STUDIES ON THE SENSITIZATION OF ANIMALS WITH SIMPLE CHEMICAL COMPOUNDS

VI. Experiments on the Sensitization of Guinea Pigs to Poison Ivy

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One of the open questions concerning skin sensitization of the "contact dermatitis" type to simple chemical compounds is in which way sensitivity spreads all over the integument when only a given area is treated. This subject has been considered in a paper by Simon (1) and studied experimentally. The skin of guinea pigs was treated with concentrated nitric acid in such manner that a complete belt severed the continuity of the epidermis. Poison ivy extract was applied on the posterior part of the animals and after 10 days the anterior and the posterior parts were tested; both halves were seen to be sensitive. According to the author the evidence "indicates that the route of distribution is not confined to the epidermis," and suggests that the spread is due to distribution of altered allergen by the blood stream or the lymphatic system.

Furthermore, Simon observed that no significant difference exists in the degree of sensitivity between the area used for the sensitizing application and other parts of the skin, and by timed excisions found that such removal of the treated area did not interfere with the general skin sensitization provided it was performed later than 18 to 24 hours after the application of ivy extracts. He also found that sensitivity was first seen after a latency period of from 4 to 6 days.

In contrast to the result of Simon, Straus and Coca (2) reported that in monkeys severance of the continuity of the skin some distance above the elbow and application of poison ivy extract to the forearm

resulted in sensitization of this part only and prevented general skin sensitization. The experiment was also performed in reversed fashion. In the authors' opinion, their results suggest that the spread of sensitization is attributable "probably to a diffusion of the oily excitant through the oily substances normally present in the skin," in harmony with a hypothesis previously advanced by Coca (3, 2).

Similar results have recently been described in a preliminary communication by Schreus (4) who treated guinea pigs with dinitrochlorobenzene, a substance found in this laboratory (5, 6) to sensitize these animals. This author assumes spread by way of the intercellular bridges connecting the cells of the epidermis.

Further investigation of the subject was desirable, particularly in view of the lack of agreement in the reported experiments.

General Methods

Male albino guinea pigs from healthy stock, caged separately, were employed in the experiments; since operative procedures were to be carried out, rather heavy animals were commonly selected (500 to 600 gm. weight). For uniformity as regards the period allowed for sensitization (10 to 14 days) a group included only the animals operated upon within a 3 day period, and these were tested at the same time; in instances of comparisons between differing methods, the operations in question were done as far as practicable in alternation to avoid technical bias. All operations were conducted under deep ether anesthesia.

Sensitizing Application.—Poison ivy extract Lederle,[1] supplied as a 12½ or 13 per cent solution in acetone of extractives from *Rhus toxicodendron radicans*, was used to sensitize guinea pigs by application to the skin on a clipped (frequently also shaved) site, spread evenly over a circular area 10 to 11 mm. in diameter with the tip of a thin glass rod. The doses ranged from 0.05 cc. of the undiluted extract to 0.025 cc. of a 1:5 dilution in acetone or less, delivered as a series of micro drops (0.05 cc. = 10 to 13 drops) from a gauge 26 Luer needle with tip ground flat, attached to a 1 cc. Dewitt and Herz record (tuberculin) syringe where the amount discharged can be accurately controlled by a check-nut on the plunger shaft. Upon full evaporation of the solvent, an even, brownish, waxy film remained on the skin.

Rather elaborate precautions were taken in the case of experimental skin barriers to ensure that no ivy material could come into contact with other parts of the skin than the desired field. To this end, several layers of bandage gauze and an outer covering of crepe tissue paper were used to shield the body, the site

[1] This material came to us through the courtesy of Dr. Arthur F. Coca of the Lederle Laboratories. It may be recalled that the active principle of poison ivy is urushiol, a catechol with a fifteen carbon, unsaturated side chain (*cf.* 6).

of application being exposed through a hole in the coverings, which were firmly held against the skin, and likewise all instruments used after application of the ivy extract were not handled again until they had been cleaned chemically (with alcohol, and then with potassium permanganate). Finally the deposit was covered with a 14 mm. square of cellophane (cut from cellophane tubing, each of the two adhering layers being about 0.002 inch thick) cemented to the skin, along its margin, by means of duo liquid adhesive (Johnson and Johnson); over this was affixed a disc, about 30 mm. in diameter, of finely woven linen, the adhesive first being applied both to the periphery of the linen and to the skin outside the cellophane patch.

On the 4th or 5th day the ivy was removed, either, in early experiments, by cleaning the site with olive oil and acetone and painting with aqueous permanganate solution, or by excision, *in toto*, of the treated skin and its protective coverings, as described below.

Test for General Cutaneous Sensitivity.—On the 10th to 14th day following the original application of ivy extract to the skin, various areas were clipped with an electric clipper, and on these sites single drops (approximately 1/60 cc.) of freshly prepared dilutions in 95 per cent alcohol of the extract were allowed to fall from a fine capillary pipette. The drops were at once spread with the tip of a thin glass stirring rod over an area about 10 to 11 mm. in diameter and dried in a gentle current of air from a small electric blower; the sites were marked by touching the centers with the moistened tip of an indelible pencil.

The highest concentration of ivy causing no, or occasionally a slight reaction with normal animals was determined for each lot of extract (a 1:15 or 1:20 dilution), and was applied to the flank and to 2 sites on the back while areas close to these were tested with half the concentration (see Figs. 1, 2); and one-half and one-third concentration were used on the belly.

