch of four animals a noticeable effect when they were tested intracutaneously a week later, and with the rubbing test after 1 more week, two strong, one marked, and one faint reaction were obtained, whereas the controls were practically negative. In a few other experiments designed to vary the method of sensitization it was found that following a single injection the effect seemed to be somewhat but not much more pronounced when tests were made after an interval of 4 weeks, than after 1. A considerably larger dose than that commonly used, namely five simultaneous intracutaneous injections of 1/100 mg., was not superior to one injection of 1/400 mg. when the animals were tested after a rest period of 4 weeks. That a course of several injections over a period of weeks gives better results than a single injection was indicated by the following experiment. Of two batches of animals given two injections of 1/400 mg. 3 days apart, one in which the injections were continued once a week for 5 weeks showed much stronger reactions than the other batch, on testing 1 week after the last injection given to the second lot.

In preliminary experiments subcutaneous or intravenous, instead of intracutaneous injections, gave only slight sensitization effects, and repeated instillations of a dilute saline solution into the eye were ineffective.

Chloro and Nitro Substituted Benzenes.—Definitely positive sensitization effects were observed with a number of chloro and nitro substitution products of benzene: 1:2:4 trinitrobenzene, picryl chloride, and four dichlorodinitrobenzenes (1:3:4:6, 1:3:2:5, 1:2:4:5, 1:4:2:6), while three dichloronitrobenzenes (3:5, 3:4, 2:5), *p*-chloronitrobenzene, *m*-dinitrobenzene, 1:3:5 trinitrobenzene, picric acid, and four

compounds containing only chlorine as substituents, namely *p*-dichlorobenzene, 1:2:4 trichlorobenzene, 1:2:4:5 tetrachlorobenzene, and hexachlorobenzene,[7] failed to yield evidence of sensitization.

Of substituted benzenes of the type $R:NO_2:NO_2$, 1:2:4, tested for sensitization, 2:4 dinitrofluorobenzene, 2:4 dinitrobromobenzene, 2:4 dinitroiodobenzene (and 1:2:4 trinitrobenzene) were distinctly active, and 2:4 dinitrophenol gave a slightly positive result which would require confirmation. With 2:4 dinitroaniline and 2:4 dinitrophenylmercaptan[8] negative results were obtained.

Among other substances marked results were obtained with *m*-chloroacetylaminophenol and definitely positive effects with chloroacetyl-*p*-chloroaniline, *p*-aminophenol, and *p*-nitrosophenol.

With some substances, excitants of human idiosyncrasies, namely quinine, resorcinol, and acetylsalicylic acid, our methods failed to give results. Likewise negative were experiments with substances chosen because of their highly irritating properties, to wit turpentine, croton oil, acridine, as well as the carcinogenic compound dibenzanthracene. Again, a number of dyes, among them azodyes obtained by coupling resorcinol with diazo compounds, were ineffective, or not definitely active. An exception to this seemed to be resorcinoldisazo-*p*-suberanilic acid.[9] Of six animals given repeated injections of 1/50 mg. of this dye for 10 weeks one showed unmistakable and one somewhat increased sensitivity on reinjection after a month of rest. Finally it is worth noting that the method of injecting minute quantities intracutaneously did not induce hypersensitivity of the skin in some preliminary experiments, using carbohydrates of *V. cholerae* and *B. pseudoanthracis* and dextran.[10] Of course, the negative results reported cannot be taken as final, since other methods may still prove effective.

Tests for Specificity.—The specificity of hypersensitiveness has been

[7] The solutions used were stabilized by the addition of some guinea pig serum.

[8] Dissolved with the aid of dilute ammonium hydroxide.

[9] Previously shown to be able to induce specific anaphylactic shock in guinea pigs sensitized with an azoprotein made from suberanilic acid (Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1933, **57**, 633.

[10] Zozaya, J., *J. Exp. Med.*, 1932, **55**, 325.

studied by several authors in human beings,[11] but, so far, not many observations have been reported in animals (Mayer (19)). We have performed such experiments, first with guinea pigs selected for their strongly developed sensitivity to *p*-nitrosodimethylaniline or 2:4 dinitrochlorobenzene, between which sharp specificity was noticed, the animals reacting to the substance with which they had been treated and not significantly with the other, as is shown in Table IV.

