IMMUNOCHEMICAL STUDIES OF ANTITOXIN PRODUCED IN NORMAL AND ALLERGIC INDIVIDUALS HYPERIMMUNIZED WITH DIPHTHERIA TOXOID

I. Relationship of Skin Sensitivity to Purified Diphtheria Toxoid to the Presence of Circulating, Non-Precipitating Antitoxin*

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The present study is the first of a series designed to use hypersensitivity of the immediate type to diphtheria toxin or toxoid as a model for the study of the hay-fever type of allergy. The use of the toxin-antitoxin system in the experimental study of allergy possesses several distinct advantages over less well characterized systems such as are encountered in patients sensitive to pollens, grasses, etc. In the first place, diphtheria toxin and toxoid are available in a high state of purity and are reasonably well characterized proteins. Secondly, it is possible to measure by means of the sensitive rabbit intracutaneous test both toxin and antitoxin with considerable accuracy even when they are present in very low concentration. Finally, it has been demonstrated that administration of a single dose of purified diphtheria toxoid into Schick-negative adults is frequently followed by a rapid and high antitoxin response. Quantitative studies (1) have shown that no other antibody is formed in appreciable amount and therefore the toxin-antitoxin reaction in human beings immunized in this way closely approximates a single antigen-antibody system.

Prausnitz and Kustner in 1921 (2) first demonstrated in an allergic individual a serum factor which was capable of causing local sensitivity of the wheal and erythema type in the skin of normal human subjects. (Sensitivity to fish was passively transferred in the original experiments.) Prausnitz and Kustner showed that the reaction was highly specific but they were unable to demonstrate either precipitating or complement-fixing antibodies in the patient's serum, nor was it capable of passively sensitizing guinea pigs to anaphylactic shock. Moreover, they were unable to demonstrate by means of skin tests neutralization of the allergen in extracts of fish by the patient's serum. Most of the original observations were subsequently confirmed and extended to include passive transfer of sensitivity to a variety of antigenic materials.

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Coca and Grove (3) later found the allergic factor, termed by them *atopic reagin*, in the serum of all subjects with hay-fever or asthma in whom a positive skin test could be elicited. Not only were Coca and Grove unable to sensitize guinea pigs to anaphylactic shock with the allergic factor, but even tests using the sensitive Schultz-Dale method of uterine contraction were negative. The same authors showed that the serum factor could be neutralized by the homologous antigen since injection of test tube mixtures of antigen and serum failed to sensitize the skin. In other words, although injection of the test tube mixture caused an immediate wheal and erythema reaction, no reaction could be elicited when the site was challenged 24 hours later provided sufficient antigen was added to combine with all the reagin. On the other hand neutralization of the *antigen* or (allergen) by reagin could not be demonstrated at skin sites. Coca and Grove also showed that local passive sensitization persisted for 4 weeks or longer and that the skin-sensitizing capacity of atopic reagin was considerably diminished by heating for 1/2 hour at 56°C.

The method most frequently used in treatment of hay-fever patients consists of repeated and increasing subcutaneous doses of allergen. Thus, treatment may be regarded as specific immunization. Levine and Coca (4) showed that treatment may result in an increased titer of atopic reagin in the patient's serum. They introduced two methods of quantitative determination of reagin. In the first, progressive serum dilutions were placed in skin sites of a normal recipient and followed by injection 24 hours later of a fixed quantity of the corresponding antigen into the sensitized areas. The second depended on the reaction produced by injecting progressively increasing amounts of antigen into areas of normal skin previously sensitized by injection of serum in constant amounts. Attempts were made later by Lippard and Schmidt (5) to further quantitate methods for titrating the passive transfer antibody. They determined the amount of antigen (in terms of mg.-N) necessary to neutralize available antibody in a given amount of serum. The procedure involved mixture of various dilutions of antigen with constant amounts of serum *in vitro*, the intracutaneous injection of resultant mixtures, and a subsequent intramuscular challenge injection of antigen 1 to 3 days later. There was close correlation between the titers obtained by serum dilution and neutralization methods.

A new concept was introduced when Cooke *et al.* (6) demonstrated that sera of treated patients occasionally contained a specific protective substance which they termed *blocking* or *inhibiting* antibody. The presence of blocking antibody was thought to be correlated with clinical benefits observed in patients following treatment by means of specific immunization. Cooke *et al.* (7) later found that blocking antibody was also formed by normal individuals injected with pollen extracts.

