

# Timothy pollen fractions in treatment of hay fever

## I. Clinical and immunological response to small and higher molecular weight fractions

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*Forty-two timothy-sensitive patients have been treated with timothy pollen fractions in alginate during the 1967 and 1968 pollen seasons. The results of these studies indicate that patients treated with a low molecular weight fraction (antigen D) showed better clinical protection than patients treated with the crude pollen extract (WST). The observed clinical protection in antigen D-treated patients was correlated with a significant drop in reagin titer rather than an increase in blocking antibody. Eighty per cent (12/15) of patients treated with antigen D showed a tenfold or greater decrease in reagin titer during the first year of treatment. The observed decrease in reagin titer suggested that the mechanism of hyposensitization may involve either in vivo neutralization of reagin or the induction of partial immune tolerance.*

Over the last decade a number of investigative studies have been directed toward the isolation and characterization of allergenic substances. A natural extension of these programs was the use of these purified fractions in the treatment of allergic patients. A number of investigations with the use of either crude ragweed extract<sup>1-6</sup> or the purified allergen E<sup>7-9</sup> of ragweed have tried to demonstrate a relationship between clinical improvement, blocking antibody production, and reagin titers with the extent of treatment. In general, the results of these studies demonstrated an increase in blocking antibody titers in many patients and only limited clinical improvement with progressive treatment over several years.

Previous studies<sup>10, 11</sup> in our laboratory have indicated that: (1) Timothy pollen contains two major allergens (A and B). (2) Allergens A and B cross-react. (3) Allergen A contains at least one allergenic determinant not shared

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with allergen B. (4) A low molecular weight, nonprecipitating antigen (antigen D) is antigenically closely related to allergen B. The biological properties of antigen D have been reported in detail elsewhere.<sup>12</sup> These include weak skin reactivity in timothy-sensitive patients and inhibition of precipitation, passive cutaneous anaphylaxis, and systemic anaphylaxis with rabbit and guinea pig antitimothy sera. The antigenic relationship to the major allergens of timothy pollen and the observed biological properties of antigen D suggested that it may have therapeutic value.

A pilot program of treating patients with antigen D has been conducted over the last 2 years. The results of this study indicated that patients treated with antigen D showed significant clinical improvement in comparison with control groups treated with the crude pollen extract (WST). The improvement observed was not related to increased levels of blocking antibody but was associated with a significant drop in reagin titer of patients treated with antigen D.

## **MATERIALS AND METHODS**

A pilot study treating 42 timothy-sensitive patients at both the University of Oregon Medical School and the Oregon State Penitentiary has been conducted during the 1967 and 1968 pollen seasons. The isolation and characterization of the antigens (WST and antigen D) used in this study have been described elsewhere.<sup>10-12</sup>

### **Selection of patients**

Patient selection was based on a history of grass pollen hay fever together with a strong wheal and flare reaction on intradermal testing with the crude timothy pollen extract (WST) at a concentration of  $10^{-6}$  Gm. of protein per milliliter. Patients were also tested with other aeroallergens which may overlap the grass pollen period. This later information was found to be helpful in the correlation of the pollen count, patient's symptoms, and antibody titer. Fourteen of the patients selected for this study had received either aqueous or emulsion therapy for at least the 2 preceding years. A second group of 14 patients had no previous treatment, and a third group of 14 patients had not received any form of treatment within the 2 years prior to this study.

### **Treatment**

At each location patients were randomly divided into two groups, one receiving treatment with antigen D and the other group, WST. Patients treated with antigen D and the group treated with WST received weekly subcutaneous injections of their respective antigens incorporated in an equal volume of "alginate" (4 per cent sodium alginate with 0.67 per cent calcium gluconate and 0.4 per cent phenol added; Consolidated Lab., Inc., Chicago Heights, Ill.). The amount of antigen (WST) utilized initially and the progression of antigen concentration used for each patient was based on the patient's degree of sensitivity. Patients treated with antigen D received 10  $\mu$ g protein nitrogen initially. With both groups of patients the antigen dosage was increased as rapidly as tolerated. Patients receiving antigen D ultimately reached an antigen level of

2.0 mg. of protein nitrogen, and WST patients reached an antigen level of 160  $\mu$ g of protein nitrogen. Both groups of patients tolerated these antigen levels without significant immediate or delayed local reactions or any systemic reactions. The total dosage of allergen which patients received during a year of treatment (January to July) was 11 mg. of protein nitrogen of antigen D and 1 mg. of protein nitrogen of WST. The total dose of WST at the close of treatment was 2 to 10 times larger in most individuals than that which would have been tolerated in aqueous solution.

