

Original Articles

FORMALINIZED POLLEN TANNATE IN DESENSITIZATION TREATMENT

HYMAN L. NATERMAN, M.D., BROOKLINE, MASS.

INTRODUCTION

VARIOUS methods to increase the ease, safety, and effectiveness of parenteral pollen desensitization have been used, such as (1) emulsification of pollen extract solutions in oil,¹ (2) addition of gelatin to pollen extract solutions,² (3) tannic acid or hydrochloric acid precipitates of pollen extract suspended in water,³ and (4) tannic acid precipitates suspended in oil with adjuvants.⁴ These methods depend upon the menstruum or upon changes in the physical state of the antigen to slow absorption.

Acid precipitates of pollen protein prepared by these methods have relatively little or no immunologic denaturation, as is shown by their ability to produce potentially dangerous, prompt, local and general reactions. A method of denaturing pollen precipitates which would reduce or eliminate immediate reactivity without destroying antigenicity would make it possible to give larger doses. Formalin, which has long been used to denature antigens without destroying antigenic functions, seemed worth trying. In 1948, fourteen ragweed-sensitive patients treated with formalinized ragweed tannate showed small local reactions with large doses. In succeeding years, partially formalinized pollen tannates were used with good desensitization. It was decided to treat a large series of patients in 1954 and 1955 with fully formalinized pollen tannates; the present article describes the methods of preparation, use, and clinical results in 355 pollen seasons, in 193 patients.

METHODS

Preparation of Formalinized Pollen Tannate (Pollen-FT).—Short ragweed pollen was used in making the ragweed extract; equal parts of timothy and orchard grass were used for the grass extract. Birch pollen and oak pollen were processed separately and the formalinized tannates were combined to make the tree antigen.

A 1:20 phenolized saline pollen extract was made in the usual manner and passed through filter paper. Zinc chloride was added to a concentration of

From the Allergy Clinic, Beth Israel Hospital, Boston, Massachusetts; the Department of Medicine, Harvard Medical School; and the Department of Medicine, Tufts Medical School.

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0.1 per cent. Tannic acid (reagent grade in freshly prepared solution) was added to make a concentration of 0.5 per cent or 1.0 per cent. Flocculation of pollen tannate occurred promptly, and the sediment was collected by centrifugation.

In 1954, the pollen tannate was resuspended in saline (sediment from 50 grams of pollen in 200 ml. of saline) and U.S.P. formalin was added to make a 2 per cent concentration. After standing at room temperature for six days, the supernatant was removed and the sediment was lyophilized. The resulting powder was passed through a 200-guage screen, and its nitrogen content was determined by the micro-Kjeldahl method. The powder, as needed, was suspended in phenolized saline with 1 per cent aluminum hydroxide, prepared in the following manner: 4 per cent aluminum chloride in saline was neutralized with sodium hydroxide and autoclaved; phenol to 1 per cent was added. Dilutions of this stock 4 per cent aluminum hydroxide were made as needed. For clinical use, enough pollen-FT was added to 1 per cent aluminum hydroxide to make 1.0 mg. of nitrogen per milliliter, tested for sterility, and called Concentration A. Tenfold dilutions in phenolized saline were made so that B = 0.1 mg. N/ml., C = 0.01 mg. N/ml., and D = 0.001 mg. N/ml.

For 1955, after collecting the tannic acid precipitate as above, the nitrogen content was determined, and the volume was adjusted so that 1 ml. = 1.0 mg. N. Formalin was added to make a 0.5 per cent concentration, and the preparations were kept at room temperature. After two weeks to six or more months of formalinization, the 1.0 mg. N/ml. concentration was diluted with an equal volume of 0.8 per cent sodium bisulfite solution in saline to neutralize the formalin. An equal volume of a suspension of 0.5 per cent aluminum hydroxide and 0.5 per cent hydrocortisone acetate in saline was then added. The final saline suspension contained:

