DELAYED HYPERSENSITIVITY

III. Specific Desensitization of Guinea Pigs Sensitized to Protein Antigens*

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A general method for experimental induction in guinea pigs and man of the delayed hypersensitive state directed against protein antigens has been decribed previously (1). Sensitization follows a single intradermal injection of a small amount of antigen in the form of a washed immune precipitate. Provided the specific precipitation is carried out in the region of antibody excess, active antibody production is suppressed and no circulating antibody can be detected for 2 to 3 weeks after the delayed type of skin reactivity has appeared. If the sensitizing injection is suspended in adjuvant containing killed acid-fast bacteria, the delayed skin reactions to a given amount of antigen are larger than those seen when mycobacteria are excluded.

The present studies were undertaken to determine the effects of a single injection of specific antigen on the subsequent skin reactivity of guinea pigs sensitized by this method. In this paper we have used the term desensitization, as have others in the past, to indicate loss of skin reactivity to a specific antigen. In the experiments to be reported it has been shown that guinea pigs sensitized to protein antigens can be completely and specifically desensitized by a single injection containing a sufficient amount of the corresponding antigen. In the following paper, the systemic effects of specific challenge are described (2).

Previous experimental studies of desensitization in delayed hypersensitivity have been restricted almost exclusively to attempts to specifically desensitize tuberculous animals and man. The literature has been extensively reviewed by Rich (3). In most of the experiments, repeated large doses of Old Tuberculin were administered over long periods of time. For example, Rothschild *et al.* (4) treated tuberculous guinea pigs

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with increasing doses of Old Tuberculin up to 2 gm. daily for several weeks. In one of their experiments only 8 per cent of the animals survived this drastic treatment. There is no evidence from their results that desensitization was specific; in fact, tuberculous animals given repeated injections of concentrated broth also showed loss of skin reactivity to tuberculin.

Materials and Methods

Antigens.—Diphtheria toxin (T) was prepared from a culture filtrate of the PW8 strain grown on Mueller and Miller's medium (5). It was partially purified by ammonium sulfate fractionation and dialysis. Purified diphtheria toxoids (To) were obtained through the courtesy of Dr. James A. McComb, Massachusetts Department of Health. KP59 contained 50 Lf/ml., 1730 Lf/mg. N, and 1:10,000 merthiolate. PT55 contained 1400 Lf/ml., was 66 per cent specifically precipitable by antitoxin 5353AD and also contained 1:10,000 merthiolate.

Of these materials, the toxin in the form of its immune complex was used for sensitization, KP59 was used for skin testing and PT55 for desensitization.[1]

Three times recrystallized ovalbumin (Ea) was kindly supplied by Dr. R. C. Warner. It was dissolved in saline, filtered, and the protein concentration calculated from the extinction in 0.25 N acetic acid at 277 mμ.

Horse gamma globulin (HGG) was a digested antitoxic gamma globulin 5353AD. The properties of this antitoxin have been described elsewhere (6).

Antisera.—Rabbit anti-horse gamma globulin was prepared in the following manner. Horse antitoxic globulin No. 5353AD containing 860 antitoxin units was flocculated with 800 Lf purified toxoid KP59. The twice washed floccules were suspended in saline and emulsified with incomplete adjuvant to give a suspension containing about 1 mg. protein per ml. Rabbits were injected subcutaneously with 2.5 ml. at various sites. One month later they were given a booster injection of the same suspension and 1 week later were exsanguinated. The serum contained 700 μg. anti-HGG specifically precipitable by antitoxin 5353AD.

Rabbit antitoxin No. 3999 was a precipitating serum from a single rabbit containing 50 units per ml. (7). The anti-ovalbumin serum was a pooled sample from 4 rabbits, each given a single injection of 20 mg. crystalline Ea in complete adjuvant, 1 month previously. It contained 3.55 mg./ml. antibody specifically precipitable by Ea. We are indebted to Dr. P. G. H. Gell for this serum.

Antibody content of anti-Ea and anti-HGG was determined by quantitative precipitation according to the method of Gitlin (8).

Endotoxin.—*E. coli* endotoxin (Difco) was dissolved in saline and heated to 80°C. for 10 minutes before use.