First readings were taken 24 hours later, some time after the sites had been cleaned with pledgets of cotton wet with acetone; second readings were made the day following. With the most sensitive animals, the reactions are strongest at 24 hours, but then turn brownish and begin to fade, while animals of lesser sensitivity may exhibit higher reactions at the second reading. In our experience, there was no difficulty in recognizing the difference between even moderately sensitized animals and normal controls similarly tested.

The intensity of the reactions was designated as follows: ++++, pink or dark pink, sometimes slightly elevated; +++, pink, but either somewhat pale or macular; ++, between faint pink and pale pink in color; +, faint pink; ±, faint pink ring; tr., trace; f.tr., faint trace.

EXPERIMENTAL

In order first to determine, under the conditions of our experiments, how long the active material must remain in contact with the skin to induce hypersensitiveness, and the length of the latency period before sensitivity appears, experiments similar to those of Simon (1; *cf.* 7)

were made. As regards the first question, sensitization was found to result if the treated area was extirpated later than 8 to 12 hours following the application (Table I).

TABLE I

Composite Table

Sensitization with poison ivy extract in relation to the excision of treated skin areas at different times (see text). The readings recorded were made 24 hours after application of the test doses on the 11th day; also observations at 48 hours are given (within parentheses) when there was an increase over the earlier reactions.

No.	Time before excision	Reactions to ivy extract			
		Dorsum		Flank	
		Dilutions		Dilutions	
		1:15	1:30	1:15	1:30
	hrs.				
1	4	f.tr.	0	0	0
2	4	f.tr.	0	f.tr.	0
3	6	0	0	0	0
4	6	0	0	0	0
5	8	± (+++)	± (±)	± (++)	tr. (++)
6	8	0	0	0	0
7	12	++++	++	++++	+
8	12	++	+	+++	+++
9	16	++ (+++)	± (++)	± (+++)	± (+)
10	16	++++	++++	++++	++++
11	16	++++	+++	++++	+++
12	16	+ (+++)	tr. (±)	+ (++++)	± (+)
13	24	++++	+++	+++	+++
14	24	++++	+++	++++	+++
		Normal controls			
15		0	0	tr.	0
16		f.tr.	f.tr.	0	0
17		f.tr.	f.tr.	tr.	0
18		± (tr.)	0	0	0
19		tr.	tr.	±	tr.
20		tr.	0	tr.	0

A linen ring having an outer diameter of about 26 mm. and an 18 mm. opening was affixed to the skin in the sacral region of the back by means of the liquid adhesive mentioned. The body of the guinea pig was shielded and only the skin

within the ring was exposed; on this site 0.03 cc. poison ivy extract was put on a circular area approximately 12 mm. in diameter. Upon evaporation of the acetone the residue was covered by a 14 mm. square of cellophane cemented at the corners to the skin and above it a linen disc 26 mm. in diameter was attached similarly to the linen ring. Cardboard collars (adapted from (8)) were fixed around the neck to keep the coverings undisturbed. After varying intervals the skin bearing the ivy and coverings was excised 1 to 2 mm. beyond the linen ring (with anesthesia); in this way not only the ivy-treated area but also a wide margin (8 to 11 mm.) outside was removed and the defect was dressed with thymol iodide. The animals were tested for sensitivity on the 11th or 12th day.

The somewhat shorter period of contact as compared with that of Simon possibly is due to the use of a larger dose of ivy extract or to some other technical difference. The necessary time of contact may be that required for absorption of a sufficient quantity of the incitant (which would vary according to such conditions as the amount and size of the treated area) or possibly an interval during which some chemical process takes place in the skin.

In our experiments on the latency period, hypersensitivity became manifest to tests made 5 days after the ivy was placed on the skin. Remarkably, the onset was quite regularly sudden with well developed reactions to the larger test dose present at the 24 hour reading and in tests made after 6 days the reactivity already was maximal (Table II).

The sudden appearance of hypersensitivity was further emphasized by the coincident and equal reactivities seen on the 6th day to the applications of the 5th day (one day reading) and the 4th day (48 hour reading), the latter having produced no significant reaction at 24 hours.

As pointed out before, the main issue of our investigation was rather the question whether an epidermal pathway is a necessary condition for the spread of sensitivity from one site to the whole body. In the first experiments, an area of skin on the back was isolated during deep anesthesia by means of a circular cautery burn roughly 5 mm. wide and deep enough to destroy the epidermis with certainty (*cf.* 1), and then was treated with ivy. The site was covered to avoid mechanical transfer and after 4 to 5 days was carefully freed from the incitant, or the whole isolated area was excised. When the animals were tested 6 to 8 days later, all were definitely hypersensitive, in varying degrees.

While this experiment in agreement with Simon's appeared to show

that interruption of epidermal continuity did not prevent general sensitization, we desired to attempt the experiment under more rigorous conditions. For this purpose a deep circular cut down to the muscles of the trunk was made on the back (or flank), thus isolating an island of skin, the edges, through contraction, being separated by a wide gap. By excision of additional strips of skin, depending upon the location, the gap was made uniformly wide (7 mm. or more).

TABLE II

Latency period preceding hypersensitivity to poison ivy. 0.05 cc. extract was applied to the sacral region and left for 4 days, covered with cellophane and linen patches; the residue then was removed with solvents and the site swabbed with $KMnO_4$ solution. Test applications on the back were made at the times noted after the ivy was placed on the skin, and concomitantly on additional animals as controls; the test doses were removed on the succeeding day. The table shows the 24 hour readings of the reactions to both dilutions, 1:20 and 1:40, separated by the slant line.

