

REPOSITORY IMMUNIZATION IN POLLEN ALLERGY

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The spectacular demonstration that hay fever can be prevented by a series of preseasonal injections with the offending pollen has been abundantly confirmed since Noon's report in 1911 (1). Unfortunately, however, many patients forego therapy because they are unable to manage the 14 to 40 visits demanded annually by today's schedules. Evidence accumulated during the past decade will now be presented to show that a single repository of emulsified antigen can essentially duplicate the results of multiple injections.

Since 1937 a number of attempts have been made to curtail the prophylactic procedure in allergy by retarding the release of allergen from the site of its injection. Zoss *et al.* (2), for example, prepared particulate antigen by adding alum to their pollen extracts, while Naterman (3) preferred tannic acid and Rockwell (4) selected HCl as the precipitant. Spain *et al.* (5), on the other hand, concluded that the simple procedure of mixing gelatin with the immunizing solution permitted a safe intensification of therapy. Still another approach was suggested by Naterman (6, 7) who emulsified pollen extract in vegetable oil, a device also exploited by Malkiel and Feinberg (8).

Meanwhile, the writer had found that thermostable, blocking antibodies are formed by normal subjects as well as by allergic individuals following a series of injections with pollen extract (9). An objective, immunologic gage was thereby made available for application to the problem of curtailing therapy. It was first necessary to determine the relationship between clinical immunity and blocking antibody output (10). The amount of this antibody in the bloodstream was estimated by a special procedure of passive transference which called for prior heating of the

serum to destroy its sensitizing antibodies. Concurrently, the acquisition of blocking antibodies by the tissues was appraised by a controlled instillation test of the patient's eye (11). Once the protective requirement had been established for the individual by comparing his immunologic with his clinical data, this requirement served as the goal of future therapy, injections being halted as soon as the desired output of blocking antibody had been achieved. Frequent examinations during immunization made it apparent that the average patient regains his protective titer following brief booster stimulation, as little as 0.1 mg of "protein" N given during seven weekly visits providing the average pollen-allergic patient with optimal clinical and immunologic results (12).

Although this represented a considerable saving in time and material, still greater curtailment was suggested by the publications of Freund's group (13) in the early 1940's. They observed that the antibody production of animals could be enhanced and prolonged if the immunizing agent were emulsified in mineral oil and placed in the muscle to provide a repository of slowly released antigen. Furthermore, Halbert *et al.* (14) found that the lethal requirement of toxin for experimental animals had to be increased 10-fold after the toxin's emulsification. With these striking results in mind, the principle was cautiously applied to human subjects in 1947 (15). Absorption from a subcutaneous depot of emulsified allergen was sufficiently retarded that up to 10 times more antigen was tolerated by hypersensitive patients than they had been able to take during forced therapy with plain extract. Since then, it has been possible to raise the ratio of tolerated dosage by factors of 20, 50 and even 100. To date, none of the 1200 repositories given allergic individuals has been fortified with acid-fast bacilli, albeit this step still further enhances the immunologic effect of Freund's adjuvant in laboratory animals.

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The purpose of this report is to make clinical and statistical comparisons between depot therapy and conventional, multi-visit treatment in ragweed hay fever. Since conclusions are more valid if each subject serves as his own control than when one patient is compared with another, only those individuals who took at least one course of customary treatment and one annual repository will be included in the analysis. The experimental period extended from 1939 through 1955, during which time the 117 patients involved in the study received 448 depots and 308 conventional courses. Therapy was alternated from one to the other type of management in a random fashion. To evaluate psychic and other nonimmunologic influences on the clinical results, placebo emulsions were added to the program during the last few years. Superficial attention has also been paid to the cosmetic matter of the localized nodule which follows depot therapy, two substitutes for mineral oil being tried in an effort to restrict indurations to the desired period of six months. The extent to which emulsification enhanced antibody output was examined in 1955.

MATERIALS AND METHODS

Experimental subjects provided a classical history of ragweed pollinosis and responded in a typical manner to tests of the skin, nose, eye and serum. Since there were no additional prerequisites, the sampling may be considered random. Half the group had received conventional therapy elsewhere. Although some members took as many as eight annual depots and others completed up to 11 conventional courses under our observation, the average individual received 3.8 repositories and 2.6 multi-visit courses. Because the findings essentially duplicated those for the ragweed reactive group, data accumulated on 746 depots and 390 multi-visit courses given 200 patients with allergies toward other pollens, house-dust, or animal danders will be omitted.

Allergen was prepared from low ragweed pollen each year in May. Alkaline saline solution at pH 8.4, ionic strength .12, was the extractant employed. After 48 hr at 4° to 6°C., the supernate was withdrawn, filtered to remove bacteria, and standardized on the basis of its phosphotungstic acid-precipitable nitrogen, 0.1 mg Pta N representing 10,000 "protein" units. Whereas most of the extract was mixed with an equal volume of sterile glycerin to stabilize its activity (16), a

portion had to be reserved in original form for use in emulsions because glycerin in some way abolishes the delaying influence of mineral oil on antigen release. A fresh series of twofold dilutions, prepared each month from the glycerinated stock supply, was employed for tests and for conventional therapy. Consecutive tests were carried out in the two eyes of 10 or more patients at the time of transfer from the expiring to the new series to make certain that no change in potency had occurred.

