

A TECHNIQUE FOR STUDYING THE NASAL ABSORPTION OF ALLERGENS IN HUMAN BEINGS*

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MANY investigators have demonstrated the passage of antigens through the nasal mucous membrane in animals. In human beings, this phenomenon has also been repeatedly demonstrated with a wide variety of substances, including insulin, pituitary extract, diphtheria toxoid and toxin, animal sera, pollens, silk, and others.

Different techniques¹⁻⁷ have been employed to demonstrate allergenic absorption. In most instances, the antigen was used in its natural state or in the form of a solution, a dry powder, or a dust. It was blown, sprayed, or instilled into the nose, or introduced on tampons. These techniques did not control the amount of material which came in contact with the nasal mucous membrane, nor the site or size of the absorptive surface. In fact, with most techniques, it was impossible to prevent the antigen, introduced into the nose, from reaching the lungs¹⁹ or alimentary tract where absorption also occurred. Hence, these techniques were of limited value in quantitative studies on nasal absorption.

The present study was undertaken in order to find a suitable technique for studying the rapidity and amount of absorption from the nasal mucous membrane. The writers are of the opinion that an ideal technique for this purpose should possess all or most of the following features: (1) its performance should not be difficult; (2) it should furnish a readily accessible site in the nose for the test; (3) it should permit the use of a constant area of nasal mucous membrane as the absorbing surface; (4) it should permit the application of a constant amount of antigen to the absorbing surface; (5) it should furnish immediate and objective evidence of absorption; and (6) its performance should not be unpleasant to the subject.

Walzer and his associates⁸⁻¹⁵ have shown that a passively sensitized cutaneous site will react following the systemic absorption of a related allergen through various portals of entry, including the mucous membranes of the alimentary and genitourinary tracts, the serous-lined body cavities, and the skin. Cohen, Ecker, Breitbart, and Rudolph⁵ and Sulzberger and Vaughan⁶ used variations of this technique to demonstrate the passage of inhaled antigen into the circulation.

A modification of the Walzer technique was adopted for the studies on nasal absorption about to be described.

TECHNIQUE

The principle involved in this technique was the one employed in studies on absorption from the alimentary tract.⁸⁻¹² The subjects to be tested were passively and locally sensitized with a suitable serum. After one or two days, the antigen

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was applied to the nasal mucous membrane. The onset of an urticarial reaction at the sensitized site indicated that absorption of the antigen had taken place.

The Sensitizing Serum:

The serum for sensitization was obtained from a patient who was markedly sensitive to peanut. Under sterile precautions, blood was obtained from the subject by venipuncture. It was allowed to coagulate and the clot to retract. The serum was then pipetted off, passed through a Seitz filter, and stored without preservative in the refrigerator.

The reagin titer of the serum was found to be 1:2048, by the dilution method of Coca and Grove.¹⁶ Although the serum was used over a period of twenty-one months, its titer remained high and relatively constant.

Passive Local Sensitization:

Passive local sensitization of the subject to peanut was accomplished by the intracutaneous injection of 0.05 ml. of serum on the upper, outer aspect of the arm. To determine the most suitable dilution of the serum for these experiments, a series of subjects were sensitized, at different sites, with undiluted serum and with dilutions of 1:4 and 1:10. Although the serum was effective in all 3 dilutions, the 1:4 dilution proved most satisfactory and was adopted for the standard technique.

In order to prevent neutralization of the sensitizing reagents by ingested antigens, all types of nuts were eliminated from the subjects' diets for twenty-four hours preceding sensitization. In addition, peas, beans, and mustard were omitted from their diets, since reagents for these foods were also present in the serum.

The Positive Reaction at the Sensitized Site:

The absorption of peanut antigen into the circulation through the nasal mucous membrane was demonstrated by the onset, at the sensitized site, of a reaction consisting of erythema and wheal, usually accompanied by pruritus. The absorption rate was measured from the time of application of the antigen in the nose to the appearance of the first objective sign of a reaction at the sensitized cutaneous site.

