The experiments described in this paper represent an attempt to inhibit sensitization of the allergic contact type with an allergen-protein conjugate. Chase (1) has already shown that when 2,4-dinitrochlorobenzene is fed to nonsensitive animals "a state of resistance may be established against subsequent experimental sensitization of the skin by the same substance". In a similar sense, Sulzberger found that previous intracutaneous injection of neoarsphenamine could prevent sensitization by intracutaneous injection of that compound (2). The present study is based upon the concept that circulating antibodies to a synthetic conjugate of pure protein and pure allergen (of any type) may inhibit subsequent sensitization to the allergen, and may depress the level of sensitization to the allergen where the allergy is already established.

To test this concept, we have chosen 3-n-pentadecylcatechol [a prototype of the poison ivy allergens (3)] as the experimental allergen, human serum albumin as the antigenic protein, and guinea pigs as the test animals. A conjugate between the allergen and protein was prepared in vitro, then injected into experimental animals. These animals were subsequently tested for susceptibility toward sensitization by 3-n-pentadecylcatechol; untreated animals and animals injected only with protein were used as controls.

**PREPARATION OF ALLERGEN-PROTEIN CONJUGATE**

To prepare the allergen-protein conjugate, synthetic 3-n-pentadecylcatechol was converted to the corresponding o-quinone (4, 5) and 0.30 gram of this quinone was dissolved in 100 ml. of anhydrous, peroxide-free dioxane. Six grams of human albumin† were dissolved in 500 ml. of water buffered at pH 9 with disodium phosphate, and cooled to 0°C. The cold dioxane solution of the quinone was then added dropwise to that of the albumin, with gentle stirring, over a period of three hours. After the dioxane had been added, stirring was continued for an additional half hour. The flask containing the reaction mixture was placed in a cold room at 5°C overnight; the resultant solution was dialyzed against frequently changed distilled water for twenty-four hours, then filtered. These operations were also carried out in a cold room at 5°C. The clear pink filtrate so obtained was frozen and vacuum-dried to an amorphous, water soluble solid.

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† The human albumin was an electrophoretically homogeneous fraction generously furnished by Professor E. J. Cohn of Harvard University.
Aqueous solutions of the conjugate were spectrophotometrically compared with albumin solutions containing the same amount of protein nitrogen. The absorption spectra so obtained showed that in the conjugation reaction the o-quinone had been converted to a bound form of 3-n-pentadecylcatechol, and on the basis of known extinction coefficients and molecular weights it was calculated that approximately four molecules of 3-n-pentadecylcatechol were bound to each molecule of albumin. A binding reaction of the type illustrated in the accompanying equation can be postulated on the basis of the studies of E. Fischer (6):

\[
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{C}_{13}H_{21} \\
\end{array}
\quad + \quad
\begin{array}{c}
\text{NH}_2 \\
\text{Protein} \\
\end{array}
\quad \rightarrow 
\begin{array}{c}
\text{HO} \\
\text{OH} \\
\text{C}_{13}H_{21} \\
\text{NH} \\
\text{Protein} \\
\end{array}
\]

**QUANTITATIVE SKIN TESTING**

The technic used for performing quantitative skin tests was a modification of that described by Dunn, Mason, and Smith (3). Experimental doses of the allergen, 3-n-pentadecylcatechol, in acetone were applied with a capillary pipette of 0.1 ml. capacity and graduated in 0.001 ml. units. This pipette was mounted in a Guthrie pipette holder, which permitted the whole delivery apparatus to be raised and lowered with facility. Rubber tubing attached to the top of the pipette was connected to a suction bottle between delivery of test doses (for cleaning and drying), and a screw clamp just above the pipette served to compress the tubing while solution was being taken up and applied. Brass rings used to confine the dose to an area of 0.5 cm² of skin were fitted with brass handles attached to the outer wall of the cylinder. A starch-glycerine sealing compound was applied to the bottom of the ring by tapping it gently on a flat piece of glass covered with a film of the material. The ring was then firmly held against the clipped abdomen of the guinea pig and a measured dose applied, in this manner, to a known area of skin.

**LEVELS OF PRIMARY IRRITANCY AND SENSITIVITY**

Using the quantitative patch test procedure described above, it was found that no guinea pig reacted to an initial 0.9 microgram application of 3-n-pentadecylcatechol per 0.5 cm² of skin. On the other hand, all guinea pigs reacted to initial applications of 25 micrograms of 3-n-pentadecylcatechol per 0.5 cm² of skin, and this level was accordingly considered to be within the level of primary irritancy. For the purposes of this study, subsequent reactivity to an application of 0.9 microgram per 0.5 cm² has been considered the experimental criterion of sensitization.

**THE COURSE OF EXPERIMENTAL IMMUNIZATIONS**

Forty-five animals were used in a preliminary experiment. These were white, male guinea pigs ranging in weight from 400–800 grams. They were divided into
three groups, as follows: group 1 received 1.0 ml. of 1.66% solution of the conjugate in physiological saline, intraperitoneally, once a week for four weeks; group 2 received 1.0 ml. of a 1.66% solution of albumin only, once a week for four weeks; group 3 was held for four weeks with no treatment. One week was allowed to elapse at the end of the final injection. All animals then received a sensitizing dose of 25 micrograms of 3-n-pentadecylcatechol (day 1) and on days 8, 10, and 12 challenging doses of 0.9 microgram were applied. Each of these was read for a reaction 48 hours after application and then observed for an additional 96 hours. Four further sensitizing doses of 25 micrograms of 3-n-pentadecylcatechol were applied on days 15, 16, 17, and 18, and tests for sensitization again performed on days 25 and 27. These were read as before. No attempt was made to grade the degree of response; and the reaction was called positive if the test site showed any erythema. The majority of reactions to the 0.9 microgram dose were faint to strong erythemas. The results of this experiment are given in Table 1. Absolute inhibition of sensitization within short periods (25 days) after exposure to irritant doses of 3-n-pentadecylcatechol was observed in the conjugate-treated group; after this, variations in the numbers of sensitized animals within the control groups made the outcome less impressive.

