

THE CLINICAL EVALUATION AND THE PREPARATION AND STANDARDIZATION OF SUSPENSIONS OF A NEW WATER-INSOLUBLE WHOLE RAGWEED POLLEN COMPLEX

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THE first extracts made from pollen were prepared by extracting the whole pollen grains with either water,¹ N/200 sodium hydroxide and 0.9 per cent sodium chloride solution,² 66 per cent glycerol solution,³ phenol and pollen suspended in buffered salt solution,⁴ or 5 per cent dextrose⁵ (Table I). Milford⁶ and Moore and associates,⁷ in carefully controlled experiments, showed that there is an allergenically active constituent in pollen oil. Most common pollens contain considerable quantities of water-immiscible oil. Because of the presence of this water-immiscible oil, extraction of the whole pollen with aqueous extracting fluids results in turbid solutions. Therefore, in 1922 Coca⁸ suggested removing the oily substances with ether, and in 1931 Stull and his co-workers⁹ removed fatty material from the pollen to facilitate aqueous extraction. Since that time, all the various modifications of the preparation of pollen extracts have started with defatted pollen¹⁰⁻¹⁹ and have been based upon the following statement by Stull, Chobot, and Cooke⁹: "The skin-active material from defatted pollen was practically insoluble in ethyl ether, petroleum ether, chloroform, benzene and absolute alcohol." Although it is obvious that an aqueous extract of pollen which before extraction has been rendered fat-free will not contain the fatty fraction, it has been erroneously assumed from the work of Stull and associates that there is no skin reactivity in the fatty fraction. Their pollen had been completely defatted before aqueous extraction, so it would have been most unlikely that any oil or activity remained in the pollen for subsequent extraction from an aqueous extract into a fat solvent.

Rockwell¹⁸ has suggested that an active fraction in ragweed pollen is a proteose, carbohydrate, flavonol complex. Extraction of pollen with an organic solvent, such as ether or acetone, which dehydrates as it defats would not only tend to disrupt this complex by extracting the flavonol but could also change the nature of the original proteose due to dehydration at room temperature. Many laboratories actually use a Soxhlet extractor to remove all oil from the pollen, and this procedure is carried out at approximately 35° C. for hours. When proteins are isolated by precipitation with alcohol or

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acetone, this is carried out in most instances in a cold room at 5° C. or lower to prevent changes in the protein moiety. Table I shows variations in the preparation of pollen extracts.

TABLE I. VARIOUS PROCEDURES IN THE PREPARATION OF POLLEN EXTRACTS

STATE OF POLLEN BEFORE EXTRAC- TION	EXTRACTING FLUID AND ADDITIONAL PROCEDURES	AUTHOR	DATE
Not defatted	Water	Noon	1911
Not defatted	N/200 NaOH and 0.9% NaCl solution	Cooke	1915
Not defatted	33% saturated NaCl solution and 66% glycerol	Clock	1917
Defatted	Coca's solution	Coca	1922
Not defatted	Olive oil, glycerin, and petroleum	Milford	1929
Not defatted	5% Dextrose and 0.1% cresol	Moore et al.	1932
Not defatted	Phenol, then pollen suspended in buffered salt solution	Murphy	1929
Defatted	Coca's solution	Stull et al.	1930
Defatted	Aqueous* solution, then formalin	Carter	1936
Defatted	Aqueous, then ultrafiltered	Spain et al.	1935
Defatted	Aqueous, then alum	Zoss, et al.	1937
Defatted	Aqueous, then Lanolin and olive oil	Naterman	1938
Defatted	Aqueous, then formalin	Stull et al.	1940
Defatted	Aqueous, then heat		
Defatted	Aqueous, then acetylation		
Defatted	Aqueous, then ultraviolet radiation		
Defatted	Aqueous, then alum		
Defatted	Aqueous, then gelatin	Spain et al.	1941
Defatted	Aqueous, then frozen, dried, in sesame oil	Taub et al.	1941
Defatted	Aqueous, then tannic acid	Naterman	1941
Defatted	N/100 NaOH in saline solution, HCl pre- cipitated	Rockwell	1953
Defatted	Aqueous, then $ZnCl_2$, tannic acid, formalin, and $Al(OH)_3$	Naterman	1957

*Referring to bicarbonate or sodium chloride in aqueous solution.