No.	Reactions to ivy extract applied at stated intervals following the primary administration					
	48 hrs.	3 days	4 days	5 days	6 days	7 days
21	0 / 0	f.tr. * / 0	tr. / 0	± / 0	++++ / ++	++++ / +++
22		0 / 0	0 / 0	++++ / +++	++++ / ++	+++ / +
23		0 / 0	0 / 0	++++ / +++	++++ / +++	
24		0 / 0	0 / 0	+++ / +	++++ / +±	
25		tr. * / 0	tr. / 0	+++ / +	++++ / +++	++++ / +++
26			f.tr. * / 0	+++ / tr.	++++ / +++	++++ / +++
27			f.tr. * / 0	++++ / +	++++ / +++	++± / +
28				+ / 0	++± / ±	+++ / +
29				++ / 0	+++ / +	+++ / ++
30				± / tr.	++++ / +++	++++ / ++±
31					+++ / ±	+++ / +
32					++++ / +	+++ / ++
33						+++ / +
34						++++ / ++++

* These reactions were less, or not greater than those exhibited by one or more of the 3 or 4 controls tested at the same time; on the average, about half of the controls showed trivial reactions (f.tr. or tr.) to the higher concentration.

With this technique the results were now different, sensitization being obtained in some cases but inconsistently and, with few exceptions, of low degree. In order to obtain more decisive results, several different procedures were tried. Finally definitely positive or negative sensitization effects across a dermal barrier were obtained almost regularly, depending upon whether or not the thin muscle layer underlying the cutis, the panniculus carnosus, was severed as well as the skin. A cut was made upon the flank circumscribing an area of skin; poison ivy

extract was applied in the center of this area, which then was carefully covered to prevent transfer of the active material to other parts of the integument. 4 or 5 days later the skin island, together with the protective coverings, was removed. By inspection it was easy to make sure at this time that no appreciable epithelial growth had taken place and that the edges of the epidermis were still wide apart; this macroscopic observation was confirmed by histological examination. Between the 10th and 14th days the animals were tested on various sites (see Figs. 1 to 4). The operations described were well tolerated, and within the 14 day period there was an actual gain in weight, averaging from 15 to 40 gm. with the different groups of animals.

The procedures will be described in some detail because they are of importance for obtaining clear cut results.

Flank Islands with Panniculus Carnosus Intact.—The position of the desired island was sketched with pencil on the clipped and shaved left flank, with the guinea pig in resting posture; after the animal under ether anesthesia had been fixed to the board on its side so as to maintain a right angle between trunk and extended hind limbs, without displacement of the loose flank skin, the outline was completed with the help of an ovoid stencil, 43 mm. by 37 mm. along the axes. (It might prove possible to make use of smaller islands in this type of experiment.) In an animal of about 550 gm. weight, the anterior midpoint of the line was, for instance, about 15 mm. posterior to the scapular angle. A second line was then drawn, to give a nearly rectangular figure, situated outside the first by 5 mm. at the dorsal and ventral midpoints, 6 to 7 mm. at the anterior and posterior midpoints, and separated by as much as 10 mm. along the diagonal diameters midway between dorso-ventral and antero-posterior diameters. (This technique assists in obtaining an island of circular shape, and a uniform width in the denuded ring.)

After the flank was sponged with alcohol, shallow incisions were made along both lines and carried cautiously down towards the panniculus carnosus, which appears as a grayish layer below the firm white connective tissue; the intervening skin was then carefully and sharply dissected from the panniculus, the procedure being facilitated by use of a binocular loupe. The field of dissection was kept moist with wet dressings, to reveal any residual bridges of dense connective tissue. Relatively little bleeding occurred. Upon contraction, the skin thus isolated becomes a nearly circular island of about 35 mm. diameter, surrounded by a ring-shaped defect about 8 mm. wide (Figs. 4, 5). When correctly done, which requires attention to detail, the operation will leave patent for the greater part the lymph vessels extending across the panniculus, as may be evidenced by intracutaneous injection into such islands of solutions of dyes (9) *e.g.*, pontamine sky blue 6 B.

With the body protected against accidental contact, the ivy extract was applied to the center of the island in the manner described previously; then the cellophane and linen coverings were cemented to the island over the ivy material.

The wound was kept dry and clean by applying thymol iodide (Merck); this treatment was repeated several times, and thereafter once daily.

The claws of the hind feet were covered with boots of adhesive tape and, besides, the animals were prevented from disturbing the bandage by attachment of a cardboard collar or, preferably, by the following device: by means of a strip of cotton twill tape attached to an adhesive tape band around the right thigh and then affixed to a collar of iron wire covered with soft rubber tubing, worn around the neck, the leg was advanced toward the neck sufficiently to keep the trunk bent slightly sidewise, away from the flank island. This arrangement, maintained until subsequent excision of the islands, prevented narrowing of the defect and kept the cut surfaces from adhering, without otherwise interfering with the free movements of the animal. The isolated skin was surrounded by an even, circular moat, which remained dry (but for exceptional bleeding) and in which a crust appeared about the 3rd day. Frequent inspections were made, and occasionally blood clots or dried exudate were removed. (In the early experiments where cardboard collars were employed, free movements of the flank sometimes led to a sticking together of the opposing cut surfaces: whenever this occurred, the animals were withdrawn from the experiment.)