The work of Loveless (8) served to further differentiate blocking antibody from atopic reagin. She showed that blocking antibody differs from reagin in that it disappears rapidly from injected skin sites. In contrast to reagin, blocking antibody retains its activity after prolonged heating at 56°C. Loveless has used the latter property to eliminate heat-labile reagin from the serum of treated hay-fever patients and permit the separate demonstration of thermostable blocking antibody. However, her experiments did not rule out the possibility that heated reagin may retain its capacity to combine with allergen while losing its wheal- and erythema-producing capacity. Earlier work of Jadassohn (9) with *Ascaris* antiserum-antigen mixtures actually showed

that heating *Ascaris*-sensitizing serum for as long as 24 hours did not remove its neutralizing power. Similar findings were also reported by Lippard and Schmidt (10) with heated serum of pollen-sensitive patients.

Loveless (11) showed that high titers of thermostable blocking antibody developed in 3 "normal" subjects who received repeated injections of ragweed pollen extracts. Only one of the subjects developed skin sensitivity to ragweed pollen. She was unable to demonstrate precipitins in the serum from any of the 3 individuals.

Recent studies with relatively pure antigens have tended to confirm earlier investigations which demonstrated antibodies of varying reactivities against hay-fever pollen. Lowell (12) has presented evidence that an immune mechanism plays a role in some cases of insulin resistance. He found a high state of resistance to crystalline beef and pork insulin in a patient who still responded to human insulin preparations. The resistance was observed to develop during periods in which beef or pork insulin was administered and to disappear when insulin was withheld. The patient was also allergic to beef and pork insulin. Injections of insulin caused swelling and generalized urticaria and serum specimens were shown to contain skin-sensitizing antibody. Of significance in this patient was the fact that the degree of resistance and hypersensitivity varied independently.

Sherman *et al.* (13) have shown that rabbits immunized by subcutaneous injection of recrystallized, alum-precipitated ovalbumin formed both precipitating antibody and non-precipitating antibody. Only the latter was capable of sensitizing human skin. Addition of equivalent amounts of antigen all at once to this serum resulted in coprecipitation of skin-sensitizing antibody. However, the latter remained in the supernatant when the serum was absorbed by repeated small amounts of antigen. It was of interest that animals similarly injected with fluid ovalbumin formed only precipitating antibody. In sera which possessed both types of antibody, the amounts of skin-sensitizing antibody present bore no relation to the precipitin content and were dependent on the amounts of so-called "univalent" or "incomplete" (14) antibody present.

The stimulus for the present investigations began when Dr. H. S. Lawrence drew to our attention the case of a 6 year old boy with a personal and familial history of allergy, who was brought into hospital in severe asthmatic crisis precipitated within a few minutes following a routine "booster" dose of diphtheria toxoid administered by his school physician. Subsequently, minute doses of purified diphtheria toxoid given intracutaneously produced marked wheal and erythema reactions with long spidery pseudopodia at the injection site. As little as 0.001 μg. toxoid caused a pronounced skin reaction. Moreover, the specific sensitivity to toxoid could be passively transferred to normal skin sites with 0.001 cc. of serum or less. Serum drawn 2 days after admission showed an antitoxin titer of 50 units per cc. by rabbit skin test but contained no precipitating antitoxin whatever (15). This behavior was quite unusual since in previous studies the sera of a large series of normal subjects hyperimmunized with purified toxoid had all shown good precipitin reactions when 5 units/cc. or more of antitoxin were present (1, 16).

In the present paper and the one that follows several instances are described

of individuals whose serum contained a high titer of non-precipitating antitoxin. It will be shown that such non-precipitating antitoxin possesses properties characteristic of atopic reagin.

Materials and Methods

Schick Test Material[1].—Highly purified diphtheria toxin diluted in buffered heated human serum albumin (17) to 0.2 guinea pig M.L.D. per cc. was used in the Schick test. Highly purified toxoid containing 0.08 Lf per cc. was used as the control. The volume injected intradermally was 0.1 cc.