TABLE IV

Guinea Pigs Tested by Intracutaneous Injection of 1/50 Mg. p-Nitrosodimethylaniline or 1/400 Mg. 2:4 Dinitrochlorobenzene

Sensitive to	Guinea pig No.	Tested with	
		Nitrosodimethylaniline	Dinitrochlorobenzene
Nitroso-dimethyl-aniline	34	9 x 7, p.p., f.el.	a. neg.
	35	12 x 9, f.p., sl.el.	3, f.p., f.el.
	36	12 x 7, p.p., el.	3, f.p., nod.
	37	11 x 10, p.p., el.	c., nod.
	38	11, p., el.	5 x 3, p.p., sl.el.
	39	11, p., el.	4 x 3, p.p., sl.el.
Dinitrochloro-benzene	40	c., nod.	9, f.p., el., liv.c.
	41	neg.	5, f.p., sl.el., liv.c. 3
	42	a. neg.	10, p.p., el., liv.c. 3
	43	a. neg.	10 x 8, p.p., el.
	44	c., nod.	9 x 8, p.p., el.
	45	3, f.p., sl.el.	6, p.p., sl.el.

Guinea pigs hypersensitive to *p*-nitrosodimethylaniline did not exhibit increased reactions to some structurally related compounds as *p*-nitrosophenol, dimethylaniline, and nitrosobenzene. One guinea pig sensitive to *p*-nitrosophenol showed a distinct reaction when tested with nitrosodimethylaniline.

In the series of substituted benzenes of the type 1:2:4 = R:NO_2:NO_2 the nitro, fluoro, chloro, bromo, and iodo compounds sensitized, as mentioned previously, and gave cross-reactions among themselves; the corresponding amino and sulfhydryl compounds (and *m*-dinitrobenzene)

[11] For bibliography see Landsteiner K., Die Spezifizität der serologischen Reaktionen, Berlin, Julius Springer, 1933, 95.

which did not sensitize gave no, or much weaker, reactions in animals sensitized with other substances of the series. The action of 2:4 dinitrophenol was equivocal both as a sensitizing agent and in the tests. Furthermore, solutions of 2:4 dinitrobenzoic acid, 2:4 dinitroanisole, 2:4 dinitrotoluene, and 2:4 dinitrodiethylaniline did not react with pigs hypersensitive to 2:4 dinitrochlorobenzene. With these substances no sensitization experiments were made. Additional tests on animals sensitized to 2:4 dinitrochlorobenzene were made with numerous chloro and nitro substituted benzenes. Definite cross-reactions were observed in several cases, namely with picryl chloride, and two dichlorodinitrobenzenes, 1:2:4:5 and 4:6:1:3. These substances belong among those found to produce sensitization. Specificity tests were performed with the following compounds, also: picryl chloride (A), 1:4:2:6 dichlorodinitrobenzene (B), 1:3:2:5 dichlorodinitrobenzene (C), 4:6:1:3 dichlorodinitrobenzene (D) and 1:2:4:5 dichlorodinitrobenzene (E), on a limited number of animals sensitized to these substances. The specific character of the reactions was evidenced by the fact that in each batch of animals the reaction with the homologous compound was stronger, or not weaker than those with other substances. Definite cross-reactions were observed in the following cases: A with B, B with A, C with A and B, D with A, and E with A and D, where the first letter refers to the substance used for sensitization, and the others to the compounds used for the skin test.

Selected animals sensitized by repeated injections with neoarsphenamine, nitrosodimethylaniline, *p*-phenylenediamine, and resorcinol-disazo-*p*-suberanilic acid respectively, were each injected with all four of the substances. The strongest reaction, in each case, was given by the homologous substance, but the guinea pigs hypersensitive to *p*-phenylenediamine showed marked reactions with *p*-nitrosodimethylaniline, as well. This last reaction was regular and quite definite.

DISCUSSION

The essential outcome of the foregoing studies is that sensitization effects have been obtained with simple compounds, which are easily reproducible and for that reason seem to offer an advantage for further studies. From the experiments it would appear that there are a large number of substances of different composition by means of which a

state of hypersensitiveness can be induced in animals. Yet substances gave negative results with the methods used although they were structurally quite similar to those yielding positive effects.

