

## IMMUNOLOGIC STUDIES OF REPOSITORY RAGWEED POLLEN THERAPY

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IT has been shown that after multiple injection therapy with pollen extracts, a thermostable blocking antibody is produced.<sup>1</sup> There is usually no change in the skin-sensitizing antibody titer during hyposensitization therapy, but one investigator<sup>2</sup> reported a temporary early increase in this antibody response. More recently, we<sup>3</sup> and others<sup>4, 5</sup> have shown that there is an increase in the hemagglutinating antibody titer following multiple aqueous pollen injection therapy as measured by either the tanned cell or bis-diazotized benzidine technique. Unfortunately, neither the significance of this antigenic response nor the apparent lack of correlation between the change in blocking, skin-sensitizing, or hemagglutinating antibodies to each other, or to the clinical response of the patients is, as yet, explained.

Repository pollen therapy has been reported by several investigators to be at least as effective in the relief of pollinosis as the conventional multiple injection therapy. Nevertheless, there has been a paucity of information on the immunologic response following pollen emulsion therapy. However, Loveless<sup>6</sup> noted that blocking antibody titers were increased and sustained by repository therapy.

In view of these facts, it was decided to critically study this phase by means of determining the change in blocking, reagin, and hemagglutination antibody titers in patients who received either the repository pollen, a placebo emulsion, or the usual multiple aqueous injection therapy.

### METHODS AND PROCEDURES

#### 1. *Source and Preparation of Emulsions.*—

As a member of the Committee on Specific Therapy of the American Academy of Allergy, we received our material from the Chairman, Dr. Samuel Feinberg. One part of defatted ragweed pollen extract was added to two parts of 10 per cent Arlacel A in Drakeol VR6 and placed in sterile vials with neo-

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prene stoppers. The final concentrations of the ragweed pollen extract in this mixture available to the investigator varied from 500 to 20,000 pollen units. These mixtures were emulsified for 30 minutes by a Conscot\* apparatus several days prior to use, and again for 10 minutes just prior to injection into patients. The emulsions were all examined microscopically, by centrifugation, and by scratch tests in sensitive individuals. The emulsions were transferred to 2.0 c.c. disposable syringes and 1.0 c.c. was injected at one time, in one site, deep subcutaneously.

## 2. Selection of Patients.—

Ragweed-sensitive patients from the Allergy Clinics of the Buffalo General and Children's Hospitals and from private practice were used in this study and were divided into two separate investigations.

A. *Double-blind study*: Patients who had been on perennial ragweed therapy were divided, at random, into three groups by one of the investigators. The groupings were unknown to the other participants† in this study who evaluated the clinical results. One group of patients received 20,000 pollen units in repository form, another group was given only a saline oil emulsion without the pollen extract, and the third group was continued on its regular aqueous perennial therapy.

B. *Pre-seasonal study*: Ragweed-sensitive patients who never had previous specific therapy, or had no therapy in 1960, were given from three to eight primer doses of aqueous extracts prior to the single injection of emulsion. The amount of pollen in the emulsion was between 25 to 50 times the concentration of the last aqueous dose. This was considered to be a safely tolerable dose on the basis of a maximum of 2 per cent free antigen (not in emulsion phase) present in a "good" emulsion. The repository therapy was given at varying intervals from 3 to 11 weeks prior to the onset of the ragweed pollen season.

Samples of sera were obtained from each patient prior to (1) aqueous primer doses, (2) the repository injection, (3) the ragweed pollen season, and (4) after the ragweed pollen season. These sera were frozen until all samples were collected and ready for immunologic investigation.

Sera from the patients in the double-blind study were taken prior to the repository injection, prior to the pollen season, and again at the end of the season.

## 3. Immunologic Methods.—

A. *Hemagglutination titers* of all samples of the sera collected were determined by the tanned cell technique as previously described.<sup>7</sup> All samples from an individual patient were tested at the same time. The reciprocal of the highest dilution of serum which still caused an agglutination of tanned red blood cells coated with ragweed pollen extract was called the titer.

B. *Reagin antibody titers*: The studies on change in reaginic and blocking antibody titers were limited to 14 patients of the pre-seasonal group. All sera

\*Manufactured by Conscot Company, Rockaway, New Jersey.