TABLE I

Grading of Immediate Reactions to Schick Test

Rating	Erythema	Intensity	Wheal	Pseudopodia
	mm.		*mm.*	
++++	Over 25	++++	Over 15	Usually present
+++	20 to 25	+++	10–15	Present or absent
++	15 to 20	++	5–10	Absent
+	10 to 15	++	0–5	Absent

TABLE II

Immediate Reaction to Schick Test in 131 Medical Students

Strength of immediate reaction to Schick control (0.008 Lf purified toxoid)	No. claiming personal or familial allergic history	No. claiming *no* personal or familial allergic history
0	24	67
+	7	3
++	9	0
+++	8	2
++++	11	0
Total.....	59	72

Reactions to the Schick Test.—Immediate reactions were read at the Schick control site 15 to 30 minutes after performing the Schick test. The size of the wheal and of the erythema were both measured and the presence of pseudopodia, pruritis, and color intensity noted. On the basis of these criteria, reactions were rated as negative, +, ++, +++, and ++++ (see Table I).

In all subjects additional skin sites were injected with 0.1 cc. buffered human albumin (Schick diluent) as a control. The volunteers also received simultaneously intracutaneous injections of old tuberculin (1:1000) and this served as a further control. Equally strong immediate reactions against both toxoid and albumin diluent are arbitrarily classified in Table II as negatives. Thus, in one group of individuals with a personal or familial history of allergy, 11 of 24 (46 per cent) negative reactors fell in this category, and 12 of 67 (18 per cent) of the non-allergic group were likewise classified as negative because they reacted in this manner.

[1] The authors are indebted to Dr. J. A. McComb and L. Levine of the Massachusetts Antitoxin Laboratory, Jamaica Plains, for this material.

The Schick test was read again at 48 hours and individuals who showed cutaneous reactions of the delayed type or who were Schick-positive were not studied further.

Allergic Histories.—All persons were questioned in regard to allergic history. Included in the survey were questions related to personal sensitivities of the immediate wheal and erythema variety and treatment given, if any. Individuals with contact or delayed types of dermatitis were not included in this category. The same questions were asked concerning the existence of allergies in relatives, including parents, grandparents, brothers, sisters, uncles, aunts, and first cousins.

Immunization.—Subjects who were Schick-negative but who did not show a delayed reaction to Schick control toxoid at 48 hours were given at this time a single injection of purified diphtheria toxoid subcutaneously. The dose was 37.5 Lf for fluid toxoid and 60 Lf when purified toxoid adsorbed on alumina cream (18) was used. 2 subjects received only 0.75 Lf of fluid toxoid and 2 received 200 Lf adsorbed on alumina cream.

Medical and dental students constituted a large proportion of the immunized group. They were divided into two categories. The first was a normal group which gave no immediate skin reaction against Schick toxoid and no personal or familial history of allergy. The second group comprised persons with positive immediate skin reactions against Schick toxoid and a personal allergic history. Additional selected patients in the allergy clinic and in hospital were also hyperimmunized. The criteria for selection were the same as those outlined above. The desired minimal qualification for the allergic group was a strong personal history of allergy. Preimmunization bleedings were taken at the time the Schick tests were given. The booster dose of toxoid was given 48 hours later. Postimmunization bleedings were usually drawn on the 10th or 14th day and in certain cases at intervals thereafter. Serum was removed and stored in small glass tubes at −30°C until used.

In Vivo Antitoxin Titer.—The rabbit intracutaneous test was used, following the technic of Fraser (19). In all cases unknowns were compared with a simultaneous titration carried out with standard antitoxin obtained from the National Institutes of Health. Dilutions were spaced in such a manner that the titrations are generally accurate to within 10 to 15 per cent.

Removal of Complement.—Complement was removed from human antitoxic sera prior to carrying out quantitative precipitin tests. An amount of anti-type III pneumococcal rabbit serum sufficient to yield 50 μg. specifically precipitable nitrogen upon addition of an appropriate amount of SIII was added per ml. of human serum. After standing in the cold for 4 days, the specific precipitate was removed by centrifugation.

Quantitative Precipitin Reaction.—The test was carried out by adding an appropriate amount of antitoxin to varying amounts of toxin. On the basis of titers obtained in the rabbit skin tests, sera were diluted or added in amounts so that about 30 units was present in each tube. The number of tubes set up for a given titration varied somewhat. As a rule, five to eight points were selected along a calculated theoretical curve, and toxin was added in increments of 5 Lf up to and slightly over the calculated point of equivalence assuming that 1 Lf = 1 antitoxic unit. The purified toxin used contained 2480 Lf/cc., and 1 Lf was equivalent to 0.46 μg. specifically precipitable N.

