STUDIES ON ANAPHYLAXIS WITH POLLEN

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(Received for publication, November 5, 1934)

Many fundamental questions regarding immunobiological reactions are as yet unanswered. However, certain working principles have been reached through long observation and research, and the terms foreign protein reactions, complement, precipitins, antibodies, anaphylaxis, etc., have acquired definite and specific significance. Attempts have been made to fit these concepts to clinically observed phenomena, but to date the so-called pollen sensitizations have never been satisfactorily elucidated on an immunological basis. Many observers have noted the wide variations in the clinical results of therapy. Others have questioned the expectation of any significant effects from the injection of so little real protein in desensitization and have found it difficult to correlate this desensitization and clinical improvement with a skin test persistently positive to the specific atopic agent. The apparent inability to obliterate these hypersensitivities, as well as their frequent multiplicity, are problems as yet unexplained.

Efforts at solving these problems have resolved themselves into (a) attempts to sensitize animals and reproduce the syndrome observed in human beings (1); (b) the study of Dale preparations of uterine strips taken from sensitized guinea pigs (2); (c) efforts to sensitize animals by upper air passage or intratracheal insufflation (1); (d) the investigation of serum reagins by Prausnitz-Küstner reaction and by precipitin tests (3); (e) studies of results of therapy in clinical cases of atopic hypersensitiveness (4). These investigations are well known to readers of the literature on the subject. As with all biological experiments the results have been inconstant, or the methods introduced elements into the problem not germane to the investigation at hand.
These and many other considerations led us to inquire into the possible mechanisms involved. What substances in the realm of immunological reagents might reproduce such a series of phenomena? Landsteiner and van der Scheer (5), Avery and Goebel (6, 7), and others, following Landsteiner’s original hapten concept, investigated the properties of certain synthesized antigens. They found that the antigen was essentially dual in structure, a larger portion possessing antigenicity, and a smaller, prosthetic portion (hapten), specificity. The union between these two was not close; there was either a loose chemical bond, or a simple colloidochemical linkage. The specific hapten portion, when united with either antigenic nucleus (or haptophore group), was capable of calling forth the specific response, whereas the hapten portion alone, while it could elicit a positive skin reaction (e.g. the pneumococcus specific soluble carbohydrate), actually inhibited the union of antigen and antibody in vitro.

It is possible that the mechanism involved in atopic hypersensitivity represents just such a hapten-like action, rather than the tacitly assumed, firmer union of true antigen-antibody complex. Certainly atopens as a rule are protein-poor substances; they elicit cutaneous evidence of sensitivity, but in general are ineffective in calling out demonstrable precipitins. However, they give a positive passive transfer phenomenon. Landsteiner has shown that these haptens may be carbohydrates, lipoids, or relatively much smaller proteins. Does some such non-specific antigen produce the allergic state referred to as underlying sensitivity, or antibody unsaturation, or protoplasmic instability (8)? Or shall we be content to accept it as some change (Kahn (9)) which is “rooted in the biologic and physicochemical structure of the chromosomes”?

It was felt that an experiment might be planned which would throw some light on a few of these questions. Various proteins were employed as basic sensitizers, always using a 1:25 extract of burweed marsh-elder (Iva) as the superimposed atopen. Experiments were done on several series of guinea pigs: one with veal broth; two with egg white; and one (the present), with horse serum. This last was found to give the most consistent and clear-cut results. We report in detail the final series which constitutes a representative study.
Materials and Method

The guinea pigs were all males, weighing between 300 and 500 gm. Injections, with Luer syringes and ¼ inch, 26 gauge needles, were made intracutaneously with some possible subcutaneous leakage. All injections were 0.4 cc in quantity.

