STUDIES ON THE SENSITIZATION OF ANIMALS WITH SIMPLE CHEMICAL COMPOUNDS. II

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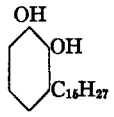
In an earlier paper (1) experiments were described in which, with the use of a suitable method, sensitization effects were produced by numerous simple compounds with satisfactory regularity. The work has been continued and also extended to include a number of substances not studied hitherto.[1]

Urushiol and Related Substances.—Urushiol is a substance contained in Japanese lacquer from *Rhus vernicifera*[2] which, like poison ivy, *Rhus toxicodendron*, produces intense dermatitis in a certain percentage of individuals.

The chemical identity of the effective substance in *Rhus toxicodendron* has been the subject of controversy, various substances having been held responsible. Acree and Syme (2) believed it to be a complex glycoside, and by others the active principle has loosely been described as an oleoresin. But a series of studies carried out by Majima (3), on the chief constituent of *Rhus vernicifera*, led him to the isolation of urushiol, a pale yellow viscous oil boiling at 200–210°C. at 0.4–0.6 mm., soluble in alcohol and petrol ether, which he showed to be chiefly *o*-dihydroxybenzene with a straight, unsaturated fatty chain, of the formula



According to Majima his preparation was not homogeneous but contained substances differing in the number and position of double bonds in the side chain, and also hydrourushiol. Urushiol was found by Toyama (4) and Majima (3) to

[1] The statement about negative results with quinine may need correction because one apparently positive and some questionable results have since been obtained in treated animals.

[2] We wish to express our thanks to the Mitsui Co. for their kind cooperation in furnishing a supply of Japanese lacquer.

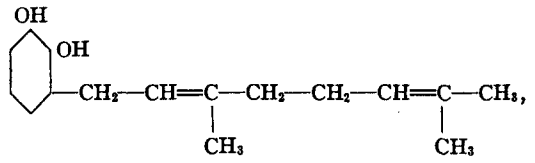
be the substance responsible for the injurious effects in human beings. In agreement with these findings McNair (5) reported that the toxic substance in poison oak, *Rhus diversiloba*, is probably an unsaturated ortho-dihydroxybenzene derivative, and later Hill and his coworkers (6) identified the active agent of poison ivy with that in Japanese lacquer, namely urushiol.

Sensitization of guinea pigs to poison ivy extracts, but not to the isolated active substance has been described by Rackemann and Simon (7), Dienes (8), and Simon (9). Therefore, the following experiments were made on guinea pigs with urushiol and related compounds.

One drop of a 3.0 per cent acetone solution of distilled urushiol was spread on the back (the hair having been clipped) with a glass rod, twice a week for 2 weeks, and tests were made after an interval of 3 weeks by gently spreading one drop of a 0.3 per cent acetone solution of urushiol on the flank; the reactions were recorded after application of a depilatory.

Distinct, pinkish reactions appeared at the sites of application by the next morning. On cross testing these animals and guinea pigs similarly sensitized to poison ivy extract, positive reactions were observed.

In order to study the influence of variations in molecular structure on sensitizations with this sort of substance, a related compound, geranyl catechol,



was synthesized according to the method of Kawai (10), and animals were sensitized as with urushiol excepting that an 0.8 per cent solution in acetone was used for testing. Some of the animals showed hypersensitiveness. In cross tests between geranyl cathechol and urushiol overlapping reactions were observed when animals treated with urushiol were tested with geranyl catechol.

With another compound, 4-tetradecyl catechol, prepared from myristic acid and catechol (11), some evidence of sensitization, although slight, was obtained in a limited number of guinea pigs, and

a few animals sensitized to urushiol gave faint reactions when treated with a 2.0 per cent solution of this compound in olive oil.