Flank Islands with the Panniculus Severed.—The location of the islands and the stencil used were the same as described above. The incision was extended downwards through the panniculus, whereupon the muscle contracted to give a gaping moat. Consequently, only narrow (3 to 4 mm.) strips of skin opposite the anterior and posterior margins of the island had to be removed in order to secure the same outer diameters of the ring (about 50 by 50 mm.) which obtained in the operations where the panniculus was left intact. Blood vessels were tied off when necessary. The isolated skin areas thus created measured about 29 mm. along the antero-posterior axis and 32 mm. dorso-ventrally (Fig. 3). (When dye is injected intracutaneously into such islands, it infiltrates the tissue and in some measure oozes out into the moat.) Application of ivy extract, protective coverings, and postoperative care followed the procedures in the section above; to be emphasized is the collar-to-leg method of restraint in order to maintain an even and dry moat despite the loose connective tissue between panniculus and trunk muscles. (By frequent application of thymol iodide, the wound dries satisfactorily, although a small area along the ventral surface may remain moist for a day or two.)

Excision of the Islands.—On the 4th day, exceptionally on the 5th, the hair near the island was clipped and, with the animal under deep ether anesthesia, the isolated skin with ivy material and coverings undisturbed was removed by dissection, usually sparing the panniculus carnosus. Vessels were clamped off and tied. Finally, the whole area was packed with thymol iodide and covered with sterile cotton cloth, which was cemented to the skin. In the case of the

islands where the skin muscle had been cut, there was edema by the 2nd or 3rd day, and during excision free fluid was present; the panniculus itself and the underlying tissues appeared thickened.

From the results of the two sorts of operations (Table III *a*), it is seen that of 25 guinea pigs in which an area of the skin had been isolated with severance of the skin muscle and the superficial lymph vessels, only two attained a marked degree of sensitivity and a few others gave some evidence of a slight sensitization; where the panniculus and the lymphatic trunks passing over its surface were spared, however, and this despite the need of care in the operation, all of the 25 animals became sensitive, almost regularly in high degree and comparable to the sensitization elicited by like treatment of normal animals (treated with group D, Table III *a*); one showed a low grade sensitivity and some a moderate hypersensitiveness. It will have been noted that a large dose was selected for the sensitizing application in these experiments (0.03 or 0.05 cc. of undiluted ivy extract); by this means the demonstration of the difference between the two operations was made more striking, but a large amount was not necessary, for smaller doses of ivy extract applied to the skin islands with intact skin muscle were sufficient to sensitize (Table III *b*).

It will be clear that the positive results can meet with no objections on technical grounds, particularly the possibility of contamination of the skin outside the island, a matter necessarily to be considered with so highly active a substance. Apart from the elaborate precautions taken, the two types of experiments were made in alternation and with identical technique (except for the difference in the cutting), and furnished convincing controls for one another.

It should be noted that a not inconsiderable percentage of positive sensitizations was obtained, in most cases, as mentioned, not of high degree, when "deep" cuts were made in other locations (principally on the back) than the one described; from some experiments with dye injections we are inclined to believe that this may be due, at least in part, to incomplete interruption of lymph passage.

Straus and Coca (2) as well as Schreus (4) have reported that in their experiments treatment of isolated parts of the skin resulted in local sensitization. Indeed that such a condition can occur seems evidenced by certain clinical experiences. Our somewhat limited attempts to achieve a local sensitivity restricted to a segregated area have so far not yielded a definitely positive result: we obtained either no sensitization at all, or a hypersensitiveness which included the

Sensitization in relation to a skin defect encircling the site of application of poison ivy on the
severed as well. The islands of treated skin were excised on the 4th (rarely the 5th) day, and bet
A, 0.03 cc. ivy extract No. 1 was applied to the isolated skin, and the animals were finally tested
No. 2 was used for sensitization, and dilutions of the same extract were employed for the tests.
on two regions of the skin are shown in the table, the readings recorded being those made at 24 and

Ivy applied to skin islands, panniculus carnosus intact						Ivy applied to skin is			
No.	Interval before test	Reaction on dorsum		Reaction on flank		No.	Interval before test	Reaction on	
		1:20	1:40	1:20	1:40			1:20	
	days						*days*		
									Grou
35	13	+, +++	±, ±	±, +++	0, tr.	41	13	0, 0	
36	13	++, ++++	±, ±	±, ±	tr., tr.	42	13	0, 0	
37	12	++, +++	±, ++	+, ++	tr., +	43	12	0, 0	
38	11	++++, +++	+++, ++	+++, +++	±, ++	44	11	0, 0	
39	11	+++, +++	±, ±	+, +++	±, ±	45	11	f.tr., 0	
40	10	+++, +++	+++, +++	+++, ++	++, ++	46	10	0, 0	
		1:15	1:30	1:15	1:30			1:15	
									Grou
53	14	++++, ++++	+++, +++	+++, +++	+, +++	61	13	±, ±	
54	12	++++, ++++	++++, ++++	+++, +++	+++, +++	62	13	±, +	
55	12	++++, ++++	+++, ++++	++++, +++	+++, +	63	12	tr., tr.	
56	12	++++, ++++	++++, ++	++++, ++++	+++, +++	64	12	+, +++	
57	11	++++, ++++	++, ±	++, ++	±, ±	65	11	f.tr., f.tr.	
58	11	++++, ++++	++++, ++++	+++, +++	+++, +++	66	11	0, f.tr.	
59	11	+, ++	tr., ±	±, ±	0, 0	67	10	±, ±	
60	11	+++, ++++	+++, ++++	++++, ++++	++, ++	68	10	0, tr.	
									Grou
73	14	+++, ++++	++, ++±	++++, ++++	++, ±	78	14	±, tr.	
74	14	++++, +++	++++, +++	++++, ++++	++++, ++++	79	14	±, ±	
75	13	++, +++	0, tr.	±, ++	0, 0	80	13	tr., f.tr.	
76	12	++++, +++	++++, +++	++++, +++	++++, +++	81	13	+, ++	
77	12	++++, ++++	++++, ++++	++++, ++++	++++, +++	82	13	f.tr., f.tr.	
									Grou
86	12	+++, +++	+, +	+++, +++	++, ++++	92	12	tr., 0	
87	12	++++, ++++	+++, +++	+++, ++++	+, ++	93	12	f.tr., 0	
88	12	++++, ++++	+++, +++	++++, ++++	++++, ++++	94	12	+++, +++	
89	12	++++, +++	+++, ++	++++, ++++	++++, ++++	95	11	f.tr., 0	
90	11	++++, ++++	++++, +++	++++, ++++	++++, ++++	96	11	tr., 0	
91	10	++++, ++++	++++, +++	+++, +++	+++, +++	97	10	tr., tr.	