Sensitization.—Preparation of the specific precipitates containing excess antibody has been previously described (1). The well washed precipitates were suspended in saline-adjuvant emulsions containing 6 μg. of antigen and (unless otherwise noted) 1 mg. of dried *Mycobacterium butyricum*/ml. 400 gm. albino guinea pigs were usually injected intraperitoneally or intramuscularly with 0.5 ml. (3 μg antigen) of the suspension.

Desensitization.—Protein antigens were usually passed through a Seitz filter just prior to use; however, in several experiments sterile technique was not utilized. Intravenous injections were made using one of the veins in the hind foot.

Skin Tests.—Guinea pigs were injected intradermally with 0.1 ml. of the antigen diluted in saline. In experiments in which antigen concentration was less than 1 μg. protein/ml., 1 per

[1] Diphtheria toxin was usually used for sensitization since animals sensitized with specific precipitates of toxoid and later injected with 4 mg. toxoid frequently showed small residual reactions on subsequent skin testing.

cent normal guinea pig serum in saline was used as the diluent to prevent surface denaturation. Skin tests were read as routine at 18 to 24 hours after challenge, but frequently at 2 hours, to determine if "Arthus-type" reactions were present. Reactions recorded in the tables, however, show diameter of erythema at 18 to 24 hours. Skin tests were usually read by one observer (JU) *without* knowledge of the pretreatment of the animal.

To exclude any effects that might result from prior intradermal challenge, animals were skin-tested *after* desensitization only. In each experiment, however, a group of untreated sensitized animals was skin-tested at the same time as the desensitized groups. The reactions in the control group were considered as the "expected" reactions of all the animals. In the recorded experiments the average diameter of the reactions in the control group was usually between 30 and 45 mm.

Antibody Detection.—Sera were obtained by bleeding from the retro-orbital space with a capillary pipette. The presence of antibody was determined by passive cutaneous anaphylaxis according to the method of Ovary (9). One tenth ml. of serum was injected intradermally into 250 gm. albino guinea pigs. A known serum diluted to contain 1 μg. antibody nitrogen per 0.1 ml. was included in each test. Five hours later the animals were challenged intravenously with 1 to 5 mg. of the antigen in 0.25 per cent Evans blue dye. Ten minutes after the intravenous challenge, the animals were sacrificed and the skin removed. The presence of a blue spot at the site of the intradermal injection was considered as evidence that circulating antibody was present in the serum.

In other animals, signs of systemic anaphylaxis after intravenous challenge were used to detect antibody. 1 to 5 mg. of the antigen were injected into one of the veins in the hind foot.

RESULTS

Specificity of Desensitization.—

Guinea pigs were sensitized simultaneously to *two* immunologically distinct protein antigens using specific complexes incorporated in adjuvant containing mycobacteria. After 1 to 2 weeks, when the animals had become highly sensitive, 2 to 4.2 mg. of one of the two antigens was injected intraperitoneally. The animals were skin-tested for the first time with 3 μg. of each antigen, 8 to 24 hours after receiving the desensitizing dose of one of them.

Table I shows the results of two experiments. In the first, animals were sensitized to both diphtheria toxoid (To) and to ovalbumin (Ea); in the second, to horse gamma globulin (HGG) and Ea. As seen from the table, no skin reactions were obtained in 11 of 12 animals after challenge with the antigen with which they had been previously treated. The small reaction to HGG in one animal might have been due to sensitivity to some contaminating protein present in the HGG preparation. In two animals desensitized to Ea, even 300 μg Ea failed to elicit a skin reaction. On the other hand, pronounced delayed skin reactions were still elicited in all animals to the second antigen to which they had been sensitized.

In order to rule out any possible effect of mycobacteria on the ease with which the animals became desensitized, experiments were carried out using guinea pigs sensitized with immune complexes suspended in incomplete adjuvant. Four guinea pigs that had been sensitized in this manner to To and two sensitized to Ea 9 days previously were skin-tested with 3 μg. of the corresponding antigen. The average diameter of the delayed reactions was 15 mm.

when read at 24 hours. The animals then received 4 mg. specific antigen intraperitoneally and 8 hours later were again skin-tested with 3 μg. of the same antigen; this time no reactions were seen.

None of the specifically challenged animals showed signs of acute or protracted anaphylaxis or of any condition resembling "tuberculin shock" after receiving the desensitizing dose of antigen.

