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# Nasal Secretory Antibody to Inhalant Allergens in Allergic and Non-Allergic Patients

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**Summary.** Nasal washings were obtained from normal and ragweed allergic (immunized and non-immunized) subjects. All specimens contained 'blocking' antibodies as measured by their ability to inhibit antigen E induced histamine release from human leucocytes. The antibody was largely of the IgA class (secretory antibody). There was little difference between the blocking activity of normal, allergic and immunized allergic subjects. All patients also had blocking secretory antibody against grass pollen group I antigens to which they were not clinically sensitive. There was no correlation between the level of anti-grass and anti-ragweed activity; moreover, no antibody activity was demonstrated against an allergen (pitressin) to which the subjects had not been exposed. A modest but significant rank correlation was found between the level of serum and nasal anti-ragweed activity in the allergic immunized patients, but no relationship obtained between the clinical severity of symptoms referable to ragweed exposure and the level of nasal antibody. It is concluded that all subjects environmentally exposed to pollen allergens develop nasal blocking antibodies; the level of this antibody bears little relationship to parenteral immunization and no detectable relationship to the clinical severity of ragweed hay fever.

## INTRODUCTION

Antibodies have been studied from a variety of external secretions including tears, saliva and urine. The structure and specificity of such secretory antibodies have recently been reviewed by Tomasi and Bienenstock (1968); at present these antibodies are felt to consist of two IgA molecules linked by a 'transport' or 'secretory' piece. The latter may play a role in the transport of antibody through mucosal surfaces (Tomasi and Bienenstock, 1968). Most recent work has been directed towards the antibody activity recovered from washings of nasal and respiratory mucosa; evidence is accumulating which suggests that such secretory antibodies play a significant role in protection against viral respiratory infection (Artenstein, Bellanti and Buescher, 1964; Rossen, Butler, Cate, Szwed and Couch, 1965; Douglas, Rossen, Butler and Couch, 1967; Waldman, Mann and Kasel, 1968). Notably, Smith, Bellenti and Chanock (1967) have shown that protection against infection with parainfluenza A is more closely related to the presence of antibody obtained from nasal washes than to serum antibody.

The pathogenesis of seasonal pollinosis is such that secretory antibody might be expected to play a role. Allergens must pass the nasal mucosa to come into contact with cell-fixed IgE (reaginic) antibodies. The resultant antigen–antibody interaction is thought to trigger a reaction in local mast cells which causes release of histamine, presumably the cause of most or all of the symptoms. One might, therefore, speculate that antibodies in nasal secretions, located in the target organ, might be more effective than serum antibodies in blocking the reaction of antigen and cell-fixed reagin.

Several studies of antibodies in secretions of patients with respiratory allergies have been reported. Reaginic antibody has been demonstrated in external secretions including nasal fluid and tears (Samter and Becker, 1947; Remington, Vosti, Lietze and Zimmerman, 1964: Settipane, Connell and Sherman, 1965). Although Prausnitz–Kustner activity was also reported in saliva of two patients with ragweed allergy by Ishizaka and co-workers (Ishizaka, Dennis and Hornbrook, 1964), parotid secretions of seventeen ragweed allergic patients were negative in passive transfer tests in the hands of Arbesman and co-workers (Arbesman, Dolovich, Wicher, Dushenski, Reisman and Romasi, 1968). Most recently, IgA 'anti-ragweed' antibodies have been demonstrated by immunodiffusion in the nasal secretions of two of six ragweed allergic patients studied (Dolovich, Arbesman and Tomasi, 1969). There has been, however, no detailed evaluation of allergic individuals with reference to the local production of secretory 'blocking' antibodies.