III *a*

ank, (*a*) when the skin muscle is not involved in the operation, and (*b*) when the skin muscle is
ween the 10th and 14th days the guinea pigs were tested for general hypersensitivity. With group
with various dilutions thereof; on the remaining animals (groups B, C, D), 0.05 cc. ivy extract
Untreated animals were included as controls with each lot of guinea pigs tested. The reactions
8 hours respectively.

uids, panniculus carnosus severed			Non-sensitized controls				
orsum	Reaction on flank		No.	Reaction on dorsum		Reaction on flank	
1:40	1:20	1:40		1:20	1:40	1:20	1:40
A							
f.tr., 0	0, 0	0, 0	47	f.tr., ±	0, 0	f.tr., tr.	0, 0
0, 0	0, f.tr.	0, 0	48	0, 0	0, 0	f.tr., ±	0, 0
0, 0	0, 0	0, 0	49	0, 0	0, 0	0, 0	0, 0
0, 0	0, 0	0, 0	50	0, 0	0, 0	tr., 0	0, 0
0, 0	0, 0	0, 0	51	0, 0	0, 0	0, tr.	0, 0
0, 0	0, 0	0, 0	52	0, 0	0, 0	0, 0	0, 0
1:30	1:15	1:30		1:15	1:30	1:15	1:30
B							
f.tr., tr.	f.tr., f.tr.	0, 0	69	f.tr., f.tr.	0, 0	f.tr., ±	0, 0
f.tr., tr.	0, 0	0, 0	70	0, 0	0, 0	0, f.tr.	0, 0
0, 0	0, 0	0, 0	71	±, tr.	0, 0	f.tr., f.tr.	f.tr., 0
tr., tr.	+++, +++	f.tr., 0	72	f.tr., f.tr.	0, 0	±, tr.	0, 0
0, 0	0, 0	0, 0					
f.tr., f.tr.	±, ±	0, 0					
0, 0	f.tr., f.tr.	0, 0					
0, 0	0, 0	0, 0					
C							
f.tr., 0	0, 0	0, 0	83	0, 0	0, 0	0, 0	0, 0
tr., 0	±, tr.	0, 0	84	tr., ±	f.tr., 0		
0, 0	0, 0	0, 0	85	0, 0	0, 0	0, 0	0, 0
0, 0	tr., ±	0, 0					
0, 0	±, tr.	0, 0					
D							
0, 0	f.tr., 0	f.tr., 0	107	+, tr.	0, 0	tr., 0	0, 0
0, 0	0, 0	0, 0	108	0, 0	0, 0	0, f.tr.	0, 0
+, +	+++, +++	+++, +++	109	f.tr., f.tr.	0, 0	0, f.tr.	0, 0
0, 0	0, tr.	0, 0					
0, 0	0, 0	0, 0					
0, 0	tr., 0	0, 0					
			Normal animals treated with ivy concurrently with group D and tested at the same time				
				1:15	1:30	1:15	1:30
			Sensitization with 0.05 cc. of undiluted extract				
			98	++++, ++++	+++, ++	+++, +++	+++, ++
			99	++++, +++	++++, ++	++++, ++	++++, ±
			100	++++, ++++	++++, +++	++++, ++++	++++, +++
			101	++++, ++++	++, ++	++++, ++++	++++, +++
			102	++++, +++	++++, +++	++++, +++	++++, +
			Sensitization with 0.05 cc. of 1:100 diluted extract				
			103	f.tr., 0	0, 0	++, +++	tr., 0
			104	f.tr., tr.	0, 0	f.tr., 0	0, 0
			105	+++, +++	+++, ++	++++, +++	++++, ++
			106	±, 0	0, 0	f.tr., f.tr.	0, 0

isolated area to which the substance (ivy extract, 2:4 dinitrochlorobenzene) had been applied, and the whole integument as well. Furthermore, when poison ivy was applied to a site of the intact skin and tests were made after a suitable interval, the intensity of the reaction on the treated site was if at all only slightly stronger than elsewhere (*cf.* 1).