TABLE I

Specificity of Desensitization

Sensitization	Desensitizing dose*	Skin reactions	
		3 μg. To	3 μg. Ea
	mg.	*mm.*	*mm.*
Ovalbumin and toxin‡	4.2 Ea	24 × 26	0
		16 × 24	0
		20 × 12	0
	4.2 To	0	20 × 16
		0	17 × 12
		0	19 × 16
		4 μg. HGG	3 μg. EA
		mm.	*mm.*
Ovalbumin and horse gamma globulin§	2.2 Ea	18 × 18	0
		16 × 12	0
		12 × 15	0
	4.2 HGG	0	26 × 21
		0	21 × 16
		9 × 8	22 × 21

* Ea, ovalbumin; To, diphtheria toxoid; HGG, horse gamma globulin.

‡ In the first experiment, guinea pigs were sensitized 7 days prior to the intraperitoneal injection of the desensitizing dose. Eight hours later all the animals were skin-tested with both Ea and To.

§ In the second experiment, the animals were sensitized 12 days prior to intraperitoneal injection of the desensitizing dose. Twenty four hours later all the animals were skin-tested with both Ea and HGG.

These experiments show that in sensitive guinea pigs a single injection containing a sufficient amount of the corresponding antigen can completely and specifically prevent subsequent delayed skin reactions to that antigen.

Amount of Antigen Required for Desensitization.—

Guinea pigs were sensitized to ovalbumin. Nine days later they were injected by various routes (intravenous, intraperitoneal, or intradermal) with decreasing amounts of Ea. Twelve hours later they were skin-tested with 5 μg. Ea.

Table II shows that three control guinea pigs that received no desensitizing injection developed skin reactions averaging 41 mm. in diameter. Of eleven

animals challenged with 1.8 mg. or more Ea, ten showed no reactivity on subsequent intradermal test. The one exception gave a 7 x 12 mm. reaction. As decreasing amounts of Ea were injected, desensitization became progressively less complete. However, as little as 18 μg. Ea appeared sufficient to decrease markedly the "expected" size of the delayed skin lesions. The route by which the desensitizing dose was administered did not appear to influence the results.

Although the order of magnitude of the amounts of antigen required for desensitization is apparent from Table II, no further quantitation is justified. It cannot be assumed, for example, that each animal had been sensitized to the same degree. Nor can it be assumed that sensitization with specific precipi-

TABLE II

Effect of the Amount of Antigen on the Completeness and Duration of the Desensitized State

No. of Animals*	Desensitizing dose	Average diameter of skin reactions to 5 μg. Ea	Reappearance of skin reactivity (days after desensitization)
	mg.	*mm.*	
3	0	41	—
4	67.5	0	6–10
2	11.5	0	5–6
2	4.7	5‡	3–4
3	1.8	0	2–4
6	0.9	6	—
5	0.09	13	—
3	0.018	24	—
1	0.001	47	—

* All animals were sensitized to Ea 9 days prior to desensitization with Ea. The desensitizing injections were given either by the intravenous, intradermal, or intraperitoneal route. All the animals were skin-tested 12 hours after desensitization.

‡ One animal had a 7 × 12 reaction.

tates had necessarily induced sensitivity to the major component only. For example, it was shown previously (10) that even animals sensitized with specific precipitates made using highly purified toxoid showed small delayed reactions when tested with supernates from which the toxoid component had been removed by specific precipitation. Such residual reactions as well as those seen in occasional desensitized animals may well be attributed to minor components present in the skin test materials.

Duration of the Desensitized State.—In order to determine how long desensitized animals remained non-reactive to intradermal challenge, animals were skin-tested with the specific antigen at intervals following the initial negative skin test. Some of the animals were skin-tested daily; others at intervals of 2 or more days. The appearance of a skin reaction was considered as indicating the return of sensitivity. The last column in Table II shows that the duration

of the desensitized state increased as the amount of Ea used in the desensitizing injection was increased. Four sensitized guinea pigs injected with 67.5 mg. of Ea remained non-reactive for 6 to 10 days. It is not surprising that skin reactivity returned, since in all of the animals depots of adjuvant containing antigen undoubtedly persisted even after the challenge dose of antigen had been eliminated (11).

