STUDIES ON HYPERSENSITIVENESS TO DIPHTHERIA BACILLI.

I. AN "IMMEDIATE" SKIN REACTION WHICH CAN BE PASSIVELY TRANSFERRED.

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PLATES 2 TO 5.

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Hypersensitiveness of man to bacteria is of general importance because of its unknown effects upon infection and resistance and upon the manifestation of disease. From at least one aspect, hypersensitivity to diphtheria bacilli seems to be especially important. While there are few diseases in which the manifestations can be referred so directly to the toxin, there are few pathogenic bacteria that are as widely distributed as the diphtheria bacillus. The question of hypersensitiveness to these bacteria, therefore, must be considered significant for it can be assumed that most city adults have probably had at some time in life such opportunities to become sensitive as may be afforded by contact with diphtheria bacilli.

The present paper describes an "immediate" skin reaction to diphtheria bacilli, and presents experimental evidence to establish it as a true hypersensitive phenomenon distinct from the "delayed" skin reaction commonly encountered in Schick tests on adults ("pseudo-reaction").

The common division of human skin reactions into "immediate" and "delayed" reactions (as reviewed in detail by Zinsser (1) and others), seems to present a separation of two fundamentally different types of antibacterial hypersensitivity. In general, the hypersensitive condition manifested in man by "immediate" skin reactions has much in common with the condition of "true anaphylaxis" (Wells (2)) obtained in the guinea pig by laboratory sensitization. In view of the infrequent reports of the "immediate skin reaction type" of hypersensitiveness in
man to bacteria, our interest was aroused when we obtained an "immediate" skin reaction in an apparently normal adult to the bacterial derivatives contained in the routine Schick test material. Since Schick tests are employed so frequently, it seemed to us that if this type of hypersensitiveness were not rare it must have been observed in the past. A thorough review of the literature, however, revealed no reports of hypersensitive skin reactions that could be referred definitely to the diphtheria bacillus itself, other than the well known "pseudoreaction" which is a classical example of the "delayed skin reaction type" of hypersensitiveness.

The "Immediate" Skin Reaction.

The "immediate" skin reaction reported in this paper was first observed in March, 1926, on one individual in a group of 40 medical students who had received the routine Schick test. The "immediate" and the "delayed" reactions exhibited by this man are described below:

"Immediate" Reaction.—Within 5 minutes after the injection of the Schick test material, the man experienced a slight itching on the areas of the injections. Within 15 minutes, a blister about 25 mm. in diameter, with definite pseudopodia became evident; the blister was surrounded by a solid zone of bright red erythema extending out 20 to 25 mm. with punctiform, scattered points of erythema extending beyond the solid zone. This reaction is essentially the same in appearance as that often observed in the common examples of the "immediate skin reaction type" of hypersensitiveness, and requires no further description since pictures of the reaction are given in Figs. 1 and 2. The reactions were essentially the same on the left arm which had been injected with heated (75°C.) Schick toxin as on the right arm which had been injected with unheated Schick toxin.

This immediate reaction began to fade within 1 hour after the intradermal injection and disappeared within 2 hours.

"Delayed" Reactions.—After the disappearance of the "immediate" reaction (Fig. 3), the further manifestations of this individual's reaction to diphtheria filtrate were the same as that frequently observed in adults who give pronounced "combined reactions" in the routine Schick test. The "combined reaction" (Fig. 4) is too well known to require a detailed description of the "delayed" reactions in this particular individual.

The individual giving the "immediate" skin reaction to diphtheria filtrate was 28 years of age. His diphtheria history is indefinite. It is possible that he had a mild attack when about 4 years old, since he experienced a sore throat at the time of a diphtheria epidemic in Corfu. He was not severely sick, but was kept in bed for 3 days by the physician. No cultures were taken. No antitoxin was given. The sore throat was not diagnosed as diphtheria by the physician in spite of the local prevalence of the disease.
He has never been immunized with toxin-antitoxin or toxoid. There is no evidence that he is a carrier; negative results were obtained in all attempts to detect diphtheria bacilli either by staining or by guinea pig inoculation of Loeffler's serum cultures made at different times from his throat, nose, and ears. Skin tests with a wide variety of substances seemed undesirable during our investigation but, as far as clinical symptoms are concerned, he has no recognizable asthma, hay fever, or food idiosyncrasies. There is, thus, no evidence that this individual has been endowed with an unusual tendency to become sensitized or that he has had any greater opportunities to become sensitive to diphtheria bacilli than have many other city adults.

Tests for "immediate" skin reactions to diphtheria filtrate (0.0008 cc.) were negative in all the immediate relatives of the diphtheria-hypersensitive individual; namely, the mother (60 years old), a sister (26 years old), and her three children (2 to 5 years old). No clinical symptoms of hypersensitiveness (asthma, hay fever, food idiosyncrasies) have ever been observed in any of them.

**Comparison of the Skin Reactions Induced by Broth.**

The material injected in the Schick test is the diluted filtrate of a broth culture of the diphtheria bacillus. Experiments were made to determine whether or not the broth culture medium itself was responsible for the "immediate" skin reaction. The first tests were made with the same amount of broth as that contained in the dose of the culture filtrate utilized in the Schick test with which the reaction was first observed. A sample of the same broth culture medium as that employed at the Massachusetts Antitoxin Laboratory in the preparation of this particular lot of Schick toxin was obtained. The equivalent amount (0.0004 cc.) of the broth contained in 0.1 cc. of salt solution was injected and found to elicit no "immediate" skin response at all.

