

POLLEN TANNATE SUSPENDED IN PEANUT OIL WITH ALUMINUM MONOSTEARATE IN THE TREATMENT OF HAY FEVER*

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TANNIC acid precipitates^{1, 2} of the active fractions of pollen extract have been used for the desensitization treatment of pollinosis since 1939, except for the years 1942 to 1945. It was shown¹ that the active principle in pollen extract solutions was precipitated by the tannic acid as indicated by inability of the supernatant to produce positive skin reactions in sensitive patients, and by the production of local and general reactions in sensitive patients injected with tannate precipitates. Desensitization after treatment with tannate precipitates was proved by reduction in skin sensitivity, the ability to tolerate large doses of regular extract, and the satisfactory clinical results. In a preliminary study in 1949, 28 patients treated with grass and ragweed precipitates suspended in peanut oil with aluminum monostearate had encouraging results. The purpose of this paper is to describe the present method of preparation of pollen tannate precipitates, and to report results of treatment in 1950 with such precipitates suspended in peanut oil with 2 per cent aluminum monostearate.

METHODS

Preparation of Materials.—Short ragweed pollen was used. The grass preparation was made from equal parts of timothy and orchard grass pollen. Pollen is extracted with ether, dried, and then extracted with physiologic saline solution to make a 1:20 solution. The extract is filtered and measured. A solution of 10 per cent zinc sulfate is added sufficient to make a 0.1 per cent concentration. Then a freshly prepared 10 per cent tannic acid solution is added to make a 0.5 per cent final concentration. The resulting flocculation is allowed to settle. The supernatant is removed. The remainder is centrifuged lightly, the supernatant removed, and the sediment of pollen tannate is rubbed into a small volume of 95 per cent ethyl alcohol to break up any clumps. At this point the sediment becomes dark brown. To remove excess tannic acid, water, and other nonprotein pollen fractions, the sediment is extracted repeatedly with alcohol until the supernatant is clear and colorless. The sediment is finally taken up in sufficient 95 per cent alcohol containing 2 per cent phenol to make a 1:1 relation, that is, if 100 Gm. of pollen were originally extracted and 1,800 c.c. of 1:20 dilution obtained, the final volume is 90 c.c. The suspension is placed in a sterile bottle, capped, and after being tested for sterility, is ready for use and stored as stock.

This method may be modified readily. If the dry pollen is extracted fractionally with small portions of saline solution, or by continuous saline drip, the active material will be practically completely removed from the pollen. The final suspension may then be considered to contain all the active material from the original weight of pollen and the volume adjusted accordingly, that is if

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100 Gm. of pollen were used originally, the final volume of 1:1 extract is 100 c.c. Although desirable, it is difficult to carry out these procedures with sterile technique, especially when dealing with large quantities. For practical purposes, sterile technique is not necessary since the use of 2 per cent phenol in 95 per cent alcohol to make the final suspension ensures chemical sterilization.

If dilutions of the suspension in saline solution are to be used for treatment, it is advisable to make the final 1:1 stock suspension in glycerin instead of 95 per cent alcohol, since a 1:10 dilution in saline solution of the alcoholic stock suspension causes pain on subcutaneous injection.

To make a suspension in oil with aluminum monostearate, using sterile technique, the desired amount of the alcoholic stock suspension is centrifuged lightly, and the supernatant removed. The sediment is taken up in ether to the original amount, centrifuged again, the supernatant removed, and the sediment is rubbed and stirred into a small volume of the oil with aluminum monostearate, making certain a homogeneous suspension is present before adding oil sufficient to making a 1:1 suspension. Dilutions are made by adding the 1:1 suspension in oil to sterile peanut oil with 2 per cent aluminum monostearate.

Standardization of Preparation.—Attempts were made to standardize the stock suspension 1:1 in alcohol by nitrogen determination of the whole suspension. The only method available was the micro-Kjeldahl, which gave very variable results. The regular Kjeldahl method would probably give more accurate, consistent results. Because of the variable nitrogen determinations on the same preparation and the use of different lots of suspensions during the course of the study, the weight-volume standardization is used here. However, it may be stated that the average content of tannic acid-precipitable N_2 was about 1.5 mg. per cubic centimeter of the 1:1 stock. It is generally estimated that 1 c.c. of 1:1 pollen extract (1.0 Gm. of pollen) should yield about 5.0 mg. of protein N_2 by phosphotungstic acid precipitation.³ However, the tannic acid precipitation was about one-third the estimated yield. This discrepancy requires further study.