Immunologic tests will be restricted to the conjunctival method in this report. Allergic susceptibility was determined on the day of the first injection each year, and was again estimated just before and shortly after the season of ragweed pollination. A fully rounded drop of 10 unit/ml extract was allowed to fall against the everted lower lid from a gage-26 hypodermic needle (pointed toward the tip of the nose, not toward the eye). At 5 min intervals, a drop of doubling strength was instilled until minimal reddening developed in the caruncle. This delicate end point was confirmed by adding one or two higher concentrations to evoke mild pruritis and bulbar conjunctival erythema. Following a similar test of the other eye, the *cul de sac* was flooded with isotonic saline solution, and a few drops of 1-1000 epinephrine solution were instilled to negate the reaction.

Diary of hay fever hours. Not only the hours of mild, bad and severe allergic symptoms but also the number of antihistamines, hours spent in an air-conditioned environment, and the existence of other factors which may have modified clinical behavior were recorded daily by the patient throughout the pollinating season. In many instances it was possible to collect this information over the telephone every week or so. The factor 1 was applied by us to the hours of mild symptoms, 2 to the hours listed as bad and 3 to severe manifestations. Thus, at the conclusion of pollination on October 1, a cumulative figure representing the total hay fever intensity hours was available for each subject. The log of this sum was employed for statistical purposes.

The patient's estimate of improvement was obtained during October. First he gave his opinion as to whether the current season had been more or less discomforting than the preceding one. Then he was requested to compare the result with those surrounding years prior to his first course

of therapy. If he felt that 90% of his original symptoms had been eliminated, the current outcome was listed as excellent, whereas 75 to 85% control was considered good, while 50 to 70% was fair, and less than 50% was a poor result. For statistical purposes, either an excellent or a good report was termed satisfactory.

Preparation of the repository varied considerably during the first 3 years when empirical trials were undertaken with a number of formulas. Thereafter, the emulsions were comprised of 1 ml of Falba, 1.5 ml of mineral oil and 0.5 ml of pollen extract. Falba is an emulsion stabilizer which contains beeswax, paraffin oils of varying viscosities and oxysterins extracted from lanolin. It is supplied by Pfaltz and Bauer Inc., of New York, New York. A light mineral oil, Atreol No. 9 U.S.P., was procured from the Atlantic Refining Co. of Newark, New Jersey. The petrolatum and Falba were autoclaved together in a rubber-capped Wasserman tube which was vented with a gage-26 needle. Subsequently, 0.5 ml of pollen extract in a strength ranging from 20,000 to 135,000 units/ml was injected through the cap into the warmed contents. After mixing the three components crudely by manual twirling of the tube, a sterility test was done. The final mixing was postponed until just before therapeutic use of the emulsion, a slender 1 or 2 ml sterile syringe being employed at this time to pump the material gently up and down for 5 min. After the designated volume had been drawn up into the syringe for the first patient's treatment, a gage-26 needle was firmly fitted onto its tip. Divided doses were injected into the same site under the skin or in a muscle. Early trials had revealed that antigen was more rapidly released from multiple than from single depots.

Isopropyl myristate (Delyl Extra of Givaudan-Delawanna, Inc. of New York, New York) was used as a substitute for mineral oil in a number of repositories. It was hoped that this nonirritating, nonallergenic, oil-like product (17) would exert the delaying influence of petrolatum without giving rise to unduly persistent nodules. Corn oil was employed as another substitute during 1955.

Therapeutic schedules were modified gradually in the direction of fewer visits each year. Whereas, for example, conventional courses called for as many as 25 weekly injections and some 40,000 units under the skin during the early 1940's, by

1943 only occasional patients received more than 10,000 units or came in oftener than seven times yearly. The doses administered in repositories, on the other hand, rose from initially low levels to considerably higher ones. Individuals of a conjunctival susceptibility which had been assigned 1000 units in 1947, for example, were given 10,000 units by 1955. Recently, dosage has been allotted without reference to the type of preparation involved, conjunctival reactivity being the sole criterion. For instance, subjects with low thresholds of response are receiving an annual total of 5000 units, regardless of whether it is to be taken in seven weekly injections in plain form or as a single repository of emulsified allergen. Similarly, patients of intermediate reactivity are assigned to a dose of 7500 units, while the least allergic members are allotted 10,000 units. The sequence of weekly dosage for conventional courses, as well as the divided doses recommended for depot therapy, are listed in Table I. This balanced scheme of dosage simplifies comparisons between the effects of emulsified and of plain extract.

Overdose reactions were described in detail in the patient's record. Although more than one untoward response was associated with some of the multi-visit courses, only the first was taken into account for statistical purposes.