Choice of Subjects:

Only nonatopic adults, free from nasal symptoms, were used in these experiments. Individuals with "colds" were rejected, as were those who had any previous nasal operations or frank evidence of chronic sinus infection. Sixty-eight of the subjects were males and 10 were females. Since nasal congestion frequently accompanies catamenia, female subjects were tested only during the intermenstrual period.

Skin tests, with an extract of peanut containing 0.01 mg. of total nitrogen per ml., were performed on all candidates several days before the experiment. Only negative reactors were accepted as subjects for these experiments.

Since it has been shown¹⁷ that atopic individuals frequently fail to accept passive local sensitization normally, such subjects were not used in the present investigation. The study of nasal absorption in atopic individuals will be the subject of a future communication.

The Antigen:

The extract of peanut employed in these studies was freshly prepared according to the method of Coca and Milford,¹⁸ and its total nitrogen content determined by the macro-Kjeldahl method. It was diluted to contain 0.05 mg. of nitrogen per ml.

Methods of Studying Absorption From Various Parts of the Nasal Mucous Membrane:

A. *A technique for studying absorption from the inferior meatus.*—With this technique, which was the first attempted, the solution of antigen was applied in the inferior meatus by means of a piece of cotton wrapped on a thin metal applicator. The size of the cotton pledget was approximately the same in each case. The pledget was moistened with that amount of antigen solution needed to wet the cotton without causing it to drip. The number of drops used was kept constant throughout the experiment. The cotton pledget containing the antigen was left in contact with the nasal mucous membrane until a reaction started at the sensitized cutaneous site.

At no time were anesthetics used in these experiments, since it was believed that their application to the nasal mucous membrane would affect the rate of absorption. The technique produced no untoward effects in the nose, and the mucous membrane at the site of application showed no gross changes.

Following the introduction of the cotton-tipped applicator moistened with antigen into the inferior meatus, the absorption reaction was regularly demonstrated. However, the presence of the probe under the inferior turbinate was painful to most subjects and this fact alone was sufficient to render this technique unsatisfactory.

B. *A technique for studying absorption from the middle meatus.*—After a number of trials with Technique A, the site of application was changed to the middle meatus. The technique remained the same, except that the metal probe was withdrawn after the pledget of antigen-soaked cotton had been tucked into the middle meatus.

From this site, absorption was also readily demonstrated. This technique was an improvement over the preceding one in that it was less painful and the site of application was more accessible. However, there were definite disadvantages connected with it. The anatomical structure of the middle meatus rendered it impossible to control the size of the absorptive area, an objection which applied to the preceding technique as well. The cotton pledget varied in its contacts with the lateral aspect of the turbinate and with the nasoantral wall. Moreover, the complex anatomy of the middle meatus, with sinus ostia opening into it, presented the possibility that antigen might enter the nasal accessory sinuses, thus increasing the absorptive area.

C. *A technique for studying absorption from the septum.*—In view of the many disadvantages associated with turbinates as a site of absorption, it was decided to investigate the possibility of utilizing the anterior inferior portion of the nasal septum for these studies. This area seemed desirable as a site for application of the allergen for several reasons. It would be readily accessible and entirely visible to the investigator. It would utilize a flat surface for the

test which would make it possible to measure accurately the area of mucous membrane which was being used as the absorptive surface.

Preliminary studies revealed that absorption from the medial wall of the nasal chamber occurred about as regularly as from the lateral wall. The size of the absorptive area on the septum was standardized by using small squares of compressed cotton,* measuring one square centimeter in area, for applying the antigen. These tabs were moistened with antigenic solution and, with the aid of a thumb forceps, were placed against the anterior portion of the septum. Contact between the tabs and the turbinates was avoided, since this would increase the absorptive surface. When exactly 0.25 ml. of solution was used, the tabs stayed in position on the septum without slipping and without losing their contents because of dripping. Because no pressure was exerted on the mucous membrane, this technique was painless. It presented no obvious disadvantages and seemed to provide suitable means for studying absorption from the nasal mucous membrane under standard conditions.