Dilutions of toxin were made in borate-NaCl buffer pH 7.4 containing 200 μg. gelatin per ml. to prevent surface denaturation. All tubes were adjusted to the same total volume (3 cc.) with borate buffer. Following the addition of antigen to antiserum, the mixture was agitated briefly, incubated at 37°C. for 1 hour and placed in the cold for 10 to 14 days. This precaution is usually unnecessary in the case of sera giving normal *in vivo/in vitro* ratios and precipitation in such instances is complete within 24 hours in contrast to human antipneumococcal sera (21) which precipitate slowly. However, antitoxins with high *in vivo/in vitro* ratios also precipitate slowly and were therefore allowed to remain in the cold for a protracted period. Merthiolate was added to concentration of 1:10,000. This was done after comparative titrations performed with and without the addition of merthiolate had demonstrated that this substance possessed no inhibitory effect upon precipitability. After centrifugation, the supernatants were drawn off and saved for further tests.

The precipitates were broken up and washed three times with cold saline. Nitrogen was determined by Markham's (20) modification of the micro-Kjeldahl method, using a Scholander burette.

The assay of supernatants was performed by use of the intracutaneous rabbit and guinea pig tests.

RESULTS

Relationship between Skin Sensitivity and Allergic History.—Immediate skin reactions of the wheal and erythema type were surprisingly frequent among a group of 131 medical students Schick-tested with purified diphtheria toxin using purified toxoid as control. As can be seen from Table II, 40 immediate reactions were observed, an incidence of 30.5 per cent. The incidence of such reactions was twice as high among the Schick-negative individuals as compared with the Schick-positives (Table III). Moreover, 4 of the 6 Schick-positive individuals

TABLE III

Immediate Reaction to Schick Test in 35 Schick-Positive Medical Students

Strength of immediate reaction to Schick control (0.008 Lf purified toxoid)	No. claiming personal or familial allergic history	No. claiming *no* personal or familial allergic history
0	8	21
+	2	1
++	1	0
+++	2	0
++++	0	0
Total	13	22

who showed an immediate wheal and erythema reaction had become Schick-negative when retested 1 month later, indicating the presence of some antitoxin at the time the first test was carried out. 35 of the 40 (87.5 per cent) showing immediate reactions gave either a personal or familial history of allergy or both. It is significant that *all* the eleven subjects whose skin reactions were rated as 4+ (*i.e.*, the most severe) were both Schick-negative and gave a history of allergy. It seems worth while to present brief histories obtained from each of these 11 subjects:

1. Fe Personal history—hay-fever 10 years.
   Family—grandmother has hay-fever.
2. Ro Personal history—0.
   Family—mother allergic to numerous foods.
3. Sa Personal—vasomotor rhinitis.
   Family—father has hay-fever.
4. Sch Personal—allergic to dusts, gets attacks of sneezing of unknown cause every spring.
   Family—father has hay-fever (under treatment).

5. Ch Personal—sensitive to timothy and egg white, has hay-fever.
Family—mother has hay-fever.
6. Ha Personal—hay-fever for a number of years.
Family—no allergic history elicited.
7. Kr Personal—hay-fever 2 years (ragweed); treated.
Family—1st cousin has hay-fever.
8. La Personal—hay-fever 21 years, treated; asthmatic attacks, allergic to grasses, trees, ragweed.
Family—many members with hay-fever and other allergies.
9. Lau Personal—hay-fever 5 years (ragweed); treated.
Family—2 cousins with hay-fever.
10. Ne Personal—allergic to dusts and a variety of vegetables, vasomotor rhinitis of long standing.
Family—no allergic history elicited.
11. Bi Personal—allergic to cat hair and other furs, attacks of idiopathic purpura.
Family—mother and brother have hay-fever.

7 of 9 Schick-negative patients tested in allergy clinics showed the wheal and erythema reaction at the Schick control site.

Immediate Reactions to Toxoid Following Immunization.—Regardless of whether or not immediate skin reactions were observed at the time of initial Schick test, almost all Schick-negative individuals developed some degree of skin sensitivity to purified toxoid following a "booster" dose of toxoid (Table IV). Thus 34 of 39 persons tested 1 month after immunization showed skin reactivity of the immediate type. This was independent of whether fluid- or alum-precipitated toxoid was used as immunizing agent. All but one of the 5 who failed to react showed less than 10 units circulating antitoxin per cc. 19 of the total group of 39 showed no skin reaction prior to immunization.