METHOD

It was our desire to prepare an allergenically active product from whole pollen so that (1) all of the original fractions in the pollen grain would be incorporated into the extract and (2) it would be in a form which would lend itself to simple standardization and easy administration even to very sensitive persons. After many trials, an extraction fluid consisting of one part pyridine and one part 0.3 per cent sodium bicarbonate solution was chosen. Pyridine is a weak tertiary base and an important solvent because of its solvent action on many organic substances. The extract was prepared as follows: Five grams of ragweed pollen was allowed to extract with 100 ml. of the pyridine-bicarbonate solution for twenty-four hours. The extract was filtered off and

then Seitz-filtered for sterilization. Under sterile conditions, to one part of sterile distilled water was added one part of the 5 per cent pyridine-bicarbonate ragweed extract, and then one part of a solution of sterile 2 per cent potassium aluminum sulfate in $\frac{1}{4}$ N sulfuric acid was added slowly with mixing causing precipitation of an allergenically active water-insoluble whole ragweed complex. The precipitate and solution were allowed to stand overnight at 5° C. Then the mixture was centrifuged and the supernatant fluid was discarded. The residue was then washed three times with large quantities of sterile saline solution, and sterile glass beads were used to separate the particles of the precipitate and facilitate washing. The final volume of this suspension was made up to a volume equal to the initial volume of the 5 per cent pyridine-bicarbonate ragweed extract used.

TABLE II. POSSIBLE STANDARDS ON THE SUSPENSIONS OF WATER-INSOLUBLE WHOLE RAGWEED POLLEN COMPLEX

Protein nitrogen unit:	
Mg. protein nitrogen $\times 100,000$	23,000
Total nitrogen unit:	
Mg. total nitrogen $\times 100,000$	23,300
Total weight unit:	
Mg. dry weight of whole ragweed complex $\times 2,000$	24,000
Noon or pollen unit:	
Mg. pollen extracted per milliliter $\times 1,000$	50,000
Weight/volume standard:	
Grams of pollen extracted per milliliter	1:20

An aliquot of the suspension was taken for a phosphotungstic-acid-precipitable nitrogen (protein nitrogen) determination as described by Cooke and Stull.²⁰ Another aliquot was carefully measured into a weighed fritted glass filter, filtered with the aid of suction, and dried at 37° C. for twenty-four hours, which gave a constant weight at this temperature and time interval. Weight and nitrogen determinations on different lots of the 5 per cent suspension show a constant proportion between the nitrogen and weight values. Stull and Cooke converted milligrams of phosphotungstic-acid-precipitable nitrogen into the protein nitrogen unit system by multiplication by 100,000. We propose to multiply our total dry weight by 2,000 to convert to the approximate protein nitrogen unit value. For example, the dry weight of one lot was found to be 12 mg. per milliliter and the protein nitrogen was 0.233 mg. If, as proposed above, 12 is multiplied by 2,000, a figure of 24,000 units is reached and 23,300 units were actually found by nitrogen determination (Table II).

ABSORPTION TIME

The difference in the rate of absorption of the ordinary aqueous ragweed extract and the new ragweed complex suspension was determined by passive transfer tests which were performed on six suitable normal nonsensitive

adults, and the time interval and degree of reaction at the passive transfer sites were noted. It was found that there was a definite decrease (threefold or more) in the rate of absorption of the ragweed complex suspension over the absorption of an ordinary ragweed extract (Table III).

TABLE III. TIME AND DEGREE OF REACTION UPON PASSIVELY SENSITIZED SITE AFTER INJECTION OF RAGWEED ANTIGEN IN OPPOSITE ARM

CASE NO.	AQUEOUS EXTRACTS, 5,000 UNITS		SUSPENSION OF WHOLE RAGWEED COMPLEX, 5,000 UNITS	
	TIME INTERVAL (MINUTES)	REACTION	TIME INTERVAL (MINUTES)	REACTION
1	30	Marked	90	Moderate
2	45	Moderate	140	Moderate
3	30	Marked	110	Moderate
4	45	Moderate	130	Slight
5	25	Marked	90	Moderate
6	22	Marked	90	Marked

Slight = moderate erythema; moderate = small, round wheal with an area of erythema; marked = large wheal, irregular, with pseudopods and an area of erythema.