The return of sensitivity suggested that free antigen had been virtually eliminated, probably in part by production of circulating antibody. The presence of excess circulating antibody was looked for in desensitized guinea pigs at the time when their skin reactions first reappeared. For example, 7 guinea pigs sensitized to Ea were completely desensitized with 1 mg. Ea. Skin tests

TABLE III

Desensitization with Antigen-Antibody Complexes

No. of animals*	Desensitizing dose	Average diameter of skin reactions to 3 μg. toxoid
		mm.
7	0	32
3	15 Lf To	16
4	15 Lf toxin-22 units antitoxin	9
2	560 Lf To	0
6	560 Lf To-570 units antitoxin	0‡

* All animals were sensitized to diphtheria toxin 8 to 12 days prior to desensitization. Horse antitoxin was used in the desensitizing complex, rabbit antitoxin in the sensitizing. Skin testing was done 1 to 6 hours after desensitization.

‡ Four of these animals were also sensitive to Ea. When skin-tested on the following day with Ea and To, reactions were only obtained with Ea, averaging 24 mm. in diameter.

with 5 μg. Ea were negative for 48 to 96 hours and then became positive. After return of sensitivity, antibody was demonstrated in 4 of these animals by fatal anaphylaxis following intravenous challenge with antigen, and in the serum of two of the three remaining animals by passive cutaneous anaphylaxis. Small skin reactions of the delayed type seemed to be the first indication of returning sensitivity. By the following day, early reactions of the Arthus type were usually present as well.

Desensitization by Antigen-Antibody Complexes.—Since small amounts of protein antigens complexed with antibody have been shown to induce the delayed hypersensitive state, it was of interest to see if large amounts of complex could effect desensitization. In the experiments summarized in Table III guinea pigs were sensitized with diphtheria toxin precipitated by excess rabbit antitoxin. The table shows that 15 Lf (40 μg.) of *toxin* complexed with *horse* antitoxin was as effective as 15 Lf of free *toxoid* in reducing the size of delayed reactions to the skin test dose of toxoid. Since the toxin used contained about

600 MLD and no toxic symptoms were observed, it is obvious that dissociated toxin did not bring about the desensitization. When 560 Lf (1.4 mg.) of toxoid was injected after mixing with excess horse antitoxin, desensitization was complete regardless of whether the injection was made before or after visible aggregation had occurred. Four of the animals desensitized in this way had also been sensitized to Ea and still reacted to skin test with Ea. The fact that desensitization can be accomplished as effectively with antigen complexed with slight excess antibody as with free antigen does not mean that the specificity of sensitized cells is directed against different configurations on the antigen molecule from those which interact with conventional antibody. There are at least 6 to 8 and perhaps more specific sites on the toxin (or toxoid) molecule capable of interacting with horse antitoxin (12). The complexes used in the above experiments were of approximate composition TA_3, and therefore sites remained available on the antigen for further interaction either with antitoxin or with sensitized cells.

If a large amount of antigen-antibody complex can specifically desensitize, it follows that a small amount should be capable of *eliciting* delayed skin reactions. Such proved to be the case. Three guinea pigs sensitized with toxoid–rabbit antitoxin complexes showed delayed skin reactions between 30 and 40 mm. in diameter following intradermal injection of 1 Lf toxoid complexed with 1.3 units horse antitoxin. These complexes did not elicit skin reactions in normal guinea pigs. Hitchcock *et al.* (13) and Lawrence and Pappenheimer (14) have previously demonstrated that incubation with immune sera did not inhibit elicitation of delayed skin reactions by living streptococci in specifically sensitized rabbits and by diphtheria toxoid in the toxoid sensitive human, respectively.

Specific Desensitization before and after Skin Testing.—In the experiments described so far, the desensitizing dose of antigen was administered 8 to 24 hours *before* skin testing. In the experiments which follow, skin reactions were studied in sensitized animals that received an intravenous desensitizing dose of antigen at various intervals both before and *after* skin test. Twenty-two guinea pigs were sensitized to Ea and an additional four to both Ea and To. Nine days later 22 animals (including those doubly sensitized) received 3.5 mg. Ea by the intravenous route. Groups of animals were skin-tested with 5 μg. of Ea at intervals from 5 hours *prior* up to 5 hours *after* intravenous desensitization. Table IV shows that the intravenous dose of Ea prevented the appearance of macroscopic skin reactions in all animals when given before or at the same time as the skin test and in nine of eleven animals 1 to 2 hours *after* the skin test. Even when given 5 hours after intradermal challenge, the desensitizing dose of Ea prevented the appearance of visible reactions in two of five animals and the reactions which developed in the remaining three were small.