†The attending physicians in the Allergy Clinics, who evaluated the clinical response, were Dr. Charles Banas, Dr. Thelma Brock, Dr. Doris Rapp, and Dr. Leonard Wolin.

were cultured for sterility and each of the donors (patients) were carefully questioned as to a previous history of jaundice or hepatitis.

Serial "five"-fold dilutions of the various serum samples were injected intradermally in 2 nonatopic recipients. Twenty-four to 48 hours later each site was tested with a constant amount of ragweed pollen extract. The reciprocal of the highest dilution of serum which positively sensitized the skin was considered the titer of the serum.

C. *Blocking antibody titers*: Blocking antibody titers were determined, in each of 2 recipients, according to the classical method of in vitro neutralization, as described by Loveless.<sup>1</sup>

D. *Scratch test titrations*: Serial dilutions of ragweed pollen extract were used for scratch tests on the patients who received the repository treatment and 13 who received placebo injections. The highest dilution of pollen extract which still gave a positive reaction was determined prior to repository therapy, prior to the pollen season, and after the pollen season.

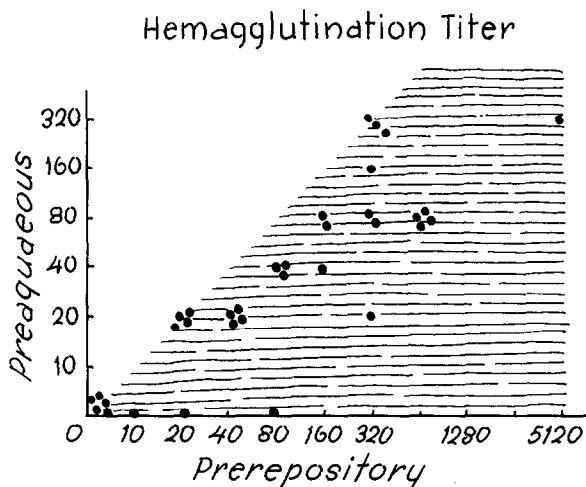


Fig. 1.—The effect of small doses of aqueous ragweed pollen extract on the tanned cell hemagglutination titer prior to the injection of the repository emulsion. Each dot represents the comparative titers. All symbols in the shaded area represent an increased titer.

#### RESULTS: IMMUNOLOGIC RESPONSE

##### A. *Hemagglutination*.—

1. *Pre-aqueous to pre-repository*: Of 38 patients from whom we obtained serum samples prior to their aqueous therapy (3 to 8 injections) and before the repository injection, 23 (60 per cent) showed an increase in titer even with this minimal dosage of pollen extract injected. Twelve of these 23 patients (34 per cent) had a fourfold increase or greater in titer (Fig. 1; Table I).

TABLE I. HEMAGGLUTINATION TITERS (PRE-AQUEOUS TO PRE-REPOSITORY)

| NUMBER OF<br>PATIENTS | INCREASE IN TITER |        |          |
|-----------------------|-------------------|--------|----------|
|                       | 2-FOLD            | 4-FOLD | > 4-FOLD |
| 38                    | 11                | 4      | 8        |

2. *Pre-repository to pre-season*: Comparison of the hemagglutination titers just prior to the injection of emulsion and before the season is graphically demonstrated in Fig. 2. Twenty-nine of the 61 patients in the pre-seasonal group had an increase in hemagglutination titer. The titer remained unchanged in 18, 11 showed a twofold decrease, and 3 showed a fourfold or greater decrease (Table II).

Groups: • = Preseasonal, x = Perennial, o = Placebo

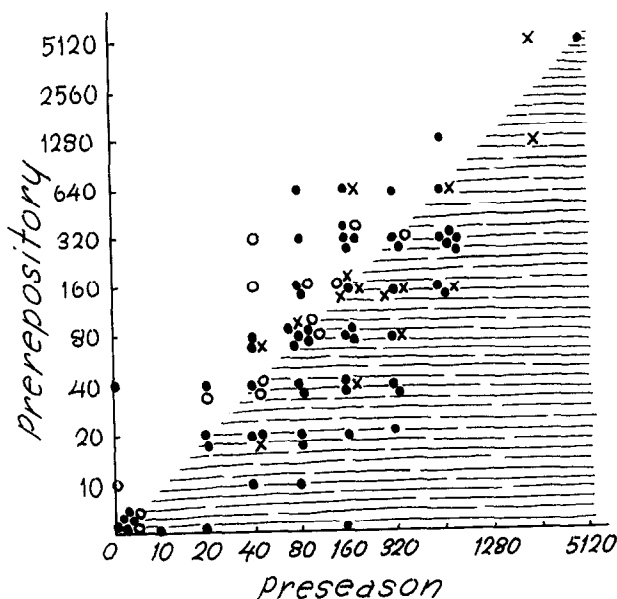


Fig. 2.—Change in tanned cell hemagglutination titer after repository pollen therapy and prior to the onset of the ragweed pollen season. Symbols in shaded area demonstrate an increase in titer.