In mid-August of 1955, twenty highly sensitive adult hay fever patients were selected for coseasonal treatment on an experimental basis by injection with the new whole ragweed complex suspension. These patients were chosen because of the fact that, due to their high degree of sensitiveness, they were unable to undergo satisfactory treatment or reach maximum* or near maximum dosages with the aqueous ragweed extract. Each patient was tested intracutaneously with the ordinary aqueous ragweed pollen extract as well as with the suspension of whole ragweed complex in dilutions of 10, 100, and 500 protein nitrogen units. These tests showed no reactions or only slight reactions in eighteen of the twenty patients tested with the new suspension of whole ragweed complex, whereas, because of highly sensitive skin (dermographism), in the remaining two patients reactions were identical to those shown with the regular aqueous ragweed extracts. The patients were classified as AA, A, or B, according to the degree of reactivity as indicated by testing with the aqueous ragweed extract; AA was the most sensitive and B the least sensitive of the group. This classification served as a guide to the average dosage schedule suitable for prophylactic treatment, which was then begun by using the pyridine alum-precipitated whole ragweed complex. Eight patients were in Class AA, seven were in Class A, and five were in Class B. Class C patients were not chosen for treatment with the new ragweed complex suspension since they present no problem when treated with the regular aqueous extracts. They demonstrate a mild degree of reactivity, as indicated by intracutaneous tests with aqueous pollen extracts, and are able to reach maximum or near maximum dosages with the aqueous extract. Since the mixture was new and untried, the standard schedule of dosages usually employed with the aqueous ragweed pollen extracts was followed (Table IV). After several injections had been administered at weekly intervals without discomfort to

*Maximum dosage is the dosage which, if exceeded, is likely to induce a marked local or constitutional reaction.

TABLE IV. PROPHYLACTIC TREATMENT; AVERAGE DOSAGE FOR VARIOUS CLASSES GIVEN IN PROTEIN NITROGEN UNITS (STULL-COOKE)

CLASS AA (1 UNIT = MARKED; 10 UNITS = MARKED PLUS) (UNITS)			CLASS A (10 UNITS = MARKED; 100 UNITS = MARKED PLUS) (UNITS)	CLASS B (100 UNITS = MARKED; 500 UNITS = MARKED PLUS) (UNITS)	CLASS C (100 UNITS = MODERATE; 500 UNITS = MARKED) (UNITS)
Test	Day	0	0	0	5
Inj.	1	2	5	10	10
Inj.	2	5	10	20	20
Inj.	3	10	20	40	40
Inj.	4	20	30	70	70
Inj.	5	30	40	100	100
Inj.	6	40	60	200	200
Inj.	7	50*	80	300	400
Inj.	8	--	100	400	700
Inj.	9	--	150	600	1,000
Inj.	10	--	200	800	1,250
Inj.	11	--	300	1,000	1,500
Inj.	12	--	400	1,250	2,000
Inj.	13	--	500*	1,500	2,500
Inj.	14	--	---	1,750	3,000
Inj.	15	--	---	2,000	3,500
Inj.	16	--	---	2,250	4,000
Inj.	17	--	---	2,500*	5,000*

*This dose continued at four- to seven-day intervals during the season.

any patient and with no reactions or only small local reactions resulting, dosages greater than those which ordinarily would be administered with the aqueous ragweed extract were given. Antihistaminic drugs were administered in addition to the immunization treatment for symptomatic relief. A significant observation during the coseasonal treatment with the new suspension was a notable absence of itching of the eyes, which is a predominant symptom in pollen-sensitive persons. No local irritation, dermatitis, or toxic effect was noticed with the use of the new extract. The local reactions from injections with the suspension attained their maximum size in about one-half to four hours after injection. Two of the twenty patients experienced untoward reactions in this initial trial with the suspension. These reactions occurred in extremely sensitive patients and were mild and delayed requiring no medication for relief. One patient (Table V, Case 17) had slight tightness of the chest and itching of the palms one hour after injection of 70 units. The second patient (Table V, Case 15) had a marked local reaction with itching of the hands one hour after injection of 200 units. These reactions occurred because the dosages were excessively increased during this study. In one patient (Table V, Case 20) an injection of 20 units of the aqueous ragweed extract was substituted one week after the same amount of the suspension had been injected without ill effect, and a severe local reaction involving the entire arm occurred. Subsequently, 30 units of the suspension were injected with no local reaction resulting.

In 1956 thirty hay fever patients were selected for treatment with the suspension since they were unable to undergo satisfactory treatment with the aqueous ragweed extracts. This group included patients who demonstrated

TABLE V. DOSAGE SCHEDULE (1955)*
(GIVEN IN PROTEIN NITROGEN UNITS)