The biological activity of urushiol may well be connected with its oxidizability; observations by Hill (6) and Toyama (4) would tend to show that methylation of the hydroxyl groups greatly diminishes activity, and hydrogenation of the double bonds in the side chain considerably reduces it, according to the latter author; Hill found only a slight diminution.[3]

Methyl Heptine Carbonate $CH_3(CH_2)_4C \equiv C.CO_2CH_3$.—Another product examined was a commercial preparation, methyl heptine

TABLE I*

Application of a 20 Per Cent Solution in Alcohol of Methyl Heptine Carbonate on the Skin

No.	Animals sensitized to methyl heptine carbonate	No.	Controls
1	p., m.el.	7	a.neg.
2	d.p., sl.el.	8	a.neg.
3	p., sl.el.	9	f.–p.p.
4	p.p.–p., sl.el.		
5	p., sl.el.		
6	p., sl.el.		

* Here and in subsequent tables the following abbreviations are used: almost colorless (a.cls.), faintly pink (f.p.), pale pink (p.p.), pink (p.), and dark pink (d.p.). Other designations are: negative (neg.), almost negative (a.neg.), slightly elevated (sl.el.), elevated (el.), markedly elevated (m.el.), slightly swollen (sl.swol.), and swollen (swol.), livid center (liv.c.), necrosis (necr.).

carbonate, which was investigated because several cases have been described (12, 13) of hypersensitiveness to this substance in human beings when cosmetics were used.[4] The substance seemed of interest because it is an aliphatic compound, containing no aromatic rings.

Ten intracutaneous injections of 1/20 mg. in 0.05 cc. olive oil on the back, at weekly intervals, were followed by 2 weeks of rest, and the animals were tested by gently spreading 1 drop of a 20 per cent alcoholic solution of the substance on the flank.

[3] Concerning the toxicity of other catechol derivatives see Toyama (4).

[4] A preliminary report has appeared (14).

It may be seen in Table I that, although controls were negative, or, in a few cases, faintly pinkish, many treated animals gave frankly pink, raised lesions, indubitable evidence of sensitization, which was confirmed by a number of specificity tests.

TABLE II

Substance	K*	Reaction with aniline	Sensitization
1:2:4 Chlorodinitrobenzene	0.110 (0°); 3.26 (15°, eth.)	+	pos.
1:2:4 Bromodinitrobenzene	1.89 (15°, eth.)	+	"
1:2:4 Iododinitrobenzene	0.455 (15°, eth.)	+	"
1:2:4 Fluorodinitrobenzene	686.0 (15°)	+	"
1:4:2:6 Dichlorodinitrobenzene	0.0248 (0°)	+	"
1:3:4:6 Dichlorodinitrobenzene	1.20 (0°)	+	"
1:2:4:6 Chlorotrinitrobenzene	Very great† (0°)	+	"
1:3:5 Dichloronitrobenzene	Reaction irregular‡	−	neg.
1:4:2 Dichloronitrobenzene	0.00000297 (0°)	−	"
1:2:4 Dichloronitrobenzene	0.0000183 (0°)	−	"
p-Chloronitrobenzene	0.000000987 (0°)	−	"
p-Dichlorobenzene	0.00019 (175°)	−	"
1:2:4 Trichlorobenzene	Very small§ (0°)	−	"
1:2:4:5 Tetrachlorobenzene	Very small§ (0°)	−	"
Hexachlorobenzene	Very small (175°)	−	"

* Constants for the velocity of decomposition of the substances by sodium methylate, or sodium ethylate (eth.) at the temperature indicated. The figures are taken from papers from the Laboratory of Organic Chemistry of the University in Amsterdam (*Rec. trav. chim. Pay-bas*, 1890–1924, Vols. **9** to **43**), mostly by Holleman and coworkers.

† Velocity too great to be measured.

‡ Formation of azoxy compounds.

§ Value found by the authors to be very small, less than or of the same order as that for 1:2:4 dichloronitrobenzene.

On repeating the tests, it was found that different samples of the commercial substance showed distinct gradations in activity. It is therefore possible that the effect is due, not to methyl heptine carbonate itself, but to some impurity. In fact, on account of the method of preparation, one may assume that it is not very easy to obtain the substance in a condition of absolute purity. (The preparations used did not contain nitrogen.)