Blood samples were obtained from all patients at the following times during the 2 years of the study: January, March, April, June, and August. The blood samples were collected one week after the last injection of their treatment preparation and prior to their next injection. Exceptions to this general procedure were blood samples obtained in August (4 to 6 weeks after completion of treatment) and in January of the second year of treatment (6 months after completion of treatment). The sera from all blood samples were withdrawn aseptically. The bulk of sera was stored in siliconized glass bottles with rubber caps, without preservative, at  $-15^{\circ}$  C. However, a small aliquot (2 ml.) of serum from each patient was removed for use in the analysis of the reagin and blocking antibody titers. Although the duration of time between drawing of blood samples and analysis of both blocking and reagin antibody titers was generally less than 10 days (never more than 18 days), each aliquot of sera was stored at  $-15^{\circ}$  C. until used. Reagin titrations were performed on human subjects by the Prausnitz-Küstner (P-K) test and in some cases on monkeys by the allergic serum transfer method of Layton and Yamanka.<sup>15</sup> Blocking antibody titrations were performed as described previously.<sup>16</sup> The serum utilized to reach the neutralization point for blocking antibody determinations was heated at  $56^{\circ}$  C. for 4 hours, dispensed into aliquots sufficient for one experiment, and stored at  $-15^{\circ}$  C. until used. All passive transfer experiments were performed on nonallergic human volunteers.

### **Clinical evaluation**

The effectiveness of this treatment program was measured by correlating daily symptoms reports, reagin and blocking antibody titers, personal interviews at the completion of the program, and pollen counts. A detailed description of our method of clinical evaluation is not included, but rather a few abbreviated remarks and a figure are offered. Once patients were randomly distributed (at both locations) into their respective treatment groups, the clinical evaluation of the data obtained from the daily diaries and personal interviews was performed as a double-blind study.

### **Daily log**

The daily diaries (log sheets) utilized in this study (Fig. 1) are a modification of the charts previously described by Norman and associates.<sup>13</sup> These diaries were supplied to each patient weekly beginning about 2 weeks before onset of the grass pollen season and collected each week at the time of the subsequent injection treatment. When these daily diaries were first distributed, each pa-



tient was oriented in the method of keeping them diligently and was made aware of the significance of symbols for scoring their symptoms. When the weekly sheets were collected, they were perused by the interviewer, and any confusing data were clarified with the patient present. The interviewer was not aware of the allergenic fraction being used.

### **Scoring of weekly summaries**

This was done from each diary sheet and at the end of the treatment program (and the grass pollen season) with the use of a scoring system similar to that described by Connell and Sherman<sup>14</sup>: Numerical rating of symptoms ranged from 0 (no symptoms) to 6 (severe symptoms). Likewise, medication needed ranged from 0 (none used) to 6 (if three or more antihistamines or any steroid therapy were required in a day).

With the use of the completed daily diaries, a total or composite treatment score was determined for each patient with a scoring system similar to that described by Connell and Sherman,<sup>14</sup> taking into account the amount of medication used and the extent of symptoms. A comparison of the effectiveness of treatment with antigen D and WST on the basis of total treatment score (symptoms and medication) and medication for the 1967 pollen season is shown in Figs. 3 and 4. The above data were evaluated by nonparametric statistical analysis with the Mann-Whitney U test and the Walsh test.<sup>17</sup> Preseasonal reaginic antibody titers of patients in both the antigen D- and WST-treated groups demonstrated a comparable distribution of patients with either high ( $\geq 1,000$ ) or low ( $\leq 100$ ) reagent titers. Also the number of patients in either antigen D or WST treatment groups was evenly distributed at both locations not only in regard to their absolute numbers but also in regard to their treatment status (untreated, no treatment within 2 years, or treated the last 2 years).

The question of incorporating a placebo group in this study was considered. It was our feeling that such a placebo group (patients treated with alginate alone without any antigens) was not warranted because: (1) The two groups of patients treated with alginate and antigen D or WST serve as an internal control. (2) The number of patients from these randomly selected groups that report spontaneous clinical improvement as a result of participation in this program would probably be equally distributed. (3) The evaluation of clinical improvement involved the objective measurement of both reagent and blocking antibody titers which are not affected by psychological stimuli. (4) The duration of this study would deny some patients beneficial treatment. A comparison was made of the changes in both reagent and blocking antibody titers between 10 patients receiving WST in aqueous solution over this same period and those patients receiving either WST or antigen D in an alginate solution. The maximal dosage of aqueous WST received by the majority of these 10 patients was 120  $\mu$ g of protein nitrogen. Protein nitrogen measurements were made by the Nessler method described by Campbell and associates.<sup>18</sup>