On considering the properties which may characterize compounds causing increased sensitivity it would seem possible that the ability to irritate the tissues plays a rôle, since most of the substances that were found to produce sensitization are irritating. Primulin used by Bloch and Steiner-Wourlisch (8) in human beings and animals, and extracts of *Rhus toxicodendron* (Rackemann and Simon (9)) were likewise found to produce local lesions. Still there are examples to the contrary, namely *p*-phenylenediamine which has little effect upon the skin of normal animals and yet readily induces the sensitization phenomenon, and other compounds that are highly irritating and do not do so.

As to the mechanism of hypersensitiveness to simple compounds, it is the simplest explanation and in line with the opinion of most authors to relate them to the familiar processes of immunization, especially in view of the specificity of the reactions. The chief difficulty in the way of adopting this view is the uncertainty still prevailing about the possibility of demonstrating circulating antibodies even in pronounced cases of human drug idiosyncrasy (see Coca (27)). In the literature there are a number of reports on successful Prausnitz-Küstner reactions in such cases, but they have been questioned by others and are not generally accepted.[12] More recent reports on passive transfers were those communicated by Frumess (28) on 2:4 dinitrophenol and by Ensbruner (29) on neosalvarsan, which, if confirmed, would be of great significance.[13]

The fact that all parts of the skin become sensitive following injection at one site would indicate either that the substance itself is transported, or that some antibody-like substance (or, possibly, a product derived from the inciting compound) manufactured at the site of injection, is responsible for the spread of sensitivity. The first

[12] Birnbaum, O., *Centr. Haut- u. Geschlechtskrankh.*, 1934, **49**, 97.

[13] The question of to what extent circulating antibodies are the necessary concomitant of immunological responses, is discussed by Zinsser, H., in Jordan, E. O., and Falk, I. S., The newer knowledge of bacteriology and immunology, Chicago, University of Chicago Press, 1928, 721.

hypothesis is rendered improbable, if, as Rackemann and Simon found, and as seems to be suggested by preliminary experiments of our own, the mode of introducing the substance, namely cutaneous application, is of significance for the success of the experiment. Another conceivable explanation for increased reactivity, namely accumulation of the substance injected into the body, appears to be excluded on quantitative grounds, since the sensitization effects are of long duration and can be obtained with amounts which altogether would not suffice to produce local lesions.

As pointed out in the review of the literature, immunization by simple substances could be explained if one might assume a combination of the compounds with protein, which is probable in the case of *p*-phenylenediamine.[14] In this connection it should be mentioned that recently Horsfall (30) reported on the sensitization of rabbits to formaldehyde by immunizing them with formalinized proteins. Such a mechanism would appear to be more probable than the supposition that the substances act as antigens by themselves since even with bacterial carbohydrates, which as a class can be supposed to have some antigenic activity, skin sensitization in guinea pigs has, so far, not been attained. With the substances dealt with in the present paper and others known to be the cause of idiosyncrasies, the assumption of the formation of antigenic conjugates in the animal is not so obvious. However it is known that in 2:4 dinitrochlorobenzene, indeed in most chloro and nitro substituted compounds mentioned in this paper, a chlorine atom or a nitro group is but loosely bound (31), so that some of these substances combine with bases, much like acyl chlorides. With the other compounds, for instance nitrosodimethylaniline, one must bear in mind that changes may occur in the animal body by which they acquire the capacity to enter into antigenic combinations with other substances;[14] and the possibility that the mechanism of sensitization may be different with various classes of compounds.

[14] For a thorough discussion and study of the chemical processes involved in dyeing with *p*-phenylenediamine and the chemical changes of aromatic bases in the animal body we refer to the studies of Cox, H. E., *Analyst*, 1929, **54**, 694; 1933, **58**, 738; 1934, **59**, 3; and Kracke, R. R., and Parker, F. P., *J. Lab. and Clin. Med.*, 1934, **19**, 799.

An investigation of these questions is under way particularly with regard to the fact that some of the substituted benzenes with easily detachable substituents did not elicit sensitization.