It seemed important, however, to compare the response of the apparently sensitive individual with the response of other individuals to larger amounts of broth than that contained in the Schick test dose. The tests were made on 12 other normal individuals, with doses of 0.02 cc and 0.001 cc. of broth. The larger amount (equivalent to 50 times the amount injected in our Schick test dose) gave a pronounced "immediate" skin reaction in the test individual and in 11 out of 12 of the control group. The reaction was of the same appearance as that usually obtained in the "immediate skin reaction type" of hypersensitiveness; it is probably not a hypersensitive reaction at all, but only the normal response to a constituent of broth which is primarily toxic in man when injected in such large amounts. The smaller dose (equivalent to 2.5 times the Schick test dose) gave no reaction in the test individual although it did give small but definite reactions in a few of the group; consisting of an erythematous rash, without definite raising of the wheal and without pseudopodia. With neither dose of broth, however, was the reaction of the test individual greater than that of the majority of the normal individuals in the control group.
The failure of the hypersensitive individual to give any "immediate" skin response to the amount (0.0004 cc.) of broth equivalent to that contained in the Schick test dose, together with his failure to give quantitatively greater reactions to larger amounts of broth than those given by a group of other individuals, is important; for it shows that he is neither truly "hypersensitive" to broth nor does he possess a low degree of "tolerance" to broth constituents which apparently are primarily toxic to other men when injected intradermally in sufficient quantity.

"Immediate" Skin Reaction to Washed Diphtheria Bacilli (Heated) and to Sterile Filtered Extracts.

Experiments were made to determine whether the "immediate" skin reaction could be produced by diphtheria bacilli when washed free from all traces of culture fluid. Two sorts of material were tested: (1) salt solution suspensions of the washed bacterial cells; (2) sterile solutions or extracts of the intracellular substances derived by repeated freezing and thawing of concentrated suspensions of the washed bacilli.

Preparation of Bacterial Suspensions.—Broth cultures (5 days old) of the Park 8 strain of the diphtheria bacillus were centrifuged and the bacilli resuspended in salt solution. They were then washed 3 times with 50 cc. of sterile salt solution; the washing removed all traces of broth remaining on the bacilli, for the centrifuged sediment was sufficiently compact to enable one to remove, after each washing, practically all traces of the supernatant fluid. The washed bacteria were finally suspended in a volume of salt solution equivalent to the original broth culture, heated for 15 minutes at 80°C., and 0.4 per cent of phenol added. Sterility was tested by culture in glucose broth, and by intradermal injection into guinea pigs.

Preparation of Solutions (Extracts) of the Intracellular Substances.—The bacterial cells from a broth culture of a recently isolated strain of the diphtheria bacillus were collected and washed as described above, and finally resuspended in a volume of salt solution equivalent to 1/20 the original volume of broth culture. The concentrated suspension of washed bacterial cells was then repeatedly frozen and thawed as described in a preceding paper for the preparation of diphtheria bacillus enzymes (3). The frozen and thawed suspensions were finally filtered through a Berkefeld candle. These extracts or solutions contained a certain amount of intracellular substances as demonstrated by a slight but definite coagulum in boiled samples of the solution and by the presence of the active maltase enzyme which is known to be an endocellular constituent of the diphtheria bacillus (3). The extracts were not heated, but were proved sterile by culture and by guinea pig inoculation.
The tests for "immediate skin reaction" were made by the intradermal injection of 0.1 cc. volumes of salt solution containing: (1) heated suspensions of washed bacterial cells equivalent to the bacteria contained in 0.0004 cc. of the broth culture; (2) 0.00005 cc. of the sterile filtered extract of the frozen and thawed suspension of washed bacilli. Salt solution was injected as control.

The results of the tests with the washed bacilli and with the sterile filtered extract prepared by physical disintegration of the washed bacilli, showed that these substances induced the same type of "immediate" skin reaction as had the filtrate of the broth culture. Similar injections in 25 other individuals gave no evidence of any "immediate" skin response to washed diphtheria bacilli. This experiment, together with the preceding one, indicated that the described "immediate" reaction was due to some constituent of the diphtheria bacillus itself, contained in broth culture filtrates, in suspensions of the washed bacterial cells, and in filtered solutions of substances derived from the washed bacteria.

The results with the washed bacilli are also of interest as proof that neither active toxin nor its heat-inactivated derivatives are involved in the hypersensitive reaction. The suspension of washed bacilli can be assumed to have been freed from soluble toxin by washing the cells, and since they were heated at once, the bacilli were killed before new toxin could have been formed if it were possible to do so in salt solution. The bacterial extracts, while unheated, were likewise devoid of amounts of toxin detectable in guinea pigs. 2.0 cc. of the undiluted and unheated extract injected subcutaneously being innocuous, and the intradermal injection of 0.15 cc. failing to give a skin reaction on normal guinea pigs. These tests for the presence of toxin show that the amount of the bacterial extract (0.00005 cc.) which gave a strong "immediate" reaction in the hypersensitive individual must have contained an absurdly small amount of toxin, if any. (The amount (0.15 cc.) injected intradermally in the guinea pig without reaction was 3,000 times the amount injected in the hypersensitive man. Since 1/500 M.L.D. is commonly accepted as giving a definite skin reaction in a guinea pig (4), then the amount of toxin contained in the unheated extract that produced the "immediate" hypersensitive reaction must have been less than 1/1,500,000 M.L.D.)