Parenteral immunization, often termed desensitization, is a time honored treatment for allergic rhinitis. We, and others, have shown by controlled studies that it has a definite, if moderate, ameliorative effect on the symptoms of this disease (Lowell and Franklin, 1963, 1965; Lichtenstein, Norman and Winkenwerder, 1968; Sadan, Rhyne, Mellits, Goldstein, Levy and Lichtenstein, 1969). The mechanism by which this occurs is, however, not clear. As one result of immunization, serum IgG antibodies, called blocking antibodies, are produced. We reported that there is a rank correlation between the level of this blocking antibody and the degree of symptom relief (Lichtenstein, Norman and Winkenwerder, 1969) although others have failed to note this relationship (Loveless, 1943; Arbesman, Kantor, Rapp and Rose, 1960). The correlation is rough, however, and IgG serum blocking antibody appears to be only one of several variables related to the intensity of allergic symptoms.

This study was designed to ascertain whether antibodies which have blocking activity could be detected in nasal secretions, and, if so, whether they had any relation to either immunotherapy or symptom relief. The experimental design utilized both normal and ragweed sensitive patients, the latter undergoing either placebo therapy or being immunized by one of several regimens. We studied the levels of antibody directed against an allergen to which the patients were clinically sensitive (ragweed antigen E) and against one to which they were not clinically sensitive (rye grass group I antigen).

## METHODS

*Patients*

Patients for study were obtained from a group of adult hay fever sufferers sensitive to ragweed and having symptoms only during the fall. They had positive intradermal reactions to ragweed, and their cells released histamine *in vitro* when challenged with antigen E (King, Norman and Connell, 1964). These patients were part of a controlled study of immunotherapy (Lichtenstein *et al.*, 1969) and were being treated with either ragweed

antigen E, antigen E plus antigen K (King, Norman and Lichtenstein, 1967), whole ragweed extract, alum precipitated extract or placebo. The immunization continued for a four month period with total doses of approximately 0·5 mg ragweed protein (8000 pnu) of whole or alum-precipitated ragweed extract, 1 mg antigen E, or 1·4 mg antigen E plus antigen K. Nasal washings were obtained in September 1968, about 6 weeks after immunotherapy had ceased. Patients used as non-allergic controls had no history of allergic disease.

A total of thirty-four patients undergoing desensitization, fifteen placebo-treated patients and eight non-allergic, non-treated patients were studied for nasal antibodies against ragweed antigen E. Of these, nineteen of the treated group, ten of the placebo group, and six non-allergics were randomly selected for study of their nasal secretory blocking activity for group I rye antigen. The latter patients had no clinical history of grass pollen hypersensitivity. Nasal secretions of six patients with high levels of antigen E blocking activity were further tested in the *in vitro* histamine release system for blocking activity against pitressin (see below).

*Specimens*

Nasal washings were obtained by instillation of 10 ml of Tris-buffered saline (Lichtenstein and Osler, 1964) into the posterior portion of the nostril following the technique of Rossen *et al.* (1965). The solution was held in the nares for 10–20 seconds and then forcefully expelled. Each nostril was washed twice using this procedure. Returns varied from 15 to 38 ml; specimens of less than 25 ml were discarded. Each specimen was tested for occult red cells with benzidene and guaiac and was negative.

The buffer–mucous mixture was homogenized in a laboratory blender at slow speed and centrifuged at high speed to remove suspended material. The supernate was concentrated by vacuum-colloidion bag apparatus to a final volume of 2–3 ml and kept frozen at −20°. Pools were prepared from these samples for use in antigen–antibody binding and absorption experiments.