Method of Treatment.—Injections were given subcutaneously in the arms every 7 to 14 days using a tuberculin syringe with an ordinary hypodermic needle (26 gauge, one-half inch). The dilutions in oil used in treatment were 1:1, 1:10, 1:100, and 1:1,000. Patients who were considered to be very sensitive by skin test, especially patients with no previous treatment, were started with 0.1 c.c. of the 1:1,000 dilution. Patients who had lesser skin test sensitivity or had had previous treatment were started with the 1:100 dilution, occasionally with the 1:10 dilution. Usually, subsequent doses were increased by 3 times, thus: Dose 1, 1:1,000, 0.1 c.c.; Dose 2, 1:1,000, 0.3 c.c.; Dose 3, 1:100, 0.1 c.c., and so on. Often a fivefold or tenfold increase in dosage was given if a very small reaction occurred from the previous injection. The maximum dose arbitrarily selected was 0.1 c.c. to 0.3 c.c. of the 1:1 suspension. If an excessive reaction occurred with a particular dose, the same dose was repeated and practically always a small reaction resulted. When the maximum dose was reached, it was repeated once if necessary to make sure the reaction was slight to mod-

erate, and then was repeated every 4 to 6 weeks until the beginning of the pollen season. Usually no injections were given during the season.

A patient sensitive to grass and ragweed pollen was treated with a mixture of equal parts of grass and ragweed suspension. After June 1, no further grass pollen was given, and desensitization was continued with ragweed alone until the early part of August. The tree preparation consisted of equal parts of 1:1 birch and 1:1 oak pollen tannate in oil.

Selection of Patients.—Only patients with pollen sensitivity of moderate or severe degree by both history and skin test were selected for treatment. The degree of skin sensitivity was determined by measuring the wheal and erythema produced by scratch tests using solutions of pollen extract in 50 per cent glycerin in dilutions of 1:50, 1:500, 1:5,000. These tests were repeated in most cases during and at the end of the course of treatment to detect desensitization, objectively.

The patients' ages ranged from 5 years to 83 years. Some were treated for 2 to 3 different pollen seasons. The number of pollen seasons treated is used as the number of patients for convenience in presenting results. The maximum dose usually was reached early and was repeated at intervals of 4 to 6 weeks until the onset of the season. The total number of injections is therefore affected by the date when the patients started treatment; the number of injections required to reach the maximum dose is not so affected. For this reason, the number of injections required to reach the maximum dose indicates the efficiency of the method. The number of injections needed to reach a certain dose in any one patient may be reduced because of persistent desensitization from previous treatment; therefore the grass and ragweed patients who had no previous treatment are presented separately.

RESULTS

An analysis of the method of treatment considers (1) the starting dose, (2) the maximum dosage, (3) the number of injections required to reach the maximum dose, and the total number of injections, and (4) the clinical results divided into the local and general reactions, and the symptomatic relief.

The Starting Dose.—The starting doses for each pollen season are presented in Table I. The dose was usually 0.1 c.c. of the particular dilution; occasionally 0.05 c.c. or 0.15 was given. The 1:1,000 dilution was usually used for new patients or patients with very large skin reactions. In general, the starting dose was about 10 times the usual starting dose when regular pollen extract is used.

The Maximum Dose.—The maximum dose ranged from 0.1 c.c. to 0.3 c.c. of the 1:1 dilution. When the concentrated 1:1 preparation is used, small differences in volume represent very large differences in units. Most, if not all, patients who were maintained on a dose of 0.1 c.c. of 1:1 dilution could have been raised higher. The smaller doses were used to observe the results with different dosage, and to conserve material. The 0.1 c.c. dose, theoretically 100,000 noon pollen units, was estimated to contain at least 10,000 to 20,000 protein nitrogen units.

Number of Injections.—The number of injections required to reach the maximum dose is very small, averaging from 4.3 to 5.8 (Table I). The patients who had had treatment in previous years, usually with pollen tannate suspended in water, had some desensitization remaining, and their degree of sensitivity was known from experience. Therefore they were usually started with a higher dose, and frequently their increase in dosage was greater. The previously untreated patients present no such factors influencing treatment, and their figures are given in Table II. The average number of injections required for the new patients was 6.3 to 7.4. To reach a similar maximum dose of regular extract would require ordinarily 3 or more times that number of injections.