The general question of toxin hypersensitiveness is of considerable theoretical interest and although considerable work has been done in the past, the question is still an open one (5). In the present instance, however, there is no reason to believe that the toxin or any of its heat-inactivated derivatives have anything at all to do with the hypersensitiveness of the individual described in this paper.
The Bacterial Specificity of the "Immediate" Skin Reaction.

The first experiments were made with heated suspensions of washed bacterial cells and consisted of a comparison of both the "immediate" and the "delayed" reactions induced by the intradermal injection of diphtheria bacilli with the reactions invoked by other bacteria. Colon bacilli, typhoid bacilli, and Type I pneumococci were used as species not closely related to diphtheria bacilli; and hofmanni and xerosis bacilli as bacteria supposed to be more closely related to the diphtheria organisms. In order to make the test of the specificity more rigid, the amount of bacterial substance was made twice as great in the test doses of the other kinds of bacteria as in the test dose with the diphtheria bacilli. The doses were based upon the bacterial substance contained in the suspensions as determined by turbidity comparisons.

Preparation of Bacterial Suspensions.—Broth cultures (18 hours old in the case of the pneumococci and 5 days old in the case of the other bacteria) were centrifuged, the bacterial cells washed 3 times with large volumes of sterile salt solution, and finally resuspended in salt solution in a volume equivalent to that of the original broth culture. The bacteria were killed by 15 minutes exposure to 80°C and the sterility of the suspensions checked by cultural tests. Since the growth of the different bacteria was not comparable, all of the suspensions were adjusted to a turbidity equivalent to that of a 1/20 dilution of the original diphtheria bacillus suspension. The adjusted suspensions, after the addition of 0.4 per cent phenol, were used as stock suspensions from which the test doses were prepared by their proper dilution.

Test Injections.—The tests themselves were made by intradermal injection of 0.1 cc. of suspensions of the different kinds of bacteria in different areas of the flexor surface of the forearms. The dose of diphtheria bacilli was equivalent to the bacteria contained in 0.0005 cc. of its broth culture; the amount of the other kinds was equivalent to twice the bacterial substance contained in the diphtheria test dose. Observations at 5 minute intervals for 1 hour were made for the "immediate" reactions; and observations at 4 hour intervals for 24 hours for the "delayed" reactions. Two normal individuals were injected at the same time with the same materials.

The results of two experiments made at different times were approximately the same. The "immediate" reactions are shown in Fig. 5.

The results of these experiments (Fig. 5) showed that the "immediate" reaction to diphtheria bacilli is specific and not invoked by all bacteria regardless of species. Although the bacterial substance was twice as great as in the diphtheria test, pneumococci, hofmanni bacilli, typhoid bacilli, and colon bacilli gave no "immediate" skin reaction at all. The xerosis bacilli, on the other hand, gave a definite "imme-
"Immediate" reaction, weaker yet of the same type as that invoked by the smaller amount of diphtheria bacilli. The reaction given by the xerosis bacilli does not detract at all from the specificity of the diphtheria reaction and in fact, is important in itself as an index of a probable immunological group relationship between our strain of xerosis and the usual diphtheria bacillus.

The fact that some of the different bacteria gave "delayed" reactions in this man and others did not, is not of particular importance in this paper, for the majority of adults give "delayed" reactions to some kinds and not to other kinds of bacteria. But it is important that the xerosis bacilli which gave a definite "immediate" reaction failed entirely to cause a "delayed" reaction. This furnishes a sharper distinction between the "immediate" and "delayed" skin reactions than can be obtained with diphtheria bacteria in this individual because a dose of diphtheria which gives only a small "immediate" reaction still gives a definite "delayed" reaction.

The following experiments differed from the preceding ones chiefly in the fact that filtrates of broth cultures of the different kinds of bacteria were employed instead of suspensions of the washed bacterial cells.

In the preceding experiments, the dose of the other bacteria was twice the diphtheria dose, while in the present ones, the amount of the filtrate of the other kinds of bacteria was increased to 5 times that of the diphtheria filtrate. The increase in the relative dose of the filtrates of the other bacteria made the test for specificity more rigid, but was chosen particularly in order to determine if the "immediate" reaction to the xerosis filtrate could be made comparable in size to the "immediate" reaction to the diphtheria filtrate without producing a "delayed" skin reaction.

Two experiments were made with similar results, which are presented in Table I. Pictures of the "immediate" and "delayed" reactions are shown in Figs. 6 and 7.

The results (Table I and Figs. 6 and 7) of the experiments with the filtrates were essentially the same as those obtained with the suspensions of washed bacterial cells. But the results with the filtrates are of additional interest as evidence that the specificity of the "immediate" reaction holds true even when the amount of culture filtrate of the unrelated bacteria (typhoid, colon, hofmanni bacilli, and pneumococci) is 5 times as great as the diphtheria dose.
### Table I.