*Assessment of blocking activity*

The techniques for *in vitro* histamine release were as previously described except that the volumes were scaled down following the method of May (Lichtenstein and Osler, 1964; May, personal communication 1968). The antigens used to stimulate histamine release were ragweed antigen E, rye grass group I (Marsh, Milner and Johnson, 1966) and commercial pitressin. Cell donors were naturally sensitive to either the ragweed or grass pollen antigens. In the case of pitressin, the cells were passively sensitized by the method of Levy and Osler (1967) with the serum of a patient known to have pitressin sensitivity. In each experiment a 1-ml sample of isolated leucocytes from a single donor was added to a series of tubes containing 0·2 ml of the antigen and 0·1 ml of each of the concentrated nasal specimens or buffer. The antigen E concentration was adjusted so as to cause 60–80 per cent histamine release in the absence of antibody. The nasal secretion and antigen had previously been mixed and allowed to incubate at room temperature for 30 minutes and then at 4° overnight (18 hours). Antigen–antibody binding experiments at various other incubation times were also performed. Per cent inhibition by blocking antibody was obtained, as described previously, by comparing histamine release in reaction mixtures containing nasal concentrate ($N$) to control tubes containing buffer in place of nasal secretions ($C$): (per cent inhibition = $(C-N/C)\times 100$) (Lichtenstein and Osler, 1966).

*Reproducibility of nasal wash blocking activity*

Three serial washings taken over a 2-hour period from each of three patients and for each of 3 successive days in two patients were processed in routine fashion and assayed for antigen E induced histamine release blocking activity.

*Serum blocking activity*

Serum blocking activity was assessed as previously described (Lichtenstein, Norman and Winkenwerder, 1968). The titre of blocking antibody is defined as the reciprocal of the dilution of serum which inhibited 50 per cent of possible histamine release.

*Absorption of nasal secretions*

Commercial anti-IgA (Hyland Laboratories, Los Angeles) was added in small amounts to the nasal concentrate and the mixture was allowed to incubate overnight. The specimen was then centrifuged at high speed and tested for residual IgA by immunodiffusion. This was continued until the IgA line was barely visible. A similar procedure was utilized in an attempt to remove IgG.

*Symptom scores*

Symptoms were evaluated by the use of symptom diaries kept by each patient and by periodic examinations by a physician. The technique has been described in detail elsewhere (Norman, Rhyne and Mellits, 1966).

## RESULTS

### TIME COURSE OF ANTIGEN E BINDING BY NASAL SECRETIONS

In previous experiments assessing the *in vitro* blocking activity in sera from ragweed sensitive donors, 30 minutes preincubation of the serum with antigen E produced essentially maximal inhibition of histamine release (Lichtenstein and Osler, 1966). This was not found to be the case when nasal secretions were used to inhibit antigen E induced histamine release. As seen in Fig. 1, for a pool of nasal secretions, only 40 per cent inhibition

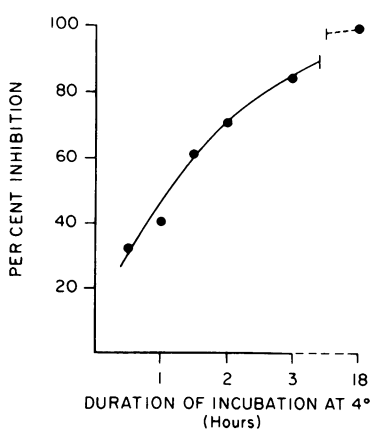


FIG. 1. Per cent inhibition of antigen E induced histamine release by pooled nasal secretions versus incubation time with ragweed antigen E. All samples were incubated initially for 30 minutes at room temperature before placement in 4° bath.

was obtained after incubation of nasal secretions and antigen E for 30 minutes at room temperature plus 1 hour at 4°; after 3 hours at 4° the inhibition was in excess of 80 per cent and, in this experiment, complete inhibition was obtained after overnight incubation. The latter procedure was followed for all subsequent studies of inhibitory activity.

## ABSORPTION STUDIES

A pool of concentrated nasal secretions showed clear immunodiffusion lines with both anti-IgA and anti-secretory piece antibody (the latter kindly supplied by Dr Richard Hong) as well as with an anti-IgG serum. After absorption with anti-IgA as previously described, the pool showed only a very faint line against IgA and none against secretory piece. The IgG line was unchanged. Complete absorption of IgA was not carried out because excess anti-IgA in test samples was found to cause some histamine release from the leucocytes used in the blocking antibody assay.