RESULTS OF TESTS FOR NASAL ABSORPTION

Preliminary studies with the 3 techniques described above were performed on 78 subjects. In all, 121 test trials were made, of which 113 gave positive results (Table I). Repeated trials on one subject gave consistently negative results which could not be accounted for. In other instances, failures were usually attributable to errors in technique or to faulty screening of subjects, resulting in the testing of atopic individuals. When such errors were recognized, the data on these subjects were omitted from the protocols, even when positive results were obtained. The influence of atopy on absorption from the nose will be the subject of another communication.

TABLE I. ABSORPTION OF PEANUT ANTIGEN FROM NASAL SEPTUM AND MEATI

DILUTION OF SERUM	INFERIOR MEATUS			MIDDLE MEATUS			NASAL SEPTUM		
	NO. OF CASES TESTED	NO. OF CASES POSITIVE	AVERAGE ABSORP- TION RATE (MIN.)	NO. OF CASES TESTED	NO. OF CASES POSITIVE	AVERAGE ABSORP- TION RATE (MIN.)	NO. OF CASES TESTED	NO. OF CASES POSITIVE	AVERAGE ABSORP- TION RATE (MIN.)
Undiluted	6	6 (100%)	11.8	14	13 (92%)	11.7	4	3 (75%)	13.6
1:4	19	19 (100%)	12.4	15	14 (93%)	15.6	34	33 (97%)	14.1
1:10	13	13 (100%)	14.3	12	11 (92%)	16.3	4	1 (25%)	

EFFECT OF SERUM DILUTION ON ABSORPTION TIME

The effects of dilution of the sensitizing serum on absorption time are best illustrated in the studies of absorption from the middle meatus. A sufficient number of tests were made at this site with the 3 strengths of serum to permit comparisons of the results (Table II). Using the same technique throughout, 14 tests were made with the undiluted serum, 15 with the 1:4 dilution, and 13 with the 1:10 dilution. There was one failure with each dilution. With undiluted serum, the absorption times ranged from 6 to 18 minutes with an

*"Cottonoid," Johnson & Johnson Company, New Brunswick, N. J.

average of 11.7 minutes (Tables I and II). The 1:4 dilution of serum yielded absorption rates ranging from 8 to 29 minutes, with an average of 15.6 minutes. The absorption rates for the 1:10 dilution ran from 9 to 31 minutes, with an average of 16.3 minutes. Hence, dilution of the sensitizing serum appeared to lengthen the absorption rate to some degree.

TABLE II. ABSORPTION RATES OF PEANUT ANTIGEN WITH DIFFERENT DILUTIONS OF SENSITIZING SERUM

SERUM DILUTION	ABSORPTION RATES IN MINUTES FROM												
	INFERIOR MEATUS					MIDDLE MEATUS					SEPTUM		
Undiluted serum	2	9	12	13	13	6	7	9	9	9	11	14	16 F
	22					11	11	13	14	15			
						15	16	18	F*				
Number of cases	6					14					4		
Average rate	11.8					11.7					13.6		
Serum dilution	6.5	7	8	8	9	8	9	9	10	14	9	10	10 11 11
1:4	9.5	10	10	11	12	14	15	15	16	16	11.5	12	12 12 12.5
	12.5	13	13	14	14.5	18	18	28	29	F	13	13	13.5 13.5 14
	16	18	21	23	F						14	14	14 14 14.5
											15	15	15 15 15
											15.5	16	16 17 18
											18	19	23 F
Number of cases	20					15					34		
Average rate	12.4					15.6					14.1		
Serum dilution	7	9	9	9.5	11	9	10	13	14	15	15.5	F	F F F
1:10	12	14	14.5	15	15	15	15	18	19	21			
	21	23	27			31	F						
Number of cases	13					12					4		
Average rate	14.3					16.3							

*F indicates failure to manifest absorption reaction.

Similar results were obtained in the studies on absorption from the inferior meatus and from the septum (Tables I and II). The same conclusion was also derived from those studies in which each subject was sensitized with the 3 dilutions of serum (Table III).