### **RESULTS**

Fig. 2 shows the pollen counts obtained in both Salem and Portland during the 1967 pollen season. Although the quantitative limitations of the gravity

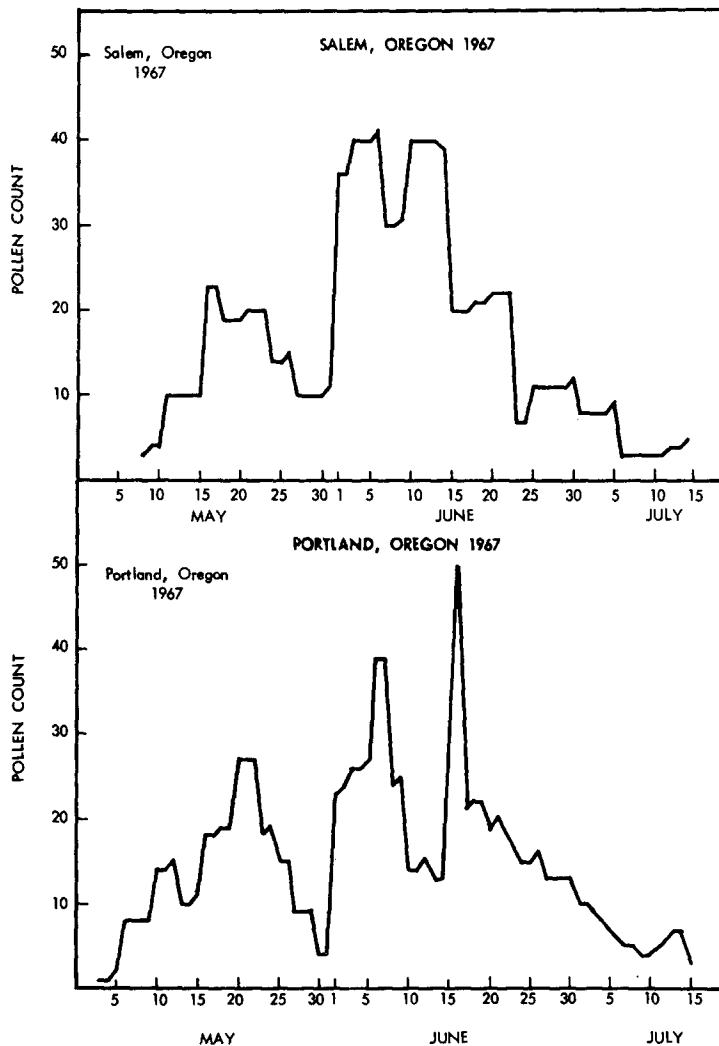
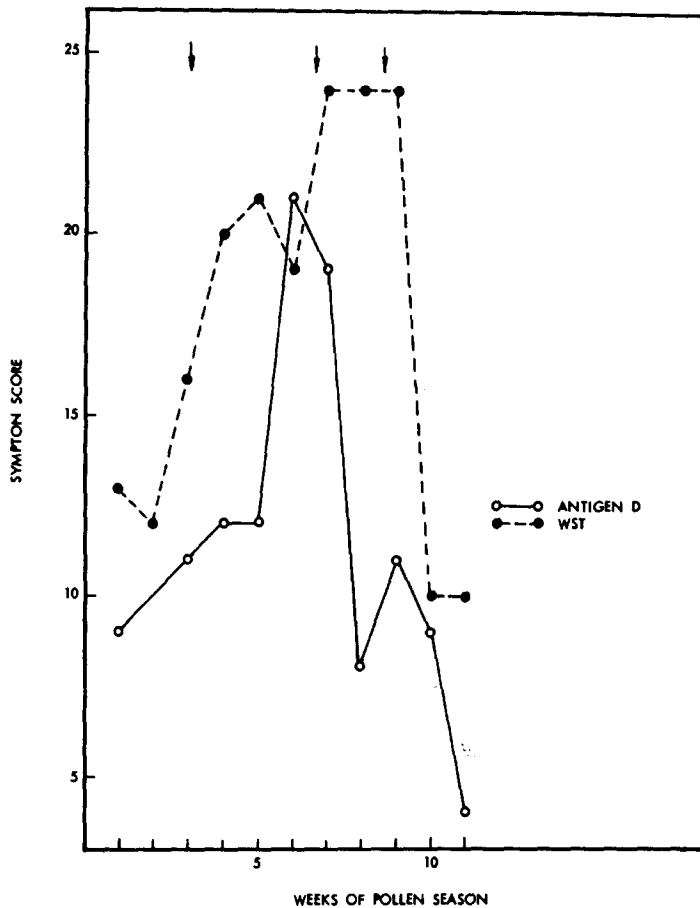


**Fig. 2**

Counts of grass pollen during the 1967 season were done by the Durham gravity method (grains per square centimeter per 24 hrs.).

slide pollen counts are well known, it is evident that in both locations there were three major peaks of pollen exposure.