Cl, NO_2 Substituted Benzenes.—Our former results suggested strongly that ability to sensitize is connected with the chemical reactivity of the substances, making it probable that they form compounds in the animal body possessing antigenic activity. Table II, presenting more detailed data, will serve to support this conclusion. The table gives the sensitizing capacity of the chloro- and nitro- substitution products of benzene tested, along with the constants calculated from the reaction with sodium methylate, indicating the rate at which Cl is split off by alkali. One sees that those substances which have a very low constant did not sensitize, and also that the lowest constant of an active substance was many times greater than the highest constant of any inactive substance.

TABLE III

Substance	K_0*	Reaction with aniline	Sensitization
1:2:4:5 Dichlorodinitrobenzene	0.326	+	pos.
1:3:2:5 Dichlorodinitrobenzene	0.145	+	"
1:2:4 Trinitrobenzene	Very great†	+	"
1:3:5 Trinitrobenzene	1.57	−	neg.
m-Dinitrobenzene	Reaction irregular‡	−	"

* Constants for the velocity of decomposition of the substances at 0°C. by sodium methylate.

† Velocity too great to be measured.

‡ Formation of azoxy compound.

Turning now to those compounds in which the NO_2 group is replaced on treatment with sodium alcoholate or which do not contain chlorine, namely 1:2:4 and 1:3:5 trinitrobenzene, 1:2:4:5 and 1:3:2:5 dichlorodinitrobenzene, and *m*-dinitrobenzene, one finds that the correlation does not hold in that 1:3:5 trinitrobenzene ($K_0 = 1.57$) had no sensitizing capacity whereas the two dichlorodinitrobenzenes ($K_0 = 0.1$ and 0.3) and some chlorine labile compounds with smaller constants, were effective (Table III).

On account of this discrepancy we investigated how the substances react with an organic base (aniline), and the same sort of experiment was made with the compounds listed in Table III.

1. The substances known to possess labile halogen (Table II) were dissolved in absolute alcohol and aniline (5 mols) added. The mixtures were heated in sealed tubes in the steam bath for 2 hours and the quantity of liberated halogen determined. In column 3 of Table II the symbol + designates almost complete liberation of halogen (more than 90 per cent) and the symbol − indicates that no or very little (less than 5 per cent) halogen was replaced.

2. Five millimols of the substances appearing in Table III were dissolved in 10 cc. of absolute alcohol and 15 millimols of aniline added. The mixtures were heated in sealed tubes in the steam bath for 15 hours, evaporated to dryness, washed with a little absolute alcohol, and recrystallized twice from the same solvent. (The same results were obtained on heating mixtures set up as in (1) for 2 hours). Column 3 of Table III summarizes the experiments, + being used to indicate the formation of a substitution compound, and − for addition compound formation.

Now it was found that 1:2:4 trinitrobenzene, 1:2:4:5 and 1:3:2:5 dichlorodinitrobenzene gave substitution compounds with aniline whereas this was not the case with 1:3:5 trinitrobenzene and *m*-dinitrobenzene.

The compound formed on treating 1:2:4 trinitrobenzene with aniline has been described by Hepp (15); from 1:2:4:5 dichlorodinitrobenzene we obtained an orange colored substance melting at 94–95°C., N calculated 9.89 per cent (for $C_{12}H_8O_2N_2Cl_2$), found 9.83 per cent; from 1:3:2:5 dichlorodinitrobenzene a compound melting at 114–115°C., N calculated 9.89 per cent (for $C_{12}H_8O_2N_2Cl_2$), found 9.71 per cent. 1:3:5 trinitrobenzene forms a highly colored addition compound (15) which gives off aniline readily when exposed to air; *m*-dinitrobenzene also forms an addition compound (16), and in both cases the original nitro compound was readily recovered unchanged.