CASE NO.	INJECTION															MAXIMUM DOSE† AQUEOUS EXTRACT (PREVIOUS YEARS)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Class B																
1	25	50	100	200	400	700 ⁶	1,000	1,500 ¹	2,000 ¹	2,500 ³	3,000 ³	4,000 ³	5,000 ³			None†
2	25	50	100	200	400	700	1,000	1,500	2,000	2,500	3,000 ¹	4,000 ³	5,000 ³			1,500
3	25	50	100	200	400	700	1,000	1,500	2,000	2,500	3,000 ¹	4,000 ³	5,000 ³	6,000 ³		2,500
4	25	50	100	200	400	700	1,000	1,500	2,000	2,500 ¹	3,000 ¹	4,000 ³	5,000 ³			2,000
5	10	20	40	70	100	200	400	700	1,000	1,500	2,000 ¹	3,000 ³	4,000 ³			2,000
Class A																
6	10	20	40	70	100	200	400	700	1,000 ⁶	200	300	400	600	800	1,000 ⁶	None
7	5	10	20	30	40	60	80	100	200	300	400	600	800	1,000 ⁶		500
8	10	20	40	70	100	200	400	700 ¹	1,000 ^{2, 6}	1,000 ^{2, 6}	1,000 ^{2, 6}	1,000 ^{2, 6}	1,000 ^{2, 6}			1,000
9	10	20	40	70	100	200	400	700	1,000	1,000	1,000	1,000	1,000			400
10	5	10	20	30	40	60	70	100	150	300	500	600	800	1,000	1,500 ²	60
11	5	10	20	30	40	60	80	100	200	300	400	600	800	1,000	1,500 ²	200
12	10	20	30	40	60	80	100	200	400	600	800	1,000	1,500 ²			30
Class AA																
13	5	10	20	30	40	70	100	150	200	250	300	400	600	800	1,000 ¹	100
14	5	10	20	30	40	70	100	200	300 ⁴	400	500	600	800	1,000 ¹		10
15	5	10	20	30	40	60	80	100	200 ^{4, 5}	300 ^{4, 5}	400 ^{4, 5}	500 ^{4, 5}	600 ^{4, 5}			None
16	2	5	10	20	30	40 ^{4, 6}	50	70	100	150	200	300	400			30
17	2	4	7	10	15	20	30	40	70 ⁵	100	150	200	300			8
18	5	10	20	30	40	50 ⁶	60	80	100	150	200	300	400			None
19	2	3	4	5	6	7	8	9	10 ⁴	15	20	30	40			2
20	5	10	20	30	40	50	60	70	80	90	100	110	120			None

*Injections were given at weekly intervals, unless otherwise noted. Exceptions and reactions are symbolized as follows:

¹Given after interval of two weeks.

²Given after interval of three weeks.

³Given after interval of four weeks.

⁴Marked local reaction.

⁵Mild systemic reaction.

⁶Patient did not return for further treatment.

⁷Aqueous extract given and marked local reaction occurred.

[†]Maximum dosage is the dosage which, if exceeded, is likely to induce a marked local or constitutional reaction.

[‡]No previous hay fever treatment.

a high degree of sensitiveness, as shown by intracutaneous testing, or who in previous years demonstrated repeated marked local or constitutional reactions and therefore could not receive maximum dosages of the aqueous ragweed extracts. The patients were classified by intracutaneous testing with aqueous ragweed extracts; ten patients were in Class AA, fourteen were in Class A, and six were in Class B. There were no Class C cases. Since treatment with the suspension in 1955 had made it apparent that a schedule with a decreased number of injections was possible, the schedule employed with the aqueous pollen extracts was not followed. In general, the schedule that was successfully employed in our clinic with the use of slowly absorbed gelatin pollen extracts¹⁵ was adopted (Table VI).

TABLE VI. PROPHYLACTIC TREATMENT, AVERAGE DOSAGE FOR VARIOUS CLASSES
SUSPENSION OF WHOLE RAGWEED COMPLEX GIVEN IN PROTEIN NITROGEN UNITS*

INJECTION	CLASS AA (UNITS)	CLASS A (UNITS)	CLASS B (UNITS)	CLASS C (UNITS)
1	10	25	25	50
2	20	50	50	150
3	30	100	100	300
4	40	200	200	700
5	60	300	400	1,000
6	80	500	700	2,000
7	100	700	1,000	3,500
8	200	1,000	1,500	5,000
9	300	1,500	2,000	7,000
10	400	2,000	2,500	8,000
11	600	2,500	3,000	10,000
12	800	3,000	4,000	
13	1,000	3,500	5,000	
14		4,000	6,000	
15		5,000	7,000	
16			8,000	
17			9,000	
18			10,000	

*1 unit = 0.00001 mg. protein nitrogen.