Statistical analysis was undertaken to aid us in reaching a valid decision as to the safety and efficacy of repository as compared to conventional therapy. It seemed likely that the chief source of discrepancy would be the biologic variable. Individuals might be expected, for example, to differ in the intensity of their underlying sensitization just as they might differ in their judgment as to what comprises a satisfactory clinical result or a recordable degree of discomfort. Such factors as the emotional attitude toward symptoms and the influence of occupation on the pattern of exposure to atmospheric pollen might also be reflected in a daily diary. It seemed essential, therefore, that the individual be his own control—that his average responses to the new type of management be compared with his average responses to the old. This longitudinal approach avoids some of the assumptions that must otherwise be made to interpret statistical data.

The method of handling the clinical data is best elucidated by an example. Patient A reported good or excellent symptomatic control during 3

TABLE I
Dosage schedules used in 1955
(Pta N units)

Threshold Requirement of Eye	Unemulsified Extract: 7 Doses at Weekly Intervals							Emulsified Extract (1000 units/0.1 ml): Doses at ½-hr Intervals					Total
units/ml													
10-30	25	100	200	400	775	1250	2250	1000	1000	1000	1000	1000	5,000
40-240	50	200	450	800	1250	2000	2750	2000	2500	3000			7,500
320-10,240	75	275	650	1000	1750	2500	3750	2000	2500	2500	3000		10,000

of his 4 years under repository treatment. Similarly, he experienced little discomfort during 5 years of conventional management. The proportion of satisfactory results (amounting to 75 and 100%, respectively) was converted to "angle," a common statistical device (18). After the angle values had been set up in this manner for the entire group of patients, a *t* test was carried out to determine the statistical significance of any overall difference noted between the two types of therapy. The incidence of overdose reactions could be similarly evaluated. In the case of conjunctival data, angles were not employed. Instead, the immunity conferred by therapy was expressed in terms of the number of tubes of antigen. If, for example, a patient's eye had responded to 20-unit extract prior to treatment and required 320-unit solution after his course, the difference amounted to four tubes in our series of doubling strengths. The *t* test gaged the significance of any difference found between the average number of tubes involved with repository and with conventional management. In the case of the hay fever intensity hours, the log of the average total hours recorded annually for the new and for the old therapeutic procedures served as the unit of comparison.

Thus, for the several criteria, the paired *t* test served as the common basis of comparison between the two methods of immunization. This statistical method was applied because it is sufficiently robust to retain validity for most experimental situations. The purpose of the *t* test is, of course, to differentiate effects referable to sampling variation from other, reproducible effects. When the calculated value of *t* exceeds the value listed at the 5% level for the appropriate number of observations (18), it can be asserted that a real difference exists between the pairs under examination. Although this assertion is at

times incorrect, one can be confident that it will be justified 19 out of 20 times, on the average. Even when the *t* test indicates a difference between two sets of data, however, it is necessary to show that factors other than the variable under consideration are not responsible. Fluctuation in the annual pollen counts, for example, had to be considered as a possible cause of change in a patient's clinical behavior during certain years.

Finally, an effort was made to distinguish clinical from statistical significance. Whereas the latter rests on deviations which exceed those attributable to random sampling variations, the former rests on differences which are large enough to be of practical meaning to the clinician. The two do not necessarily coincide. With small samples, for example, an effect which has clinical implications may not be found to have statistical significance. Conversely, with large samples, it may be possible to demonstrate statistical significance for differences which seem negligible to the physician. Ideally, significance will be indicated by both criteria.

Pollination fluctuated normally during the experimental period, with the exception of 1941 which had an unusually high count. Furthermore, a sustained reduction in the amount of pollen in the air occurred after the New York City Public Health Department instituted its weed eradication program in 1946. This campaign lowered the ragweed index from an average of 46.8 for the 1937 to 1945 period to 32.5 for the years 1946 to 1954 (19). Fortunately, all our repositories and 58% of the conventional courses covered by this article were administered after 1945. During the year of lowest count, 1947, the number of repositories given was balanced by the number of multi-visit courses; and during 1941 the conventional courses were few, amounting to only 2.3% of the aggregate involved in this survey. In any

event, the longitudinal approach, combined with random alternation of therapy, minimized any influence which the pollination level might have had on the clinical data. In spite of these facts and the opinion of Durham (20) that the annual pollen count must change more than 50% before clinical responses are appreciably altered, we repeated statistical analyses on all the hay fever intensity hours after applying a correction factor for the pollen variable.

Obviously neither the conjunctival tests nor the overdose reactions could have been influenced by pollination, since they were recorded prior to the blossoming dates.