Of the substances given in Table II those which sensitized, formed substitution compounds; in all these cases 90–100 per cent of the theoretical amount of halogen was liberated. The nonsensitizing substances treated in the same way gave off no or but little chlorine.

The difference in sensitizing activity of 1:2:4 trinitrobenzene (active) and 1:3:5 trinitrobenzene (inactive) mentioned before, but without details, was reinvestigated with a larger number of animals, since it seemed to be particularly striking on account of the structural similarity of the two compounds, different only in the position of the NO_2 groups. Parallel experiments with the two compounds were carried out as given below.

In both cases, a batch of white guinea pigs was injected with 1/400 mg. (0.1 cc. of a solution made by adding 0.5 cc. of a 0.3 per cent alcoholic solution to 60 cc. saline) intracutaneously, on the back, at weekly intervals for 10 weeks and after 2 weeks of rest the animals were tested by gently spreading 1 drop of a 1 per cent olive oil solution, or by an injection similar to those used for sensitization, on the flank. The first mode of application, namely to the surface of the skin, gave the most striking effects, the controls being, as a rule, com-

TABLE IV

Treated and tested with	Treated animals			Controls		
	No.	Application of a 1 per cent solution in olive oil on the skin	Intracutaneous injection of 1/400 mg. in 0.1 cc. saline	No.	Application of a 1 per cent solution in olive oil on the skin	Intracutaneous injection of 1/400 mg. in 0.1 cc. saline
1:2:4 Trinitrobenzene	10	f.p.	8,* p.p., el.	21	neg.	neg.
	11	p., el.	10, f.p., el., liv.c. 3	22	"	5, p.p., el.
	12	f.p.	9, p.p., el.	23	"	neg.
	13	p.p.	8, p., el.	24	"	a.neg.
	14	p., sl.el.	9, p.p., el.			
1:3:5 Trinitrobenzene	15	neg.	neg.	25	a.neg.	neg.
	16	"	"	26	neg.	a.neg.
	17	"	"	27	"	a.neg.
	18	"	"	28	a.neg.	neg.
	19	"	"			
	20	"	a. neg.			

* The figures give diameters of the lesions in millimeters.

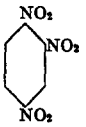
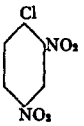
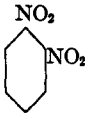
pletely negative. The results show that the 1:2:4 substituted compound is an active sensitizing agent, producing lesions similar to those obtained with 2:4 dinitrochlorobenzene (1), while the symmetrical compound proved to be entirely inactive (Table IV).

The above comparison of the substances demonstrates a parallelism, in the compounds examined so far, between sensitizing capacity and chemical behavior. In most cases a correspondence was evinced between lability of Cl and NO_2, when treated with alkali, and sensitizing effects; and in all cases tested there was agreement between

ability to sensitize and formation of substitution compounds with an organic base. It seems reasonable to assume that in the animal a reaction takes place by which the substances are converted into antigens. How and with what substances, proteins or others, a combination of the active compounds occurs, remains to be ascertained. For some of the sensitizers dealt with herein, compounds with amino acids or peptides have been reported (17, 18); also with thiols (19, 20) and phenols (21).

TABLE V

Application of One Drop Each of a 2.0 Per Cent Solution of 1:2:4 Trinitrobenzene and 1.0 Per Cent of 2:4 Dinitrochlorobenzene in Olive Oil on the Skin

Treated with	No.	Tested with	
			
	29	p., el.	p., el.
	30	f.p.	f.p.
	31	p., el.	p., el.
	32	p.p.–p., el.	p.p.–p., el.
	33	f.p.	f.p.
	34	p., el.	p., el.
	35	p., el.	p., el.
	36	p.p., el.	p., el.
Controls	37	neg.	neg.
	38	"	"
	39	"	"

In keeping with the foregoing was the fact that the two substances 2:4 dinitrochlorobenzene and 1:2:4 trinitrobenzene gave overlapping reactions, as has already been noted (1); in fact, they were practically interchangeable, a result to be expected inasmuch as both compounds will, after removal of Cl or NO_2, respectively, yield the same conjugate (Table V).