(1) Pollen-FT	0.25 mg. N/ml.
(2) Aluminum hydroxide	0.25 per cent
(3) Hydrocortisone acetate	0.25 per cent
(4) Sodium bisulfite	0.20 per cent
(5) Phenol	0.88 per cent

In making the 1954 preparations, a 2 per cent concentration of formalin was selected arbitrarily; pollen tannate treated with this concentration for one day showed moderately decreased skin reactivity on intradermal injection and, after three or more days, marked reduction to complete absence of skin reactivity. A six-day period of formalinization was used. In making the 1955 preparations, it was determined that pollen tannate in formalin concentrations of 0.5 per cent and 0.25 per cent for six days showed marked reduction in skin test reactivity; lesser concentrations were increasingly unreactive. Therefore, a 0.5 per cent concentration of formalin was used. Prolonged contact with this concentration of formalin does not destroy antigenicity; a preparation of ragweed tannate standing in 0.5 per cent formalin at room temperature for six months was capable of producing a systemic allergic reaction.

Dosage.—In 1954, the starting dose for previously untreated patients was usually 0.1 ml. of the C dilution. Some patients with large skin reactions were

started with the D dilution. Many patients with previous treatment or with smaller skin reactions were started with the B dilution and some were started with A. The maximum dose was 0.25 ml. to 0.5 ml. of A. The usual schedule of doses (in milliliters) was: (1) C 0.1, (2) B 0.1, (3) A 0.1, (4) 0.25 or 0.5. In 1955, the usual dose schedule (in milliliters) of the 0.25 mg. N/ml. concentration was: (1) 0.1, (2) 0.35, and (3) 1.0. In a few instances, when marked sensitivity was suspected, one-half the usual doses were given. For some patients not prone to general reactions, the 1955 schedule was: (1) 0.2 ml., (2) 1.0 ml. Occasionally a dose was repeated because of a large local reaction.

Injections were given subcutaneously in the upper arms every one to three weeks, with increasing dosage. When the maximum dose was reached, it was repeated once at the usual one- to three-week interval, and thereafter at intervals of one to three months, usually two months, until the season began. The last preseasonal injection usually was within four weeks of the expected onset of the season.

Selection of Patients.—Hay fever patients, many with varying degrees of seasonal asthma, were skin tested by the scratch method with pollen extract in 50 per cent glycerin-saline in dilutions of 1:50, 1:500, and 1:5,000. Only patients with moderate to marked skin reactions were selected for treatment. Patients with asthma alone who usually showed slight skin reactions were excluded. Patients able to tolerate large starting doses of pollen extract solutions were also excluded. The tests frequently were repeated during and after treatment. The patients were about equally divided as to sex, and ages were from 4 to 79 years. Each pollen season was considered as a unit; thus, a patient treated for both grass and ragweed pollen was considered statistically as two patient seasons. One hundred and four patients treated in 1954, representing 128 pollen seasons, were also treated in 1955.

RESULTS

Number of Injections.—Thirty ragweed-sensitive patients treated in 1953 with a partially formalinized tannate were given ragweed-FT without a prolonged waiting period. They were classified as perennial, and the injections

TABLE I. AVERAGE NUMBER OF POLLEN-FT INJECTIONS GIVEN TO 118 PATIENTS IN 1954
REPRESENTING 162 PATIENT SEASONS

		NUMBER OF PATIENT SEASONS	AVERAGE NUMBER OF INJECTIONS TO MAXIMUM DOSE	AVERAGE TOTAL INJECTIONS
Ragweed	Perennial:	30	---	10.1
	Preseasonal:			
	No previous treatment	26	4.3	9.6
	Previous treatment	36	4.05	8.1
	Total	62	4.15	8.8
Grass	No previous treatment	15	4.4	6.6
	Previous treatment	29	4.4	6.7
	Total	44	4.4	6.7
Trees	No previous treatment	12	3.9	5.1
	Previous treatment	14	4.2	6.0
	Total	26	4.06	5.6

given from October, 1953, to October, 1954, were considered the season's treatment; the average number of injections was 10.1 (Table I). From October, 1954, to October, 1955, sixty-six patients were given perennial ragweed treatment; the average number of injections was 6.5 (Table II). Seven grass-sensitive and six tree-sensitive patients had perennial treatment in 1954 and 1955; the average number of injections was 5.4 and 6.0, respectively (Table II).