It is of interest that some of the subjects who showed the greatest degree of skin sensitivity following immunization were those who gave *no* personal or familial history of allergy and who showed no immediate reaction at the time of the first Schick test. While most of the more severe skin reactions following immunization occurred among the group with allergic histories, the correlation was considerably less striking than that shown in Table II.

Non-Precipitating Diphtheria Antitoxin.—The evidence for presence or absence of non-precipitating antitoxin in sera was based on whether a discrepancy was observed between the unitage determined by the rabbit skin test (*in vivo*) and the quantitative precipitin test (*in vitro*). The number of units as determined by the precipitin reaction was calculated by dividing the antitoxin nitrogen found at the point of maximal precipitation by 2.5. In a previous study (1) of sera from 12 hyperimmunized "normal" human subjects close to 2.5 μg. nitrogen was precipitated per unit of antitoxin. In order to obtain maximal precipitation it was necessary to add only 0.75 $\pm$ 15 per cent Lf toxin for each unit of antitoxin found by rabbit skin test. This deviation from unity was shown to be dependent on the *in vivo/in vitro* ratio of the horse serum used as

TABLE IV

Immediate Skin Reaction, Rabbit Skin Titer, and Quantitative Precipitin Titer in 39 Hyperimmunized Normal and Allergic Schick-Negative Individuals

Subject No.	Toxoid (immunizing dose)	Allergic history	Immediate reaction—Schick control	Postimmunization antitoxin titer		*In vivo* / *In vitro* ratio	Immediate reaction—Postimmunization Schick test
				In vivo,* units/cc.	*In vitro*,‡ units/cc.		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1. Go	60 Lf alum	0	0	10	+	N	0
2. G'r	200 Lf alum	0	0	10	+	N	0
3. Ho	37.5 Lf fluid	0	0	20	0		+++
4. Hu	200 Lf alum	0	0	80	0		++++
5. Lev	37.5 Lf fluid	0	0	10	+	N	0
6. O'c	37.5 Lf fluid	0	0	60	20	3.0	+++
7. Kr	60 Lf alum	0	+++	80	15	5.3	++±
8. Dav	60 Lf alum	0	0	10	+	N	±
9. Fo	37.5 Lf alum	0	0	140	100	1.4	+
10. Hi	60 Lf alum	0	0	10–20	+	N	+
11. Leh	37.5 Lf fluid	0	0	10–20	+	N	±
12. Lu	37.5 Lf fluid	0	0	10	+	N	+
13. Mu	37.5 Lf fluid	0	0	20	0		++
14. Sk	60 Lf alum	0	0	10–20	+	N	±
15. We	37.5 Lf fluid	0	0	15	8	1.9	++++
16. Pa	37.5 Lf fluid	0	++	20	15	1.3	++
17. Pi	37.5 Lf fluid	0	0	10	−		0
18. Sc	60 Lf alum	+	++++	10	+	N	++++
19. Lar	60 Lf alum	+	++	10	+	N	+
20. Be	60 Lf alum	+	++++	10–20	+	N	+++±
21. La	60 Lf alum	+	++++	60	20	3.0	++++
22. Ro	37.5 Lf fluid	+	0	140	120	1.2	++
23. Ba	37.5 Lf fluid	+	0	80	60	1.3	++
24. He	0.75 Lf fluid	+	+++	15	8	1.9	+++
25. Si	0.75 Lf fluid	+	+++	10	8	1.3	+++
26. Ap	37.5 Lf fluid	+	++	60	30	2.0	++
27. Do	37.5 Lf fluid	+	+++	40	25	1.8	+++
28. Fe	37.5 Lf fluid	+	++	20	−		++
29. Ja	37.5 Lf fluid	+	0	10	−		+
30. Ra	37.5 Lf fluid	+	+	10	−		+
31. We	37.5 Lf fluid	+	+++	20	−		+++
32. Ch	37.5 Lf fluid	+	+++	10	−		++++
33. Le	37.5 Lf fluid	+	++	80	80	1.0	++
34. Ca	37.5 Lf fluid	+	++	20	20	1.0	++
35. Ma	37.5 Lf fluid	+	+±	100	55	1.8	+++
36. Br	60 Lf alum	+	++	10	−		++
37. Maz	60 Lf alum	+	++±	10	−		++±
38. Da	60 Lf alum	+	++±	80	40	2.0	+++
39. Al	60 Lf alum	+	0	40	−		0

* Rabbit intracutaneous test.