Various ways to sensitize intracutaneously with 2:4 dinitrochlorobenzene[5] have been tested simultaneously in batches of 5 animals each: 16 injections, 2 a week (the poorest, perhaps by chance); 16 daily injections; 8 weekly injections; and 8 injections, 2 each week; all of 1/400 mg.; also 16 rubbings, 2 a week, of a 1 per cent olive oil solution. The animals were tested 2 weeks in this experiment, after the last injection, before testing by application of a 1 per cent olive oil solution. All of the above treatments resulted in distinct sensitization although 8 weekly injections seemed to be somewhat the superior method, by a slight margin. At another time a very satisfactory effect was seen after 8 daily injections; the animals were tested after an interval of 25 days.

The striking superiority of cutaneous sensitization, already mentioned in our recent paper, had previously been emphasized by Sulzberger (23) who also reported observations on variations in efficiency of intracutaneous and other methods of administration, in ordinary anaphylaxis. Attempts to sensitize guinea pigs intravenously or subcutaneously to 2:4 dinitrochlorobenzene were almost ineffective in three experiments. However, since one experiment gave doubtful results, possibly due to faulty technique, further confirmation would be desirable.

Benzyl Chlorides.—From the point of view suggested, it should be possible to find substances producing sensitization merely on the basis of their chemical properties. Consequently, it appeared promising to examine benzyl and acyl chlorides, containing easily detachable chlorine, although these substances have not yet been reported as sensitizing agents in human beings.

The experiments on benzyl chlorides were in general similar in their outcome to those observed with the substances already discussed. Marked results were obtained with *o*-chlorobenzyl chloride and 2:4 dinitrobenzyl chloride, and positive effects were noted likewise with *p*-chlorobenzyl chloride, benzyl chloride, and *p*-nitrobenzyl chloride.

The solutions for injection were prepared as above. In each case 1/100 mg. was injected twice a week for 12 weeks, followed by 2 weeks of rest. Tests were made with the solution used for sensitization, or olive oil solutions, a 20 per cent solution being used for *o*-chlorobenzyl chloride, and a 1.0 per cent solution for 2:4 dinitrobenzyl chloride. The results with these two substances are summarized in Table VI.

[5] Since our communications Wedroff and Dolgoff (22) have reported the experimental sensitization of human beings with the substance.

When 2:4 dinitrochlorobenzene was compared with 2:4 dinitrobenzyl chloride a distinct difference was noted although cross reactions were observed; 2:4 dinitrochlorobenzene was different from *o*-chlorobenzyl chloride (Table VII). The two benzyl chlorides, on the other hand—2:4 dinitrobenzyl chloride and *o*-chlorobenzyl chloride—interacted.

TABLE VI

Application of a 1 Per Cent Solution of 2:4 Dinitrobenzyl Chloride in Olive Oil on the Skin

No.	Animals sensitized to 2:4 dinitrobenzyl chloride	No.	Controls
40	p.	56	neg.
41	p.p.	57	"
42	d.p.	58	"
43	d.p.	59	"
44	p.p.–p.	60	"
45	p.p.	61	f.p.–p.p.
46	p.p.		
47	p.p.		

Application of a 20 Per Cent Solution of o-Chlorobenzyl Chloride in Olive Oil on the Skin

No.	Animals sensitized to *o*-chlorobenzyl chloride	No.	Controls
48	p.p., sl.el.	62	neg.
49	p., sl.el.	63	"
50	d.p., el.	64	a.neg.
51	p., sl. el.	65	a.neg.
52	p.p.	66	a.neg.
53	p., sl.el.		
54	p.p.–p., el.		
55	p., el.		