TABLE I. 1950 TREATMENT WITH POLLEN TANNATE SUSPENSION IN OIL WITH 2 PER CENT ALUMINUM MONOSTEARATE

	NUMBER OF PATIENTS	STARTING DOSE DILUTION			AVERAGE NUM- BER OF INJEC- TIONS TO MAXIMUM DOSE	AVERAGE TOTAL INJECTIONS
		1:1000	1:100	1:10		
Trees	9	0	6	3	4.3	5.5
Grass	31	4	23	4	5.7	7.6
Ragweed	82	18	49	15	5.8	9.1

The total number of injections for each pollen season are given in Tables I and II, and ranged from 5.5 to 10.6. This factor is influenced by the time of starting treatment; the earlier treatment was started, the longer the period for repeating the maximum dose. It is probable that desensitization was increased by repeated maximum doses.

TABLE II. PATIENTS WITH NO PREVIOUS HAY FEVER TREATMENT

	NUMBER OF PATIENTS	AVERAGE NUMBER OF INJECTIONS TO MAXIMUM DOSE	AVERAGE TOTAL INJECTIONS
Grass in oil	8	6.3	7.9
Ragweed in oil	30	7.4	10.6

Local and General Reactions.—The peanut oil with 2 per cent aluminum monostearate in the amounts used had no gross tissue reaction. Whenever a local reaction occurred, it seemed related to the pollen tannate. The local reactions in general were similar to the local reactions produced by regular extracts. They developed much more slowly, however, with the greatest reaction occurring 1 or 2 days after injection. The slight to moderate reactions disappeared in 1 to 4 or 5 days, with no residual. The larger reactions showed diffuse swelling for several days, and frequently showed a small induration, which gradually disappeared, at the area of injection for 7 days or more thereafter. Occasionally a small, barely palpable, fibrotic nodule remained at the site of the more intense local reactions for several weeks. Usually even very large local reactions were not accompanied by general reactions. General reactions occurred very seldom, started 1 to 3 hours after injection, developed gradually, were mild enough to be treated by the patient with an antihistamine or ephedrine tablet, or required no treatment.

Two patients developed sterile abscesses at the site of tree pollen in oil injections. One, a ragweed-sensitive patient, received by error an injection of 0.1 c.c. of 1:1 tree pollen tannate in oil. He developed a walnut-sized induration which became fluctuant and healed without incident after incision and drainage done 6 weeks after the injection. The second patient, who had severe chronic atopic dermatitis, was sensitive to tree, grass, and ragweed. In the arm which received tree tannate in oil, she developed 3 nodules which became fluctuant and required incision and drainage 3 months after the last tree pollen injection. No complications appeared in the arm which received the grass and ragweed preparations. The tendency to induration was much greater with the tree preparations. It is likely there was some nonspecific irritant extracted from the tree pollen which accounted for this. The induration in the patient not sensitive to trees suggests this. About 1,000 grass and ragweed injections showed no such complications.

Skin Tests.—Objective evidence of desensitization is the effect of treatment on the degree of sensitivity measured by the skin test. Practically all patients had scratch skin test measurements done before treatment and most were repeated at the end of treatment. In general, most patients showed definite decrease in skin test sensitivity, and in many cases the tests became practically negative.

Symptomatic Relief.—There is no objective method of evaluating symptomatic relief. The patients were questioned about their symptoms and medication at intervals during and at the end of the season. They were classified as having satisfactory symptomatic relief, or not. In 6 instances the results were equivocal and could not be placed in one or the other category. The satisfactory group ranged from definite improvement with the help of occasional small doses of antihistamines to practically complete absence of symptoms without any medication. The results according to size of maximum dose given are not presented since it is not possible at this time to state with certainty the variations in results. Eleven patients did not report after their season had started, and results in their cases could not be evaluated. The results in the others are presented in Table III; 90.0 per cent of the cases had satisfactory results, 5.4 per cent were equivocal, and 4.5 per cent had unsatisfactory results. It is our impression that even the patients with unsatisfactory results had some degree of relief, and would have been worse without treatment. They showed evidence of desensitization by skin test, and tolerated their doses well. Factors other than the specific pollen sensitivity probably influence the symptoms of

TABLE III. SYMPTOMATIC RELIEF

	NUMBER OF PATIENTS	UNSATISFACTORY	EQUIVOCAL	SATISFACTORY
Ragweed	74	4	4	66
Grass	29	1	2	26
Trees	8	0	0	8
Total	111	5 (4.5%)	6 (5.4%)	100 (90.0%)

patients⁴ and it is unlikely that specific desensitization can completely relieve all patients. The results obtained compare favorably with regular methods of treatment.