RESULTS

Overdose reactions were encountered after 5% of the repository treatments and 7% of the conventional courses, as indicated in Table II. In view of the preliminary observation that the incidence was comparable for the intracutaneous and the subcutaneous routes, and was only slightly higher for the intramuscular than for the subcutaneous injection of emulsions, such data have been paired. The calculated value for t being half that listed for 116 degrees of freedom at the 5% level, it was appropriate to attribute the higher rate of untoward responses during conventional therapy to sampling variation. The difference was noted to be even smaller when only those courses given under the skin were included in the comparison. By either analysis, no evidence

was found for an increased reaction rate with depot treatment, despite the large amount of allergen injected during the single visit. The slowed release of antigen accounted not only for this situation but also for a relative delay in the onset and disappearance of manifestations. It was noted that only occasional and atypical symptoms, such as mild giddiness and tachycardia, were described by placebo-treated individuals.

Conjunctival data were available for both types of therapy in 109 members of the group. Whereas the 385 repositories given them elevated the threshold requirement by an average of 2.2 tubes, 231 conventional courses raised the antigenic requirement by an average of 2.7 tubes, as shown in Table II. Although statistical analysis indicated that the two sets of data differed significantly, the deviation was too small to have clinical meaning, inasmuch as the end point established for a given patient on a given day often proves to be reproducible only within half a tube of antigen.

Hay fever hours were faithfully recorded by only 89 members. Table II shows that the data for depot treatments did not differ significantly from those surrounding multi-visit courses, as judged by the t test. From the clinician's viewpoint, a difference of only 7 hay fever intensity hours in the seasonal totals of about 10 times this figure did not seem significant, especially in view of the extensive (six-week) pollinating period.

TABLE II

Effect of emulsified allergen in a subcutaneous depot compared with serial injections of nonemulsified allergen in 117 ragweed allergic subjects given at least one course of each between 1939 and 1955

Type of Course	Overdose Reactions (117 Patients)				Acquired Immunity of Eye (109 Patients)				Hay Fever Intensity-Hours (89 Patients)				Patient's Estimate of Improvement (109 Patients)			
	Number of courses	Incidence of reactions %	Calculated t	Listed t .05	Number of courses	Average increase in threshold requirement (tubes of antigen)	Calculated t	Listed t .01	Number of courses	Average total per season (intensity hours)	Calculated t	Listed t .05	Number of courses	Proportion of courses with satisfactory clinical result %	Calculated t	Listed t .05
Single depot...	403*	5.2	0.76	1.98	385†	2.2	2.90	2.63	283†	62.2	0.63	1.99	412†	70.6	1.89	1.98
Multivisit....	308‡	7.3			231	2.7			219	69.2			299	81.8		

* Includes 49 courses of mineral oil emulsion given by the intramuscular route to 47 patients; subcutaneous route used for others.

† In 38 of the emulsions, mineral oil was replaced by isopropyl myristate which provided comparable clinical control.

‡ 103 courses were given intracutaneously, the rest under the skin.

When the data were reexamined statistically following the application of a factor of correction for the annual pollen counts, the index for 1941 being arbitrarily taken as unity, the calculated value for t amounted to 1.52 but was still not significant at the 5% level. The inference could therefore be drawn that it was unnecessary to make allowance for the variations in pollination which occurred during the experimental period. The rather large number of observations, the longitudinal approach, and the essentially normal fluctuations in the seasonal pollen counts probably contributed to this situation.

Clinical effectiveness was judged by the October statements of the 117 patients after their treatment with 441 repositories and 308 conventional courses between 1939 and 1955. The statistical analysis carried out for 109 of the group, who also had come in for conjunctival study just prior to pollination after most of their immunizations, is summarized in Table II. The calculated value of t was smaller than the tabular value, indicating that the difference was probably due to sampling variation. From the practicing allergist's viewpoint likewise, two methods providing satisfactory clinical results in 71 and 82% of trials would seem not to differ materially in effectiveness.

Because a number of variables had been eliminated by 1953, a separate analysis was set up with the clinical information of this and the succeeding 2 years. During this period no isopropyl myristate was employed, the formula of the petrolatum emulsion had become essentially standardized, and the pollen count remained consistently above the mean of the weed eradication era. But the most significant changes were the introduction of statistical design into the assignment of therapy and the routine inclusion of placebo emulsions in the program. Total doses involved in specific emulsions had also risen, ranging from 2750 to 5500 units in 1953, from 2750 to 8250 in 1954, and from 5000 to 10,000 in 1955. Meanwhile, multiple injections with plain antigen called for 5000 to 10,000 units annually. The experimental group contained 51 members who were separated into pairs by means of conjunctival tests. One was then assigned to repository therapy and the other to weekly injections by the flipping of a coin. There was usually a third partner who took placebo emulsion (see below).

Although 68% of the 70 antigenic depots given

this group and 79% of their 53 multi-visit courses provided satisfactory clinical control in the ensuing pollination periods, the difference did not carry statistical significance. (The calculated value for t amounted to 1.03 whereas the listed value at the 5% level was 2.01.) The result resembled, therefore, that yielded by the more comprehensive analysis of 1939 to 1955 both from the clinical and the statistical viewpoints.

Placebo repositories were identical in appearance with the standard emulsions. During 1953 both types of preparation were injected in the "double blind" manner, each batch being code-labeled so that its composition remained obscure until the administering physician had entered all his findings for the year in the patient's record. Since the experiment will be described in detail in a separate publication (21), only brief reference will be made here to the effectiveness of placebo therapy.