The Class AA patient received 10 units as an initial injection, and the Class A or B patient received 25 units. The schedule was often modified to suit the individual's tolerance for the suspension (Table VII). The Class B patient usually reached 2,500 units at the tenth injection with the suspension, as compared to at least seventeen injections with the aqueous extract and 5,000 units at the thirteenth injection of the suspension. One patient classified as B reached 10,000 units in eighteen injections. The Class A patient usually reached 2,000 units at the tenth injection and an approximate average of 3,000 units in twelve injections. The extremely sensitive Class AA patient reached an average of 600 units at the eleventh injection. It is difficult to achieve such high dosages in very sensitive patients with the aqueous extract. Some of the patients might possibly have taken greater doses, but no attempt was made to increase the dosage further as the ragweed concentration was often twice the amount attained with the aqueous extracts. Another important advantage is the ability to administer the suspension in the prescribed doses

at intervals greater than one week, in some cases as long as four weeks. In Fig. 1 a comparison is made of the average dosage schedule when the suspension is used and the schedule followed with the standard aqueous extracts.

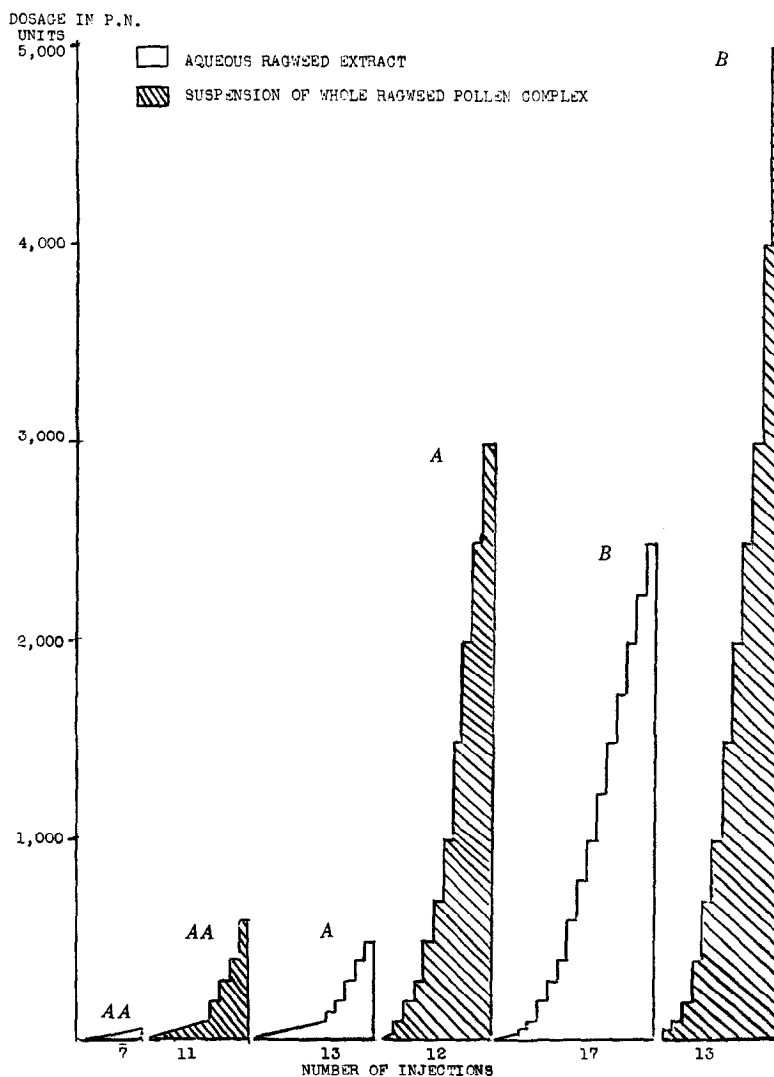


Fig. 1.

Twenty-six, or 86.7 per cent, of the thirty patients who received pre-seasonal treatment with the whole ragweed complex obtained satisfactory relief, against twenty-three, or 76.7 per cent, in a control group of thirty less sensitive patients treated with the standard aqueous extracts and with anti-histaminic drugs and other medications (Table VIII). These results were determined by a study of the daily symptoms recorded by each patient. An

TABLE VII. DOSAGE SCHEDULE (1956)*
(GIVEN IN PROTEIN NITROGEN UNITS)

[illegible]

Class AA													
21	10	20	30	40	70	100	200	300	400	600	800	1,000	200
22	10	20	30	40	60	80	100	200	300	400	600	1,000	100
23	10	20	30	40	60	80	100	200	300	400	600	1,000	100
24	10	20	30	40	60	80	100	200	300	400	500	700 ⁴	100
25	10	20	30	40	60	80	100	200	300	400	600	800	150
26	10	20	30	40	60	80	100	200 ⁵	300	400	600	800	20
27	10	20	30	40	60	80	100	200	300	400			None
28	10	20	30	40	60	80	100	200	300	400	600		200
29	10	20	30	40	60	80	100	200 ⁴	300	400			70
30	10	20	30	40	60	80 ⁵	60		100				10

*Injections were given at weekly intervals unless otherwise noted. Exceptions and reactions are symbolized as follows:

¹Given after interval of two weeks.

