

Effect of immunotherapy on appearance of antibodies to ragweed in external secretions

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Studies on 50 nasal washings, 16 parotid salivas from ragweed-allergic patients, and 10 secretions from normal subjects showed considerable variation in protein and immunoglobulin concentrations. Only 2 immunoglobulins, A and G, were found in nasal washings. Of 26 parotid salivas studied (allergic and controls), IgA was found in 26, IgG in 4, and IgM in 3 specimens. Anti-ragweed antibodies in the serum and exocrine secretions of allergic patients were examined by means of Prausnitz-Küstner (PK) test, radioimmuno-electrophoresis, radioimmunodiffusion, and tanned cell hemagglutination (TH) tests. The incidence of IgE, IgA, and IgG antibodies to ragweed in the sera and nasal washings in a group of 22 patients with previous immunotherapy was compared to that of 28 patients without immunotherapy. The immunotherapy seemed to affect the incidence of serum IgE and IgG antibodies but has no influence on the incidence of anti-ragweed antibodies in the nasal washings. PK activity of nasal washings could be removed by absorption with anti-IgE but not anti-IgA or anti-IgG immunosorbents. Anti-ragweed antibodies could be detected in parotid salivas only by the TH test.

Since the recognition of a unique immunoglobulin system in human exocrine secretions,¹ a great deal of interest has been generated concerning the possible significance of the immunoglobulins in the external secretions in relation to health and disease.^{2, 3} In view of the close relationship between exocrine secretions and the respiratory tract mucosa, antibodies in the external secretions may play an important role in the pathogenesis or modification, or both, of the allergic process in pollen hay fever. The protein content and immunoglobulin profile in nasal and bronchial secretions have been described by several investigators.⁴⁻⁷ Reports concerning antibody activity in the exocrine secretions in allergic diseases are also available.⁸⁻¹¹ The present studies were undertaken to determine the effect of immunotherapy on the appearance of anti-ragweed antibodies in the nasal and parotid secretions as well as in the sera of patients allergic to ragweed.

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Supported in part by United States Public Health Service Research Grant 5-R01-AI-01303 and in part by United States Public Health Service Training Grant 5-T01-AI-00051 of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.

Presented in part at the twenty-sixth annual meeting of the American Academy of Allergy, New Orleans, La., February, 1970.

Received for publication Aug. 22, 1972.

MATERIALS AND METHODS

Selection of subjects and collection of specimens

Fifty ragweed-allergic patients and 10 apparently healthy, nonatopic individuals were studied. The criteria for the selection of these patients and the methods for the collection of serum and nasal and parotid secretions have been described previously.¹¹ Nasal washings and sera were collected from all 50 ragweed-allergic patients, and parotid saliva was obtained from 16 of them. Nasal, parotid, and serum specimens were obtained from 10 healthy, nonatopic individuals and used as controls. All collected secretions were concentrated about 20 times by negative pressure dialysis and stored at -20°C .

Determination of total protein and immunoglobulins in secretions

Concentrated secretion specimens were used for these determinations, and values of total protein and immunoglobulin content in the original unconcentrated state were obtained by extrapolation. Total protein concentration was determined according to the method of Lowry and associates.¹² The presence of an individual class of immunoglobulin in nasal secretions was identified by immunoelectrophoresis¹³ and double-diffusion gel precipitation in agar,¹⁴ using monospecific antisera. The source and methods of preparation of monospecific antisera to the 5 classes of human immunoglobulins have been described previously.^{15, 16} In addition, goat antiserum to secretory IgA* was used. This antiserum was rendered specific for the secretory piece by absorption with normal human serum.

The presence of immunoglobulins in parotid secretions was detected by double-diffusion gel precipitation (DDGP), and the quantitation of immunoglobulins was performed by single radial immunodiffusion (SRID).¹⁷ The 11S IgA used as standard was isolated from human colostrum according to the method of Tomasi and associates.¹

Determination of antibodies in secretions and serum

Radioimmunoassay (RIA) and radioimmunoassay (RIE). The procedures for these assays have been described previously.⁸ Specificity of antibody activity in these tests was determined by blocking experiments with unlabeled antigen.⁸ Serum IgG anti-ragweed antibody activity was assessed semiquantitatively in RID by placing serial dilutions of serum in the peripheral wells and the antiserum and labeled antigen in the center well. The reciprocal of the highest dilution of serum that still showed binding of ^{125}I -labeled antigen was defined as the titer.