TABLE II. HEMAGGLUTINATION TITERS (PRE-REPOSITORY TO PRE-SEASON)

| GROUP      | NUMBER OF PATIENTS | INCREASE |        |          | SAME | DECREASE |        |          |
|------------|--------------------|----------|--------|----------|------|----------|--------|----------|
|            |                    | 2-FOLD   | 4-FOLD | > 4-FOLD |      | 2-FOLD   | 4-FOLD | > 4-FOLD |
| Pre-season | 61                 | 14       | 9      | 6        | 18   | 11       | 2      | 1        |
| Perennial  | 15                 | 4        | 3      |          | 5    | 2        | 1      |          |
| Placebo    | 14                 | 0        |        |          | 8    | 4        | 2      |          |

Of the 15 patients who had been on perennial therapy and were then given a pollen emulsion injection, the titer increased in 7, was unchanged in 5, and decreased in 3.

The hemagglutination titer of those patients previously treated with perennial ragweed therapy who received the oil emulsion without pollen remained unchanged in 8 and decreased in 6. There was no increase of titer in this group of patients (Fig. 2).

3. *Pre-season to post-season*: In order to determine any effect on the hemagglutination titer by either the inhalation of pollen during the season or

any further delayed antigenic response from the repository injection, the titers just prior to the pollen season and following the disappearance of ragweed from the air were compared.

Of the 62 patients in the pre-season group, the titers remained the same in 26, decreased in 31, and increased in only 5 (Table III). Of the perennial group, 10 of 14 had a decrease, and of the placebo group, 10 of 16 had the same titer (Fig. 3).

Groups: • = Preseasonal, x = Perennial, o = Placebo

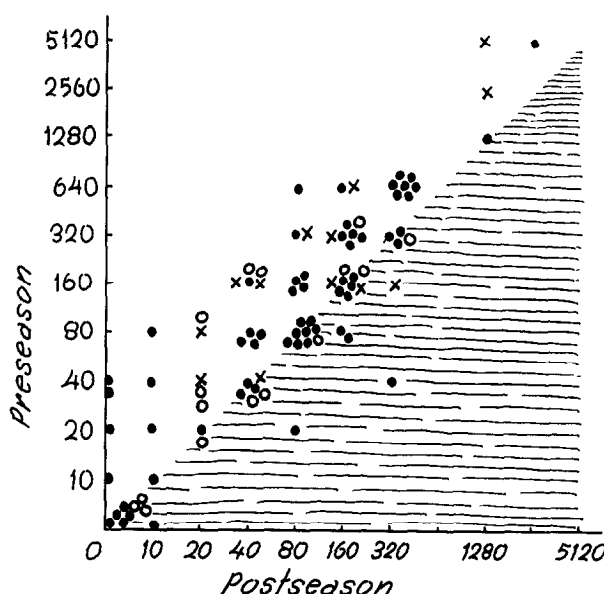


Fig. 3.—Change in tanned cell hemagglutination titer prior to and after the ragweed pollen season. The symbols in the shaded area indicate an increase in titer.