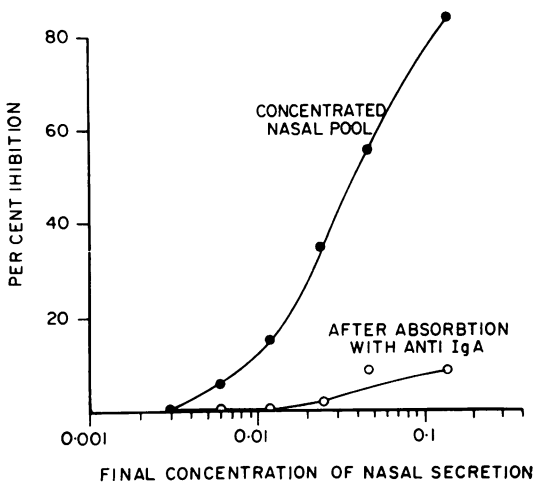


FIG. 2. Effect of absorption with anti-IgA on per cent inhibition of antigen E induced histamine release by nasal secretions.

As shown in Fig. 2, IgA absorbtion decreased the inhibitory activity of the nasal secretions by about 90 per cent. The same type of experiment was attempted with anti-IgG; no reduction in blocking activity was observed but the anti-IgG serum, at the high concentrations found to be necessary to significantly decrease the IgG immunodiffusion line, caused some histamine release itself. We cannot, therefore, definitely rule out the presence of blocking activity in the IgG found in nasal secretions.

## BLOCKING ACTIVITY OF SERIAL NASAL WASHINGS

In order to test the reproducibility of serial nasal washings, successive samples over a 2-hour period from each of three patients and for each of three successive days in two patients were made. The antigen E blocking activity of the serial washings is shown in Table 1.

TABLE 1

BLOCKING ACTIVITY OF SERIAL NASAL WASHINGS

| Patients | Per cent inhibition of antigen E stimulated histamine release | | |
|---|---|---|---|
| | Wash 1 | Wash 2 | Wash 3 |
| FK* | 17 | 21 | 15 |
| LML* | 79 | 81 | 89 |
| NFA* | 42 | 47 | 53 |
| SF† | 29 | 35 | 27 |
| FK† | 32 | 17 | 27 |

* Washes over 2-hour period.
† Serial daily washes.

## ANTIGEN E BLOCKING ACTIVITY OF NASAL SECRETIONS

Fig. 3 shows the per cent inhibition by nasal secretions of antigen E stimulated histamine release for the patients studied. Values ranged from 5 to 100 per cent with most in the range from 40 to 80 per cent. Values for histamine release inhibition over 90 per cent may be higher than graphically shown since the inhibition curve is asymptotic in this area.

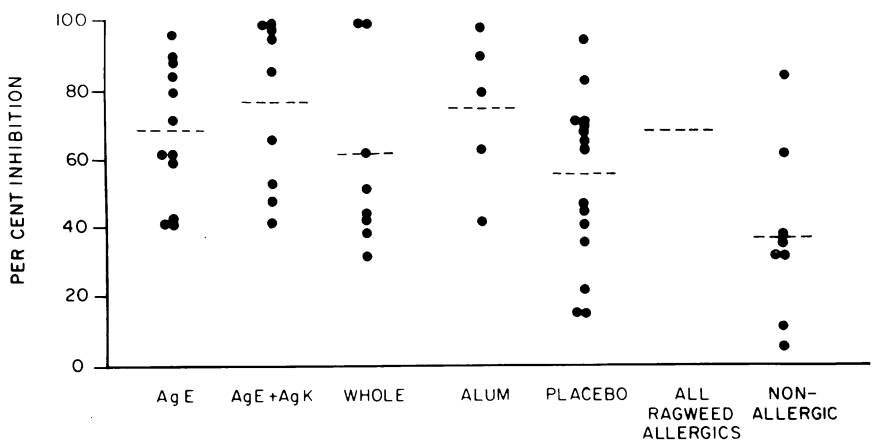


FIG. 3. Inhibition by individual nasal wash specimens of antigen E induced histamine release for the various treatment regimens described in the text. In this figure the average shown for 'All Ragweed Allergics' includes the genuinely treated patients only.