Tanned-cell hemagglutination (TH) and inhibition of tanned-cell hemagglutination (ITH) tests. These tests were performed as described previously.¹⁸ The erythrocytes were coated with 15 per cent short ragweed extract, the same reagent as used for Prausnitz-Küstner (PK) and other tests in these studies. The specificity of the TH activity in serum and secretions was determined by the ITH, using a ragweed extract.

PK test. Passive transfer tests were performed on nonallergic volunteers, as described previously.⁸

Adsorption of secretions. Nasal washings with positive PK activity were adsorbed with copolymerized sera containing anti-IgE, anti-IgA, or anti-IgG. The preparation of the immunoabsorbent and the adsorption techniques was the same as described previously.^{15, 16} The specimens after adsorption were re-examined by the PK test.

*Kindly supplied by Dr. T. B. Tomasi, Jr., Department of Medicine, School of Medicine, Buffalo, N. Y.

TABLE I. Total protein and immunoglobulin concentrations in nasal and parotid secretions of allergic and nonallergic individuals

	Nasal washings			Parotid saliva		
	Specimens positive	Range of concentration ($\mu\text{g/ml.}$)	Mean value ($\mu\text{g/ml.}$)	Specimens positive	Range of concentration ($\mu\text{g/ml.}$)	Mean value ($\mu\text{g/ml.}$)
Total protein	60	30-536	194	26	74-443	217
Immunoglobulins						
IgA	60	20-350	122	26	14-120	40
IgG	60	5-200	24	4	6-15	8
IgM	—	—	—	3*	Not measurable	—
IgD	—	—	—	—	—	—

— = Not detected.

*Detected only qualitatively by double-diffusion gel precipitation.

RESULTS

Total protein and immunoglobulin content in nasal and parotid secretions

The total protein concentration varied greatly between individual specimens (Table I). The values for unconcentrated nasal washings ranged from 30 to 536 μg per milliliter, and for unconcentrated parotid secretions the range was 74 to 443 μg per milliliter. All nasal washings contained IgA and IgG but not IgM or IgD. All parotid salivas contained IgA; IgG was detected in 4 and IgM in 3 out of 26 salivas. Immunoglobulin D was not detected in any of the specimens. The IgA in nasal and parotid fluids was a secretory IgA as detected by immunoelectrophoresis, using antiserum to the secretory piece. The IgA content in unconcentrated nasal washings ranged from 20 to 350 μg per milliliter, and the IgG from 5 to 200 μg per milliliter. In the unconcentrated parotid salivas, IgA concentration ranged from 14 to 120 μg per milliliter and the IgG from 6 to 15 μg per milliliter. The concentration of IgM in parotid salivas was below the measurable level of the SRID, and the presence in 3 parotid salivas was detected only qualitatively in DDGP test. There was no difference in the ranges of the concentrations of total proteins or immunoglobulins between the allergic and nonallergic individuals.

The range of IgA concentration in secretions was narrower than that of total protein concentration. Since IgA is the predominant immunoglobulin in external secretions,¹ IgA content was chosen as the reference standard to determine the degree of concentration for each individual specimen that was used for detecting specific antibody activity as described below. All secretions were concentrated until the IgA content was in the range of 1 to 2 mg. per milliliter. For most specimens it was a twentyfold concentration.

Antibody activity in secretions and sera

RID test. Antibodies to ragweed in serum and nasal secretions were compared by RID (Fig. 1). Precipitation lines of identity between the IgG and IgA in the serum and nasal secretion were observed (Fig. 1, A). Similar reactions of the antibodies to ragweed associated with the IgG and IgA were seen in the corresponding radioautograph (Fig. 1, B). The electrophoretic mobility of the IgG