**Bacterial Specificity of the "Immediate" Skin Reaction in Tests with Broth Culture Filtrates of Different Kinds of Bacteria.**

<table>
<thead>
<tr>
<th>Kind of bacteria</th>
<th>Amount of filtrate</th>
<th>&quot;Immediate&quot; reactions (20 min. after time of injection)</th>
<th>&quot;Delayed&quot; reactions (24 hrs. after time of injection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria bacilli</td>
<td>0.0001</td>
<td>Strong {30 × 35 mm. intensely red, solid area; surrounded by additional slight speckling}</td>
<td>Strong {20 × 30 mm. intensely red and slightly swollen area; increased since 16 hr. reading}</td>
</tr>
<tr>
<td>Xerosis bacilli</td>
<td>0.0005</td>
<td>Strong {35 × 40 mm. intensely red, solid area; slightly greater than the reaction to the smaller amount of diphtheria}</td>
<td>None {No redness and only faint mark of needle point}</td>
</tr>
<tr>
<td>Typhoid bacilli</td>
<td>0.0005</td>
<td>None. Indistinguishable from broth control</td>
<td>Strong {15 × 20 mm. moderately red and slightly swollen area; reduced since 16 hr. reading}</td>
</tr>
<tr>
<td>Colon bacilli</td>
<td>0.0005</td>
<td>None. Indistinguishable from broth control</td>
<td>Strong {15 × 20 mm. moderately red and slightly swollen area; reduced since 16 hr. reading}</td>
</tr>
<tr>
<td>Pneumococci</td>
<td>0.0005</td>
<td>None. Indistinguishable from broth control</td>
<td>None {No redness and only faint mark of needle point}</td>
</tr>
<tr>
<td><em>Hofmanni</em> bacilli</td>
<td>0.0005</td>
<td>None. Indistinguishable from broth control</td>
<td>None {No redness and only faint mark of needle point}</td>
</tr>
<tr>
<td>Broth control</td>
<td>0.0005</td>
<td>Only a faintly pink area (5 × 6 mm.) at site of original injection; a faint trauma reaction</td>
<td>None {No redness and only faint mark of needle point}</td>
</tr>
</tbody>
</table>
The most significant evidence produced by these experiments, however, consists of the relations between the skin reactions to diphtheria and the reactions to xerosis. In the previous experiment, it was shown that with the doses used the xerosis and diphtheria washed bacilli were alike in that both produced "immediate" skin reactions but differed in that only diphtheria produced a "delayed" skin reaction. The present experiment (Table I) showed that by changing the ratio of the xerosis and diphtheria doses the "immediate" reaction to the xerosis filtrate was made larger than the "immediate" reaction to the diphtheria filtrate; and that even under these conditions, the xerosis filtrate failed to cause the "delayed" skin injury produced by the smaller dose of diphtheria filtrate. These relations indicated that while both "immediate" and "delayed" reactions were produced by diphtheria bacteria or filtrate, they were due to different constituents of this bacillus. The substance responsible for the "immediate" reaction was also possessed by our strain of xerosis bacilli. The constituent responsible for the "delayed" reaction was either not possessed by the xerosis bacilli or the two substances were present in them in a ratio much different from the ratio in which they were present in diphtheria bacilli since a dose of xerosis material which failed to produce a "delayed" reaction produced a larger "immediate" reaction than did a dose of diphtheria material which also produced a pronounced "delayed" reaction.

The production of "immediate" reactions by the xerosis bacilli and filtrates presents further evidence that diphtheria toxin is not involved in the hypersensitivity. This strain of xerosis although immunologically related to diphtheria bacilli is absolutely non-toxicogenic and is avirulent for guinea pigs and rabbits. A number of both kinds of animals have been injected with 10 cc. of broth culture, and rabbits have been injected with suspensions containing the concentrated bacilli from 750 cc. of broth culture with apparently no harmful effect.

The Influence of Heating the Filtrate.

The filtrate of a 7 day broth culture was diluted 8 times with sterile salt solution and then divided into 3 equal portions. The first portion was heated for 10 minutes at 80°C. as in the usual inactivation of the heated control used in the Schick test; the second portion was heated at 100°C. for 1 hour; and the third at 120°C. for 30 minutes. Phenol in physiological salt solution was then added so that the filtrate was finally diluted 10 times in salt solution containing 0.4 per cent.
phenol. (The phenol was added last in order that it might not be present during the heating processes.)

In the particular experiment presented in Figs. 8 and 9 the test doses employed were 0.0004 cc. of filtrate, which is as much as that usually contained in the Schick test dose. The same amount of the same broth as that utilized in growth of the culture was injected as the control.

The results (Figs. 8 and 9) of these experiments were always the same: exposure of diphtheria culture filtrate to temperatures above 80°C. had a much greater effect upon its ability to induce the "immediate" reaction than upon its ability to invoke the "delayed" reaction. Heating the filtrate at 100°C. for 1 hour weakened its reactivity in respect to the "immediate" reaction but had no detectable effect upon the "delayed" reaction; exposure to 120°C. for 30 minutes seemed to destroy its ability to induce the "immediate" reaction, but caused only a quantitative decrease in the "delayed" reaction. These results present further evidence that the "immediate" and the "delayed" reactions represent two distinctly different types of hypersensitive phenomena, the "immediate" response being brought about by a diphtheria constituent which is more heat-labile than is the constituent responsible for the delayed injury of the usual "pseudoreaction" of the Schick test.