TABLE II. AVERAGE NUMBER OF POLLEN-FT INJECTIONS GIVEN TO 162 PATIENTS IN 1955 REPRESENTING 193 PATIENT SEASONS

		NUMBER OF PATIENT SEASONS	AVERAGE NUMBER OF INJECTIONS TO MAXIMUM DOSE	AVERAGE TOTAL INJECTIONS
Perennial	Ragweed	66	--	6.5
	Grass	7	--	5.4
	Tree	6	--	6.0
Preseasonal	Ragweed	65	3.0	6.1
	Grass	36	3.0	5.9
	Tree	13	2.8	5.7

In considering the 1954 preseasonal patients, those with no desensitization the previous year, or who never had desensitization treatment, are presented separately to eliminate the possible persistence of desensitization from the previous treatment (Table I). No significant difference is noted when compared with those patients who did have previous treatment. The average number of injections to reach the maximum dose was ragweed, 4.15; grass, 4.4; and trees, 4.06. The average total number of injections was ragweed, 8.8; grass, 6.7; and trees, 5.6. In 1955 (Table II), because it was found safe to start with bigger doses and because of the experience gained in 1954, the number of injections was reduced. The average required to reach the maximum dose was ragweed, 3.0; grass, 3.0; and trees, 2.8. The average total number was ragweed, 6.1; grass, 5.9; and trees, 5.7.

Local Reactions.—The injections of pollen-FT were as painless as pollen solutions. The local reactions developed slowly and usually reached a maximum twenty-four to forty-eight hours after injection, which was slight to moderate in degree. The edematous swelling usually cleared in two to four days, leaving a central firm area which gradually shrank to a pea-sized nodule in one week. If there was a marked local reaction, the central nodule would be larger and persist for four to eight weeks, and at times a bluish discoloration of the skin would develop, suggesting a deep-seated ecchymosis. No necrosis or other complications have developed. A dose which gave a large reaction, usually the maximum dose, when repeated invariably caused a much smaller reaction. In 1955, hydrocortisone acetate was added to reduce the induration resulting from intense specific local reactions, with great success.

General Reactions.—Some patients who were highly reactive and prone to general reactions had mild general reactions with the maximum dose, consisting of a few wheals which might be scattered or localized to the opposite arm. Occasionally mild nasal congestion also occurred. Such reactions often did not recur with repetition of the maximum dose. No reduction in dose was made

since the reactions were so mild. They occurred from thirty minutes to several hours after injection and subsided spontaneously in from one to several hours, or were relieved promptly by an antihistamine tablet. In 1954, when increases in dosage were five- or tenfold, three female patients had moderate to severe generalized urticaria several hours after injection. No patient had the acute respiratory difficulty characteristic of severe general reactions following injection of pollen extract solution.

TABLE III. CLINICAL RESULTS IN 350 OF 355 PATIENT SEASONS TREATED IN 1954* AND 1955† WITH POLLEN-FT

		NUMBER OF PATIENT SEASONS	SATISFACTORY		UNSATISFACTORY	
			NUMBER	PER CENT	NUMBER	PER CENT
1954	Ragweed	92	88	96	4	4
	Grass	44	41	93	3	7
	Trees	26	24	93	2	7
1955	Ragweed	126	107	85	19	15
	Grass	43	43	98	1	2
	Trees	19	18	95	1	5
Total		350	320	91	30	9

*Representing 118 individual patients

†Representing 161 individual patients.