Normal horse serum was obtained from Eli Lilly Co.; the extract of burweed marsh-elder (Iva xanthifolia) was made in our laboratory as follows:

The following solvent was prepared:

\[
\begin{align*}
\text{KH}_2\text{PO}_4 & \quad 0.54 \text{ gm.} \\
\text{Na}_2\text{HPO}_4 & \quad 2.26 \text{ gm.} \\
\text{NaCl} & \quad 9.00 \text{ gm.} \\
\text{Phenol} & \quad 2.5 \text{ gm.} \\
\text{Glucose} & \quad 25.0 \text{ gm.} \\
\text{Water, sufficient to make} & \quad 500.0 \text{ cc.}
\end{align*}
\]

To each 100 cc. of solvent was added 4 gm. of clean pollen (1931). The mixture was shaken for 24 hours, centrifuged, and the supernatant fluid filtered through standard, fine filter paper. This was next filtered through a Seitz filter and tested for bacterial growth; found sterile, it was kept on ice when not in use.

The mixture of equal volumes of horse serum and pollen extract was allowed to stand at room temperature for 24 hours; at various times during the 3 weeks previous to use it was taken from the ice box for repeated room temperature exposures. The mixture produced no precipitate.

Throughout the experiment separate syringes were used for each material. These were recorded by their serial numbers etched on the barrel and plunger, and their identities constantly maintained. After use they were cleaned separately.

Quantitative analysis (Kjeldahl) of the horse serum showed it to contain 7.35 gm. per cent protein. Average determinations of protein content of the Iva extract yielded about 50 mg. per cent. In the latter there were wide variations, due either to the batch of extract, or the method of analysis.

3 weeks after the final sensitizing injections blood samples were drawn by venipuncture for precipitin titration with Iva and horse serum, and shock experiments done with both of these substances. For this the jugular vein was exposed, blood first taken by syringe, and 0.4 cc. of the shocking material slowly injected. Each animal was observed for a minimum of 1 hour following this procedure, and the reactions carefully recorded.

EXPERIMENTAL

Six guinea pigs were sensitized by three injections (0.4 cc. each) of whole horse serum at intervals of 4 days. After 3 weeks these (Group B0) and six normal guinea pigs (Group B) were given three injections (0.4 cc. each) of pollen
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extract (*Iva xanthifolia*), at 4 day intervals. At the same time twelve normal guinea pigs were injected with three inoculations (0.4 cc.) of equal parts of horse serum and pollen extract (Group A), and a fourth group (Group C) of six animals were sensitized by injections of horse serum alone (0.4 cc.). 3 weeks after the final inoculation the precipitins for pollen extract and for horse serum were investigated, and the anaphylactic response to these substances studied. These are set forth in Table I.

As shown in the table, marked differences were obtained between the several groups of animals, most notably in their response to the test of anaphylaxis. All the guinea pigs of Groups A and B0 which had received both horse serum and pollen, reacted to intravenous pollen extract with moderate to severe anaphylaxis. One such animal, No. 1-3, was shocked fatally (4+), and only one, No. 1-2, showed a negative response, having received only half the regular shock dose (or 0.2 cc.).

In Group B (animals prepared with pollen extract alone), anaphylaxis to pollen extract could not be demonstrated, even though in one instance the intravenous dose was doubled. It might be pointed out that the absolute amount of pollen extract given in the preliminary injections in this group was actually twice that received by animals of Group A.

Anaphylaxis to horse serum was almost uniformly severe in Groups A, B0, and C. One animal, No. 9-1, showed only a mild reaction. Group B pigs were quite negative to intravenous horse serum on several trials, and pollen extract elicited no response in the animals sensitive to horse serum alone (Group C).

The skin tests in all groups of this series were inconclusive. However, in the three previous series, at a similar date the skin reaction to pollen extract intracutaneously was by objective measurement definitely greater in the Group A animals than in those of Group B. There were no Group B0 pigs in the earlier experiments.

Precipitin tests in all four series were carried out in the manner described by Hektoen. The results were not sufficiently conclusive to permit any inferences. They are reported, however, as a matter of possible investigative interest.

**DISCUSSION**

By means of the above and similar experiments we have shown that in the presence of an underlying sensitization, a substance not in
**TABLE I**

*Series IV*

Group A. Pollen-horse serum, equal parts.
Group B. Pollen extract alone. 0.4 cc. injected Aug. 21, 24, and 29.
Group C. Horse serum alone.