The relative heat labilities of the substances involved in the two types of reactions were confirmed with other test doses as well as with the dose (0.0004 cc. of filtrate) used in the described experiment.

The amount of the filtrate which failed to give any "immediate" reaction in the described experiment after it had been heated at 120°C. is at least 10 times as great as the minimum amount required to give a definite reaction to the same filtrate which had been heated at only 80°C. It was impossible to determine whether the inactivation at 120°C. was complete to the extent that much larger amounts would still fail to give any "immediate" reaction, because of the marked and somewhat painful "delayed" reactions given in this individual by large doses of the filtrate even after this treatment.

The Passive Transfer of the "Immediate" Skin Reaction.

Attempts to passively sensitize local skin areas (6) in the arms of normal individuals were made by the intradermal injection of the serum of the person who gave the "immediate" skin reaction to diphtheria. The serum of another individual who gave a pronounced "delayed" but no "immediate" reaction to diphtheria,
was also injected into another local area in order to determine whether or not the “delayed” reaction could be passively transferred. The tests of the passive transfer were made 48 hours after the introduction of the serum, and consisted of the injection of equal doses of diphtheria filtrate into each of the areas prepared by previous injection of the two foreign sera and into a control area previously injected with sterile salt solution. Although most of the individuals were tested with diphtheria broth culture filtrate, some were tested with heated salt solution suspensions of the washed bacilli.

During our investigation we have made transfer tests with over 100 normal individuals and a detailed analysis of the results will be given in a following paper. It is desired in the present paper to present simply the fact that the “immediate” skin reaction was passively transferred to a significant percentage of the individuals of the group; and that the “delayed” reaction failed to be transferred to a single one of the large group tested. The “immediate” reactions in the passively sensitized skin areas of the normal people when injected either with filtrate (Fig. 10) or washed bacilli (Fig. 11) were of significant dimensions and intensity and qualitatively of exactly the same appearance as those shown by the donor of the serum when injected himself with diphtheria material.

The Bacterial Specificity of the Passive Transfer.

Experiments to determine whether or not the bacterial specificity of the “immediate” reaction held true in tests on passively sensitized people were made by sensitizing a number of areas on the arms of normal individuals and subsequently injecting them with equal amounts of the filtrates of different kinds of bacteria.

The results (Fig. 12) of these experiments which were the same with the 4 normal individuals tested, showed that the reaction was just as specific in passively sensitized individuals as in the hypersensitive individual; i.e., the filtrates of the bacteria apparently unrelated to diphtheria bacilli (typhoid, colon, hofmanni bacilli, and pneumococci) gave no “immediate” reactions, while the xerosis filtrate gave a definite “immediate” reaction which was of the same type, but less in intensity and extent than that invoked by diphtheria filtrate. The “immediate” reaction to xerosis was less in comparison to the “immediate” reaction to diphtheria filtrate in the passive transfer experiments (Fig. 12)
than in the previous tests with the same filtrates on the hypersensitive individual himself (Fig. 6); but this is due simply to the fact that equal doses of diphtheria and xerosis were used in the passive transfer experiments, while in the previous experiments with filtrates on the hypersensitive man the xerosis dose was 5 times greater than the diphtheria dose.

The Influence of Heating Treatment of Diphtheria Culture Filtrate upon "Immediate" Reactions in Passively Sensitized Skin Areas.

The influence of previous heating treatment of diphtheria culture filtrate upon its capacity to produce "immediate" reaction in passively sensitized skin areas was studied by injecting equal doses of filtrate heated at different temperatures into different locally sensitized areas of a normal individual.

The results (Fig. 13) were essentially the same as in the analogous experiment (Fig. 8) upon the diphtheria-sensitive individual—i.e., 1 hour's heating of the diphtheria filtrate at 100°C. caused a marked decrease in, and 30 minutes at 120°C. caused an apparently complete loss of, the property of producing "immediate" skin reactions in locally sensitized areas on normal individuals. The fact that previous heating treatment of diphtheria culture filtrate had the same effect upon its property of producing "immediate" skin responses in passively sensitized individuals as in the diphtheria-hypersensitive man, is important as evidence that the same bacterial constituent is responsible for the skin reaction in both instances.

Other Experiments upon the Passive Transfer.

One passive sensitization sufficed in the more responsive normal individuals for the production of another "immediate" reaction to a second injection of the same dose of diphtheria filtrate made about 1 week after the production of the first "immediate" reaction. The second injection was not always successful, but usually was in individuals who gave pronounced reactions to the first injection.

Most of our tests have been made 48 hours after the injection of the sensitive serum. This time was chosen partly for convenience and partly in order to allow all traces of the serum injection or of trauma, to disappear from the passively prepared skin areas. A longer time can elapse between the injection of the sensitive serum and the injection of the bacterial filtrate, for in the only individual tested, a marked "immediate" reaction was manifested when the filtrate was injected 6 weeks after the injection of the serum.
DISCUSSION.