Symptomatic Relief.—After careful questioning regarding the nature, frequency, and severity of symptoms and the amount of medication taken after treatment as compared with previous years, the patients were divided into satisfactory and unsatisfactory groups (Table III). The satisfactory group had relief of symptoms ranging from complete with no medication needed to moderate with small doses of antihistamines needed at times. Patients who had little or no change, as compared with previous years, and needed full doses of antihistamines regularly were placed in the unsatisfactory group. In 1954, the results known in 162 patients show satisfactory results in 96 per cent of ninety-two ragweed-sensitive patients, 93 per cent of forty-four grass-sensitive patients, and 93 per cent of twenty-six tree-sensitive patients. In 1955, satisfactory results were obtained in 85 per cent of 126 ragweed-sensitive patients, 98 per cent of forty-three grass-sensitive patients, and 95 per cent of nineteen tree patients. The results in five patients were not obtained. Among 350 patient seasons, therefore, the results were satisfactory in 91 per cent.

DISCUSSION

The usual pollen desensitization therapy depends on the subcutaneous injection of specific antigen in increasing quantities so that the patient eventually tolerates the injection of a larger amount of antigen with the same or less local reaction than when treatment was begun. The rate of increasing dosage is restricted by the need to avoid distressing local and dangerous general reactions. The methods currently used to slow absorption and thus avoid these undesirable reactions are not entirely satisfactory. They depend on physical or chemical measures which do not alter the reactivity of the antigen. Excessive reactions, therefore, may occur due to unpredictable changes in the physical

state of the preparation on standing or variation in the rate of absorption after injection. For example, radioactive protein in Freund's adjuvant had an increased absorption rate in the first twenty-four hours averaging up to 30 per cent of the total injected and a much slower rate thereafter.⁵ The use of an antigen whose capacity for immediate reaction has been reduced or eliminated is safer. An antihistamine in the preparation would increase this safety.

Formalin has long been used to make vaccines and toxoids without loss of antigenicity. Its principal action is thought to be the production of methylene bridges between free protein amine groups, binding protein molecules together. When pollen tannate was formalinized for three days or more, and intradermal skin tests were done, the immediate reactions were absent or greatly reduced. The same material injected subcutaneously did produce reactions after a period of several hours, suggesting that the specific antigen links were blocked by formalinization and gradually released by enzymatic action. Because of this gradual release, the possibility of an overwhelming acute anaphylactic reaction is greatly reduced. That formalinized pollen tannates do desensitize is indicated by the following observations: (1) A large local reaction becomes much smaller when the dose is repeated; (2) frequently the local reaction to an increased dose is less than to the previous smaller dose; (3) frequently the scratch skin test with pollen solutions is markedly reduced after treatment; and (4) satisfactory clinical relief is obtained.

In 1954 pollen-FT was stored in the lyophilized state; in 1955 pollen-FT was stored as 1 mg. N per milliliter in 0.5 per cent formalin at room temperature. No apparent difference could be detected in clinical use. A preparation stored in 0.5 per cent formalin for more than six months, when processed for clinical use, still showed activity. By storing lyophilized pollen-FT, the deterioration of activity in solutions may be avoided with less danger of reactions when different preparations are to be used. It is likely that the same advantage will be evident with the preparations stored in 0.5 per cent formalin.

When pollen-FT is suspended in saline, some of the particles are small enough to form a colloidal suspension. It is likely that the small colloidal particles disperse more readily and react more quickly than larger aggregates. Aluminum hydroxide was used to counteract this and to produce a flocculent type of precipitate.

In 1954, with larger doses of lyophilized FT, intense local reactions would occur at times with resulting large nodules absorbing slowly. Such reactions were specific and not of the "foreign body" type since, when the same dose was repeated, the reaction was invariably much less. In 1955, the addition of hydrocortisone in 0.25 per cent concentration practically eliminated local nodule formation. The maximum quantity of hydrocortisone injected was 2.5 mg., a quantity too small to exert any significant general effect.