The mean values of per cent inhibition are close for all the treated groups and, with the number of determinations available, no significant differences can be discerned among these populations. The distribution of placebo-treated patients is over the same range as the combined treated group, but a marginally significant difference is noted between the means (Student's $t$-test, $P = 0{\cdot}05$). The nasal secretions of all non-allergic patients inhibited antigen E stimulated histamine release. The mean value of inhibition is significantly lower ($P<0{\cdot}05$) than in the treated group but, with the number of patients studied, no significant difference between the non-allergic and placebo treated groups is seen. Thus, all specimens studied demonstrated nasal blocking activity. This is in contrast to serum IgG blocking antibody which is detectable only in allergic patients (Lichtenstein and Osler, 1966).

GROUP I RYE GRASS ANTIGEN BLOCKING ACTIVITY OF NASAL SECRETIONS

As might be expected from the above data, when the nasal secretions of these donors were tested for inhibiting activity against rye grass pollen group I antigens, an allergen to which the patients were not sensitive, all specimens had activity. There was no difference between the blocking activity in any of the groups (Fig. 4). The average level of anti-grass

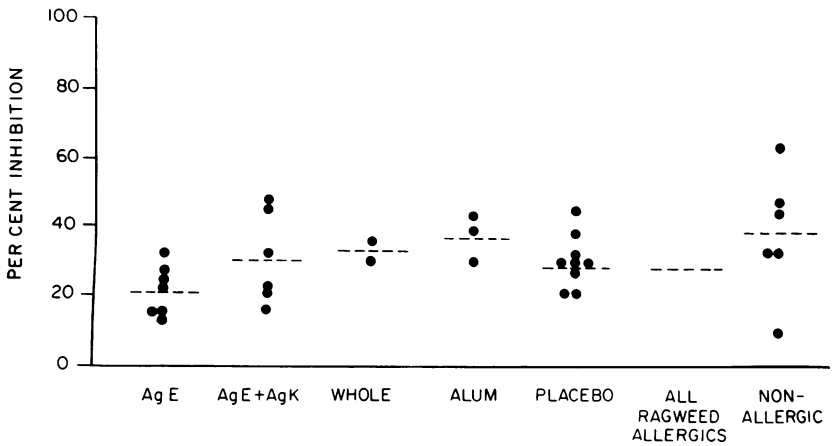


FIG. 4. Inhibition of group I rye antigen induced histamine release by nasal secretions from the individuals described in Fig. 3.

activity was, however, significantly less ($P<0{\cdot}01$) than the anti-ragweed activity. In this context it should be remembered that the specimens were taken just *after* environmental exposure to ragweed but several months following the season of grass pollination. There was no correlation between the levels of nasal blocking antibody to antigen E and to group I rye antigen and often patients with high anti-antigen E activity had low anti-group I activity and vice-versa.

PITRESSIN BLOCKING ACTIVITY OF NASAL SECRETIONS

Since it appeared likely that all exposed individuals develop antibody against the common inhalant pollen antigens another type of antigen was necessary in order to demonstrate that the blocking activity was specific. For this purpose the serum of a patient with diabetes insipidus who was clinically sensitive to crude pitressin was used to passively sensitize normal leucocytes. The nasal secretions of five donors with the highest levels of activity against antigen E were incubated with pitressin and then added to the passively sensitized cells. No inhibition of histamine release was observed. The serum of the pitressin-sensitive patient did, however, contain blocking antibody against pitressin (Lawrence and Lichtenstein, unpublished data).