The four animals sensitized to both Ea and To were skin-tested with 5 μg. of each antigen; two of them were injected intravenously 1 hour later, and two 5 hours later with a desensitizing dose of Ea. Table IV shows that all four animals reacted to To but showed no visible delayed reactions to Ea. This control was necessary in view of evidence to be presented below that specific challenge can produce a transient non-specific inhibition of skin reactivity under certain conditions.

The results summarized in Table IV help to give some concept of the speed of immunologic events which occur in the delayed skin reaction. Up to 2 hours and even longer after intradermal introduction of antigen, its interaction with antigen-specific cells in the host tissues has progressed so slowly that its interruption by a desensitizing dose of antigen given at this time may still prevent

TABLE IV

Desensitization before and after Skin Testing

No. of animals*	Time of desensitization before (−) or after (+) skin testing	Skin reactions to 5 μg. EA
	hrs.	*mm.*
4	Not desensitized	42 × 40, 55 × 40, 70 × 50, 55 × 40
3	−5	0, 0, 0
3	0	0, 0, 0
6	+1	0, 8 × 6, 0, 0, 0,‡ 0‡
5	+2	10 × 5, 0, 0, 0, 0
5	+5	10 × 12, 24 × 18, 17 × 19, 0,‡ 0‡

* All animals were sensitized to Ea 9 days prior to desensitization with 3.5 mg. Ea intravenously.

‡ These animals were also sensitized to To and were skin-tested with 5 μg. To simultaneous to skin testing with Ea. All showed delayed skin reactions to To averaging 27 mm. in diameter.

the appearance of macroscopic inflammation. It follows that if a desensitizing dose of specific antigen given intradermally to sensitive animals is absorbed rapidly enough, no visible skin reaction should appear. The following experiments show that such is indeed the case. Twenty-seven guinea pigs which had been sensitized 10 days previously to Ea were used. Groups of 2 to 4 of these animals were then skin-tested with increasing doses of Ea, ranging from 0.0016 to 2400 μg. contained in a volume of 0.2 ml. In Fig. 1, the logarithm of the intradermal dose is plotted against the average diameter of the skin reactions read at 24 hours. Maximal reactions 40 to 50 mm. in diameter were obtained with 1.6 to 16 μg. Ea. However, of nine animals that received 1600 μg. Ea or more *no* visible reactions occurred in five and the remaining four guinea pigs showed small skin reactions of doubtful significance. Even one millionth this amount of Ea (0.0016 μg.) elicited delayed skin reactions whose average diameter was 15 mm. Reactions of this size provoked by such minute amounts of antigen suggest an extremely high degree of sensitivity in these animals.

The Effect of Specific Desensitization to One Antigen upon Skin Reactions to a Second in Animals Sensitive to Both.—In the initial experiments involving animals sensitive to *two* antigens, skin testing with both antigens was done 8 to 24 hours after administration of a desensitizing dose of one of them. In the following experiments desensitizing and skin test doses were given at the same time in doubly sensitized animals. Table V summarizes the results of an experiment in which 24 guinea pigs were sensitized to both Ea and To. In addition, three animals were sensitized to Ea only and three to To alone. Ten days after sensitization six of the animals sensitive to both antigens were skin-tested with Ea and four with To. The skin reactions when read at 24 hours averaged 36 and 42 mm. in diameter respectively. The three animals sensitive

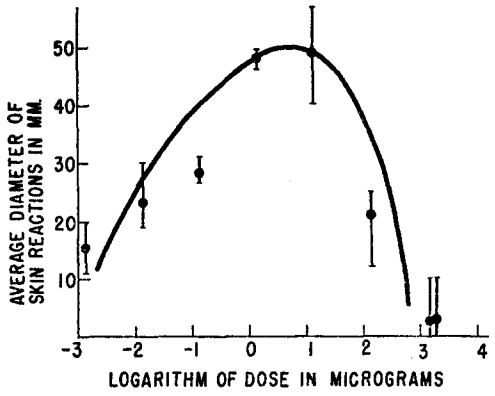

FIG. 1. Relation between amount of antigen injected intradermally and resultant skin reactions. Animals were sensitized to Ea 10 days prior to skin testing with Ea. Each point on the curve represents the average diameter of the skin reactions of 2 to 6 animals. The vertical lines indicate the range of values.