With the use of the information obtained from the daily diaries, a total treatment score (based on symptoms and medication used) was compiled for each patient weekly during the pollen season. Evaluation of the relative clinical protection of patients receiving either antigen D or WST treatment was made by comparing the two groups of patients against the compiled treatment score (Fig. 3). The data indicate that patients receiving antigen D treatment had less severe pollinosis than those patients treated with WST. Only during the seventh week of the pollen season did patients treated with antigen D show a greater treatment score than the WST-treated patients; however, this difference

**Fig. 3**

Comparison of treatment with antigen D and WST on the basis of total treatment score. Arrows ( $\downarrow$ ) indicate the peaks of pollen exposure. Statistical analysis was performed utilizing the Mann-Whitney U test ( $p = 0.044$ ) and the Walsh test ( $p = 0.005$ ).

was not considered significant (statistical analysis of the data in Fig. 3 resulted in a probability of 0.044 and 0.005, respectively, for the Mann-Whitney U test and the Walsh test). When a similar comparison between antigen D- and WST-treated patients was made on the basis of medication required (Fig. 4), we found that, except for the first week of the pollen season, patients treated with antigen D required less medication than those patients treated with WST. Statistical analysis of the data in Fig. 4 resulted in a probability of 0.036 and 0.003, respectively, for the Mann-Whitney U test and the Walsh test.

Blocking antibody titers (Table I) measured from serum samples obtained during the treatment program demonstrated that both groups of patients receiving injections of timothy pollen fractions in an alginate solution produced both a low incidence (5/42) and low level of blocking antibody (1/3). In contrast, patients receiving aqueous therapy with WST showed a more uniform

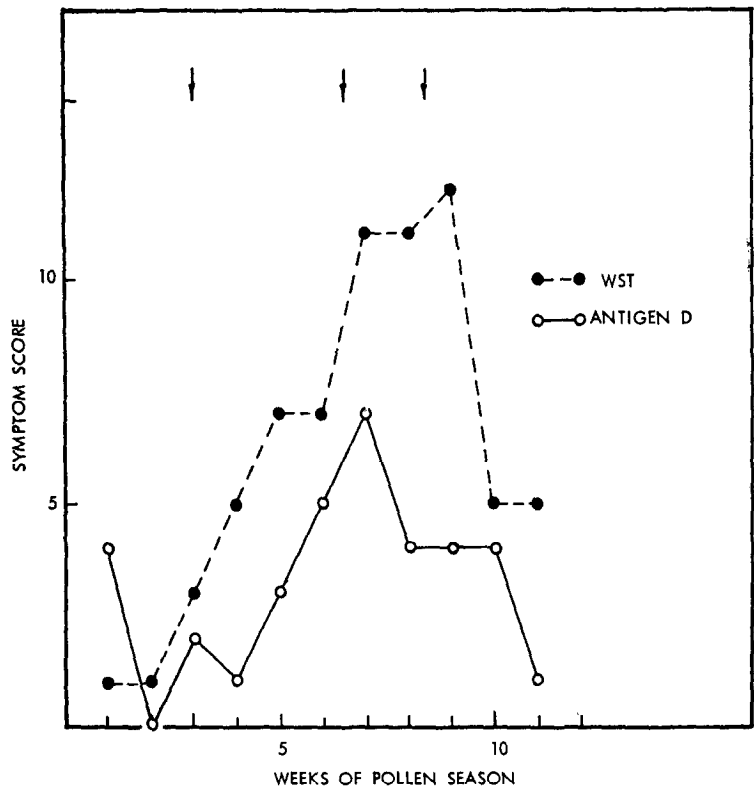


**Fig. 4**  
Comparison of treatment with antigen D and WST on the basis of medication score. Arrows (↓) indicate the peaks of pollen exposure. Statistical analysis was performed with the use of the Mann-Whitney U test ( $p = 0.036$ ) and the Walsh test ( $p = 0.003$ ).

**Table I.** Comparison of blocking antibody titers produced by alginate and aqueous therapy

| Antigen   | Treatment | No. of patients | Blocking antibody titer* |   |    |    |     |
|-----------|-----------|-----------------|--------------------------|---|----|----|-----|
|           |           |                 | 3                        | 9 | 27 | 81 | 162 |
| Antigen D | alginate  | 23              | 2                        | 0 | 0  | 0  | 0   |
| WST       | alginate  | 19              | 3                        | 0 | 0  | 0  | 0   |
| WST       | aqueous   | 12              | 1                        | 0 | 3  | 6  | 0   |

\*Represents the reciprocal of the lowest dilution demonstrating blocking antibody activity.