²Given after interval of three weeks.

³Given after interval of four weeks.

⁴Marked local reaction.

⁵Mild systemic reaction.

[†]Maximum dosage is the dosage which, if exceeded, is likely to induce a marked local of constitutional reaction.

[‡]No previous hay fever treatment.

evaluation of the results of the treatment of the two groups of hay fever patients was feasible, since the less sensitive group treated with the standard aqueous extracts accepted high dosages which in many instances approximated the maximum dosages (unit per unit of protein nitrogen) attained by the group of more sensitive hay fever patients treated with the whole pollen suspension. For example, the least sensitive Class C patient usually reached 5,000 units when treated with the aqueous extracts, and this same dosage of 5,000 units was attained by the more sensitive Class B patient treated with the whole pollen suspension.

TABLE VIII. RESULTS OF PRESEASONAL TREATMENT IN 1956

TREATMENT	NO. OF PATIENTS	NO OR SLIGHT HAY FEVER		NO SEVERE HAY FEVER		FIVE OR LESS SEVERE DAYS		SEVERE HAY FEVER		TOTAL EFFECTIVE RELIEF	
		NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
Suspension of whole ragweed complex	30	14	46.7	8	26.7	4	13.3	4	13.3	26	86.7
Aqueous ragweed extract*	30	8	26.7	6	20.0	9	30.0	7	23.3	23	76.7

*This group of patients received antihistaminics when hay fever symptoms occurred.

Again, as noted during the hay fever season of 1955, there was a conspicuous absence of itching of the eyes in the pollen-sensitive patients treated with the whole pollen complex suspension; this symptom had given them great discomfort during previous pollen seasons. Itching of the eyes was a major complaint of many patients treated with the defatted pollen.

The local reactions from injections with the ragweed complex suspension attained their maximum size in about one-half to four hours after injection. No local irritation or induration at the sites of the injection occurred. In only three highly sensitive Class AA patients did mild systemic reactions, such as itching of the palms, stuffiness of the nose, sneezing, cough, or slight tightness in the chest, appear following treatment (Table VII, Cases 21, 26, and 30). These reactions were not severe enough to require medication.

In 1957 twenty-eight highly sensitive hay fever patients were treated pre-seasonally with the suspension. Eight patients were evaluated as Class AA, seventeen as Class A, and three as Class B. In treating these patients, we generally followed the schedule with a decreased number of injections employed in 1956 (Table VI). The Class AA patients received 10 units as an initial injection, and the Class A and Class B patients received 25 units. The number of injections required to reach maximum dosages for these highly sensitive patients was almost identical with the number of dosages of the suspension administered to hay fever patients in 1956, and the ragweed concentration was often twice the amount attained with the aqueous extracts (Table IX and Fig. 1).

Ten other highly ragweed-sensitive patients who had reached maximum or near maximum dosages of the suspension at the end of the hay fever season in 1956 continued their treatment throughout 1957, receiving maximum dosages ranging from 2,500 to 5,000 units (Table IX, Cases 29 to 38). In previous years these highly pollen-sensitive patients were unable to undergo the perennial form of treatment, since they could not attain maximum dosages with aqueous extracts. It was noted that with the suspension a change from the old to the new suspension could be made without lessening the dosage and with no untoward reactions.

Twenty-four, or 85.7 per cent, of the twenty-eight patients treated pre-seasonally with the suspension obtained satisfactory relief as compared with twenty-one, or 75 per cent, in a control group of twenty-eight less sensitive patients treated with aqueous extracts and antihistaminic drugs (Table X).

Of the ten hay fever patients treated by the perennial method with injections of maximum doses of the suspension, nine, or 90 per cent, experienced effective relief, as compared with seven, or 70 per cent, in a control group of ten patients treated with aqueous pollen extracts.

No local irritation, induration, or dermatitis was noted at the sites of the injections. Again, as noted in the two previous years, there was a remarkable absence of itching of the eyes in the pollen-sensitive patients treated with the suspension.

One extremely sensitive patient (Table IX, Case 28) complained of slight tightness in the chest one-half hour after injection of 40 units of the suspension. This mild systemic reaction disappeared within ten minutes without any medication. The dosage was subsequently reduced to 30 units and then increased to a maximum of 60 units with no ill effects. Another patient (Table IX, Case 10) who had received 2,500 units of the suspension with no untoward effects was accidentally given 2,500 units of the aqueous ragweed extract. There followed immediately a severe local and systemic reaction which required repeated epinephrine injections, ephedrine compounds, and antihistaminics. The patient experienced severe hay fever symptoms, generalized urticaria, and asthma which persisted for three days and required urgent medical treatment. Two weeks later 2,500 units of the suspension was injected with safety, and these injections were repeated each month.