DISCUSSION

Antihistaminic drugs are valuable in the treatment of pollinosis. Adrenal cortical hormones may prove effective and practical. However, desensitization by injections continues to be a necessary procedure in most patients with more than slight sensitivity. Since the introduction of desensitization therapy by Noon 40 years ago, no significant change in the preparation or administration of pollen extracts has occurred. In general, the greater the desensitization, the greater the relief of symptoms, and the greater the chance for persistent desensitization approximating a "clinical cure." Attempts to obtain more effective desensitization have been concerned mostly with methods of delaying and prolonging absorption of the pollen extract. One effective method is the emulsification of solutions of pollen extract in oil using as the emulsifying agent cholesterol substances such as lanolin, Aquaphor, or Eucerite.^{5, 6} Another method converts the water-soluble pollen extract to an insoluble precipitate by means of tannic acid and zinc,^{1, 2} or hydrochloric acid.⁷ The preparation used in this report combines the important features of both these methods with greatly increased effectiveness. The same principles applied to penicillin very successfully prolong absorption and the effect of each injection. The very large quantities of pollen extract tolerated by these means and the comparatively few injections needed to accomplish significant desensitization are striking.

It is likely that increasing the viscosity of the preparations by adding cholesterol agents such as lanolin, Aquaphor, and Eucerite, or making solid pellets for implantation, will increase the effectiveness still further, so that 1 or 2 injections may suffice for desensitization. Suspensions of pollen tannate in gelatin⁸ may be effective and should be tried. There is some indication that treatment of pollen tannate precipitates with formalin reduces the capacity to produce immediate reactions, but may nevertheless desensitize. Dry lyophilized pollen extract suspended in oil⁹ probably cannot be as effective as the method presented since the suspended material is water soluble, whereas pollen tannate is not.

Another advantage in using the oil suspension concerns the factor of safety. The dreaded severe acute general reaction due to the unpredictable rapid absorption of water solutions of pollen extract is much less likely to occur with oil suspensions. Many patients cannot exceed a certain dose of solutions without developing general reactions, and often this tolerated dose may not desensitize effectively. No such instances have been encountered with the use of oil suspensions. A patient treated with solutions who skips a weekly visit usually must have the last dose repeated; with oil suspensions the next scheduled increase may be given safely, and thus time is saved. With the use of large doses, a greater degree of desensitization results as indicated by the reduction in size of skin tests and by the smaller number of injections needed to reach the same

or larger dosage in successive years of treatment. The possibility of a clinical "cure" is thus greatly increased by a method which permits the administration of larger dosage.

Several patients had coseasonal treatment with oil suspensions given twice a week. Although in general such patients claimed improvement, the number is too small to warrant drawing any conclusions. No perennial treatment was given since the number of preseasonal injections was smaller than the usual number of perennial injections. However, it may be possible to give perennial treatment with oil suspensions at intervals of 8 weeks, or longer, in which case perennial treatment may be more desirable.

A major problem with solutions of pollen extract concerns the deterioration of activity which makes it unwise to prepare large quantities of material, makes it difficult to change treatment from an old preparation to a fresh one, and necessitates refrigeration of extracts. The factors, possibly enzymes, responsible for the deterioration apparently are eliminated in the process of precipitation. The precipitates, in a dehydrated state in alcohol, and the oil suspensions used in treatment were stored at room temperature. In large scale commercial production, the storing of the precipitate in the dry powdered state might be more practical.

House dust precipitates have been made and used, but not sufficiently to warrant a report. The methods described should be applicable to any pollen, mold, and other antigens.

A patent on the use of tannic acid to make pollen precipitates has been issued and assigned to Eli Lilly and Company.¹⁰ Since the patent application followed the publication of the original paper, the right to sublicense under the patent has been secured by the author, who will grant sublicenses freely.

SUMMARY AND CONCLUSIONS

1. Water suspensions of the active fractions of pollen extract precipitated by tannic acid and zinc have been used effectively for specific pollen desensitization since 1939. Suspensions of pollen tannate in peanut oil with 2 per cent aluminum monostearate were used in 1949 and 1950.

2. The methods of preparing such suspensions are described.

3. The advantages of using pollen tannate suspensions in peanut oil with 2 per cent aluminum monostearate depend on slowing and prolonging the absorption of the active material resulting in (a) increased safety and tolerance of larger doses, and (b) increased stability of the active material due to its dehydrated state, and possible elimination of enzymes.

4. The results of treatments, in 1950, of 122 patients with pollinosis with pollen tannate suspended in peanut oil with 2 per cent aluminum monostearate are presented. A final dose of 0.1 c.c. to 0.3 c.c. of a 1:1 suspension in oil, representing about 15,000 to 45,000 protein nitrogen units, was attained by an average number of 4.3 to 7.4 injections, with 90.0 per cent satisfactory results.

5. This new method of preparing and administering desensitizing antigens presents opportunities for further experimentation.

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