**Table II.** The effect of treatment on reagin titer during the first year of treatment

| Treatment | Reagin titers |           |          |
|-----------|---------------|-----------|----------|
|           | Increase      | No change | Decrease |
| Antigen D | 1/23          | 7/23      | 15/23    |
| WST       | 6/19          | 12/19     | 1/19     |

production of blocking antibody both with regard to the numbers responding (10/12) and the levels produced (up to 1/81 titer).

An interesting observation was made, however, when we compared the reagin titers of patients receiving antigen D and WST treatment (Table II). Fifteen of the 23 patients treated with antigen D showed a significant decrease in the reagin titer during the first year of treatment, while 12 of 19 patients treated with WST showed no change, and only one patient showed a slight decrease in reagin titer. The reaginic antibody titers of patients treated with antigen D, rather than dropping progressively during treatment, dropped sharply early in the course of the program and remained at a low level through the remainder of the year. These findings suggest that patients receiving antigen D treatment experienced *in vivo* neutralization of reagin which blocked the antibody-combining sites. Such blocked or inhibited antibody would not be able to react with the larger pollen allergens upon exposure during the season.

The following experiment (Table III) was designed to determine the extent of antigen D neutralization of reagin. The sera of 4 patients obtained in January, 1967 (pretreatment), were each divided into two aliquots. Five milligrams of protein of antigen D was added to one aliquot, and saline was added to the second aliquot. Both the antigen D- and saline-treated sera were diluted to a final concentration of 1/10 with saline and were incubated at room temperature for one hour then overnight at 4° C. The next morning all sera were diluted (as indicated in Table III) and implanted into nonallergic volunteers. Immediate skin reactions at the site of injection of antigen D serum mixtures were not observed in any of the nonallergic volunteers. After 48 hours each site was challenged with 10<sup>-5</sup>Gm. of protein of WST. The data clearly indicate that incubation of sera containing high titers of reaginic antibody with antigen D markedly inhibited the passive transfer reactions, thus supporting the concept that clinical protection afforded patients treated with antigen D may involve an *in vivo* neutralization of reagin.

**Table III.** *In vitro neutralization of reagin with antigen D*

| Patient | Treatment* | Serum dilutions† |      |     |     |       |
|---------|------------|------------------|------|-----|-----|-------|
|         |            | 10               | 50   | 100 | 500 | 1,000 |
|         |            | P-K reactions‡   |      |     |     |       |
| A. G.   | Saline     | ++++             | ++++ | +++ | ++  | +     |
|         | Antigen D  | ++               | +    | ±   | -   | -     |
| K. F.   | Saline     | +++              | +++  | +++ | ++  | ++    |
|         | Antigen D  | ++               | -    | -   | -   | -     |
| E. W.   | Saline     | +++              | +++  | ++  | ++  | +     |
|         | Antigen D  | +                | +    | ±   | -   | -     |
| W. B.   | Saline     | +++              | +++  | ++  | +   | +     |
|         | Antigen D  | ++               | +    | -   | -   | -     |

\*Serum was treated with either saline or 5 mg. protein of antigen D and diluted to a final concentration of 1/10. See text for additional details of treatment.

†Serum dilutions were the reciprocal of indicated value.

‡Reactions of wheal size were graded as follows: negative, -; less than 5 mm. diameter, ±; 6 to 10 mm. diameter, +; 11 to 15 mm. diameter, ++; 16 to 20 mm. diameter, +++; greater than 20 mm. diameter, +++++. Tests were done in triplicate and mean reactions recorded.

Earlier it was noted that patients (15/23) treated with antigen D showed an abrupt drop in the reagin titer rather than a more progressive decline during the course of treatment. An example of this drop in reagin titer and the specificity of the residual antibody is illustrated in Table IV. The sera obtained from an antigen D-treated patient (A. G.) at various stages during the treatment program were passively transferred into nonallergic volunteers. Each pair of recipients was sensitized with the sera collected in January, 1967 (pretreatment), and the sera from one of the three other blood samples collected during the first year of the treatment program. Forty-eight hours later, recipients were challenged with  $10^{-5}$  Gm. of protein of WST, purified allergen A, and purified allergen B. The data demonstrate that the reaginic antibody titer dropped twentyfold (January to April) early in the treatment and that the antibody directed against allergen B is the reaginic antibody primarily affected. However, with further treatment there was also a slight reduction of the reaginic antibody titer directed against allergen A, which presumably is due to the cross-reacting antigenic determinants shared between allergen A and B. It was also noted that after the completion of treatment in July, the reaginic antibody levels did not change between August and January. Thus, such a patient approached the start of a second year of treatment with an antibody titer a hundredfold lower than that at the beginning of the first year. Although the data presented in Table IV are for only one patient, corresponding experiments were performed with the sera of 8 other antigen D-treated patients; and the pattern of results were essentially identical. Patients treated with WST showed no decline in reagin titer. Table V shows the extent