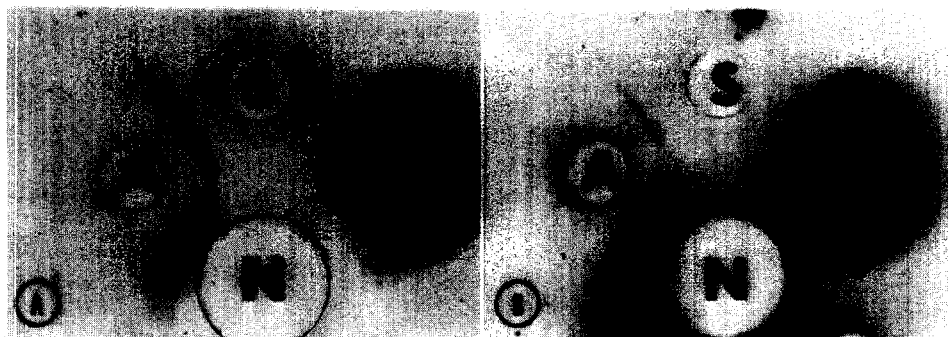


FIG. 1. Comparison of antibodies to ragweed in serum and nasal washing by radioimmunodiffusion. **A**, Immunodiffusion. **B**, Corresponding radioautograph. **S** = serum; **N** = nasal washing; Δ **A** = rabbit antiserum to human IgA (7S); Δ **G** = rabbit antiserum to human IgG. Reaction of identity between nasal and serum IgA and IgG antibodies is shown.

TABLE II. Incidence of anti-ragweed antibodies in nasal secretions and sera of allergic patients

Immunotherapy	Samples tested	Specimens	Antibodies to ragweed examined by				
			Radioimmunodiffusion			PK	TH
			Anti-IgE	Anti-IgA	Anti-IgG		
Yes	22	Nasal washings	3 (14%)	16 (73%)	20 (91%)	9 (53%)*	13 (59%)
		Serum	15 (68%)	15 (68%)	22 (100%)	22 (100%)	22 (100%)
No	28	Nasal washings	5 (18%)	20 (71%)	25 (89%)	13 (68%)†	15 (54%)
		Serum	25 (89%)	16 (57%)	28 (100%)	28 (100%)	15 (54%)

*Only 17 out of 22 specimens examined.

†Only 19 out of 28 specimens examined.

and IgA antibodies of serum and nasal secretions was compared in IEP and RIEP (Fig. 2). The immunoelectrophoretic patterns of the serum and nasal IgA are very similar (Fig. 2, *A*); the shorter precipitation line obtained with nasal IgG as compared to that of serum (Fig. 2, *C*) is most likely due to the differences in the concentrations and lesser heterogeneity of the nasal IgG. Similar minor differences can be seen in the corresponding radiographs (Fig. 2, *B* & *D*), expressing the ragweed antibody activity in these specimens. The same serum and nasal secretion were examined by IEP and RIEP using antiserum to secretory IgA, which also contained antibodies to lactoferrin (Fig. 3). In the IEP (Fig. 3, *A*) the secretion demonstrated two precipitation lines, one to lactoferrin (closer to the anode) and one to secretory IgA. In the corresponding radiograph (Fig. 3, *B*), the nasal secretory IgA showed ragweed antibody activity.

The incidence of IgE, IgA, and IgG anti-ragweed antibodies in the nasal washings and sera of treated and untreated patients is shown in Table II. Nasal IgE antibodies, reported in detail in Reference 11, were found in 3 out of 22 treated patients and in 5 out of 28 untreated patients ($P > 0.05$). Serum IgE