Table III summarizes the findings of 51 ragweed-allergic subjects who received depots of antigenic as well as of placebo emulsion between 1947 and 1955. It will be noted that nearly 90% of the active preparations provided satisfactory clinical control. On the other hand, 44% of the blank mixtures given the group likewise were followed by comfortable pollinating seasons. Although the difference was clearcut and carried statistical significance at the 1% level, it was surprising to encounter this degree of success with placebos. Closer examination revealed that 20 individuals were largely responsible for the situation. In fact, they reported uniformly good results after all placebos, despite receiving as many as four. Sixteen of them had also been consistently pleased with the effects of active emulsion, as many as seven having been taken by one member. In marked contrast to these individuals who seemed to respond to the knowledge of therapy rather than to the nature of the inoculum, there were 10 subjects who expressed disappointment with placebo management after their first experience with it. They had, on the other hand, never failed to procure good relief after an active repository. Whereas the larger group might be described as placebo-responsive, these 10 patients had qualified as "discriminators." There were three others for whom therapy of either type was a clinical failure. Perhaps they were incapable of producing effective amounts of blocking antibody. Alternately, there may have

been some obscure allergen or other factor in addition to ragweed pollen which contributed to their seasonal symptoms. The remainder of the 51 members gave somewhat inconsistent appraisals of the two types of management, while favoring the antigenic depot.

Taken in the aggregate, these findings indicate that one-third of our previously treated ragweed-reactive patients can be managed successfully for at least 1 year on placebo repositories whereas another fifth promptly regress toward their original symptoms. Others relapse after a second or a third placebo repository.

These subjective findings were inconsistent with the associated immunologic data, as may be seen in Table III. Forty-six of the group, who returned for early August eye-tests after their placebo repositories, revealed a mean increase in the threshold requirement of only one-third of a tube of antigen. After their specific depots, on the other hand, the conjunctival requirement increased by an average of 2.3 tubes, or nearly fivefold. The difference between the two was highly significant according to the *t* test.

As might have been anticipated, the more subjective yardstick represented by the recorded hay fever hours revealed less difference between the two types of emulsion. Nevertheless, the figure of 42 associated with specific repositories was found to differ significantly from the average seasonal total of 75 that characterized the placebos.

The foregoing synopsis makes it apparent that the roles of blocking antibody and of nonimmunologic factors in the control of allergy vary with the individual and can be appraised by means of placebo therapy.

Substitutes for mineral oil were Delyl and

corn oil, both of which can presumably be destroyed by the body in contrast to petrolatum, which appears to depend on gradual phagocytosis rather than degradation for its disposal. The two types of vehicle were compared by noting the size and persistence of nodules under the skin. Without biopsy, it was impossible to determine whether these indurations represented the vehicles themselves or fibrotic reactions. Examinations were only casually undertaken but in most cases were made at about 3 months, 5 months, 1 year and at such other times as the patient came into the office for other purposes.

Isopropyl myristate is commonly employed as a substitute for oil in cosmetic preparations. It has also been used in experimental animals as a repository vehicle for antibiotics and for estrogens (17). When employed in volumes ranging from 0.01 to 0.05 ml in lieu of mineral oil in 1950, isopropyl myristate exhibited a tendency to disappear more rapidly than the same volume of petrolatum given a conjunctival partner (Table IV). In fact, only 11% of the 81 petrolatum depots placed under the skin were found lacking in nodule formation when a number of the sites were examined during the first 30 days, whereas 27.5% of the Delyl repositories had disappeared. By 60 days, one fifth of the petrolatum-injected areas had lost their palpable indurations while one-half the Delyl nodules had disappeared. There was less difference in the disappearance rates of the two vehicles after this time, 75% of the petrolatum depots leaving no detectable residues by the end of the first year, whereas 85% of the isopropyl repositories had become impalpable. It will be noted that by the beginning of the second year 83% of the patients given standard emulsions had lost their nodules, and 97.5% of

TABLE III

Effect of emulsified allergen compared with emulsified saline solution in 51 ragweed allergic patients given at least one depot of each between 1947 and 1955

Type of Depot (Emulsion)	Immunity Acquired by Eye (46 Subjects)				Hay Fever-Intensity Hours (44 Subjects)				Clinical Effectiveness Estimated by Patient (51 Subjects)			
	No. of courses	Average increase in threshold requirement (tubes of antigen)	Calculated <i>t</i>	Listed <i>t</i> .01	No. of courses	Average total hours/ season/ per patient	Calculated <i>t</i>	Listed <i>t</i> .05	No. of courses	Proportion of courses satisfactory	Calculated <i>t</i>	Listed <i>t</i> .01
Ragweed. . . .	168	2.3	9.93	2.69	140	42.1	2.26	2.02	182	% 89.3	5.34	2.68
Placebo. . . .	74	0.3			67	74.5			79	44.2		