**Table IV.** *Specificity of reaginic antibody remaining after completion of treatment program*

| Patient | Treatment*     | Challenge†<br>antigen | Serum dilutions‡ |      |     |      |     |       |       |
|---------|----------------|-----------------------|------------------|------|-----|------|-----|-------|-------|
|         |                |                       | 10               | 50   | 100 | 250  | 500 | 1,000 | 5,000 |
|         |                |                       | P-K reactions§   |      |     |      |     |       |       |
| A. G.   | Antigen D—Jan. | WST                   | ++++             | ++++ | +++ | N.D. | +++ | ++    | +     |
|         |                | A                     | +++              | ++   | +   | N.D. | ±   | -     | -     |
|         |                | B                     | +++              | +++  | ++  | N.D. | ++  | +     | ±     |
| A. G.   | Antigen D—Apr. | WST                   | ++++             | +++  | ++  | +    | -   | -     | -     |
|         |                | A                     | ++               | ++   | ±   | -    | -   | -     | -     |
|         |                | B                     | ++               | +    | -   | -    | -   | -     | -     |
| A. G.   | Antigen D—Aug. | WST                   | ++               | +    | -   | -    | -   | -     | -     |
|         |                | A                     | +                | +    | -   | -    | -   | -     | -     |
|         |                | B                     | +                | ±    | -   | -    | -   | -     | -     |
| A. G.   | Antigen D—Jan. | WST                   | ++               | +    | -   | -    | -   | -     | -     |
|         |                | A                     | +                | +    | -   | -    | -   | -     | -     |
|         |                | B                     | +                | ±    | -   | -    | -   | -     | -     |

\*Sera obtained from patient (A. G.) treated with antigen D at various stages during treatment program.

†Each site challenged by intracutaneous injection with  $10^{-5}$  Gm. protein of WST, purified allergen A, or purified allergen B.

‡Dilutions of serum are represented by the reciprocal of dilution tested.

§P-K reactions were graded as follows: not done, N.D.; negative, -; less than 5 mm., diameter ±; 6 to 10 mm. diameter, +; 11 to 15 mm. diameter, ++; 16 to 20 mm. diameter, +++; greater than 20 mm. diameter, ++++.

of decrease in the reagin titer of the antigen D-treated patients. Eighty per cent (12/15) of the patients treated with antigen D had a tenfold or greater drop in reagin titer during the first year of treatment.

During the 1968 pollen season, 10 new patients were added to the treatment program. Five patients were treated with WST, and the remainder were treated with antigen D. Neither the antigen D- or the WST-treated group produced demonstrable levels of blocking antibody. On the other hand, those patients treated with antigen D showed a ten- to hundredfold decrease in the reagin antibody levels. Patients treated with WST showed no change in the reagin antibody during the treatment program. Those patients treated a second year with antigen D showed either a slight decrease of reagin titer or remained at the same low level. However, those patients who were treated initially with antigen D and then, in the second year, were treated either with

**Table V.** *Decrease in reagin titer of antigen D-treated patients*

| <i>Drop in reagin titer</i> | <i>No. of patients</i> |
|-----------------------------|------------------------|
| 2                           | 2                      |
| 5                           | 1                      |
| 10                          | 6                      |
| 50                          | 1                      |
| 100                         | 3                      |
| 500                         | 1                      |
| 5,000                       | 1                      |