‡ Figures in column 6 refer to units calculated from specifically precipitable antitoxin nitrogen. (+) designates sera which produced a precipitate with an added amount of toxin at the equivalence point calculated from the rabbit skin test—a saline control tube was run with each serum tested. If the precipitate appeared of sufficient bulk, as judged by inspection, the *in vivo/in vitro* ratio was considered normal and is marked N in column 7.

a standard in carrying out the *in vivo* titrations in the rabbit skin. In the present study we have encountered very much larger deviations in certain sera which cannot be accounted for in this way. Thus 12 of 30 sera from hyperimmunized subjects showed marked discrepancies with *in vivo/in vitro* ratios of 1.8 or greater. Moreover, sera from 3 persons failed to precipitate at all with toxin despite the presence of 20 or more units of antitoxin per cc. by *in vivo* titration.

Relationship between Skin Sensitivity and Non-Precipitating Antitoxin.— Thirty subjects comprised the group whose sera were tested for antitoxic activity both by rabbit skin test and by specific precipitation. Of the 16 who claimed no history of allergy, only 2 gave an immediate reaction to the Schick control toxoid prior to immunization. Following immunization, 3 subjects showed no precipitation whatever with toxin despite antitoxin titers of 20 to 80 units/cc. In 3 other cases there was only 0.5 to 1.3 μg. specifically precipitable nitrogen per unit while in the remaining 10 individuals, either the expected amount of precipitation was found (*i.e.* 2 to 2.5 μg. nitrogen per unit) or good precipitation was observed with amounts of toxin or toxoid equivalent to the rabbit titer. The 6 subjects whose sera showed *in vitro/in vivo* antitoxin discrepancies all had negative immediate skin reactions to toxoid before immunization. Following immunization positive reactions occurred in 5 of them. In general the severity of the immediate skin reaction to toxoid following immunization was correlated with the antitoxin titer and with the magnitude of the *in vivo/in vitro* discrepancy (see Table IV).

Of 14 subjects who comprised the group claiming a personal or familial history of allergy, 12 showed skin sensitivity to Schick toxoid which was rated moderate to severe prior to immunization. Following immunization the severity of the wheal and erythema response to toxoid remained the same or increased somewhat. Six sera showed *in vivo/in vitro* ratios of 1.8 or higher with only 0.8 to 1.6 μg. specifically precipitable antitoxin nitrogen per unit instead of the expected 2 to 2.5 μg. nitrogen per unit.

DISCUSSION

Hypersensitivity of the immediate type following injection of materials used in the Schick test has received little attention in the past. When immediate reactions of the wheal and erythema type have been noted it has generally been assumed that sensitivity was directed against constituents present in the original culture medium or against the peptone used as stabilizing agent. Nonetheless, Neill and Fleming (22) demonstrated that certain individuals showed the immediate type of hypersensitivity to diphtherial proteins and produced evidence that in some subjects at least this hypersensitivity was specifically directed against the toxin itself.

In the present study a high proportion (30.5 per cent) of 131 medical students showed reactions of the wheal and erythema type 15 to 45 minutes following

intracutaneous injection of 0.008 Lf highly purified diphtheria toxoid. The high incidence of skin sensitivity to purified diphtheria toxoid may, at first glance, appear surprising. However, it will be recalled that until recently it has been customary to dilute Schick materials in buffers containing various peptones as stabilizing agents. It was shown by Hooker (23) that a large percentage of young adults show skin sensitivity to the peptones present in Schick diluent and our own experience fully corroborates that of Hooker. Evidence of hypersensitivity specifically directed against diphtherial products would thus have been masked in most cases by reactions caused by the diluents used. The use of human serum albumin as stabilizing agent, as in the present study, obviates this difficulty.

The incidence of immediate cutaneous reactions was twice as high in Schick-negative as in Schick-positive subjects and reactions were more intense in members of the Schick-negative group than in those of the Schick-positive group. It is of interest that 4 of the 6 Schick-positive subjects who showed immediate reactions proved Schick-negative on retest 1 month later, indicating the presence of some antitoxin at the time the first test was performed. Additional evidence that the immediate type skin reactions observed in this study are caused by the specific diphtheria toxin or toxoid will be presented in the following paper.