A counterpart to the experiments described is the sensitization of a segregated skin area (made by a "deep" cut on the flank) when the active agent is administered on another part of the skin. Since under these conditions the island can be sensitive also, the spread through the circulation either of the allergen, probably transported in a changed state, or of antibodies is indicated. The reactions on the island were relatively weak, which may be due to alteration of the tissues. These experiments bear some relation to the "belt operation" of Simon (1).

TABLE III *b*

Same as Table III *a*, except that a smaller amount of ivy extract was applied to the skin islands (0.025 cc. of a 1:5 dilution in acetone of ivy extract No. 2). The animals were tested on the 10th or 11th days with dilutions of the same extract.

Ivy applied to skin islands, panniculus carnosus intact					Controls				
No.	Reactions on dorsum		Reactions on flank		No.	Reactions on dorsum		Reactions on flank	
	Dilutions		Dilutions			Dilutions		Dilutions	
	1:15	1:30	1:15	1:30		1:15	1:30	1:15	1:30
110	+++, ++++	++, +++	+, ++	±, +	116	tr., tr.	0, 0	tr., ±	0, tr.
111	+++, ++++	+++, ++++	+++, +++	+, +	117	f.tr., 0	0, 0	0, 0	0, 0
112	+++, +++	tr., tr.	+, +	±, ±	118	tr., tr.	0, 0	tr., ±	tr., f.tr.
113	+++, ++++	++, ++	++++, +++	±, ±	119	±, tr.	0, 0	0, 0	0, 0
114	±, ++	tr., 0	±, ±	tr., ±	120	tr., tr.	tr., f.tr.	±, +	tr., 0
115	++++, ++++	+++, ++	+++, +++	+++, ++					

We next proceeded to investigate whether a cut through the skin and the superficial muscle would also interfere with anaphylactic sensitization to a common protein antigen if injected intracutaneously into the isolated area. Actually this was the case when small doses (0.00005 cc., 0.0002 cc.) of horse serum were used (Table IV); with larger amounts (0.0006 cc. or more) such an effect did not occur. This may show that there are other ways by which high molecular substances can be distributed from a skin site than the lymphatic vessels that were severed along with the skin muscle in our experiments; indeed this idea may be considered for the infrequent instances of positive sensitization with ivy from flank islands similarly isolated.

The positive results just mentioned with large doses of horse serum

may possibly be ascribed also to leakage of some of the protein into the defect from the cut lymphatic trunks. For it was seen that when diluted horse serum (0.0025 cc. contained in 0.3 cc.) was applied with

TABLE IV

Hindrance by a defect in skin and panniculus of anaphylactic sensitization to horse serum injected intracutaneously in small amounts. Skin islands on the flank with severance of the panniculus carnosus, as described for poison ivy, were prepared in guinea pigs weighing between 450 and 500 gm., and 0.1 cc. of diluted horse serum was injected intracutaneously into the center of the isolated skin or in the corresponding position on normal animals as controls. The islands, together with underlying panniculus, were excised on the 4th day. After 21 or 25 days the animals were injected intravenously with 0.3 cc. of horse serum contained in a volume of 1.0 or 1.5 cc. Figures in parentheses indicate change in temperature (°C.).

Injection into skin islands, panniculus severed		Controls	
No.	Intravenous injection of horse serum	No.	Intravenous injection of horse serum
	Sensitization with 0.0002 cc. serum		
121	No symptoms (+0.3)	127	Severe shock, recovered (−1.2)
122	Slight symptoms (−1.4)	128	† 9 min.*
123	" " (−1.0)	129	† 4 "
124	Severe shock, recovered (−2.8)	130	† 3 "
125	No symptoms (+0.5)		
126	" " (+0.9)		
	Sensitization with 0.00005 cc. serum		
131	No symptoms (−0.4)	138	Moderate to severe (chronic type) (−3.5)
132	Very slight symptoms (+0.3)	139	† 4 min.
133	Slight to moderate symptoms (−0.5)	140	Slight to moderate symptoms (−2.3)
134	No symptoms (+0.8)	141	† 4 min.
135	" " (−0.5)	142	† 3 "
136	" " (+1.0)	143	Severe symptoms (chronic type) (−3.5)
137	Slight symptoms (−0.4)	144	† 3 min.

* The symbol † signifies death; the autopsy findings were typical in all cases.

a glass rod, slowly and with pauses, to the fresh wound surrounding flank islands that were made with severance of the panniculus carnosus, anaphylactic sensitivity frequently ensued, although not always with acute lethal shock (subsequent reinjection of horse serum as in

Table IV). It should be noted that the 0.0025 cc. amount used constitutes a rather large dose.

Similar exploratory experiments were then undertaken with another sensitizing substance of known structure, namely salvarsan (10), which induces skin sensitivity of a type different from the contact dermatitis of poison ivy. Here again, the barrier proved effective, for sensitization did not ensue when 0.15 mg. of salvarsan in 0.1 cc. was injected intracutaneously into flank islands (with the panniculus severed); the islands were excised on the 4th day, and tests for dermal sensitivity were made after 3 to 4 weeks by intracutaneous injection of a like amount.

DISCUSSION

From the experiments made in guinea pigs, which still leave problems for further investigation, the conclusion seems inescapable that continuity of the skin is not required for the development of general dermal sensitization to simple chemical compounds, since a broad defect in the entire thickness of the skin surrounding the area to which ivy extract is applied does not prevent hypersensitivity all over the skin. The significance of the question is clear from the literature reviewed. That there are differences as regards the route of distribution of the agent in various species would in our opinion seem quite improbable.

