

ABSORPTION PATTERN OF RAGWEED ANTIGEN AND ACTIVE SENSITIZATION IN MAN FOLLOWING REPOSITORY POLLEN INJECTION

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THE CONCEPT of treatment of pollen-sensitive patients with an emulsified antigen in repository form has been heralded by Loveless¹ and Brown.^{2, 3} In evaluating the advantages to the patient of this method of therapy, one must consider its efficacy when compared to conventional perennial and preseasonal inoculation, the safety in its immediate and long-range effects, the mechanism of its action, and the possible adverse consequences when used indiscriminately.

The purpose of the present study was to obtain data on the absorption and release of ragweed antigen from an inoculated site by means of a reverse passive transfer technique described by A. and M. Walzer.^{4, 5} The authors are aware that the small number of subjects used in this study does not serve to give complete answers to some of the fundamental problems in this form of therapy, but, with the use of this particular technique, certain observations have been made which serve to confirm other findings made by different avenues of study. It is hoped that investigations of this type with long periods of observation will be included in the final evaluation as to the effectiveness and safety of depot therapy.

MATERIALS, METHOD, AND PROCEDURE

A. Repository.—The ragweed repository was prepared using certain of the modifications from the original method of Brown,² as suggested by Feinberg and his colleagues.⁶ One part of Arlacel A was added to nine parts of Drakeol 6-VR white mineral oil and 2 ml. of this mixture were placed in a Neoprene-stoppered vial. Seitz filtration was done to insure sterility. To this mixture was added the aqueous ragweed extract. Our preparation consisted of two parts of giant to one part of short ragweed pollen extract. The amount of ragweed extract which was added was calculated so that the final concentration when injected would contain 0.2 mg. of total nitrogen of ragweed pollen extract in 1 ml. of repository emulsion. When ready for use the vials were shaken by hand to make a crude mixture and the contents was then drawn into a sterile 10 c.c. Luer Lok syringe. The filled syringe was then attached to one

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Received for publication Feb. 17, 1961.

end of a U-shaped emulsifying valve, and an empty 10 c.c. syringe was attached to the other end. Then by means of a mechanical emulsifier the mixture was forced through the fine pores of the valve. After 20 minutes of mechanical mixing, a drop of the emulsion was examined for uniformity and particle size and, if satisfactory, the emulsion in amount of 1.5 c.c. (ragweed 0.3N) was then transferred to a disposable syringe. A deep subcutaneous injection was made immediately into the subject's left arm, over the deltoid muscle. Seventy per cent ethyl alcohol had been used as the skin antiseptic.

B. Serum.—The sensitizing serum for skin testing was obtained from an asthmatic male patient found to be Class A sensitive to ragweed and whose reagin titer to ragweed was 1:512 by the dilution method. The serum was Seitz filtered and exposed to ultraviolet light. From 1/200 to 1/100 c.c. of undiluted serum was injected intradermally just prior to the repository or the aqueous (see later) injection into the right arm in the deltoid region, and was repeated daily thereafter. The serum tests were patterned in a manner so that reinjection at the same sites would be avoided.

C. Subjects.—Persons were selected who could be available for observation and testing for at least several months. Screening of subjects was time consuming and many had to be rejected. Four subjects were used initially for evaluation of technical procedure and optimum dosage. These were not included in the final report. Of 4 additional subjects reported in this study, 3 gave negative past and family histories for atopy and showed negative intracutaneous skin tests to ragweed extract 0.1N, timothy 0.1N, and house dust extract 1:10. The fourth subject gave a negative past history for atopy, a positive family history of pollinosis, and initially an equivocal skin test to ragweed 0.1N, but a negative skin test to timothy 0.1N and house dust extract 1:10. In addition, all subjects had been screened with a skin test of undiluted reaginic serum. A test with the undiluted serum gave either a negative-plus (0) wheal reaction with slight circumscribed erythema or a slightly indurated ill-defined borderless erythema. If the intensity of reaction to the serum was found to be greater than described, the subject was not used. It was thus possible to differentiate this type of serum reaction from the immunologic wheal occurring subsequently from "internal channels"²⁵ at the same site.

The local cutaneous reaction resulting from the serum was small and rather mild as compared with the immunologic wheal. The immunologic skin reaction often developed before the local reaction of serum had completely disappeared and usually lasted between 15 to 35 minutes. The erythema and wheal was never more than of slight plus degree (1+ to 2+).