This paper reports a study of a hypersensitive condition toward diphtheria bacilli, which is not common in man. The hypersensitivity was first observed by the "immediate" skin reaction invoked in an individual by the intradermal injection of the diluted broth culture filtrate utilized in the routine Schick test. This skin reaction (Figs. 1 and 2) seems to be identical with the "immediate" reactions commonly produced by the intradermal injection of horse serum or of ragweed pollen in individuals sensitive to those substances. It is invoked not only by filtrates of broth cultures but also by heat-killed suspensions of the bacilli freed of broth and toxin by thorough washing, and by solutions of the bacterial substances liberated when washed diphtheria bacilli are partially disintegrated by repeated freezing and thawing. The Park 8 strain of diphtheria bacilli was used in all but one of the experiments reported in this paper. However, in other tests "immediate" reactions of the same type have been produced by a number of other strains of different origin.

This skin manifestation is specific and is not invoked by unrelated bacteria, for the diphtheria-sensitive individual does not give any "immediate" reaction either to the washed bacterial cells or to the broth culture filtrates of typhoid, colon, hofmanni bacilli, or pneumococci, even when the amount of bacterial substance is much greater than that required for pronounced reactions with diphtheria bacteria. Reactions of the same quality are invoked by our strain of xerosis bacteria (either by washed bacilli or by broth culture filtrates) but larger amounts are required to give reactions of the same extent and

1 In this paper we have used "xerosis bacillus" as a convenient term to refer to the particular strain used in our experiments and we wish to emphasize the fact that there is no reason to believe that immunological properties possessed by it would be common to all diphtheroids included in the loose use of C. xerosis in the species sense. The strain used in our experiments was of unknown origin; it agrees with the strain of C. xerosis in the American Type Culture Collection, in respect to the properties (sugar fermentations, etc.) generally used in the separation of the diphtheria group; we have not tested the latter strain to determine whether or not it will cause the "immediate" skin reaction. However, we have isolated a diphtheroid from the individual reported in this paper; and although it agreed in sugar fermentations with the usual definition of C. xerosis, it failed to produce the "immediate" skin reaction.
intensity as those produced by diphtheria bacilli (Fig. 6). The “immediate” reaction to xerosis is not an argument against the bacterial specificity of the hypersensitive skin reaction, but in all probability is an index of an immunological group relationship between our strain of xerosis and the usual diphtheria bacillus. The diphtheria bacterial substance which is responsible for the “immediate” skin reaction is relatively heat-labile. While exposure of the filtrate to 80°C. for 10 to 20 minutes does not materially diminish its capacity to invoke the reaction, exposure to 100°C. for 1 hour markedly lessens this capacity and exposure to 120°C. for 30 minutes seems to destroy it entirely (Fig. 8). Previous heating treatment of xerosis culture filtrate had the same effect, which indicates that the bacterial substance responsible for the “immediate” skin reaction to xerosis has the same degree of heat lability as has the kindred substance of diphtheria bacteria.

One of the most important characteristics of the described bacterial hypersensitiveness is its passive transfer to local areas of the skin of normal individuals by the intradermal injection of the diphtheria-sensitive individual’s serum. After the hypersensitiveness has been passively transferred, manifestations of the same character and appearance are invoked by the same materials in the locally sensitized skin areas as in the skin of the hypersensitive individual himself. That is, the “immediate” skin reaction is produced either by broth culture filtrate (Fig. 10) or by washed diphtheria bacilli (Fig. 11); to a less extent by xerosis bacilli or their culture filtrates (Fig. 12), but not at all by broth or by the culture filtrates or washed cells of unrelated bacteria.

2 Our explanation of the “immediate” skin reaction to both xerosis and diphtheria upon the basis of an immunological relationship between these two kinds of bacteria is not an unjustified one. We have found in experiments to be published later, that there is a serological relationship between our strain of xerosis and at least some strains of diphtheria bacilli, and hence, there is reason to believe that an individual sensitized by one of these bacteria might manifest hypersensitiveness toward both of them. The fact that larger amounts of the xerosis bacilli are required to give the “immediate” reaction may be due either to a lower concentration of an antigen identical with the particular one of the diphtheria bacilli or to a somewhat less degree of reactivity of a xerosis antigen which is related to but not identical with the one contained in the diphtheria bacilli.

Since the conditions of acquisition of the hypersensitiveness are entirely un-
bacteria (Fig. 12); and is similarly affected by previous heating treatment of the diphtheria filtrate (Fig. 13). All these facts furnish convincing evidence that identical bacterial substances are responsible for the "immediate" reaction whether it is invoked in the skin of the diphtheria-hypersensitive man or in the locally sensitized skin areas of normal men.

The described hypersensitiveness to diphtheria bacilli is not a transient condition in this individual. The "immediate" skin reaction has been manifested in tests covering a period of 28 months with no appreciable differences in intensity. His serum has likewise retained its capacity to transfer the hypersensitiveness with no detectable difference in the various samples of serum obtained in bleedings made at different times during 18 months.

Broth is not in any way responsible for the "immediate" reaction of this man to diphtheria culture filtrate. Skin reactions of the same type can be elicited in some people by small amounts of broth and in most normal individuals by large amounts, but this particular man does not respond at all to the small amount contained in the test doses of filtrate nor does he exhibit a less degree of "tolerance" to large doses of broth than do the majority of normal adults. Further facts to eliminate broth are furnished by the reactions induced by the washed bacilli and solutions prepared from washed bacilli; and by the heat (120°C.) stability of the substance responsible for the "immediate" skin reactions in the individuals who do give pronounced reactions to broth.