Acyl Chlorides.—In experiments made on a small scale, indications of sensitization were obtained with some acyl chlorides, such as benzoyl chloride, *p*-bromobenzenesulfonyl chloride, *m*-nitrobenzenesulfonyl chloride, and 2-nitrotoluene-4-sulfonyl chloride.

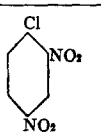
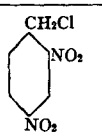
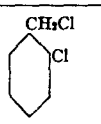
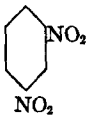
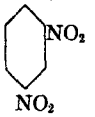
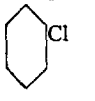
One of the acyl chlorides, *p*-chlorobenzoyl chloride, which exhibited unusually strong reactions, well developed after 24 hours, after the third or fourth injection of 1/80–1/100 mg. in olive oil solution, with

edema and pinkness over an area several centimeters in diameter and central necrosis, was investigated more extensively.

In such an experiment four weekly injections of 1/100 mg. *p*-chlorobenzoyl chloride in 0.05 cc. olive oil were given intracutaneously on the back, when necrotic lesions began to develop following new injections. The guinea pigs were then rested for 2 weeks and tested by spreading

TABLE VII

Combined Table. Application of One Drop of an Olive Oil Solution on the Skin

Treated with	No.	Treated with		
				
	67	f.p.	neg.	neg.
	68	p., el.	"	"
	69	p., el.	"	"
	70	p., el.	f.p.	"
	71	p., el.	neg.	"
	72	p.	p., sl.el.	p.
	73	neg.	p., sl.el.	f.–p.p.
	74	p.p.–p.	p.	p.p.
	75	f.p.	p.	f.–p.p.
	76	a.neg.	p.	f.p.
	77	neg.	f.p.	p.
	78	"	p.p.	p.
	79	"	f.–p.p.	p., el.
	80	"	f.p.	p.
	81	"	p.p.	p.

1 drop of a 10 per cent olive oil solution on the flank with a glass rod, or by an injection like those used for sensitization. In the surface applications, areas in contact with the olive oil solution developed pinkish to pink skin reactions of the usual appearance (Table VIII). Following injections with oil solutions the sensitized guinea pigs showed lesions similar to those observed during the treatment (Table IX).

TABLE VIII

No.	Skin reactions in sensitized animals after application of 10 per cent *p*-chlorobenzoyl chloride in olive oil	Amount injected	Intravenous injection of *p*-chlorobenzoylated guinea pig serum
		mg.	
82	p.p.	10	Typical anaphylaxis † 6 min.
83	p.p.–p.	5	" " † 5 "
84	p., sl.el.	2	" " † 5 "
85	p., el.	2	" " † 5 "
86	p.	2	" " † 7 "
87	p., sl.el.	1	" " † 15 "
88	p.p.	1	" " † 2 "
89	p.p., el.	1/4	" " † 6 "
90	p.p.	1/10	Coughs, eyes running; recovered
	Controls		Controls
91	neg.	10	No symptoms
92	"	10	" "
93	"	10	" "
94	"	2	" "
95	"	1	" "
96	"	1/4	" "
97	"	1/10	" "

† Death of animal.

TABLE IX

Intracutaneous Injection of 1/80 Mg. p-Chlorobenzoyl Chloride in 0.05 Cc. Olive Oil

No.	Animals sensitized to *p*-chlorobenzoyl chloride	No.	Controls
98	35,* p., swol., necr. 6	106	9, a.cls., m.el.
99	25, p.p.–p., swol., necr. 7	107	11, p.p., el.
100	40, p.p.–p., sl.swol., necr. 7	108	8, p.p., m.el.
101	40, p.p.–p., sl.swol., necr. 9	109	10, p.p., m.el.
102	45, p.p., swol.	110	9, p.p., el.
103	40, p.p.–p., sl.swol., necr. 8		
104	40, p.p.–p., sl. swol., necr. 8		
105	40, p.p.–p., sl.swol., necr. 6		

* The figures give diameters of the lesions in millimeters.