TABLE III. HEMAGGLUTININ TITERS (PRE-SEASON TO POST-SEASON)

| GROUP      | NUMBER<br>OF<br>PATIENTS | INCREASE |        |          | SAME | DECREASE |        |          |
|------------|--------------------------|----------|--------|----------|------|----------|--------|----------|
|            |                          | 2-FOLD   | 4-FOLD | > 4-FOLD |      | 2-FOLD   | 4-FOLD | > 4-FOLD |
| Pre-season | 62                       | 3        | 2      |          | 26   | 31       |        |          |
| Perennial  | 14                       | 1        |        |          | 3    | 4        | 6      |          |
| Placebo    | 16                       | 0        |        |          | 10   | 3        | 3      |          |

4. *Pre-repository to post-season:* The changes in hemagglutination titer of the sera taken just prior to the repository injection and following the ragweed pollen season are illustrated in Fig. 4 and tabulated in Table IV. Of the 61 patients in the pre-seasonal group, 11 had a twofold increase in titer and 6 had a fourfold or greater increase. The titers in 23 patients remained unchanged, but 21 showed a decrease in titer. Of the perennial and placebo groups, there was not one patient who had a fourfold increase in hemagglutination titer. However, 8 of the 14 patients who had stopped perennial aqueous therapy and had a placebo injection had a decrease in titer.

TABLE IV. HEMAGGLUTINATION TITERS (PRE-REPOSITORY TO POST-SEASON)

| GROUP      | NUMBER OF PATIENTS | INCREASE |        |          | SAME | DECREASE |        |          |
|------------|--------------------|----------|--------|----------|------|----------|--------|----------|
|            |                    | 2-FOLD   | 4-FOLD | > 4-FOLD |      | 2-FOLD   | 4-FOLD | > 4-FOLD |
| Pre-season | 61                 | 11       | 3      | 3        | 23   | 13       | 5      | 3        |
| Perennial  | 15                 | 2        |        |          | 9    | 2        | 2      |          |
| Placebo    | 14                 | 1        |        |          | 5    | 3        | 2      | 3        |

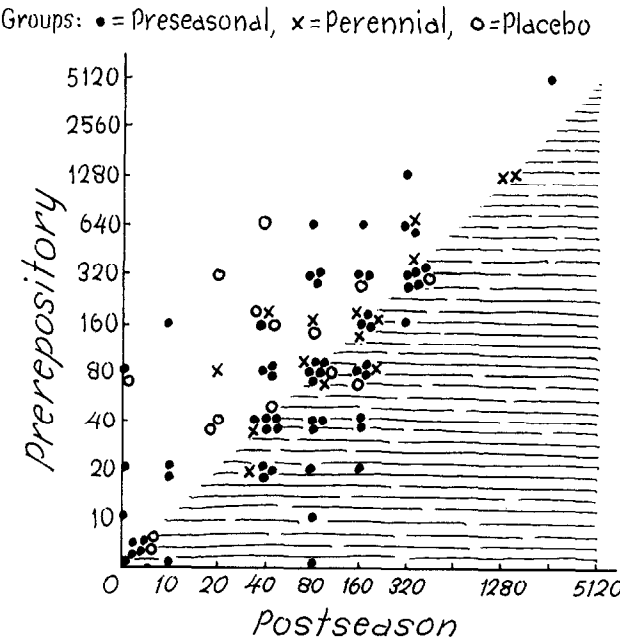


Fig. 4.—Comparative tanned cell hemagglutination titers prior to repository therapy and after the ragweed pollen season. The symbols in the shaded area indicate an increase in titer.

5. *Clinical response and hemagglutination titers:* Thirty-five patients in the pre-seasonal repository group had a satisfactory clinical response. Of these, only 6 had a fourfold or greater increase in hemagglutination titer (pre-repository to pre-season). Seven of the remaining 28 patients who did not benefit from repository treatment likewise had an increase in hemagglutination titer (Table V).

TABLE V

| CLINICAL RESPONSE | NUMBER OF PATIENTS | CHANGE IN HEMAGGLUTINATION TITER* |      |          |
|-------------------|--------------------|-----------------------------------|------|----------|
|                   |                    | INCREASE†                         | SAME | DECREASE |
| Not improved      | 35                 | 6                                 | 28   | 1        |
| Improved‡         | 28                 | 7                                 | 20   | 1        |

\*Pre-repository to pre-season.

†At least a fourfold increase.

‡At least 50 per cent benefit.

These data confirm our previously reported studies that the hemagglutination response had no relationship to the clinical response.

*B. Reagin (Skin-Sensitizing Antibody).—*

1. The skin-sensitizing antibody titers of the 14 patients of the pre-season group treated with repository ragweed extract were determined in each of 2 recipients. These patients were arbitrarily chosen on the basis of repository dosage, so as to represent the range of 500 to 10,000 pollen units. As can be seen in Table VI, 4 patients showed an increase in reagin titer, 3 a decrease, and 7 remained unchanged. There was no relationship between dosage of repository given and change in skin-sensitizing antibody titer.