In view of the theoretical importance of the mooted question (7) of

known, there is no proof that the "immediate" reaction to xerosis as well as to diphtheria is not due to individual sensitization with both these bacteria. It is simpler, however, to assume that he has been sensitized with only one of them,

for it would be an improbable coincidence for this particular man to have become sensitized at different times with two different bacteria that are serologically related in the antiserum of rabbits and to have failed to have become sensitized against the other unrelated, but common bacteria that we have tested. The fact that with dosage controlled, he gives a larger "immediate" reaction to diphtheria than to xerosis bacterial material, together with the fact that he gives a pronounced "delayed" ("pseudo") reaction to diphtheria, makes it more likely that diphtheria rather than xerosis bacilli were the sensitizing agents.
toxin hypersensitiveness, considerable attention was given to the possibility that toxin might be involved in the diphtheria hypersensitiveness of this individual. However, the active toxin is not involved at all, for heated (80°C.) filtrates in which the toxin is inactivated, give reactions comparable to the unheated filtrates containing active toxin. The possibility that heat-inactivated toxin derivatives might be responsible is ruled out by the pronounced "immediate" reactions produced by filtered solutions of the bacterial substance derived from washed, diphtheria bacilli, which although unheated contain no detectable traces of toxin. Further evidence is furnished by the "immediate" reaction given by our strain of xerosis bacilli, which although immunologically related to the diphtheria bacillus is totally devoid of the property of toxin production.

The preceding evidence seems to establish the described "immediate" skin reaction as a manifestation of true hypersensitiveness to diphtheria bacteria, of a type entirely distinct from that manifested by the common "delayed" reaction to the same bacteria. There are always objections to drawing analogies between bacterial hypersensitive phenomena in man and those in laboratory animals: in man the conditions of acquisition of the hypersensitiveness are almost always known and in other animals different methods of test are employed to detect the hypersensitive state. But, in spite of these limitations, the bacterial hypersensitiveness indicated by the "immediate" skin reaction of this man to diphtheria bacilli, like the so called bacterial "anaphylactic" condition of the guinea pig, appears to be an example of the same general type of hypersensitiveness as that obtained in a guinea pig systematically immunized with a chemically known antigenic protein; for the hypersensitive condition can be passively transferred, and the manifestation (or response) is produced by the injection of bacterial substances that are more heat-labile than those employed in the tuberculin test. On the other hand, the type of bacterial hypersensitiveness indicated by the "delayed" reaction of this man to diphtheria is like the bacterial hypersensitive condition responsible for the positive tuberculin test in a tuberculous guinea pig; for the condition of hypersensitiveness has not been passively transferred, and the manifestation (or response) is
produced by the injection of bacterial substances that are relatively heat-stable. Whether or not the above general analogy is justified, the experimental facts show that the "immediate" skin reaction of this individual involves an immunological system that is different from that involved in the production of his "delayed" reaction to the same bacteria. The injection of the entire diphtheria bacillus (or its culture filtrate) produces both reactions, but in all probability the bacterial constituent causing the "immediate" skin response is actually a different substance from the one causing the "delayed" reaction.

Many adults exhibit the "delayed skin reaction type" of hypersensitiveness to diphtheria bacilli but only one of those we have tested exhibits the "immediate skin reaction type." There is an equal lack of evidence that this man possesses greater tendency toward sensitization than that shown by others, or that he had greater opportunities to become sensitized to these particular bacteria. Although one always considers the individual's inherited tendency toward sensitization and his opportunities for contact with the antigen, all hypersensitive phenomena are influenced by a great number of such continually variable factors that one might conclude, as Cole (7) does for the causation of pneumonia, that either the acquisition or the manifestation of hypersensitiveness is usually an "accident." That is, the sensitization of this individual to diphtheria bacilli and the nonsensitization of others, like the production of pneumonia in one person and not in another, is probably due to no one factor alone but is more likely the result of a peculiar combination or coincidence of circumstances (for example, the presence of the particular bacillus at a time when certain tissues chanced to be unusually receptive).

Some authorities believe that the type of hypersensitiveness ("bacterial allergy") manifested by the tuberculin and other "delayed" skin reactions is important from the standpoint of resistance to the specific organism. But the "immediate" skin reaction represents a different immunological condition which has received little attention from the standpoint of its possible importance either in infection and resistance or in the manifestation of disease. It is not impossible that specific bacterial hypersensitiveness, while certainly not responsible for the definitely recognized symptoms of diphtheria, may account for
the occasional occurrence of the rashes\(^3\) reported for diphtheria by observers who would not confuse them with the rashes of scarlet fever or of serum sickness.

The active immunization of people against diphtheria, especially with toxoid, introduces the question of the practical importance of the recognition of the “immediate skin reaction type” of hypersensitiveness to material derived from diphtheria organisms. This point will be discussed in a later paper but it should be noted here, that this type of hypersensitiveness is not being taken into account by those engaged in active immunization. The skin reaction now being used to detect individuals hypersensitive to the bacterial material of toxoid is the same “delayed” reaction as the well known “pseudo” of the Schick test; and the type of hypersensitiveness detected by it is not the same as that indicated by the “immediate” reaction described in this paper.