TABLE IV
Disappearance times of nodules caused by repository therapy

Year	No. of Depots	Volume of Oil	Maximal Diameter of Nodule		Days after Repository Was Placed under the Skin													
			Range	Median	30	60	90	120	150	180	270	365 (12 mo.)	396 (13 mo.)	520 (17 mo.)	635 (21 mo.)	730 (2 yr.)	820 (2¼ yr.)	6 yr.
		ml	mm	mm	Per cent of injection sites which lacked nodules													
					Emulsions containing mineral oil													
1950	81	.01-.05	2-20	5	11	21	35	51	55½	64	68	75	76½	78		83	84	85
1953	26	.075-.2	3-20	10	0	15	27	31	50		54	77	80	88½				
1954	53	.1-.3	2-30	20	4	11	21		25	32		42	49	53	55			
1955	23	.375-.5	2-33	15	0	0	0	4	13	17½	22	43½	53	61				
					Emulsions containing Deltyl or corn oil													
1950	40 Deltyl	.01-.05	2-20	5	27½		50	55		75		85		95		97½		
1955	38 Corn oil	.375-.5	2-30	20	18	21	37	47		66	69	81	84					

the Deltyl-inoculated members had returned to normal. One member retained a small induration until nearly the sixth year after his petrolatum depot, bringing the total disappearance rate for the group to 85%. Of the remainder, 4% had small fibrotic areas when last examined between the first and second years, whereas the balance have not returned since sometime during the first 12 months when they exhibited palpable indurations. The patient responsible for the final 2.5% of the Deltyl group also has not returned since her nodule was palpated 270 days after therapy. The maximal diameters of the Deltyl nodules will be seen to be identical with those of the standard emulsions given in 1950, the range being from 2 to 20 mm whereas the median diameter amounted to 5 mm.

Although only standard emulsions were injected during 1953 and 1954, the data for these years are included in Table IV to show the influence of the larger volume of petrolatum on the persistence of nodules. The proportion of patients in 1954, for example, who exhibited negative indurations at various intervals up to 1 year, was about half that noted for the standard emulsion in 1950. Presumably, this was because the volume of oil had been increased 6 to 10 times over the earlier year. At 17 months, 53% of the depot sites could no longer be palpated, as compared to 78% for the year 1950. The size of the nodules also tended to be greater in 1954, ranging from 2 to 30 mm in maximal diameter,

with a median size of 20 mm. The findings for 1953 were more or less intermediate.

Corn oil was the substitute tried in 1955. The volume of vegetable or of mineral oil amounted to 0.375 ml for patients of medium conjunctival reactivity and to 0.5 ml for those of less marked susceptibility. After all members of the experimental group had been paired according to eye-test response, assignment to vegetable or mineral oil was made by randomization. Table IV indicates that nodules associated with the substitute oil disappeared more rapidly than those provoked by petrolatum emulsions. Indeed, no negative indurations were encountered during the first 3 months following treatment with the standard emulsion whereas the substitute mixture provided 37%. During the fourth month, the disappearance rate was 10-fold higher for corn oil than for petrolatum inoculated areas. Although the difference gradually narrowed, there was still a 2-fold discrepancy at 1 year. When the last examinations were made during 1956, 84% of the substitute's nodules had disappeared, while only 61% of the standard emulsion's had done so. The maximal diameters of the nodules did not, however, differ much for the two vehicles.

A glance at the 1950 Deltyl data will now make it apparent that isopropyl was associated with a somewhat more rapid loss of nodules than was the case for corn oil. However, after the first 3 months, the difference amounted to less than 10%. Larger volumes of oil had been used.

Although it is obvious from the above findings that Delyl and vegetable oil were both superior to mineral oil as far as the disappearance of their nodules after 6 months was concerned, these substitutes gave rise to distinctly more overdose reactions. Delyl, for example, when employed in 53 depots between 1949 and 1952 caused untoward responses in 18% of the recipients. The corresponding incidence was only 3% for 196 petrolatum repositories given the same individuals during this period. The clinical efficacy of the substitute emulsion was slightly inferior to that of the standard preparation, the former being productive of satisfactory control in 58% of the trials whereas the corresponding figure for petrolatum repositories was 67%. The difference would probably not, however, carry statistical significance.

Similar relationships were found to exist between the substitute, corn oil, and petrolatum when the paired trials of 1955 were studied. The 24 subjects assigned to emulsion containing vegetable oil developed $2\frac{1}{2}$ times as many overdose reactions as their partners who took the same amount of allergen emulsified in mineral oil. The median time of appearance of these induced manifestations was 17 min after a corn oil preparation, whereas it was 58 min after a petrolatum mixture. (A third partner given plain extract developed his overdose response within 7 min, indicating that both the oils exerted a delaying influence on the release of activity.) Corn oil also provided a somewhat smaller number of satisfactory clinical results, 59% of the group experiencing good or excellent control of symptoms during the ensuing pollinating season whereas 66% of their partners receiving standard emulsion reported satisfactory outcomes. The difference did not, however, carry statistical significance.