Animals sensitized to *o*-chlorobenzyl chloride were rather sharply distinguishable in their reactions from those treated with *p*-chlorobenzoyl chloride. A cross test with animals sensitized one each to the following substances:

p-bromobenzenesulfonyl chloride, *m*-nitrobenzenesulfonyl chloride, *p*-chlorobenzyl chloride, and benzyl chloride showed a marked degree of specificity except for cross reactions between the two sulfonyl chlorides.

Thus, while in discussions of the subject one encounters the opinion that there is no connection between the ability of substances to sensitize and their chemical nature, it appears from the foregoing that whole groups of substances—benzyl chlorides, acyl chlorides—characterized by a certain chemical reactivity, have the capacity to produce sensitization.[6]

With acyl chlorides there was the possibility of preparing protein compounds containing the acyl radicals and it was clearly of interest to investigate the effects of such conjugates in animals sensitized with the chlorides. This was done in the case of *p*-chlorobenzoyl chloride.

To 30 cc. of guinea pig serum (or horse serum) was added 15 cc. normal sodium carbonate solution and 1 millimol of *p*-chlorobenzoyl chloride in 5 cc. chloroform. The mixture was shaken vigorously for 10 minutes, centrifuged, the water layer acidified to maximum precipitation, centrifuged, and washed several times. The substance was redissolved in saline solution with alkali, neutralized, and made up to a 2 per cent solution.

When small quantities of the protein preparation were injected intravenously into guinea pigs sensitized to *p*-chlorobenzoyl chloride and showing positive skin reactions, a month after the skin tests, it was found that they died in acute, typical anaphylactic shock, as shown in Table VIII.

Injected into the skin, the acylated protein produces an immediate flare and wheal, followed, in stronger concentrations, by large pinkish edematous reactions on the next day.

The anaphylactic experiment described resembles to some extent the production of an anaphylactic state by injections of diazonium compounds (25) with the distinction, however, that in the latter case no skin reactions were observed of the type of "contact dermatitis" as is seen with the acyl chlorides.

This result, together with the before mentioned skin reactions with the substance, indicate that the two forms of hypersensitiveness observed in this case, sensitization of the skin and anaphylaxis, are

[6] In this regard reference may be made also to the work of Mayer (24).

intimately associated states. Thus there may be produced in an animal by one and the same treatment an allergic state with the following manifestations: pink, sometimes elevated eruptions on the treated site resulting from superficial skin applications of the simple substance; large, pinkish, edematous lesions following its intracutaneous injection in small quantities; an immediately noticeable flare and swelling, developing into rather large lesions within a day, and typical anaphylaxis, on intracutaneous and intravenous injection, respectively, of the protein compound.

Benzoyl chloride behaved, in all respects, much like *p*-chlorobenzoyl chloride.

Further evidence relating to anaphylaxis induced by chemicals was afforded by experiments with arsphenamine, to be reported later, in which typical anaphylactic shock was produced in a high proportion of the guinea pigs treated.

The chemical experiments were carried out with the assistance of Mr. Robert A. Harte.

SUMMARY

In continuation of previous work sensitization experiments have been made with various substances such as urushiol, benzyl chlorides, and acyl chlorides. In the case of a series of substituted benzenes (Cl, NO_2) a connection between sensitizing capacity and lability of the Cl or NO_2 groups has been shown, indicating the formation of conjugated antigens in the animal. This led to the study of benzyl and acyl chlorides which, actually, were found to have sensitizing capacity. Most informative as to the relationship between reactions of the skin surface and anaphylaxis were experiments with acyl chlorides. Guinea pigs sensitized with *p*-chlorobenzoyl chloride showed, on the one hand, the usual surface lesions after application of the substance, and on the other typical anaphylactic shock following intravenous injection of a compound of *p*-chlorobenzoyl chloride and guinea pig serum; from which it may be inferred that the two types of allergic manifestation are closely related conditions.

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