TABLE VI. REPOSITORY DOSAGE TO REAGIN ANTIBODY RESPONSE

| DOSAGE<br>(POLLEN UNITS) | NUMBER OF<br>PATIENTS | INCREASE | DECREASE | SAME |
|--------------------------|-----------------------|----------|----------|------|
| 500                      | 3                     | 1        |          | 2    |
| 1,000                    | 3                     |          | 1        | 2    |
| 2,500                    | 3                     | 2        |          | 1    |
| 5,000                    | 2                     | 1        | 1        |      |
| 10,000                   | 3                     |          | 1        | 2    |
| Totals                   | 14                    | 4        | 3        | 7    |

2. There was, likewise, no relationship between the clinical response and change in reagin titer (Table VII). Of the 9 patients who were clinically improved, 3 demonstrated an increase in skin-sensitizing antibody. One of the 5 patients who did not improve, likewise, showed an increase in reaginic antibody. The inhalation of pollen during the ragweed season had little or no effect on this antibody titer.

TABLE VII. RELATIONSHIP OF CHANGE IN REAGIN TITER TO CLINICAL RESPONSE

|                                      | NUMBER OF<br>PATIENTS | REAGIN TITER |          |      |
|--------------------------------------|-----------------------|--------------|----------|------|
|                                      |                       | INCREASE     | DECREASE | SAME |
| <i>Pre-repository to pre-season</i>  |                       |              |          |      |
| Improved                             | 9                     | 3            | 2        | 4    |
| Not improved                         | 5                     | 1            | 1        | 3    |
| <i>Pre-repository to post-season</i> |                       |              |          |      |
| Improved                             | 9                     | 4            | 2        | 3    |
| Not improved                         | 4                     | 1            |          | 3    |
| <i>Pre-season to post-season</i>     |                       |              |          |      |
| Improved                             | 9                     | 2            | 1        | 6    |
| Not improved                         | 5                     | 1            |          | 4    |

3. There apparently was no relationship between the time the repository therapy was given and the change in reagin titer (Table VIII).

TABLE VIII. RELATIONSHIP OF TIME OF REPOSITORY INJECTION TO CHANGE IN REAGIN TITER

| FOLLOWING REPOSITORY THERAPY<br>(WEEKS) | NUMBER OF<br>PATIENTS | REAGIN TITER |          |      |
|-----------------------------------------|-----------------------|--------------|----------|------|
|                                         |                       | INCREASE     | DECREASE | SAME |
| 10                                      | 5                     |              | 2        | 3    |
| 8                                       | 5                     | 2            | 1        | 2    |
| 5                                       | 2                     | 1            |          | 1    |
| 3                                       | 1                     |              |          | 1    |
| 2                                       | 1                     | 1            |          |      |

4. Of these 14 patients who were so studied, 3 had an increase of fourfold or greater of their hemagglutination titer, but the reagin titer remained the same. Contrariwise, of the 11 patients whose hemagglutination titer remained unchanged, 4 demonstrated an increase in reagin titer, 3 decreased, and 4 remained the same (Table IX).

TABLE IX. RELATIONSHIP OF CHANGE IN HEMAGGLUTINATION TITER AND REAGIN TITER

| HEMAGGLUTINATION |    | REAGIN TITER |          |      |
|------------------|----|--------------|----------|------|
|                  |    | INCREASE     | DECREASE | SAME |
| Increase         | 3  |              |          | 3    |
| Same             | 11 | 4            | 3        | 4    |

### C. Blocking Antibody.—

1. Eight of these 14 patients showed an increase in blocking antibody. Three patients who received 500 pollen units, and 3 who received 10,000 pollen units, did not demonstrate an increase in blocking antibody (Table X).

TABLE X. REPOSITORY DOSAGE TO CHANGE IN BLOCKING ANTIBODY TITER

| DOSAGE (POLLEN UNITS) | BLOCKING ANTIBODY TITER |      |
|-----------------------|-------------------------|------|
|                       | INCREASE                | SAME |
| 500                   | 0                       | 3    |
| 1,000                 | 3                       |      |
| 2,500                 | 3                       |      |
| 5,000                 | 2                       |      |
| 10,000                |                         | 3    |

2. There, likewise, was no correlation between the clinical response and increase in blocking antibody. Five of the 9 patients who were clinically improved had an increase in blocking antibodies. Three of the 5 patients who were not benefited by treatment also showed an increase in blocking antibody (Table XI).