Enhancement of antibody output by petrolatum was suggested by the finding, during the early years of experimentation, that small repository doses frequently duplicated the clinical and immunologic effects of much larger amounts of allergen injected in the conventional manner. Deliberate study of the extent of the enhancement was, however, postponed until 1955. The 81 subjects available for immunization at this time were segregated into triads, each member of which exhibited the same conjunctival reactivity. The uniform dose then assigned the three was

taken by one in original aqueous form during 7 weekly visits, by another in a petrolatum emulsion, and by the third in a vegetable oil emulsion. Examination of the accumulated data for the 81 subjects at the end of the season revealed that the mineral oil repository was responsible for the highest incidence of satisfactory clinical control, the lowest incidence of overdose reaction, the fewest hay fever intensity hours and the greatest elevation in the conjunctival requirement. Multi-visit therapy took second place, and corn oil emulsion stood third. Most of the differences among the three types of management were not, however, of sufficient magnitude to carry statistical significance, perhaps because of the limited number of observations. It seemed permissible to conclude, nevertheless, that the superiority of the standard water-in-oil emulsion rested on its enhancement of antibody output.

DISCUSSION

Customarily, allergists judge the success of pollen therapy by the patient's estimate of the hay fever season. However, with so subjective an index, the possible contribution of psychic and other nonimmunologic influences must be taken into account. During the past several years, the problem has been explored by means of placebo emulsions. Over 40% of such trials were associated with satisfactory clinical control of hay fever symptoms. Under this circumstance, not only the patient's opinion but the number of hours of hay fever he recorded resembled those accumulated during other years when specific depots had been involved. On the other hand, the objective test of his conjunctiva revealed that he produced little or no blocking antibody when allergen was omitted from the emulsion. Must we infer from this situation that a sizable segment of the pollen-allergic population can be successfully controlled by the mere knowledge that an injection has been given, its composition being irrelevant? Do such patients have a unique capacity to avoid seasonal discomfort without depending on blocking antibody? Whatever the explanation, this type of individual affords a sharp contrast to his confrere who promptly relapses when specific emulsion is replaced by placebo. The rest of the members of our group stood midway between these two extremes, responding to the two types of repository in a somewhat inconsistent manner, albeit they usually fared better after specific than

after blank emulsions. Although each of the three criteria revealed a significant superiority of allergen-containing repositories over placebo preparations, when the aggregated data of the entire group were analyzed, only the conjunctival test could discriminate with dependable regularity between the specific and the blank material for the individual patient. Indeed, the recorded hay fever hours proved disappointing in this regard, showing a closer correlation with the patient's subjective impression than with the objective immunologic test. Nevertheless, it represents a sorely needed additional yardstick, and also provides a continuous picture of the patient's progress which should increase the meaning of his end-of-the-season opinion.

In the present examination of data surrounding antigenic repositories and multi-visit management, neither the patient's impression nor his recorded hay fever hours revealed any significant difference between the new and the standard procedures. Although the two sets of conjunctival data differed sufficiently that statistical significance was involved, the difference was so small that it approximated the error of method of the eye-test and hence had little meaning from the practical viewpoint. Provisionally, therefore, it is justifiable to conclude that the old and the new methods are interchangeable as far as subjective and immunologic effectiveness, and also safety, are concerned. The ultimate decision must await wider application of the repository method.

Placebos should also be an integral part of such a program. Their use not only serves to measure the role of specific antigen in clinical control, but permits one to weed out placebo-responsive subjects before two forms of therapy are compared. Among our group of 117 patients, for example, 14 of the members subjected to placebo therapy have demonstrated a capacity to distinguish between specific and blank emulsions during a first experience with the latter. Obviously, such discriminators would be ideal subjects for an experiment which aims to compare multi-visit with single depot management from the viewpoint of the patient's clinical impression. With continued application of the placebo principle, we hope to accumulate a larger colony of discriminators. Meantime, the factors responsible for clinical control of hay fever among the placebo-responsive segment of our group

deserve further study, both from the immunologic and psychologic aspects.

On the whole, isopropyl myristate and vegetable oil were unsatisfactory substitutes for mineral oil in emulsions. Their chief disadvantage was the tendency of the substitutes to release allergen so rapidly that an undue number of overdose reactions developed. This, in addition to a slight inferiority in their clinical control of seasonal symptoms, more than counterbalanced the advantage of fewer persistent nodules. Indeed, many of the Delyl and corn oil nodules disappeared too soon—within the first month or two. The ideal persistence period for mineral oil emulsions has been placed at about 6 months, three of which are required for antibody output to reach its maximum. The other three carry the patient well past his pollen exposure season. If, of course, it were possible to sustain antibody output for two pollination periods, $1\frac{1}{2}$ years would be the optimal persistence time. However, there is little assurance thus far that a repository will provide control for more than one season.