The experience of 1954 indicated that a starting dose of 0.01 mg. N and a maximum dose of 0.25 mg. N, would give satisfactory clinical results. This spread of dosage could be obtained with the one concentration of 0.25 mg. N per milliliter used in 1955. The usual starting dose was 0.1 ml. (0.025 mg. N);

in rare instances, when previous susceptibility to general reactions was known or suspected, the starting dose was 0.05 ml. (0.012 mg. N). Later in the year, patients who had had treatment previously and who were not susceptible to general reactions were started with 0.2 ml. (0.05 mg. N); one patient, by error, was given initially 1.0 ml. with no excessive reaction. With threefold to fivefold increases in dosage, the maximum dose of 1.0 ml (occasionally 0.5 ml.) was reached with the second or third injection. The total number of injections given was about six for either the preseasonal or perennial forms of treatment; there is no indication that one type of treatment was better than the other. Many patients who started treatment late did not have the maximum dose repeated as often, and obtained satisfactory results with three or four injections.

Hitherto, only patients with moderate to severe sensitivity have been selected for treatment with pollen-FT. This includes the majority of pollen-sensitive patients. The smaller group of patients with slight sensitivity, who presumably require large doses for effective desensitization, may not get enough antigenic stimulus from the slowly absorbing pollen-FT. However, this group of patients can be treated easily with regular pollen extract solutions. They reach a high level of dosage, up to 0.5 c.c. of a 1:20 pollen dilution, with comparatively few injections and slight risk of general reactions. Probably nothing would be gained by treating such patients with pollen-FT unless much larger doses than those used here were tried.

The possibility of using pollen-FT in various media, such as oil with adjuvants, or gelatin, to delay absorption further has been considered and has been tried to some extent. The difficulties involved in making and injecting such combinations seem to outweigh any advantages. Furthermore, it is uncertain that they would delay significantly the absorption of pollen-FT. For example, radio-iodinated serum albumin processed like pollen-FT and suspended in various media showed about the same rate of absorption from saline suspensions as from oil or gelatin suspensions.⁶ Implanting a pellet of pollen-FT might slow absorption enough to permit a one-dose treatment.

The application of this formalinized tannate technique to other preparations is possible. House dust and other allergens may be found suitable. Protein hormones, such as pitressin, insulin, and adrenocorticotrophin, if their specific activity is not destroyed, might have a prolonged action. The danger of inducing or increasing sensitivity when animal proteins (for example insect antigen) are used in this way must be considered carefully. There is no indication that increased sensitivity to the pollens used has occurred.

Four main advantages of pollen-FT over the solutions generally used in pollen desensitization are: (1) one concentration may be used, rather than three or more; (2) a more flexible time schedule of injections can be followed; (3) the danger of general reactions is markedly reduced; and (4) the number of injections is decreased to one-third or less. The clinical results with pollen-FT compare favorably with the results obtained by any other method of pollen

desensitization. Controlled studies on a large scale would be required to determine the relative merits. The large number of patients successfully treated in this study over a two-year period suggests that the satisfactory clinical results were not coincidental and that a controlled study is warranted.

SUMMARY AND CONCLUSIONS

1. Pollen antigens precipitated from aqueous solutions by tannic acid and zinc and then formalinized (pollen-FT) showed reduced immediate reactivity and prolonged absorption, permitting the injection of large doses.

2. Pollen-FT was used in 1954 and 1955 in the treatment of 193 patients representing 355 tree, grass, or ragweed pollen seasons. The usual maximum dose of 0.25 mg. N (25,000 protein N units) could be reached in three injections. The total number of injections per season was reduced to about six. Satisfactory clinical results were obtained in 91.4 per cent of 350 patient seasons.

3. The advantages of pollen-FT suspensions over other methods of slowing absorption are discussed. The advantages over the pollen extract solutions commonly used are: (1) the use of a single concentration; (2) a more flexible time schedule of injection; (3) reduced danger of general reactions; and (4) reduction of number of injections to one-third or less.

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