TABLE XI. RELATIONSHIP OF CHANGE IN BLOCKING ANTIBODY TITER TO CLINICAL RESPONSE

| CLINICAL RESPONSE | NUMBER OF PATIENTS | BLOCKING ANTIBODY TITER |          |      |
|-------------------|--------------------|-------------------------|----------|------|
|                   |                    | INCREASE                | DECREASE | SAME |
| Improved          | 9                  | 5                       |          | 4    |
| Not improved      | 5                  | 3                       | 1        | 1    |

3. There was no relationship between the time of repository injection and blocking antibody. However, patients who received the emulsion 2 or 3 weeks previously probably did not have time to develop more blocking antibodies (Table XII).

TABLE XII. RELATIONSHIP OF TIME OF REPOSITORY INJECTION TO CHANGE IN BLOCKING ANTIBODY TITER

| FOLLOWING REPOSITORY THERAPY (WEEKS) | BLOCKING ANTIBODY TITER |          |      |
|--------------------------------------|-------------------------|----------|------|
|                                      | INCREASE                | DECREASE | SAME |
| 10                                   | 3                       |          | 2    |
| 8                                    | 3                       |          | 2    |
| 5                                    | 2                       |          |      |
| 3                                    |                         | 1        |      |
| 2                                    |                         |          | 1    |

4. Two of the 3 patients who had an increase in hemagglutination antibody titer, likewise, had an increase in blocking antibody. These 2 patients had satisfactory relief of symptoms; the other did not. Of the remaining 11 patients whose hemagglutinating antibody titer was unchanged, 4 had an increase in blocking antibody but only 2 had clinical relief of symptoms (Table XIII).

TABLE XIII. RELATIONSHIP OF CHANGE IN HEMAGGLUTINATION TITER AND BLOCKING ANTIBODY TITER

| HEMAGGLUTINATION |    | BLOCKING ANTIBODY TITER |          |      |
|------------------|----|-------------------------|----------|------|
|                  |    | INCREASE                | DECREASE | SAME |
| Increase         | 3  | 2                       | 1        |      |
| Same             | 11 | 4                       |          | 7    |

5. A comparison was made of the change in titer of skin-sensitizing antibodies and blocking antibodies following repository therapy. Of 4 patients who demonstrated an increase in reagins, 3 likewise developed a higher blocking antibody titer. However, the blocking antibody titer also increased in 3 patients in whom the reagin titer remained unchanged (Table XIV).

TABLE XIV. RELATIONSHIP OF CHANGE OF REAGIN TO BLOCKING ANTIBODY TITER

| REAGIN TITER |   | BLOCKING ANTIBODY TITER |          |      |
|--------------|---|-------------------------|----------|------|
|              |   | INCREASE                | DECREASE | SAME |
| Increase     | 4 | 3                       |          | 1    |
| Decrease     | 3 | 2                       |          | 1    |
| Same         | 7 | 3                       | 1        | 3    |

#### D. Scratch Test Titrations.—

##### 1. Pre-seasonal group of patients:

A. Serial threefold dilution scratch tests were performed on 44 patients of this group prior to the injection of the repository pollen extract and again just prior to the ragweed pollen season. Twenty-eight of these 44 patients had a decrease in skin reactivity. However, there was no correlation noted between the amount of repository extract injected and the response to skin reactivity. Nine of 12 patients who received 1,000 pollen units had a decrease in skin reactivity, whereas 3 of 5 receiving 10,000 pollen units also reacted in the same manner (Table XV).

TABLE XV. DOSAGE TO CHANGE IN SCRATCH TESTS\*

| DOSAGE IN REPOSITORY THERAPY | SKIN REACTIVITY |      |          |
|------------------------------|-----------------|------|----------|
|                              | INCREASE        | SAME | DECREASE |
| 500                          |                 | 1    | 3        |
| 1,000                        | 2               | 1    | 9        |
| 2,500                        | 4               | 2    | 6        |
| 5,000                        | 2               | 1    | 7        |
| 10,000                       |                 | 2    | 3        |
| 20,000                       |                 | 1    | 1        |
| Totals                       | 8               | 8    | 28       |

\*Pre-repository to pre-season.