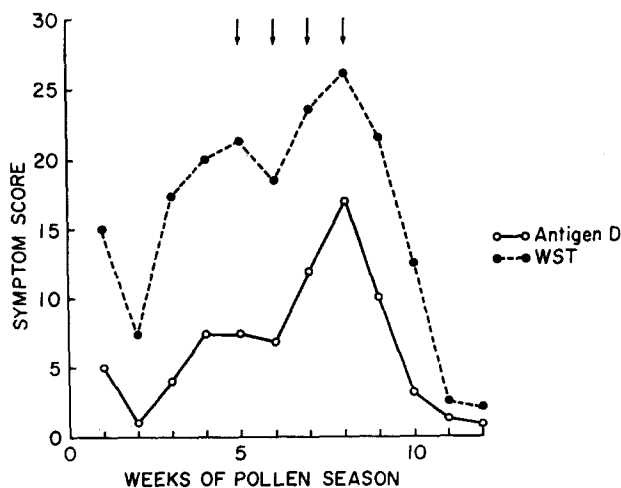


**Fig. 5**

Comparison of treatment with antigen D and WST during the 1968 pollen season on the basis of total treatment score. Arrows ( $\downarrow$ ) indicate the peaks of pollen exposure. Statistical analysis was performed with the use of the Mann-Whitney U test ( $p = 0.028$ ) and the Walsh test ( $p = 0.002$ ). A total of 48 patients were treated during this pollen season.

WST (6 patients) or received no treatment (4 patients) showed an increase in reagin titer usually to the pretreatment level observed at the beginning of the first year. Information obtained from personal interviews of antigen D-treated patients indicated that their "pollen season" was much shorter in duration than that of the WST-treated patients. Antigen D-treated patients indicated that their symptoms were restricted primarily to the period of June 15 to June 27. In contrast, patients treated with WST appeared to have significant clinical symptoms over a longer period of time (Fig. 5).

## DISCUSSION

Attempts to demonstrate a correlation between clinical protection and an increase of blocking antibody resulting from treatment with either antigen D or WST were unsuccessful. Patients treated with antigen D in an alginate suspension showed significant clinical protection despite the low incidence of blocking antibody production. The 10 patients treated with an aqueous preparation of WST showed high levels of blocking antibody. However, these patients appeared to have more severe clinical symptoms than either group of patients (antigen D or WST) treated with alginate suspensions. Previous studies<sup>19</sup> demonstrated that intracutaneous injection of nonallergic human beings with an aqueous solution of antigen D induced significant levels of blocking antibody. Similarly, patients treated with an aqueous preparation of WST produced both a high frequency and significant levels of blocking antibody. In contrast, patients treated with either antigen D or WST in alginate showed both a low incidence and low levels of blocking antibody production. It would appear that treatment with antigen D and WST in alginate permitted slow release of both antigens but failed to attract sufficient numbers of cells capable of synthesizing blocking antibody.

Clinical evaluation of each patient's treatment based on a composite score of symptoms and medication tabulated from their daily diaries and personal interview indicated that patients treated with antigen D (1) had significantly lower composite scores (Fig. 3); (2) required less medication (Fig. 4); and (3) had less symptoms throughout the pollen season than patients treated with WST. The clinical improvement noted by antigen D-treated patients correlated with a significant drop in reagin titer (Fig. 3 and Table V). Connell and Sherman<sup>14, 20</sup> previously have reported finding a correlation between clinical improvement of ragweed-sensitive patients with a decrease in reagin titer. They reported that during the first year of treatment with aqueous ragweed injections reagin titers showed no significant decrease. However, a significant decrease occurred during the next 3 years of treatment. Similarly, in the studies of Lowell and Franklin<sup>1</sup> and Lichtenstein, Norman, and Winkenwerder,<sup>6</sup> clinical improvement occurred at some stage after the second year of treatment. Connell and Sherman also reported that patients with the highest titers ( $> 1,000$ ) failed to respond to injections of ragweed extract and those patients with lower pretreatment titers ( $< 1,000$ ) showed the largest decrease in reagin during therapy.

The observations made in this study demonstrate that patients treated with

antigen D showed a significant drop in the reagin titer during the first year of treatment. Table IV illustrates both the specificity and the decrease in reagin titer with treatment. Patients treated with antigen D showed an abrupt drop in reagin titer early in the course of treatment and remained at this low level throughout the treatment. From July to January, patients received no treatment, but pretreatment titers at initiation of the second year of treatment revealed that the reagin titers remained at the low levels observed in July. Consequently, patients receiving treatment for a second year started with significantly lower pretreatment titers. It is apparent from Table IV that the reagin directed against allergen B is lost most rapidly, and with continued treatment a gradual reduction in the reagin directed against allergen A is observed. The data in Table V show that eighty per cent (12/15 patients) of the antigen D-treated patients showed a greater than tenfold decrease in reagin titer during the first year of treatment. In general, patients with high pretreatment titers were responsive to treatment with antigen D to the same extent as those patients with low pretreatment titers.

The observed *in vitro* neutralization (Table III) and the abrupt drop in reagin titer early in treatment suggest that co-seasonal treatment of patients with antigen D might be beneficial. In general, co-seasonal treatment with the use of crude pollen extracts has not been very effective and is not generally advocated. The presence of large amounts of antigen in the air during the pollen season limits the amount of antigen that can be administered co-seasonally. However, the weak allergenicity of antigen D would permit the injection of high concentrations without adverse effects.