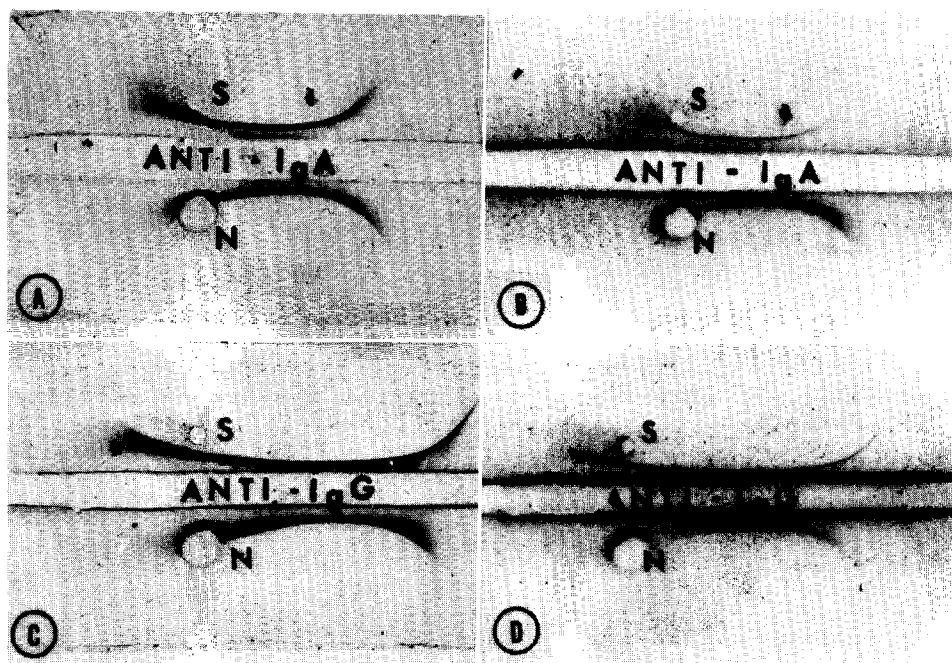


FIG. 2. Comparison of antibodies to ragweed in serum and nasal washing by radioimmuno-electrophoresis. **A** and **C**, Immunoelectrophoresis. **B** and **D**, Corresponding radioautographs. **S** = serum; **N** = nasal washing. **Anti IgA** = rabbit antiserum to human IgA (7S); **Anti IgG** = rabbit antiserum to human IgG. The secretory IgA has very similar mobility to that of serum. Slightly shorter precipitation line was observed with nasal IgG as compared to that of serum. (See text for explanation.)

antibodies were found in 15 out of 22 treated and in 25 out of 28 untreated patients ($P < 0.05$). Nasal IgA antibodies were found in 16 out of 22 treated and in 20 out of 28 untreated patients ($P > 0.05$). Serum IgA antibodies were found in 15 out of 22 treated and in 16 out of 28 untreated patients ($P > 0.05$). In 9 patients IgA antibodies were found only in the nasal washing but not in the corresponding serum, and in 4 other patients the reverse situation was observed. Nasal IgG antibodies were found in 20 out of 22 treated and in 25 out of 28 untreated patients ($P > 0.05$). All sera from allergic patients, both treated and untreated, contained IgG antibodies. However, the group of treated patients had higher serum IgG antibody titers, as shown in Fig. 4.

The parotid salivas from all 16 patients did not have antibody activity when examined by RID. Sera from control individuals did not contain IgE or IgA antibodies, but 8 out of 10 normal sera contained IgG anti-ragweed activity in a dilution less than 1:5 as determined by RID. Nasal washing and parotid salivas from these individuals did not show anti-ragweed antibody activity in RID.

PK test. Among the nasal washings from allergic patients, 9 out of 17 specimens tested from the treated group and 13 out of 19 specimens from the untreated group demonstrated PK activity ($P > 0.05$) (Table II). All the sera from allergic patients had PK activity, but the group of patients with previous immunotherapy had one dilution lower mean PK titer (160) than the group of

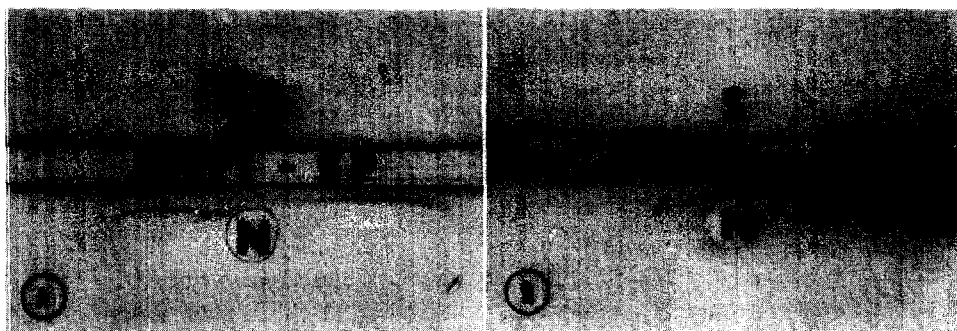


FIG. 3. Detection of antibodies to ragweed in nasal secretory IgA by radioimmuno-electrophoresis. **A**, Immunoelectrophoresis. **B**, Corresponding radioautograph. **S** = serum; **N** = nasal washing. **Anti-SP** = goat antiserum to secretory IgA (11S). This antiserum also contained antibodies to lactoferrin. The nasal washings but not the serum showed the presence of lactoferrin (closer to anode) and secretory IgA. The corresponding radioautograph shows antibody to ragweed associated with the secretory IgA.