**SUMMARY.**

The paper describes an “immediate” skin reaction to derivatives of the diphtheria bacillus which is shown to be distinct from the “delayed” or “pseudoreaction” commonly seen in Schick tests on adults. The

\(^{3}\) These rashes are transient, erythematous or urticarial in nature, and distinct not only from the lesions of actual diphtheritic infection of the skin but also from the purpura or ecchymotic lesions often seen in grave cases of the disease. They are of sufficient frequency to be included in the general reviews of diphtheria by Robinson (8), McCollom and Place (9), Gee (10), Jochmann (11), and others. The reports of their occurrence may be divided into three historical periods: (1) the early clinical observations, especially by American and English workers during the eighteenth century (12); (2) from the time of the clear definition of diphtheria in Bretonneau’s classical papers to the time of the use of antitoxin; (3) the period of general use of antitoxin. The reports during the second of these periods are less complicated by the likelihood of confusion of the rashes of diphtheria with those of scarlet fever (13) or of serum sickness.

Jochmann (11) analyzed the question of these rashes and concluded that they occur in diphtheria uncomplicated by serum rash since they have been observed recently in cases not treated with antitoxin as well as in the period before the use of antitoxin. He interprets them as “toxische Erytheme, bedingt durch das Diphtherietoxin,” but if the rashes were hypersensitive manifestations, the hypersensitiveness might not be toward the toxin but toward a non-toxic substance like the bacterial constituent involved in the hypersensitive individual studied in the present investigation.
“immediate” reaction was passively transferred to local areas of the skin of other people.

REFERENCES.


EXPLANATION OF PLATES.

PLATE 2.

FIG. 1. “Immediate” skin reaction to injection of the heated diphtheria filtrate employed as control in the Schick test; 15 minutes after injection.

FIG. 2. “Immediate” skin reactions to the Schick test; unheated filtrate injected in right arm and heated filtrate in left arm; 20 minutes after injection.

FIG. 3. Arms of the individual after the “immediate” reactions have disappeared and before the appearance of the “delayed” reactions; 3 hours after injection.

FIG. 4. “Delayed” reactions of this individual to the same injections showing a “combined” Schick reaction; larger reaction on right arm due to toxin in the unheated filtrate; 4 days after injection.
Plate 3.

Fig. 5. Specificity of the “immediate” reaction in tests with heated suspensions of washed bacteria; injections as follows: (a) typhoid bacilli; (b) diphtheria bacilli; (c) colon bacilli; (d) salt solution; (e) xerosis bacilli; (f) hofmanni bacilli; (g) pneumococci; dose of other bacteria 2 times as great as diphtheria dose; 30 minutes after injection.

Fig. 6. Specificity of the “immediate” reaction in tests with broth culture filtrates; injections of filtrates as follows: (a) typhoid; (b) diphtheria; (c) colon; (d) broth; (e) xerosis; (f) hofmanni; (g) pneumococci; doses of other filtrates 5 times as large as diphtheria dose; 30 minutes after injection.

Fig. 7. “Delayed” reactions to injections described for Fig. 6; “delayed” reactions to typhoid and to colon filtrates had decreased at time of picture, but there was at no time a “delayed” reaction to xerosis; 24 hours after injection.

Plate 4.

Fig. 8. Effect of previous heating treatment of diphtheria filtrate upon the “immediate” reaction; injections as follows: (a) filtrate heated 1 hour at 100°C.; (b) filtrate heated 30 minutes at 120°C.; (c) filtrate heated 20 minutes at 80°C.; (d) broth; 30 minutes after injection.

Fig. 9. Effect of previous heating treatment of diphtheria filtrate upon the “delayed” reactions to injections shown in Fig. 8.

Fig. 10. Passive transfer of “immediate” skin reaction in tests with diphtheria filtrate; injections as follows: (a) diphtheria filtrate into area previously sensitized with hypersensitive individual’s serum; (b) broth into normal skin area; (c) diphtheria filtrate into area previously injected with serum from another person; (d) diphtheria filtrate into area previously injected with salt solution; 30 minutes after injection.

Plate 5.

Fig. 11. Passive transfer of “immediate” skin reaction in tests with heated washed diphtheria bacilli; skin areas prepared as described for Fig. 10, but heated bacteria used as test material instead of filtrate and salt solution control substituted for broth control in area (b).

Fig. 12. Specificity of passive transfer of “immediate” skin reaction; injections into previously sensitized skin areas, as follows: (a) typhoid filtrate; (b) diphtheria filtrate; (c) colon filtrate; (d) broth; (e) hofmanni filtrate; (f) xerosis filtrate; (g) pneumococcus filtrate; 30 minutes after injection.

Fig. 13. Effect of previous heating treatment of diphtheria filtrate upon the passive transfer of the “immediate” reaction; injections into previously sensitized skin areas as follows: (a) filtrate heated 1 hour at 100°C.; (b) filtrate heated 30 minutes at 120°C.; (c) filtrate heated 20 minutes at 80°C.; (d) broth; 30 minutes after injection. (Small triangular scar between areas (a) and (b) due to a burn.)
(Neill and Fleming: Hypersensitivity to diphtheria bacilli. 1.)
(Neill and Fleming: Hypersensitivity to diphtheria bacilli. I.)