Despite the fact that undiluted serum yielded the most rapid absorption rates, its use presented certain disadvantages. In some cases, the positive cutaneous reaction which it produced was quite severe and discomforting to the subject. Furthermore, the amount of serum available was limited and its use in the undiluted form would have exhausted the supply before these studies could be completed. For these reasons, a dilution of serum for sensitization seemed desirable for the standard technique. However, the 1:10 dilution could not be relied upon since the occasional failure to demonstrate the absorption reaction occurred most frequently with this dilution (Tables I, II, and III). Since absorption occurred almost as regularly and as rapidly with the 1:4 dilution as with the undiluted serum, it was decided to use the 1:4 dilution almost exclusively in the remainder of the preliminary experiments. For this reason, there is a paucity of data, pertaining to studies with the undiluted serum and the 1:10 dilution, in some of the categories charted in the tables.

EFFECT OF SITE OF ABSORPTION ON ABSORPTION RATE

The preliminary studies of absorption from the inferior and middle meati and from the septum were each made with different techniques. Despite this

fact, differences in the rates of absorption from the 3 sites were comparatively small (Table II).

With the 1:4 dilution, the absorption rates for the inferior meatus seemed, on the average, to be about 3.2 minutes faster than for the middle meatus (12.4 as compared to 15.6 minutes). With the 1:10 dilution of serum, the difference averaged only 2 minutes (14.3 as compared to 16.3 minutes). The septal rate of absorption with the 1:4 dilution averaged 14.1 minutes, which fell between the absorption rates for the inferior meatus (12.4) and the middle meatus (15.6).

TABLE III. ABSORPTION RATES WITH VARYING SERUM DILUTIONS IN THE SAME SUBJECTS

SITE OF APPLICATION OF ALLERGEN	SUBJECT	ABSORPTION TIME IN MINUTES		
		SERUM UNDILUTED	SERUM DILUTION 1:4	SERUM DILUTION 1:10
Middle meatus	33	15	15	no reaction
	34	18	18	31
	35	15	16	21
	37	9	9	9
	42	9	8	10
	43	7	9	14
	45	13	14	18
Septum	44	16	16	no reaction
	45	13	14	18
	46	14	15	no reaction
	48	11	12	
	139		14.5	15.5
Inferior meatus	83		7	7
	84		11	11
	85		9.5	9.5
	86		14.5	14.5
	87		18	27
	89		10	12
	90		14	15
	91		6.5	9
	92		10	15
	93		16	21
	94		12.5	14
	96		21	23
	97		9	9

In view of the fact that a cotton pledget placed in either the middle or inferior meatus made contact with a larger absorptive area than that covered by a cotton tab on the septum, the rate of absorption from the septum compared favorably with the rates from the meati. Hence, the technique using the septum furnished information which appeared to be fairly representative of the respiratory nasal mucous membrane as a whole.

UNIFORMITY OF RESULTS OBTAINED WITH SEPTAL TECHNIQUE

With the recommended septal technique, the rate of nasal absorption in a given individual proved to be fairly constant. In connection with subsequent studies on nasal absorption in atopic subjects, it was necessary to make repeated use of several normal subjects as controls. It was found that the rate of absorption from the nasal septum in a given control, tested at intervals of several weeks or months, varied in repeated trials by only a few minutes. In

one subject, the absorption rates of tests made at different times were 14, 11, 14.5, 15, and 15 minutes. In another subject they were 10, 12.5, and 11.5 minutes. In a third they were 16 and 14.5 minutes, and in a fourth, 13.5 and 15.5 minutes.

SUMMARY

1. A technique which employs the anterior portion of the nasal septum as the site of application of antigen is presented for the study of absorption of allergens through the nasal mucous membrane.

2. The ready accessibility and visibility of the septum, and its flat surface which permits the use of constant amounts of antigen on constant absorptive areas, are among the outstanding advantages of the technique.

3. With the recommended technique, the absorption rate from the septum seems to be fairly representative of that from the anterior part of the respiratory nasal mucous membrane.

4. With the recommended technique, absorption of peanut antigen from the septum was demonstrated in 33 out of 34 subjects tested.

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