CORRELATION OF NASAL BLOCKING ACTIVITY WITH SERUM BLOCKING ACTIVITY AND SYMPTOM SCORES

The Spearman rank correlation test was used to assess the possible association between the per cent histamine release inhibition by nasal secretions, the serum blocking antibody

TABLE 2

| Immunotherapy | Patient No. | Per cent nasal inhibition | Symptom scores | Serum blocking Ab titre |
|---|---|---|---|---|
| Antigen E | 1 | 42 | 8·2 | 116 |
| | 2 | 42 | 3·5 | 83 |
| | 3 | 43 | 4·9 | 83 |
| | 4 | 60 | 8·5 | 32 |
| | 5 | 62 | 10·9 | 164 |
| | 6 | 62 | 10·0 | 347 |
| | 7 | 72 | 6·5 | 90 |
| | 8 | 80 | 10·9 | 72 |
| | 9 | 85 | 6·8 | 166 |
| | 10 | 89 | 8·2 | 175 |
| | 11 | 90 | 6·2 | 140 |
| | 12 | 97 | 7·8 | >8000 |
| Antigen E and K | 13 | 42 | 3·5 | 27 |
| | 14 | 48 | 0·1 | 256 |
| | 15 | 53 | 5·0 | 22 |
| | 16 | 66 | 14·5 | 162 |
| | 17 | 86 | 1·9 | 250 |
| | 18 | 96 | 4·2 | 294 |
| | 19 | 99 | 2·6 | 332 |
| | 20 | 100 | 6·1 | 228 |
| | 21 | 100 | 9·0 | 172 |
| Whole extract | 22 | 32 | 10·9 | 17 |
| | 23 | 39 | 4·9 | 23 |
| | 24 | 43 | 3·8 | 124 |
| | 25 | 44 | 1·0 | 33 |
| | 26 | 52 | 16·1 | 194 |
| | 27 | 62 | 10·9 | 29 |
| | 28 | 100 | 10·2 | 71 |
| | 29 | 100 | 8·9 | 7200 |
| Alum precipitate | 30 | 42 | 5·9 | 67 |
| | 31 | 63 | 7·2 | 112 |
| | 32 | 80 | 3·3 | 35 |
| | 33 | 90 | 3·4 | 279 |
| | 34 | 99 | 5·8 | 18 |
| Placebo treated | 35 | 15 | 8·0 | <10 |
| | 36 | 15 | 6·6 | <10 |
| | 37 | 22 | 2·5 | <10 |
| | 38 | 36 | 20·6 | <10 |
| | 39 | 41 | 11·3 | <10 |
| | 40 | 45 | 2·1 | <10 |
| | 41 | 47 | 7·5 | <10 |
| | 42 | 64 | 6·6 | <10 |
| | 43 | 65 | 11·6 | <10 |
| | 44 | 69 | 13·5 | <10 |
| | 45 | 70 | 6·4 | <10 |
| | 46 | 72 | 14·8 | <10 |
| | 47 | 72 | 9·3 | <10 |
| | 48 | 84 | 9·7 | <10 |
| | 49 | 96 | 3·6 | <10 |
| Non-allergic | 50 | 5 | – | 0 |
| | 51 | 11 | – | 0 |
| | 52 | 32 | – | 0 |
| | 53 | 32 | – | 0 |
| | 54 | 36 | – | 0 |
| | 55 | 38 | – | 0 |
| | 56 | 62 | – | 0 |
| | 57 | 85 | – | 0 |

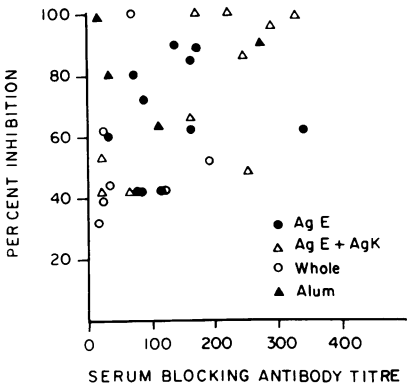


FIG. 5. Per cent inhibition by nasal wash specimens of antigen E stimulated histamine release versus serum blocking antibody titre in the treated patients. These parameters are associated with a Spearman Rank Coefficient of 0·51 and $P < 0{\cdot}01$.