Group B0 received same injections as Group B, but 4 weeks after injections of horse serum, 0.4 cc. on July 16, 20, 24.

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal No.</th>
<th>Precipitin test for</th>
<th>Anaphylaxis to</th>
<th>Remarks</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Pollen</td>
<td>Horse serum</td>
<td>Pollen</td>
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<tr>
<td>A</td>
<td>1</td>
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<td>Pos. (wk.)</td>
<td>Pos.</td>
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<tr>
<td></td>
<td>2</td>
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<td>Pos.</td>
<td>++</td>
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<tr>
<td></td>
<td>3</td>
<td>Pos.</td>
<td>Pos.</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Pos. (++)</td>
<td>Pos. (+)</td>
<td>+</td>
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<td></td>
<td>5</td>
<td></td>
<td></td>
<td>+</td>
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<td></td>
<td>8</td>
<td>Pos.</td>
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<td>Neg.</td>
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<td></td>
<td>1-3</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>1-4</td>
<td>Pos. (+)</td>
<td>Pos. (+)</td>
<td>+++</td>
</tr>
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<td>Pos. (+)</td>
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<td>Pos.</td>
<td>Pos.</td>
<td>+++</td>
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<td>Neg.</td>
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<tr>
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<td>1-1</td>
<td>Pos. (wk.)</td>
<td>Pos. (wk.)</td>
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<td>+</td>
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<tr>
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<td>2-4</td>
<td></td>
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<td>?+</td>
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<tr>
<td></td>
<td>2-5</td>
<td></td>
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<td></td>
<td>+</td>
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<td>C</td>
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<td>7-7</td>
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<td>Pos.</td>
<td>+++</td>
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<td>1-00</td>
<td>Neg.</td>
<td>Pos.</td>
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Anaphylactic response

+++ = lethal, with jactitation, convulsions, etc.
++ = dyspnea, convulsions, prostrations with survival.
+ + = dyspnea, cyanosis, wheezing, cough, loss of posture.
+ = cough, sneeze, scratching of nose, cyanosis, slight dyspnea, micturition, defecation, nervousness.

Precipitin test—wk. = turbidity only slightly greater than control.
itself capable of sensitizing can induce the hypersensitive state, and, when properly administered, produce anaphylactic shock. Hektoen (10) and Parker (11) have both done similar experiments but reported no tests on the anaphylactic response.

Rothschild, Friedenwald, and Bernstein (12), working on desensitization of tuberculin-sensitive guinea pigs (sensitized by inoculation with avirulent strain of Koch's bacillus), found that Koch's old tuberculin (O.T.) in suitable doses could produce complete desensitization by all the criteria they were able to apply. They found also that the broth control produced almost as complete a desensitization, whereas a tuberculoprotein (Seibert) of equal skin test potency produced very little diminution in sensitivity. The difference apparently lay in the beef protein content of the O.T. This finding suggests the important rôle that non-specific immunological reactions may play in the evolution and subsequent course of the allergic state. In this connection may be mentioned the frequent improvement noted in patients with allergic disease during acute infections, and following vaccines and non-specific protein therapy.

How closely this experimentally induced condition approximates the actual clinicoimmunologic mechanism can only be surmised. Further work along these lines is being undertaken with a view to clinical application.

**SUMMARY AND CONCLUSIONS**

1. Guinea pigs injected intracutaneously and subcutaneously with extract of the pollen of burweed marsh-elder in relatively small amounts did not show anaphylactic response to intravenous shock doses of this material 3 weeks later.

2. If, however, the animals were sensitized with horse serum either before, or along with the same pollen injections, they could then be shocked after an interval of 3 weeks with pollen extract alone.

3. The possible rôle of this underlying sensitivity is discussed.

The author wishes to express his appreciation of the helpful criticism given by Dr. O. H. Robertson.

**BIBLIOGRAPHY**