D. Skin Testing.—The rate and duration of absorption of antigen from the repository site was then followed by daily serum skin tests. When the immunologic wheal was no longer demonstrated during the repository phase of the experiment, an interval of 1 week of persistent negative reactions was allowed to elapse. A longer interval would have been preferred but for the inconvenience to the subjects. Then an injection of aqueous ragweed extract

in the same concentration as the repository was given the subject in the left arm. The absorption pattern of the aqueous ragweed solution was followed in similar fashion to that described above.

All subjects were likewise followed with weekly direct skin tests with ragweed 0.1N, in order to determine whether a delayed or immediate wheal reaction occurred. Tests with timothy 0.1N and house dust 1:10 extracts were done as controls.

RESULTS

A. Serum Tests.—No immediate reaction was observed at the serum test site following injection of the repository, but in all subjects there developed a pruritic, erythematous indurated response after a period of 2 to 3 hours. This interval was in contrast to a similar response at the serum site which took place in 30 to 45 minutes after injection of the aqueous extract.

In the repository study the daily serum skin tests in the 3 nonatopic subjects continued to show an immunologic skin reaction for a period of from 29 to 35 days. In the atopic subject the immunologic reaction disappeared after the fifteenth day (Table I).

In the aqueous ragweed study an immunologic wheal in the 3 nonatopic subjects continued for a period of between 8 to 15 days. In the 1 atopic individual, the reaction was not elicited past the third day (Table I).

TABLE I

	DAY OF DISAPPEARANCE OF REACTION	
	NONATOPIC (3)	ATOPIC (1)
Repository ragweed	29-35	15
Aqueous ragweed	8-15	3

It should be reiterated that with the concentration of ragweed extract used in both the repository and aqueous injections (0.3N) the immunologic reaction was distinct from the local serum reaction and that a discernible gradual decrease of response was readily evident, finally resulting in complete disappearance in the time interval just cited. In the preliminary pilot studies, a lesser concentration of ragweed extract had been used and the findings were not as clear cut.

B. Direct Skin Tests With Ragweed Extracts.—While the subjects were being followed during the various phases of this study with skin tests with ragweed extract, one of the 3 nonatopic subjects showed a delayed-type reaction about the size of a quarter piece on the ninth day after injection of the repository. In this subject an immediate wheal reaction of moderate-minus (3+) intensity was produced by test with ragweed extract on the twenty-second day. In the other 2 nonatopic subjects an immediate wheal reaction of slight plus to moderate-minus intensity (2+ to 3+) was evident by the fifteenth and thirty-second days, respectively, following onset of experiment. No delayed reaction was observed.

In the atopic subject, the initial equivocal skin test to ragweed extract (negative-slight [$\pm$]) was intensified to moderate degree (3+) and sustained, as in the others, without clinical effects.

All subjects had been followed with weekly skin tests since the appearance of the immediate-type skin wheal to ragweed extract. In all nonatopic subjects, the skin tests became negative by 4 to 4½ months. At this point the reaction in the atopic subject had returned to a negative slight ($\pm$) intensity. At the time of conversion of the ragweed skin test to positive, or of its intensification, as in the atopic subject, simultaneous skin tests with timothy extract 0.1N and house dust extract 1:10 were negative, indicating that skin test reactivity to ragweed extract was specific. This has been verified by testings at repeated intervals during the period of ragweed reactivity.

C. Passive Transfer Studies.—Passive transfer studies were done with the sera of all subjects in order to ascertain whether direct positive tests to ragweed 0.1N extract could be transferred. Only the serum taken from the atopic subject showed transfer and this to a minimal degree.

DISCUSSION

The investigation of the absorption pattern of ragweed extract from repository and aqueous media by the reverse passive transfer technique served to confirm the findings made by Feinberg and co-workers,⁶ employing the radioactive tagging of repository antigen. Traces of radioiodination were shown to exist for as long as 4 weeks with the emulsion and up to 3 weeks with the aqueous extract. It was further suggested in their study that radioiodination might be shown to persist for a longer period if the natural decay of I^{131} did not place limitations on its practical use.

The reverse passive transfer technique, using serum of high reaginic titer, was capable of establishing the presence of antigen in circulation following repository injection up to 35 days and also revealed the interesting finding of the persistence of ragweed antigen in the circulation up to 15 days after the aqueous injection.