It is true that a "deep" cut down to the muscles of the trunk, made as described, inhibited sensitization almost regularly, and in this respect there is conformity with the results reported by Straus and Coca (2) for monkeys, and recently by Schreus (4) for guinea pigs. But upon a change in the experimental conditions the outcome was strikingly different, that is, when a strip of skin 5 to 10 mm. in width and comprising the whole thickness was excised in such manner as to spare the underlying skin muscle. The fact that the results differ depending upon the integrity of this muscle is apparently referable to the location of the lymph vessels draining the skin on the surface of the panniculus carnosus, with the consequence that cutting the muscle layer interrupts the lymphatics.[2] This can be demonstrated

[2] The anatomy of these vessels and the lymph nodes in the guinea pig are well described by Keller (11) whose drawings picture the superficial ramification of the lymph vessels. In this connection we may quote the conclusion of McMaster (12) that "every intradermal injection is truly intralymphatic."

by injecting intracutaneously into a freshly isolated island a solution of a colloidal dye, such as pontamine sky blue 6 B, for when the muscle is severed the dye chiefly is held locally, penetrating into the connective tissue, and to some extent oozes out from the severed lymph vessels; whereas, if the muscle is left intact, one sees the dye streaming across the moat through the lymphatics, and shortly the superficial and even the deeper regional lymph nodes are found upon dissection to be blue.[3]

Concerning the reasons for the interference with sensitization through severance of the lymph vessels, the most obvious is prevention or hindrance of transportation of the active material, if the latter is not such as to pass easily into the blood stream. In this respect it may be pointed out that sensitizing substances of simple constitution are probably in many instances not carried as such but rather in the form of some sort of conjugates (*cf.* 6), and indeed some of the compounds by their very instability (diazomethane (13), acyl chlorides (6)) must react rapidly when brought into contact with tissues; with poison ivy, as Simon (1) has reported, and this holds in our experience for other sensitizers of simple chemical constitution, direct introduction of the extract into the blood stream fails to induce skin hypersensitivity, from which fact he suggests that the active agent, if it is distributed by the blood, must have undergone some prior transformation.

In support of mechanical causes in preventing sensitization from skin islands isolated by a "deep" cut are experiments which show that anaphylactic sensitization by proteins, when one injects not too large amounts into such islands, also is definitely impaired (Table IV). Aside from hindrance to lymph flow, loss of some of the protein by leakage into the wound will occur, as with dye injected intracutaneously into the island.[4] Whether much effective antigen is lost in this way is doubtful for dilute horse serum placed on the freshly made circular defect is in some measure taken up, since anaphylactic sensitization resulted from this treatment.

In the case of experimental ivy sensitization, there are in all probability factors additional to those which operate in the experiments

[3] We are indebted for this technique to Drs. Austin L. Joyner and Philip D. McMaster.

[4] In the experiments on poison ivy, with animals suitably restrained from activity, the base of the operated area remained dry and there was no indication of seepage from the cut edges.

with proteins, for with poison ivy, despite the use of an excess of ivy left in place on the isolated skin of such islands for several days, we find not merely a decrease in sensitization but in most cases nearly complete inhibition. That an essential difference exists in the mechanism is indeed known since proteins sensitize by any route while in order to induce skin sensitization towards simple compounds application of the incitant to or into the skin plays a special but probably not entirely mandatory,[5] rôle. It is of interest that with a substance neither protein nor fat-soluble, salvarsan, sensitization was also seen to be entirely prevented following intradermal injection into an island, and here the resulting sensitization is not of the contact dermatitis type.

Why in the case studied of hypersensitiveness to poison ivy free lymph circulation is a necessity for the sensitization process we are not in a position to say. The existence of the highly developed lymph system of the skin (15, 9) may come into consideration,[6] and also the altered state of the tissues in the skin island (*cf.* 8), as evidenced by a rather persistent edema; yet there well may be involved a disturbance of a special mechanism which is still unknown. A unique importance of the lymph glands themselves cannot be concluded without specific evidence, in view of other immunological knowledge, although the importance of these in the production of certain antibodies was demonstrated in experiments of McMaster *et al.* (18, 12).

To some extent the special conditions obtaining in the skin seem to play a part in sensitization with common protein antigens also. Thus Sulzberger (19) observed that the minimum amount of horse serum needed for anaphylactic sensitization was smaller when administered by the intradermal than the subcutaneous route, a result which we were able to verify in more extensive tests, although the ratio of the minimum doses varied from one experiment to another.

The production of a local sensitivity by application of ivy to an island has so far not succeeded, and the question may still be open

[5] Experiments in progress appear to show that a dermal sensitization may be induced by intraperitoneal injection with the aid of adjuvants, *e.g.* with picryl chloride (*vide* 14) and killed tubercle bacilli.

[6] As regards peculiarities of the immunological rôle of the skin tissues, see (16, 17).

whether the process of sensitization can be completed in or by the dermal tissues alone; on the other hand, a part of the skin isolated with interruption of the lymphatics can take part in the general sensitivity produced upon treatment of the skin outside, and this is hardly to be explained otherwise than by the intermediacy of the blood stream.

SUMMARY

Experiments are described on the latency period in sensitization to poison ivy and on the time necessary for the agent to remain in contact with the skin. The chief matter of investigation concerned the manner in which the whole skin becomes sensitive following treatment at a particular site, and especially whether this is effected by way of the epidermis.