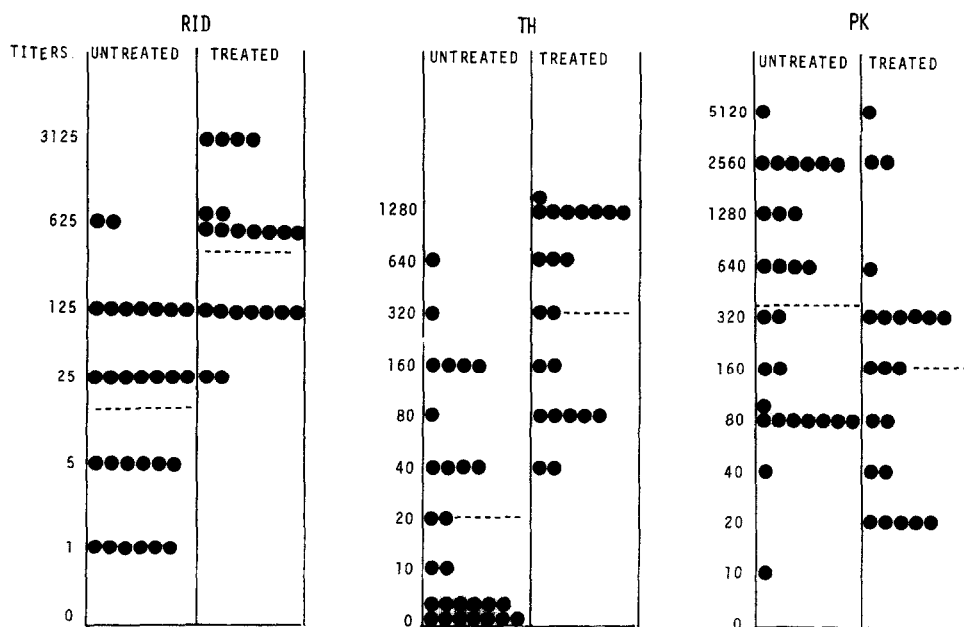


FIG. 4. Comparison of antibody titers in sera of treated and untreated patients. The titers in the RID test are of IgG antibodies to ragweed. The dotted lines indicate the geometric mean titer of the examined group.

untreated patients (320). No PK activity was detected in nasal washings of the control specimens or from any of the parotid secretions examined. Ten specimens of nasal washings with positive PK activity (4 of which were positive in RID using anti-IgE) were adsorbed with immunoabsorbents containing anti-IgE, anti-IgA, or anti-IgG. Only anti-IgE immunoabsorbent removed the PK activity from these nasal specimens.

TH test. Thirteen out of 22 nasal washings from the group of treated patients and 15 of 28 nasal specimens from the untreated patients ($P > 0.05$) contained

hemagglutinating antibodies to ragweed (Table II). Hemagglutinating antibodies were present in higher incidence (100 per cent) and higher titer (mean 320) in the sera of treated patients as compared with the sera of untreated patients (54 per cent) ($P < 0.01$), in which group the mean titer was 20 (Fig. 4). Nasal washings and sera from control individuals were negative.

Only 5 out of 16 specimens of parotid saliva showed TH antibody activity against ragweed. The titers (20-5,120) did not show any correlation with the degree of allergic symptoms or the status of immunotherapy.

The specificity of antibody activity in the TH test was confirmed by inhibition experiments. Ragweed extract was able to inhibit the hemagglutination but not other unrelated antigens such as crystalline egg albumin, *Alternaria* extract, or A and B blood group substances.

DISCUSSION

The considerable variation in protein and immunoglobulin content among individual samples of nasal and parotid secretions observed in our study is in accord with that reported by other investigators.^{6, 7, 19} The variation certainly will depend on the dilution factor and the amount of secretion present in the nasal passages at the time of irrigation. Parotid salivas were collected by suction through the opening of the parotid ducts. The amount of stimulation provided by chewing of hard candies during saliva collection probably also influences the concentration of the saliva. The comparison of antibody activities among secretions was made by adjusting the specimens to a similar immunoglobulin content. The exocrine IgA was chosen as the reference standard.