The relatively high incidence of hypersensitivity to diphtheria toxoid is in keeping with the findings of other workers who tested other materials. Grow and Herman (24) performed intracutaneous tests on a group of 150 normal persons using thirteen common allergens as testing substances. Under the conditions of their experiment, 83 or 55.5 per cent gave positive reactions to one or more of the allergens. 46 per cent gave reactions to horse dander alone. Somewhat similar results were obtained by Rackemann and Simon (25) and by Berger (26).

There was a remarkable correlation between personal or familial history of allergy and the occurrence of immediate reactions to the Schick test. Thus of 59 medical students who gave either a personal or familial history of hay-fever or other allergic manifestations 35 (60 per cent) showed wheal and erythema reaction to the toxoid used as Schick control and only slight or no immediate reaction to the tuberculin test administered simultaneously or to the diluent used in the Schick test. Only 5 (7 per cent) of the 72 students claiming a completely negative allergic history showed a slight or moderate degree of skin sensitivity.

Further investigation was concerned with the occurrence of non-precipitating antitoxin in the serum of hyperimmunized subjects and its association with the immediate skin reaction. In a previous paper (1) studies on the precipitin reaction between diphtheria toxin and antitoxin in the serum of 12 apparently normal human subjects were reported. It was shown that human antitoxic sera contained close to 2.5 μg. specifically precipitable nitrogen per unit of

antitoxin and the mean ratio of *in vivo* titer as determined by intracutaneous rabbit assay to *in vitro* titer (quantitative precipitin) was 1.3. One or two antitoxic sera were encountered in the earlier study[2] which showed unexplained discrepancies between *in vivo* and *in vitro* titers. Such atypical sera contained far less specifically precipitable antitoxin than expected.

A relatively large proportion of the antitoxins examined during the present study showed such discrepancies. Moreover, the proportion of individuals showing discrepant *in vivo/in vitro* ratios was no greater among the allergic group than among those who disclaimed all knowledge of familial or personal allergy. In fact, the 3 individuals who produced no precipitating antibody whatever despite high titers of non-precipitating antitoxin, denied any history of personal or familial allergy. This is not particularly surprising in view of the findings of others (27, 28) that the development of allergy in patients under treatment with liver extract, insulin, and other materials cannot be predicted on the basis of the patient's past history.

Although most subjects developed some degree of skin sensitivity following the booster dose of toxoid there was marked parallelism between the severity of the skin reactions observed and the presence of demonstrable amounts of non-precipitating antitoxin (see Table IV). Thus, of 12 individuals whose sera showed *in vivo/in vitro* ratios of 1.8 or greater, all but one showed +++ and ++++ immediate skin reactions when tested subsequent to immunization. One subject with 20 units per cc. of non-precipitating antitoxin showed a ++ reaction. Of 7 individuals whose sera showed ratios ranging from 1.0 to 1.4, 6 gave immediate reactions of only + or ++ intensity. One subject with 10 units showed a +++ reaction. Additional confirmatory evidence was provided by the behavior of the 10 hyperimmunized subjects who had no history of allergy and whose sera gave visible precipitates with toxoid despite a relatively low antitoxin titer. None of these persons showed more than slight skin sensitivity (0 to +).

A detailed comparison of the *in vivo* and *in vitro* properties of human precipitating and non-precipitating antitoxin will be presented in the following paper.

SUMMARY

Among a group of 131 young adults tested, there was a high degree of correlation between the occurrence of immediate skin reactions of the wheal and erythema type produced by purified diphtheria toxoid and personal or familial history of allergy of the hay-fever type.

Of 39 Schick-negative subjects who received a "booster" dose of purified diphtheria toxoid, 19 showed no immediate skin reactions before immunization.

[2] Unpublished observations.

The development of skin sensitivity in these subjects was associated with the production of non-precipitating circulating antitoxin.

Three subjects produced 20 units or more antitoxin per cc. serum following immunization, without demonstrable precipitins. Despite the fact that none showed immediate skin reactions to Schick toxoid prior to immunization, all 3 possessed a high degree of skin sensitivity to toxoid following immunization.

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CORRECTION

In Vol. **95,** No. 4, April 1, 1952, the following sentence should be inserted in the paper by Drs. Kuhns and Pappenheimer on page 365, in the fifth line from the bottom:—

The high antitoxin titer found at this time is explained by the fact that the boy had already received an earlier "booster" dose of toxoid a few months previously.