B. There was no apparent relationship between the change in skin reactivity following repository therapy and clinical response. Of the 28 patients, whose skin reactivity was decreased, exactly one half clinically benefited from repository therapy. Likewise, of the 8 patients whose skin titers remained the same, an equal number was helped (Table XVI).

TABLE XVI. SKIN REACTIVITY TO CLINICAL RESPONSE

| CLINICAL RESPONSE        | SKIN REACTIVITY |      |          |
|--------------------------|-----------------|------|----------|
|                          | INCREASE        | SAME | DECREASE |
| Total number of patients | 28              | 8    | 8        |
| Total number improved    | 14              | 4    | 6        |

## DISCUSSION

1. *Immunologic Response.*—

In previous studies<sup>3</sup> we have shown that 68 per cent of the patients receiving multiple injection treatment for ragweed pollinosis had at least a four-fold increase in hemagglutinating titer. In our present investigation, only 24 per cent of the patients treated by pollen emulsion (no matter what the dosage) had a similar type of response. Although the group of patients (38) who had only 3 to 8 small aqueous doses produced a greater antigenic response than did those on repository therapy, they did not show as great a hemagglutination antibody response as did those patients who received many aqueous injections over a longer period of time. This difference could conceivably be explained by the fact that there was a much smaller amount of extract, fewer dosages, and a shorter time interval to develop these antibodies.

This very low antigenic stimulation of hemagglutinating antibodies with the ragweed repository therapy in human beings is quite puzzling. Similar findings were reported by other investigators.<sup>8, 9</sup> One would anticipate a greater response on the basis of previous reports of emulsion therapy in humans with influenza virus vaccine<sup>10</sup> and in animals with multiple antigens.<sup>11</sup> Perhaps the explanation might be that either we did not use enough pollen extract in our mixtures or that the antigenicity of the ragweed extract was not comparable to more potent substances.

Although it is true that we did not find any correlation between the increase in hemagglutinating titer and clinical response in either our present or previous studies, the hemagglutination activity is, nevertheless, an indication of some type of antigenic response. Whether this type of response is important as far as the explanation of the mechanism of hyposensitization therapy is concerned is still in doubt. We also could find no correlation between the skin-sensitizing and blocking antibody titers to each other or to clinical response. Hence, if none of these antibodies play a role in relief of symptoms, either by multiple injection or emulsion therapy, how do these treatments work? It is conceivable that there are changes at the tissue (shock organ) level that we are, as yet, unable to measure and determine. Certainly, clinical experience has demonstrated, in the past 50 years, that hyposensitization therapy, although not perfect, is beneficial.

Although a good percentage of our patients did well on placebo therapy, this was not a good enough controlled study, as there may have been a carry-over from previous multiple injection therapy. It would be very difficult to explain all of these good results on psychogenic factors alone.

Hennessy and Davenport<sup>12</sup> have shown that with two injections of influenza virus emulsion in small doses at a 4 to 6 week interval there is a much larger protective antibody response developed than if only one large dose of influenza emulsion is given. Sheldon and associates<sup>13</sup> recently reported using this same technique with grass hay fever patients and claimed good clinical results. Unfortunately, there were no reported antibody studies. Perhaps, utilization and application of the two-injection small-dosage technique for pollinosis should be investigated further. Patients will have to be carefully selected with adequate placebo controls and careful observation. Additional serological studies of the various antibody responses should be determined. Likewise, a group of investigators, competent and critical, should treat patients in the exact manner, using the same materials, methods of preparation, and dosage schedule as described by Brown.<sup>14</sup> It is only by these methods, over a period of several years, that we will be able to establish whether pollen emulsion therapy is effective, safe, and free of subsequent complications.

#### SUMMARY AND CONCLUSIONS

1. The repository therapy did not produce as good an antigenic response (as determined by hemagglutination titers) as did multiple injection therapy.
2. There was no relationship found between the change in skin-sensitizing or blocking antibody titers and the clinical response or hemagglutinating antibody titer following repository therapy.
3. The need for more intimate knowledge of the mechanism of specific hyposensitization therapy is stressed.
4. Carefully controlled studies over a period of several years is still necessary to establish the true value and safety of repository therapy.

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