A predominance of IgA in nasal and parotid secretions was observed in our material. It is generally recognized that an exocrine IgA standard should be used for the quantitation of IgA content in exocrine secretions when examined by SRID² as a serum 7S IgA standard would grossly underestimate the actual IgA concentration. The presence of a low concentration of IgG and the inability to detect IgM and IgD in the examined nasal fluids are consistent with the findings reported by others.^{5, 6} The immunoglobulin profile of parotid saliva is slightly different from that of nasal secretion. A small number of the examined saliva contained IgG and IgM but only in minute quantity. Other investigators have made similar observations.^{19, 20} It is generally agreed that the major portion of IgA in exocrine secretions is synthesized locally in the mucosa.^{2, 21} The origin of IgG and IgM in external secretions has not been fully studied. Synthesis of IgA, IgG, and IgM by human salivary glands was observed in tissue culture, suggesting the possibility that local production was the origin for the 3 immunoglobulins.²² However, a considerable quantity of IgG in the nasal secretions might also be derived from serum by transudation, particularly in the inflamed mucosa of allergic patients.

Ragweed antibodies associated with IgA and IgG were found in the nasal washings from ragweed-allergic patients by the RIEP and RID tests. We have shown that the nasal IgA antibodies belong to the secretory IgA (Fig. 3, B). In 9 allergic patients, IgA antibodies were found only in the nasal washings but not in the sera, and in 4 other patients a reverse situation was observed. The fact that the ragweed IgA antibodies belong to the secretory IgA class and

the dissociation of IgA antibody activity between serum and nasal secretion in these 13 patients indicate that these IgA anti-ragweed antibodies might be locally produced in the mucosa. A discordance in the IgA antibody activity between nasal secretions and serum was recently reported by Ogra and associates.²⁵

In spite of the consistent higher concentration of IgA than IgG in individual nasal washing specimens in the present experiments, the radioimmunodiffusion revealed a higher incidence of nasal IgG (90 per cent) than IgA (72 per cent) anti-ragweed antibodies.

Nasal IgE anti-ragweed antibodies in allergic patients were detected by RID and PK tests. This was reported previously in detail.¹¹ Adsorption experiments with immunoadsorbents containing anti-IgE, anti-IgG, or anti-IgA have shown that the PK activity in nasal washings could be removed only by the anti-IgE immunoadsorbents. This would indicate that nasal IgE was the carrier of reaginic activity. Although IgA is known to be the predominant immunoglobulin in external secretions, the functional significance of exocrine IgA antibodies in allergy has not been fully studied. Since IgE in the examined specimens was the carrier of reaginic antibodies, it is possible, that the IgA antibodies could function as blocking antibodies. Turk, Lichtenstein, and Norman,¹⁰ using whole nasal secretion but an unpurified IgA fraction, have shown that IgA antibodies did seem to have such function.

The effect of immunotherapy was analyzed, and it was found that the incidence of serum IgE ragweed antibodies was significantly lower ($P < 0.05$) in the group of patients with previous immunotherapy in comparison with the group of untreated patients. This is consistent with similar observations previously reported by other investigators.^{23, 24} No significant ($P > 0.05$) difference between these two groups of patients in the incidence of different nasal IgE anti-ragweed antibodies was observed. This tends to suggest that, unlike the effect on serum antibodies, parenteral immunotherapy with pollen extracts had little influence on various antibodies in nasal secretions. Admittedly, a better way to assess the effect of immunotherapy on exocrine antibodies would be to study secretions from the same individuals before and after immunotherapy. This is the subject of a prospective study being at present carried out in our laboratory. Despite this shortcoming in the present study, our observation regarding the lack of effect of parenteral immunotherapy on antibodies in the nasal secretions is in agreement with the results of other investigators using different antigens for immunization. Local immunization with polio²⁵ or influenza vaccines²⁶ or diphtheria toxoid²⁷ was shown to induce significant antibody response in both the sera and external secretions, whereas parenteral immunization was found to be relatively ineffective in inducing anti-body response in the exocrine secretions.

Antibody activity against ragweed was detected by the TH test in a small number of the parotid salivas. The hemagglutination inhibition experiments indicated that these antibodies had specificity for the ragweed antigens. The positive saliva specimens did not show any correlation with clinical severity of symptoms or the status of immunotherapy. Our data differed from the much higher incidence of salivary hemagglutinating antibodies reported by Callies and Duncan,²⁸ possibly because a more sensitive hemagglutinating method was

used by these investigators. The absence of salivary antibody activity when examined by RID could most likely be due to the difference in the degree of sensitivity between the TH and RID tests. Further studies are necessary to clarify the actual incidence of different salivary antibodies and their functional role in pollen hay fever.

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