Accordingly, a second experiment, with larger numbers of animals and more rigorous courses of immunization, was undertaken. One hundred and twenty white male guinea pigs ranging in body weight from 400–800 grams were divided into three groups: Group 1, received intraperitoneally, once a week for a period of five weeks, 1.0 ml. of a 2% solution of the conjugate in physiological saline; group 2 received intraperitoneally, once a week for five weeks, 1.0 ml. of a 2% solution of albumin; group 3, held for five weeks without treatment. One week was allowed to elapse at the end of the last injection. All animals then received a 25 microgram sensitizing dose of 3-n-pentadecylcatechol, and then tested for sensitization 8, 10, and 15 days later with 0.9 microgram of allergen. A further attempt to sensitize was made 14, 15, and 16 days after this, and final testing

| TABLE 1 |

*The susceptibility toward sensitization by 3-n-pentadecylcatechol of guinea pigs previously treated with 86.4 milligrams of albumin-3-n-pentadecylcatechol conjugate, compared with albumin-treated and untreated animals, expressed as the percentage of animals which were sensitizable.*

<table>
<thead>
<tr>
<th>DAYS AFTER LAST PROTECTIVE INJECTION</th>
<th>DAYS AFTER LAST ATTEMPT TO SENSITIZE</th>
<th>CONJUGATE TREATED ANIMALS</th>
<th>ALBUMIN TREATED ANIMALS</th>
<th>UNTREATED ANIMALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>8</td>
<td>0%</td>
<td>40%</td>
<td>46%</td>
</tr>
<tr>
<td>17</td>
<td>10</td>
<td>0%</td>
<td>20%</td>
<td>39%</td>
</tr>
<tr>
<td>32</td>
<td>7</td>
<td>0%</td>
<td>14%</td>
<td>23%</td>
</tr>
<tr>
<td>34</td>
<td>9</td>
<td>13%</td>
<td>43%</td>
<td>23%</td>
</tr>
</tbody>
</table>

Number of animals at end of experiment... 15 14 13

Number of animals lost in the course of the experiment.................. 0 1 2
The susceptibility toward sensitization by 3-n-pentadecylcatechol of guinea pigs previously treated with 100 milligrams of albumin-3-n-pentadecylcatechol conjugate, compared with albumin treated and untreated animals, expressed as the percentage of animals which were sensitizable.

<table>
<thead>
<tr>
<th>DAYS AFTER LAST PROTECTIVE INJECTION</th>
<th>DAYS AFTER LAST ATTEMPT TO SENSITIZE</th>
<th>CONJUGATE TREATED ANIMALS</th>
<th>ALBUMIN TREATED ANIMALS</th>
<th>UNTREATED ANIMALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>8</td>
<td>8%</td>
<td>44%</td>
<td>45%</td>
</tr>
<tr>
<td>17</td>
<td>10</td>
<td>30%</td>
<td>69%</td>
<td>76%</td>
</tr>
<tr>
<td>19</td>
<td>12</td>
<td>11%</td>
<td>36%</td>
<td>42%</td>
</tr>
<tr>
<td>43</td>
<td>36</td>
<td>24%</td>
<td>61%</td>
<td>57%</td>
</tr>
<tr>
<td>45</td>
<td>38</td>
<td>30%</td>
<td>74%</td>
<td>79%</td>
</tr>
</tbody>
</table>

Number of animals at end of experiment ................. 37 39 39

Number of animals lost during the course of the experiment .................. 3 1 1

was carried out a week later. Each test was read 48 hours after application of allergen, then daily for an additional 96 hours. Results are given in Table 2.

The percentage of each group of animals found susceptible to sensitization varied considerably over the experimental period. However, the group of animals which had received injections of allergen conjugate always showed much lower incidence of sensitivity than either of the control groups.

DISCUSSION

Clearly, the parenteral injection of 3-n-pentadecylcatechol-albumin conjugate confers upon guinea pigs some resistance to subsequent contact sensitization by 3-n-pentadecylcatechol. In guinea pigs, as in humans, there is a great variability in susceptibility toward sensitization as well as in capacity to maintain sensitivity once it is induced. This is, perhaps, due to inheritable differences (7). It is probable that our results reflect this variability, but in any case the protective effect of allergen conjugate is sufficiently marked that no statistical analysis of the data is necessary.

It seems reasonable that similar effects of longer duration may be obtained by better methods of inciting circulating antibodies to allergen-protein conjugates, i.e., Freund's adjuvant technic.

SUMMARY

The parenteral injection of conjugates of 3-n-pentadecylcatechol with human serum albumin into guinea pigs resulted in resistance to subsequent sensitization (of the allergic contact type) by 3-n-pentadecylcatechol.

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REFERENCES