DISCUSSION

The treatment of hay fever and the standardization of pollen extracts do not fulfill all requirements, probably due to the type of extract used. Since it was assumed that the oil fraction was not responsible for hay fever symptoms, practically all pollen extracts for therapeutic or experimental use are prepared by first defatting and dehydrating the pollen with ether. Rockwell¹⁸ suggested that the active component of pollen was a flavonol-carbohydrate-proteose complex. When pollen was treated with ether to eliminate the oil prior to aqueous extractions, such a complex would be broken, due (1) to removal of the flavonol and (2) to some denaturation through dehydration and would thus lead to a changed antigenic component in aqueous solution.

A suspension of a new water-insoluble whole ragweed pollen complex has been prepared. This preparation is in a form which lends itself to easy administration and slow absorption. It can be standardized by a weight method which takes into account all antigenic fractions present. A direct correlation between the nitrogen value and weight of the complex is obtained. The initial extraction is made upon whole pollen which has not been previously treated with an organic solvent, such as ether for defatting purposes. Since this paper was read, Cohen and Michelini²¹ have reported that there is a qualitative and quantitative antigenic superiority in the whole ragweed pollen grain which could not be duplicated with immunizing procedures using preparations of pollen extracts. They also showed that ragweed pollen oil contains one antigenic fraction. The extracting fluid consisting of one part pyridine and one part 0.3 per cent sodium bicarbonate was chosen for the initial step in this preparation, so that the oil fraction in the pollen grain could be readily dissolved with the proteinaceous fraction. Experimental data on the antigenic components of this alum-precipitated pyridine ragweed complex will be reported upon at a later date. Even though this suspension of whole ragweed complex was not suitable for intracutaneous skin testing, it was felt that this was due to its slow absorption since constitutional reactions did occur in sensitive persons when the dosage was unduly increased.

Seventy-eight ragweed hay fever patients, highly sensitive to pollen, were treated with the suspension during a three-year period (1955, 1956, and 1957). The patients selected for treatment with the suspension consisted of those who were unable to undergo satisfactory treatment with aqueous extracts because of their marked degree of sensitiveness. A dosage schedule with a reduced number of injections was generally followed, since a greater tolerance for this new suspension was noted. Longer intervals between dosages were found feasible. The local reactions from injections with the suspension attained their maximum size in about one-half to four hours after injection. While this new suspension seems to be a suitable material for slow absorption, it does not entirely eliminate constitutional reactions. When untoward reactions do occur, they are usually mild and delayed, occurring one to four hours after treatment when the patient is no longer within reach of his physician. Some of the patients might possibly have taken much greater top doses, but no attempt was made to increase the dosage as the ragweed concentration was often twice the amount attained with the aqueous extracts. Occasionally, there may be an extremely sensitive patient who will not tolerate more than 2 or 3 units of the aqueous ragweed extract. Such a sensitive person should be treated conservatively, even with the suspension, and the dosages should not be excessively increased. Although Milford⁶ and Moore and associates⁷ showed that there was an allergenically active component in pollen oil, Cooke and Stull²⁰ stated that the complete removal of the ether-soluble material in pollen was indicated in order to avoid the danger of producing dermatitis in extremely susceptible patients and to facilitate the extraction of the active material. In our series of seventy-eight highly sensitive patients no local induration, irritation dermatitis, or toxic effects were noted.

Ten highly ragweed-sensitive patients who had completed a course of pre-seasonal treatments with the suspension continued to receive their maximum dosages at monthly intervals thereafter. These patients could not attain such high dosages with aqueous extracts. It was possible to change directly from the old suspension of whole ragweed pollen complex to a new suspension without lessening the dosage.

Treatment with the suspension seems to have given more effective relief than the standard aqueous pollen extracts. A significant observation following treatment with the new suspension of whole ragweed complex was a notable absence of itching of the eyes, which is a predominant and discomforting symptom in hay fever patients. The symptom of itching eyes may be caused by the oil fraction of pollen; therefore, the absence of this symptom following treatment with this special type of whole ragweed pollen product may possibly be explained on the basis of successful immunization with this whole pollen complex suspension which also contains the oil fraction.

SUMMARY

1. A suspension of a new water-insoluble whole ragweed pollen complex, believed to be more representative of all the allergenic components as they appear in the pollen and in a form which lends itself to slow absorption, was prepared.