titre and symptom scores of each patient. No correlation between symptom scores and the inhibition of the histamine release by nasal secretions could be detected for either the entire group of study patients or placebo or genuinely treated patients alone. The inhibition of histamine release by the nasal secretions and serum blocking antibody levels of genuinely treated patients were positively associated, $r_s = 0{\cdot}51$ and $P < 0{\cdot}01$ (Fig. 5). The correlation between symptom scores and serum blocking antibody has previously been reported (Lichtenstein *et al.*, 1969).

## DISCUSSION

The ability to block *in vitro* histamine release stimulated by two common inhalant allergens—ragweed antigen E and rye grass group I antigen—has been found in the nasal secretions of all individuals tested. This activity is believed to be due to antibody since absorption with anti-IgA removed about 90 per cent of the blocking activity. Its specificity is shown by the failure of potent anti-antigen E preparations to inhibit histamine release by another antigen (pitressin) to which the patients had not been exposed. Specificity is further demonstrated by the lack of correlation between the anti-antigen E and anti-group I activity in single specimens. We assume, therefore, that we are dealing largely with secretory IgA although we have not completely ruled out a contribution by transudated serum IgG.

In previous studies of serum blocking activity we could find no activity in isolated IgA even when highly concentrated (Lichtenstein, Holtzman and Burnett, 1968). Thus, the activity noted is not likely to be contributed from this source. Moreover, the demonstration of IgA blocking activity in nasal secretions together with our previous failure to demonstrate such activity in serum IgA strongly suggests that the secretory antibody is locally produced. In this study, as in others published to date, there is no standardization of the nasal wash specimens. Serial washings of individuals were reasonably reproducible and we were interested primarily in group rather than individual comparisons. Also, we can think of no perfectly adequate standard which, if used to normalize the individual specimens, would reflect the 'true' level of secretory antibody. There is nothing to suggest a bias

in the sampling technique and in our calculations we have assumed that the nasal wash specimens reflect random samples of the populations studied.

Although there are some differences between the level of secretory antibody found in the several groups studied, the important biological observation is that all specimens had activity. This is not unexpected since all patients had the same environmental exposure to the pollens in question. It might have been anticipated, however, that allergic individuals would have had a higher level of secretory antibody inasmuch as they were exposed to antigen during a period when their mucous membranes were undergoing an inflammatory response.

Salvaggio, Cavanaugh, Lowell and Leskowitz (1964) have shown that when allergic and normal individuals are exposed to a new antigen by the mucosal route, the allergic individuals develop IgE antibodies (as judged by skin test) more commonly than the normals. We have reported that all allergic individuals have serum (IgG) blocking antibodies against ragweed antigen E but we could find no activity in the serum of normal donors (Lichtenstein *et al.*, 1966). It would seem, therefore, that the absorption of antigen transmucosally in amounts sufficient to induce secretory antibody does not imply that there will be systemic production of other types of immunoglobulins, specifically IgE (reagins) or IgG serum blocking antibodies. The basic lesion of the allergic diathesis is thus not likely to be in the musosal handling of antigen but is more probably related to a genetic capacity to make a wide variety or high levels of IgE antibody.

The finding of secretory antibody in all individuals suggests that it may not play a role in the pathogenesis of allergic pollinosis. Moreover, since parenteral immunization raised the antibody level only slightly, if at all, it would seem that a protective effect by secretory antibody is not an important part of the mechanism of the symptom relief achieved by this type of therapy. Whether a means of mucosal immunization can be developed which will increase the level of secretory blocking antibody and provide some clinical protection remains to be seen.

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