A closer study of a variety of properly selected compounds, as well as a search for antibodies and desensitization effects may serve to throw light upon the pending questions.

SUMMARY

Experiments on the sensitization of guinea pigs with simple chemical compounds are described. Positive effects were obtained by the administration of small quantities, namely fractions of milligrams, with 1:2:4 chlorodinitrobenzene, *p*-nitrosodimethylaniline, 1:2:4 trinitrobenzene, picryl chloride, four dichlorodinitrobenzenes, and a number of other aromatic compounds. Several substances chemically similar to those enumerated gave negative results. The first named compound is known to produce hypersensitiveness in human beings, a large number of cases having been observed in factory workers.

The mechanism of these effects is discussed.

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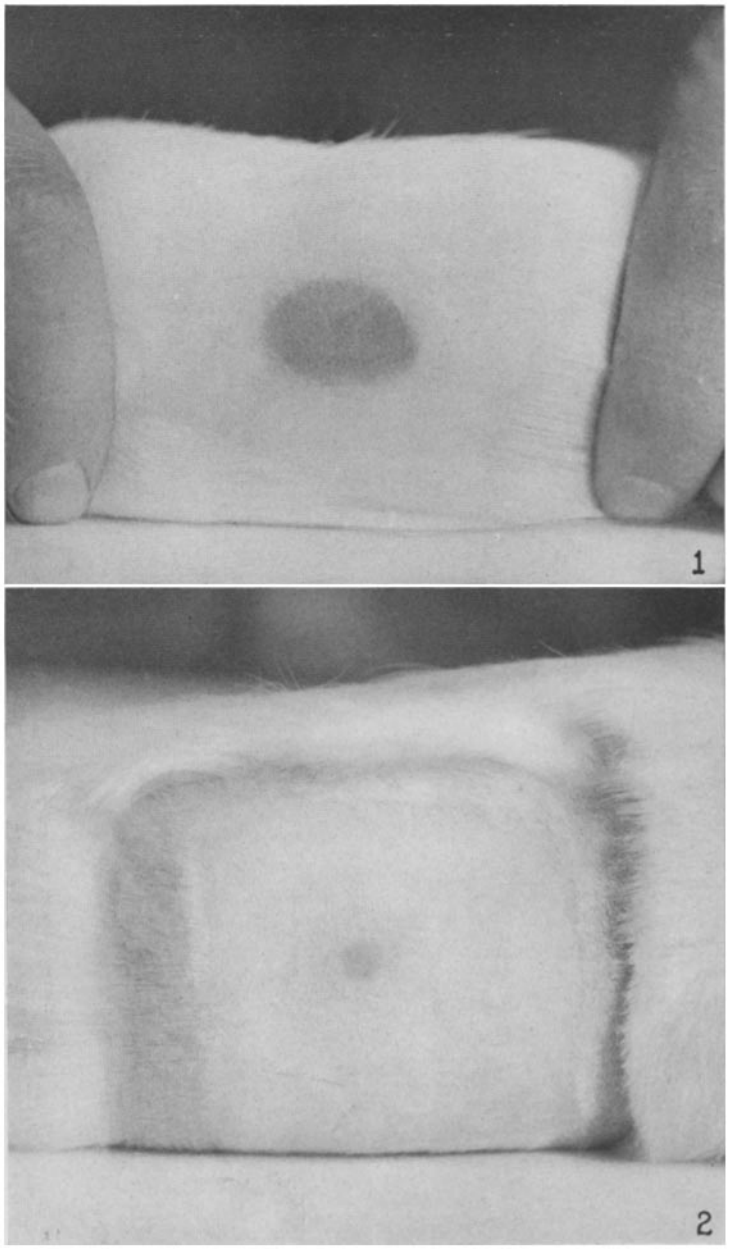
EXPLANATION OF PLATE 30

FIG. 1. Animal sensitized to 2:4 dinitrochlorobenzene and injected intracutaneously with 1/400 mg. of the substance.

FIG. 2. Untreated animal injected in the same way.



Photographed by Joseph B. Haulenbeek

(Landsteiner and Jacobs: Sensitization with simple chemical compounds)