2. The suspension can be standardized by a weight method which takes into account all antigenic fractions present.

3. A direct correlation between the nitrogen value and weight of the alum-precipitated pyridine whole ragweed complex is obtained.

4. A series of seventy-eight adult patients, highly sensitive to ragweed pollen, were treated with the suspension. Ten patients of this group continued their treatment on a monthly basis after having completed a course of pre-seasonal injections.

5. A greater tolerance for the suspension was noted (often twice the amount attained with aqueous extract was given with safety and often with one-half the number of injections).

6. Constitutional reactions were mild, delayed, and infrequent.

7. Excellent clinical relief was obtained following treatment of extremely sensitive patients treated with the suspension.

8. There was a notable absence of itching of the eyes in the hay fever patients treated with the suspension.

9. The symptom of itching eyes may be caused by the oil fraction of pollen, and the observed absence of this symptom following treatment may have been the result of successful immunization with the whole pollen extract which also contained the oil fraction.

REFERENCES

1. Noon, L.: Prophylactic Inoculation Against Hay Fever, *Lancet* 1: 1572, 1911.
2. Cooke, R. A.: The Treatment of Hay Fever by Active Immunization, *Laryngoscope* 25: 108, 1915.

3. Clock, R. O.: A Stable Pollen Antigen, *J. Infect. Dis.* **21**: 387, 1917.
4. Murphy, J. A.: Pollen Suspensions; a Preliminary Report, *J. Lab. & Clin. Med.* **15**: 158, 1929.
5. Moore, M., and Unger, L.: Studies on Pollen Extracts. IX. A New Extracting Solution, *J. ALLERGY* **4**: 92, 1932.
6. Milford, E. L.: Studies in Allergy. I. The Specific Activity of Pollen Oil, *J. ALLERGY* **1**: 331, 1930.
7. Moore, M. B., Cromwell, H. W., and Moore, E. E.: Studies on Pollen and Pollen Extracts. IV. The Allergenically Active Constituent in Pollen Oil, *J. ALLERGY* **2**: 6, 1930.
8. Coca, A. F.: Studies in Specific Hypersensitiveness; the Preparation of Fluid Extracts and Solutions for Use in the Diagnosis and Treatment of the Allergies With Notes on the Collection of Pollens, *J. Immunol.* **7**: 163, 1922.
9. Stull, A., Chobot, R., and Cooke, R. A.: Chemical and Clinical Studies on Pollen (Preliminary Report), *J. ALLERGY* **1**: 470, 1930.
10. Carter, E. B.: Detoxified Pollen Extract, U. S. Patent No. 2,019,808, *Chem. Abstr.* **30**: 575, 5, 1936.
11. Spain, W. C., and Newell, J. M.: The Ultrafiltration of Ragweed Pollen Extracts, *J. ALLERGY* **5**: 455, 1934.
12. Zoss, A. R., Koch, C. A., and Hirose, R. S.: Alum-Ragweed Precipitate: Preparation and Clinical Investigation; Preliminary Report, *J. ALLERGY* **8**: 329, 1937.
13. Naterman, H. L.: The Treatment of Hay Fever by Injections of Pollen Extract Emulsified in Lanolin and Olive Oil, *New England J. Med.* **218**: 797, 1938.
14. Stull, A., Cooke, R. A., Sherman, W. B., Hebard, S., and Hampton, S. F.: Experimental and Clinical Study of Fresh and Modified Pollen Extracts, *J. ALLERGY* **11**: 439, 1940.
15. Spain, W. C., Fuchs, A. M., and Strauss, M. B.: A Slowly Absorbed Gelatin-Pollen Extract for the Treatment of Hay Fever, *J. ALLERGY* **12**: 365, 1941.
16. Taub, S. J., and Rubens, E.: Pollen in Oil; a Preliminary Report on a New Slowly Absorbed Medium for Use in Hay Fever Treatment, *J. ALLERGY* **13**: 138, 1941.
17. Naterman, H. L.: The Treatment of Hay Fever by Injection of Suspended Pollen Tannate, *J. ALLERGY* **12**: 378, 1941.
18. Rockwell, G. E.: A Whole Pollen Antigen Hydrochloride, *Ann. Allergy* **11**: 175, 1953.
19. Naterman, H. L.: Formalinized Pollen Tannate in Desensitization Treatment, *J. ALLERGY* **28**: 76, 1957.
20. Cooke, R. A., and Stull, A.: The Preparation and Standardization of Pollen Extracts for the Treatment of Hay Fever, *J. ALLERGY* **4**: 87, 1932.
21. Cohen, S. G., and Michelini, F. J.: Immune Responses to Ragweed Pollen Antigens, *J. ALLERGY* **29**: 446, 1958.