to To only were injected with 4.2 mg. Ea intraperitoneally and skin-tested with To; and the three sensitive to Ea only were given 4.2 mg. To and skin-tested with Ea. Skin reactions were comparable in size to those seen in the first control group. In contrast to the behavior of both control groups, Table V shows that in six guinea pigs sensitive to *both* Ea and To, an intraperitoneal desensitizing dose of one antigen markedly reduced the size of skin reactions to the other. The average diameter of 24 hour skin reactions to the second antigen in this group was only 16 mm. Results were even more striking in three animals in which a desensitizing dose of Ea was given *intradermally*. No visible reaction to simultaneous skin test with To occurred in two of the three animals. The third showed a delayed reaction measuring 20 × 19 mm.

Table V also includes a group of five doubly sensitized animals who received 5 μg. of endotoxin instead of a specific desensitizing dose of one of the antigens. Despite the fact that the dose of endotoxin caused severe weakness and prostra-

tion in all animals lasting for several hours, the skin reactions to Ea and To were comparable in size to those seen in the controls. This experiment confirms the conclusion already drawn, namely, that since the transient unresponsiveness

TABLE V

*Effect of Simultaneous Desensitization to One Antigen on the Skin Reactions to a Second in Animals Sensitized to Both**

Sensitization	No. of animals	Desensitizing dose	Average diameter of skin reactions	
			3 μg. ovalbumin	3 μg. toxoid
			mm.	*mm.*
Ea + To	6	0	36	—
	4	0	—	42
To	3	4.2 mg. Ea	—	40
Ea	3	4.2 mg. To	34	—
Ea + To	3	4.2 mg. Ea	—	16
	3	4.2 " To	16	—
	3	1.6 " Ea‡	—	7
Ea + To	2	5 μg. endotoxin	36	—
	3	5 " "	—	35

* All animals were sensitized 10 days prior to challenge They were skin-tested with one antigen at the same time as the desensitizing injection of the second was given.

‡ Administered intradermally. All other desensitizing injections were given intraperitoneally.

TABLE VI

Return of Skin Reactivity to an Unrelated Antigen Following Specific Desensitization

No. of animals*	Interval between desensitization with To and skin testing with Ea	Average diameter of skin reactions to 3 μg. Ea
	hrs.	*mm.*
3	−96	24
3	−24	25
2	0	9
3	Not desensitized	30

* All animals were sensitized to both Ea and To 12 days before skin testing with Ea.

only followed *specific* challenge, contaminating endotoxin by itself could have played no role.

This state of anergy is of short duration compared with the unresponsiveness towards the antigen with which the animals have been specifically desensitized. Table VI shows that almost full skin reactivity to the second antigen returns within 24 hours.

It is probable that a desensitizing dose of antigen causes a temporary decrease in the availability of one or more factors necessary for expression of delayed inflammatory skin reactions. The size of individual lesions may be markedly reduced when multiple skin tests, even with small amounts of antigen, are performed on the same animal. Furthermore, we have repeatedly observed that in doubly sensitized animals, multiple skin tests of as little as 3 μg. each of one antigen, may reduce the expected diameter of the reaction to the second antigen to one-half or less. These findings serve to emphasize the difficulties involved in interpretations based on size of observed inflammatory skin reactions when more than one test is carried out on the same animal.

DISCUSSION

Perhaps the most striking finding that has emerged from these studies is the observation that the delayed inflammatory process can be interrupted by a single "desensitizing" dose of specific antigen even when it is administered several hours after skin challenge of sensitive guinea pigs. It follows that when a desensitizing dose is given intradermally even to highly sensitive animals, no visible skin lesion should occur. Thus 1 mg. of ovalbumin failed to produce a visible reaction when injected into the skin of sensitized guinea pigs, although other animals sensitized in the identical manner all reacted to one millionth this dose of Ea (0.001 μg.) with marked delayed skin lesions. The failure to observe a local lesion following injection of a desensitizing dose of specific antigen does not signify absence of an immunologic reaction. On the contrary, such large doses produce systemic reactions of fever and lymphopenia as discussed in the following paper (2). Moreover, challenge with antigen stimulates a secondary antibody response (15).