### **Mechanism of hyposensitization**

The observed decrease in reagin titer and the maintenance of this reagin titer at low levels over a 6 to 18 month period suggest several mechanisms of hyposensitization. One possible explanation for the decreased reagin titer could involve *in vivo* neutralization of reagin by antigen D. However, an alternative explanation is that prolonged treatment with antigen D causes a suppression of reaginic antibody formation. Connell and Sherman<sup>14</sup> suggested that the observed reduction of reaginic antibody in patients treated two or more years with crude ragweed extract might be explained by the induction of partial immune tolerance. The observed decrease in reagin titer might have been due to the presence of low molecular weight hapten-like fragments present in their ragweed extract preparation (see below). The possibility of suppression of reaginic antibody formation leading to either complete or partial immune tolerance resulting from hyposensitization therapy with hapten-like fragments, however, should be considered. The use of simple haptenic materials to induce tolerance has been the subject of much investigative study and was recently reviewed by De Weck and Frey.<sup>21</sup> The experiments of Chase, Battisto, and Ritts,<sup>22</sup> Battisto and Chase,<sup>23</sup> and Frey, De Weck, and Geleick<sup>24</sup> indicate that the unresponsive state induced by hapten treatment involves both immediate and delayed types of hypersensitivity and is quite specific.

If the decrease in reaginic antibody titer observed in patients treated with

antigen D is an example of the induction of partial tolerance, then the information below could be interpreted as follows. The 6 patients treated with antigen D during the first year and WST during the second year showed a significant decrease in reagin titer during the first year of treatment. However, early in the second year of treatment (prior to pollen season) these patients showed a rapid increase in reagin titers usually to the pretreatment level observed at the beginning of the first year, and this could represent an example of the breaking of tolerance with a cross-reacting antigen (allergen A). Patients (4) treated with antigen D who did not receive treatment during the second year showed a similar rapid increase of reagin titer, and this observation would suggest that once tolerance has been established the maximum interval between antigen D injections should be about 6 months. At this time, we would favor the less complex mechanism of in vivo neutralization as the basis of the observed hyposensitization with antigen D. However, the idea of suppression of antibody formation or the induction of tolerance by treating allergic patients with low molecular weight fragments containing allergenic determinants remains an attractive hypothesis which should be given more attention.

### **General significance**

The isolation of antigen D and its successful use in the hyposensitization of timothy-sensitive patients raises the question of the applicability of this approach to other allergenic materials. Stated in another way: Do other allergenic extracts contain analogous hapten-like fragments? Richter and associates<sup>25</sup> previously reported the isolation of a dialyzable component of ragweed extract with weak allergenic activity. Similarly, Malley, Heimlich, and Campbell<sup>26</sup> and Feigen, Sanz, Meyers, and Campbell<sup>27</sup> reported the isolation of a dialyzable fraction of ragweed extract that was capable of inhibiting precipitation and Shultz-Dale reactions of rabbit antiragweed extract antibodies. More recently a dialyzable fragment has been isolated from green peas which is capable of inhibiting precipitation (> 70 per cent inhibition) and passive cutaneous anaphylaxis of rabbit antigreen pea antisera.<sup>28</sup> The green pea dialyzable fragment (1 mg. protein per milliliter) mixed with sera from individuals allergic to green peas will almost completely inhibit the P-K reactions of sites subsequently challenged with a green pea extract. Similarly, a dialyzable fraction of house dust<sup>29</sup> has been isolated that is capable of neutralizing the reaginic antibodies present in the sera of house dust-sensitive patients.

Although haptenic substances have been isolated in all four of the above allergenic materials that we have examined, it is quite possible that not all allergenic extracts will possess readily extractable natural breakdown products that possess haptenic properties. Rye grass pollen extracts apparently do not contain natural haptenic fragments. However, Peterson and associates<sup>30</sup> treated rye grass pollen extract with trypsin and found that the resultant fragments were capable of neutralizing reaginic antibody directed against rye grass allergens. It is possible that a further refinement of the approach taken by Peterson and co-workers would be applicable in those cases in which allergenic extracts do not contain natural fragments with hapten-like properties. From our studies

it would seem likely that a great many other allergenic materials upon extraction will be found to contain low molecular weight fragments that possess hapten-like properties analogous to those described for antigen D, the green pea, and house dust dialyzates. The use of these low molecular weight fragments in the treatment of allergic patients may provide the basis of an alternative approach to treatment of allergic patients.

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