Two methods were used to interrupt the continuity of the skin, one by cutting through both skin and the underlying thin muscular layer, the other by removing a strip of skin so as to spare the skin muscle. These procedures led to different results when poison ivy extract was applied to the areas thus isolated. In the first case, sensitization was mostly prevented, whereas with the second method generalized hypersensitiveness occurred almost uniformly.

An explanation is to be found in the severance of the lymph vessels lying on the surface of the muscular layer, pointing to the necessity of a free lymph passage. On the other hand the experiments prove that general sensitization is not dependent upon maintaining the integrity of the skin around a treated area.

An inhibition of sensitization by incisions extending through the panniculus carnosus was seen to some extent in anaphylactic sensitization with protein antigens, namely when sufficiently small amounts were employed.

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EXPLANATION OF PLATE 42

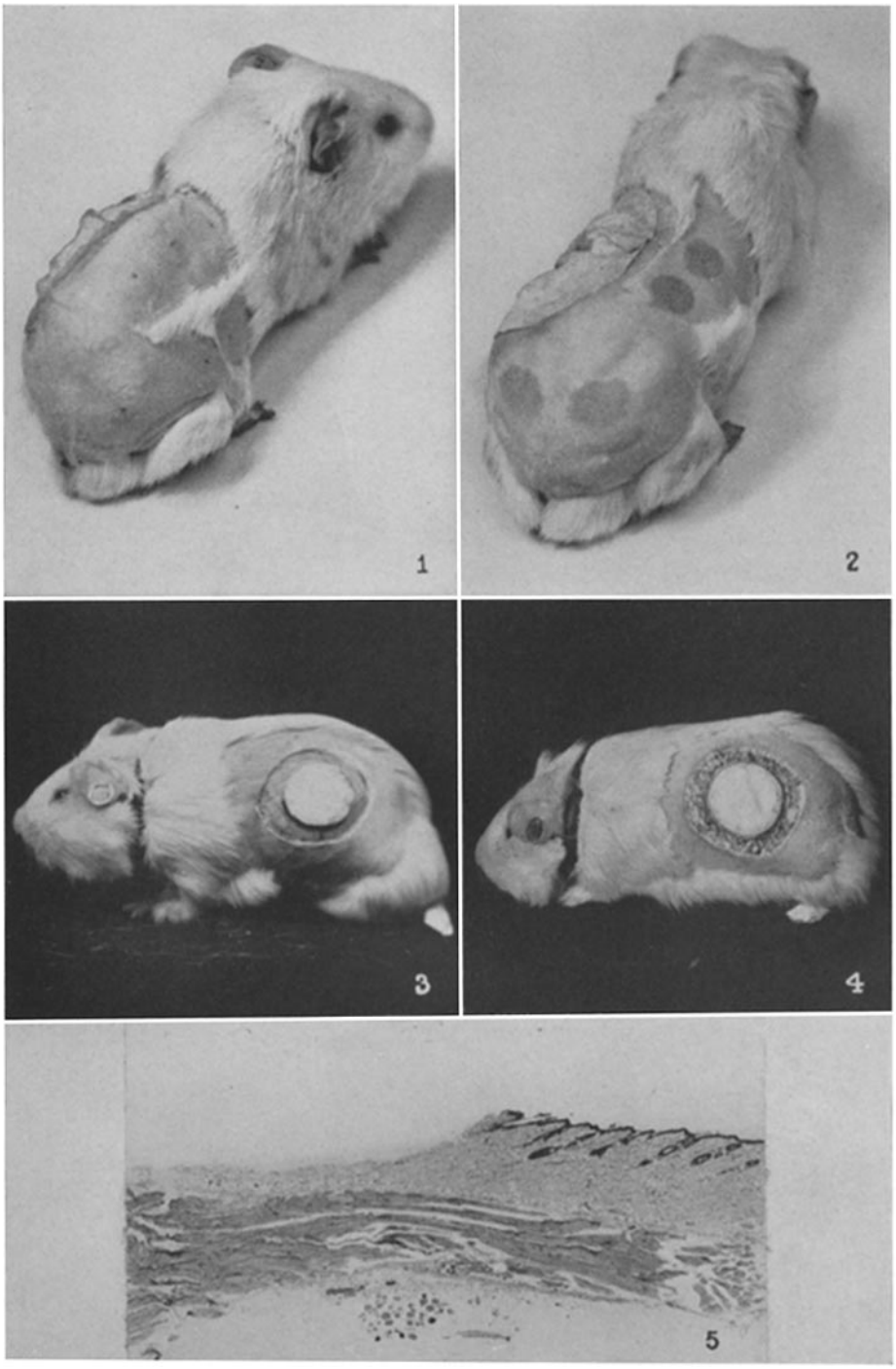
FIG. 1. Test for hypersensitiveness on the 14th day after operation (and ivy treatment) as shown in Fig. 3, with two different concentrations of ivy extract.

FIG. 2. Similar test after the operation as shown in Fig. 4.

FIG. 3. Isolated area of skin on the flank, with severance of the panniculus carnosus, after applying poison ivy and dressing.

FIG. 4. Skin island as above, but with panniculus carnosus spared.

FIG. 5. Section across the marginal part of the skin defect made as in Fig. 4. The skin muscle is intact and is covered on the larger (central) part with only a thin layer of loose connective tissue. ×8.



Photographed by Joseph B. Haulenbeek

(Landsteiner and Chase: Sensitization with chemical compounds. VI)