A second finding of considerable interest is our observation that injection of a desensitizing dose of antigen into sensitized guinea pigs results in a temporary state of anergy, so that over a period of several hours the animals not only fail to respond to the particular antigen used for their desensitization, but also show markedly diminished delayed skin reactivity against other antigens to which they were sensitized. This unresponsiveness towards heterologous antigens is of short duration, however, and skin reactivity is usually regained within 24 hours. On the other hand, lack of skin reactivity against the antigen used for desensitization persists for several days and usually returns at the time when circulating specific antibody makes its first appearance. The transient lymphopenia caused by the desensitizing antigen (2) may be responsible for the temporary state of anergy by simply decreasing the number of mononuclear leucocytes available for participation in a local delayed inflammatory reaction. At this point it is well to recall that a transient state of anergy characterizes certain diseases of man (16–18). This is particularly striking in measles when it has often been observed that tuberculin skin re-

activity disappears during the exanthemous phase of the disease. It was von Pirquet (16) who first suggested that the skin lesions in measles might be an allergic response of the host to collections of viral particles in the skin. The rash in measles is accompanied by a pronounced lymphopenia, possibly due to sequestration of lymphocytes in the perivascular collections of inflammatory cells in the skin. In measles, as well as in certain other viral infections, the mechanism underlying the temporary anergic state seen during the acute stages of the disease may be analogous to that which causes the transient unresponsiveness observed in specifically challenged guinea pigs.

In contrast to experience with tuberculous guinea pigs, animals sensitized to single protein antigens have proved surprisingly easy to desensitize. It is likely that sensitization by infection or by means of killed bacteria results in sensitivity to many different bacterial antigens. As we have seen, the amount of antigen required for desensitization is large as compared with that necessary to induce sensitivity or to elicit a skin reaction in the sensitized animal. It is possible, therefore, that desensitization to major components in a mixture such as Old Tuberculin containing degraded proteins and polysaccharide, may be masked by skin reactivity to minor components against which the tuberculous animal is also sensitive. Moreover, the presence of contaminating endotoxin in Old Tuberculin (19) together with the known hyperreactivity of tuberculous animals to endotoxin (20, 21) may serve to complicate still further the interpretation of the reactions which take place.

The mechanism of desensitization is not understood. Nevertheless, it seems clear that the desensitizing dose of antigen must act in such a way as to render specific complementary binding sites on sensitized cells unavailable for further interaction with antigen.

At least three ways in which this may occur have been considered.

1. Antigen may specifically destroy sensitive cells. The conflicting evidence concerning the cytotoxic effects of antigens on sensitive cells has been reviewed recently by Waksman (22).

2. All of the antigen-binding sites may be released from cells leaving intact "desensitized" cells. Such a mechanism was suggested by Lawrence and Pappenheimer (23) who showed that the factor responsible for transfer of tuberculin sensitivity from man could be released from sensitive donor cells *in vitro* by treatment with PPD. The present studies on desensitization in guinea pigs have failed to provide further support for their hypothesis. Numerous attempts to transfer delayed sensitivity using serum from partially and from completely desensitized guinea pigs have been unsuccessful.

3. The desensitizing dose of antigen binds specific sites on sensitized cells so that none are left available for further interraction with antigen.

At the present time insufficient evidence is available to permit us to exclude any of the above possibilities.

SUMMARY

Guinea pigs rendered hypersensitive (delayed-type) to protein antigen can be completely and specifically desensitized by a single injection containing a sufficient amount of the corresponding antigen. Although 1 to 2 mg. of specific antigen are required for complete desensitization, as little as 20 μg. suffices to decrease the size of specific skin reactions in sensitized animals. The duration of non-reactivity lengthens as the amount of antigen in the desensitizing injection is increased, but skin reactivity eventually returns and is accompanied by the appearance of excess circulating antibody. Desensitization can be accomplished with the antigen-antibody complex as well as by "free" antigen. The appearance of delayed skin reactions can be prevented in fully sensitized animals by intravenous desensitization 2 or more hours *after* intradermal challenge or by simply skin testing with a desensitizing dose of specific antigen. Injection of a desensitizing dose of antigen into specifically sensitized animals also results in a transient anergic state, the implications of which are discussed.

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