It is the contention that the effectiveness of repository therapy relates to the prolonged presence of antigen in the circulation of the host. However, the above data suggest that the depot form of administration of antigen, if effective, might be so because of factors other than the persistence of antigen alone.

Dixon and colleagues,⁸ using radioiodinated bovine gamma globulin in rabbits, showed the virtual disappearance of the antigen by the fourth day and complete disappearance by the seventh day. Weigle and Dixon⁹ studying bovine gamma globulin, bovine serum albumin, and human serum albumin all labeled with I^{131} in rabbits showed half-lives of 2.3, 4.0, and 4.3 days, respectively. Weigle,¹⁰ using many radioiodinated homologous and heterologous serum proteins in different experimental animals, found that, although the half-life of heterologous serum proteins differed greatly in different hosts, the

average half-life varied between 1.1 to 5.5 days. An investigation by H. Sherman¹¹ of the rate of excretion in the urine of ragweed antigen, following subcutaneous injection of the aqueous extract in 2 human subjects, revealed that the atopen first appeared in the urine in 24 hours, reached its greatest concentration in 48 hours, and was usually gone by 96 hours. In relation to the present study we must ask whether the prolonged presence of ragweed antigen in the circulation is peculiar to this antigen alone.

The finding of an accelerated elimination of ragweed antigen from the circulation in the atopic subject confirms the study by Feinberg and co-workers⁶ employing radioactive tagged antigen.

Feinberg⁶ reports the production of delayed hypersensitivity in several nonatopic subjects. We have shown induced delayed hypersensitivity in 1 subject followed by an immediate-type skin reaction. In 2 subjects, an induced immediate wheal test was observed. In 1 atopic subject, the skin test to ragweed extract became stronger in intensity.

The problems these findings raise in the entire question of repository therapy is basic. What is the significance of the induced immediate-type reaction in the nonatopic subject? To our knowledge, it appears to be on a nonreaginic basis. However, it is necessary to bear in mind that the skin sensitizing reagin is easily destructible, its presence may be fleeting, its titer inconstant, and it may be missed many times with passive transfer studies, until finally demonstrated.

The transfer of the ragweed reagin in the atopic subject suggests that the depot antigen can stimulate the natural mechanism of reagin formation. It warns of the possible consequences of therapy by the repository method with antigen in atopic subjects with positive skin tests in the absence of clinical symptoms recommended by Chambers and Glaser.¹² These authors had recommended specific treatment of atopic patients with positive pollen skin reactions in the absence of symptomatology.

Since the work of Freund in perfecting the adjuvant agents used in immunology the observation has been made as to its safety and noncarcinogenic nature.¹³ On the contrary, Feinberg and his group⁶ have shown the production of erythema nodosum lesions in 1 of their subjects with induced delayed sensitivity to ragweed. Moreover, one must seriously consider the implications of treatment by repository method with extracts of house dust, ragweed, insects, and others whose antigenic constituents are not yet fully revealed, or with mixtures of atopens, such as assorted trees or grasses, to some of which the patient may not be clinically sensitive. Furthermore Pearson¹⁴ and Waksman and co-workers¹⁵ have induced autoimmune disease in rats using mycobacterial adjuvant. The question may be asked can the adjuvant in loose complex with the antigen combine with connective tissue at the injected site to cause the production of autoimmune bodies against the host?

These are some of the basic questions that must be answered after the effectiveness of repository therapy is evaluated, for these answers are vital over and above the simple question of relief from atopic disease.

SUMMARY

1. The reverse passive transfer technique was used to study the absorption pattern of ragweed antigen from the site of injection of the repository as well as the conventional aqueous solution.

2. It was found that the immunologic reaction following repository ragweed injection remained positive in the nonatopic subjects for a period of 29 to 35 days as compared to 15 days in an atopic subject.

3. It was demonstrated that the ragweed antigen persisted in the circulation following aqueous injection for a period of 8 to 15 days in nonatopic subjects and 3 days in the atopic individual.

4. The production of an induced immediate type wheal reaction following a ragweed skin test was demonstrated in the nonatopic subjects. This was not transferred by the serum passive transfer technique. However, skin test to ragweed in the atopic subject was intensified and transferred.

5. A delayed reaction in 1 nonatopic subject was followed by an immediate-type reaction.

6. The induction of hypersensitivity is discussed as to its significance in the evaluation of this mode of therapy.

7. Further investigation of the mechanism of adjuvant therapy is basic, over and above the simple clinical effectiveness of repository therapy.

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