As to the production of local cysts or abscesses by petrolatum emulsions, none have been encountered to date although 1200 depots have been administered. According to Davenport (22), the Commission on Influenza of the Armed Forces Epidemiological Board observed only four cysts following 45,000 vaccinations with influenza adjuvant in 1954 and 1955. Three of our patients have described sudden pruritis and induration without fluctuation at the site of a former petrolatum repository. The condition arose after some weeks, subsided spontaneously within a few days, and may represent an allergic response.

The adjuvant agents employed in standard emulsions are noncarcinogenic and carry minimal risk of sensitization, according to the experience of Salk and others which has been reviewed by Francis (23).

From these remarks, it is obvious that the disadvantage of the unduly persistent nodule is chiefly cosmetic. With the exception of 12 subjects who participated in a limited ancillary experiment with intracutaneous repositories in 1948, few have complained about the indurations. Six of the intracutaneously placed repositories have, however, persisted to date in the form of small, elevated, faintly pigmented scars. Most of the unduly persistent nodules associated with

subcutaneous repositories could probably have been obviated had smaller proportions of oil been employed in the emulsions or had smaller, and perhaps equally effective, annual doses of allergen been prescribed. Finally, the problem is nonexistent when the intramuscular route is selected. Whether the lack of palpable material in the muscle several days after therapy is due to the actual disappearance of the petrolatum or to the difficulty of palpation in this deep location can not be stated. The latter explanation is consistent, however, with the finding that patients responded to intramuscular therapy as they did to subcutaneous repositories, with the exception that there were a few more overdose reactions after the intramuscular type and that the peak of antibody output occurred within 2 rather than 3 months. Presumably the release of antigen was only slightly less retarded than from the subcutaneous area.

Although the antibody production of our ragweed-allergic patients appeared to be enhanced by emulsification of the antigen, the degree of change was less striking for pollen than has been noted for adjuvant influenza vaccine (23). Blocking antibody development was estimated by means of conjunctival tests which were done 2 weeks after the completion of a conventional course and 3 months after the placing of a standard repository under the skin. Both were carried out a week or two before pollination began.

The fact that all our dosage schedules have been oriented to the conjunctival reactivity of the individual patient may serve as a barrier to the acceptance of the repository method by other allergists, few of whom employ tests other than the intracutaneous or scratch variety to classify subjects for therapeutic dosage. Since, however, it is common practice to segregate individuals into sensitivity categories of marked, moderate, and slight on the basis of their responses to such tests, it seems likely that our three dosage schedules might be applied accordingly. If this is attempted, it would be conservative to divide the allotted total doses into several more injections than we have advocated. Or the prescribed amounts of allergen might be reduced by half until the patient's tolerance and needs have been revealed by experience. It goes without saying that a tourniquet, epinephrine 1:1000, and facilities to place the individual in the head-low

Trendelenburg position should be available to counter any untoward effects. Epinephrine base in oil is also indicated for some overdose responses, particularly those following repository therapy, since the latter tends to evoke more prolonged manifestations than are usually encountered with plain allergen.

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SUMMARY

1. In a longitudinal study extending over 17 years and associated with statistical analysis, 117 ragweed-allergic individuals have been given 749 annual courses which alternated between conventional, multi-visit injections and single-visit repositories of the Freund type.

2. In comparing the effectiveness of these two methods of immunization, three criteria were applied: the customary clinical estimate obtained from the patient at the end of each pollinating season, his daily diary of hay fever hours, and a controlled variety of conjunctival test which gaged the acquisition of blocking antibody by the tissues. Whereas these served as yardsticks of clinical and immunologic effectiveness, the incidence of untoward reactions afforded the clue to the relative safety of the new and old procedures.

3. The two procedures were found to be comparable in effect by all the criteria except that based on the conjunctival test. Here, the older method proved to be more effective by an amount that carried statistical—but not, however, practical—significance. The repository technique offers, therefore, a great saving in time without decreasing the practical advantages of therapy or increasing the risk of overdose reactions.

4. It is possible that our schedules of dosage, which have depended on conjunctival classification of each patient, can be utilized by allergists who employ only the endermal or scratch methods of classification.

5. A sizable minority of our ragweed-allergic group appeared to respond as well to placebo repositories as to active emulsions, according to their clinical reports and recorded hay fever hours. At the same time, no acquisition of blocking antibody was indicated by eye-test. Obviously, such placebo-responsive subjects should be

excluded from experiments which compare the clinical efficacy of two therapeutic procedures. These patients stood in contrast with other individuals whose clinical reports promptly reflected any change from specific to placebo management. Such subjects should be responsible for the ultimate clinical opinion on repository therapy.

6. No abscesses, cysts, or malignant growths have resulted from 1200 repositories used during the past 9 years.

7. When either isopropyl myristate or corn oil was substituted for petrolatum in the repository, the number of overdose reactions was increased owing to a more rapid release of antigen. Clinical results were somewhat inferior with the substitutes. However, fewer nodules persisted beyond the season of pollination. This minor cosmetic problem surrounding the standard emulsion can probably be met by reducing the volume of mineral oil in repositories.

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