

The Journal of

Allergy

VOL. 44, NO. 3

SEPTEMBER, 1969

AMERICAN ACADEMY OF ALLERGY

25th Anniversary Series

Special presentations and reviews from the postgraduate course

A rational approach to desensitization

Philip S. Norman, M.D., Baltimore, Md.*

In order to give credit where it is due, my title was not devised by me but by Dr. Samuel Freedman who, in his invitation to speak, mentioned it as a topic which immediately caught my imagination. As allergists, we intensely desire that desensitization be rational, sensible. To my mind this means that we must understand its mechanism, as we understand that insulin lowers blood sugar by allowing glucose to enter cells. As other specialists have forged ahead in their precise knowledge of the pathophysiology of particular diseases and their management, allergists come under criticism because of their empirical acceptance of desensitization on the simple basis that it works. A more reasoned rationale of treatment, backed by adequately controlled clinical and laboratory observations, is the only way to answer such criticism.

It is clear that, as of now, one cannot describe a completely rational way of accomplishing desensitization. A body of laboriously obtained information, however, points the way to the development of a more rational approach. I wish to

From the Allergy and Infectious Diseases Division, Department of Medicine, The Johns Hopkins University and Hospital.

Supported by Grants AI04866 and AI08270 from the National Institute of Allergy and Infectious Diseases.

Presented at the American Academy of Allergy 25th Anniversary Meeting, March 16, 1969, at Bal Harbour, Fla.

Received for publication May 29, 1969.

*Recipient of a Research Career Development Award from the National Institute of Allergy and Infectious Diseases.

report the status of such development and to perhaps set up a few signposts for future goals.

Prophylactic inoculation was introduced in 1911.^{1, 2} The scientific method was introduced in its clinical study in 1955, when Frankland³ divided 154 grass hay fever patients into two groups and treated one group with grass extract and gave the other group placebos. In the treated group, 88 per cent reported a satisfactory result, and in the placebo group 43 per cent reported a satisfactory result. Such results have since been obtained a number of times with grass pollen and ragweed pollen hay fever in carefully controlled studies,^{4, 5} but it is an indication of the complexity of the subject that, when one turns to desensitization with bacterial vaccines, 6 different studies have been unable to find a significant difference between treated and control patients.⁶⁻¹¹ Nevertheless, results of studies with hay fever give one courage to deal with symptom relief by desensitization in human allergies as a real phenomenon, even though they provide little of the information required to develop a rational approach.

Three developments provide improved tools for elucidation of the problem of desensitization. First is the perfection of an adequate system for assessing allergic disease by symptom reporting by Lowell and Franklin⁴ and by Rhyne.¹² Second is the isolation of several purified allergens from pollens and their identification as the principal source of allergic sensitization and symptoms upon subsequent exposure.¹³⁻¹⁵ Third is the demonstration that histamine release from isolated washed leukocytes *in vitro* represents a laboratory counterpart of the disease which can be dissected in some detail in the laboratory by accurate procedures.^{16, 17}

Part of the problem in assessment of allergic disease is that there has been no laboratory measurement relevant to the disease, such as blood sugar in diabetes, or a simple test of progress, such as the chest x-ray in pneumonia. In the case of hay fever, the symptoms of the disease largely depend on the degree of swelling and hyperemia of the nasal mucosa, which, when they are increased, lead to hypersecretion of mucus and obstruction of the nasal airway. It is true that the resistance of the nasal airway can be measured with great accuracy at any point in time. Furthermore, with a color scale, the hyperemia of nasal mucosa can be graded accurately. Nevertheless, nasal obstruction, as everyone who has suffered a common cold well knows, is a transient condition which can change every few minutes. Hence, a few casual observations, no matter how accurate at the moment, do not reflect the state of the disease, hay fever, over days or weeks. A good example of infrequent but accurate observations failing to reflect disease has been given in asthma by the elegant demonstration of Chai and colleagues who measured the maximum expiratory flow rate of hospitalized patients 4 times a day, as shown in Fig. 1. Such frequent observations clearly demonstrate the changing status of the patient's airway obstruction over a number of days. Fig. 2 shows, superimposed, the result of plotting only the observations taken once a day. It is evident that all sense of the changing status is lost as single daily observations may catch the individual at almost any place on the up or down of the curve.¹⁸

In hay fever what the patient and physician perceive as disease is the result

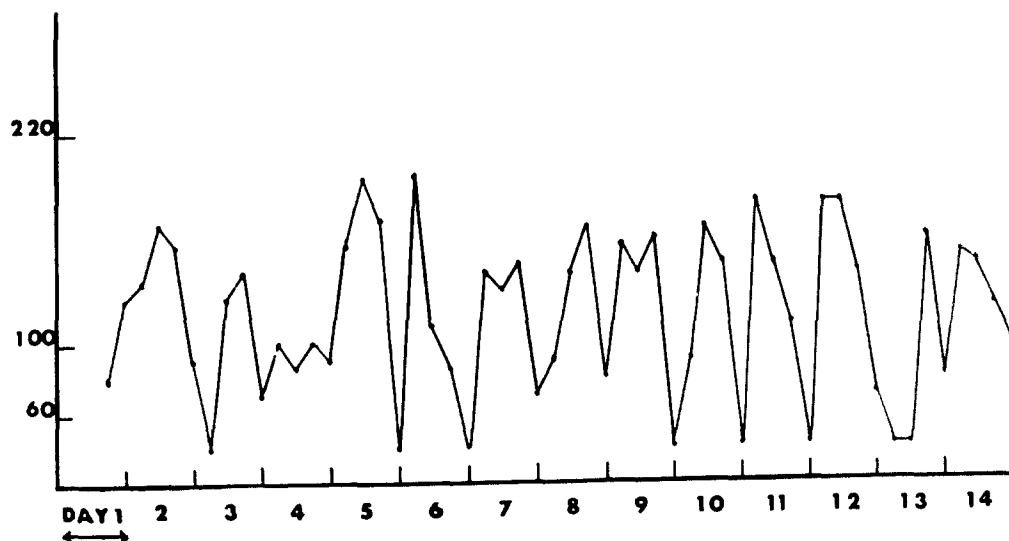


Fig. 1

Peak expiratory flow rate (liters per minute) performed 4 times daily. (J. ALLERGY 4: 23, 1968.²³)

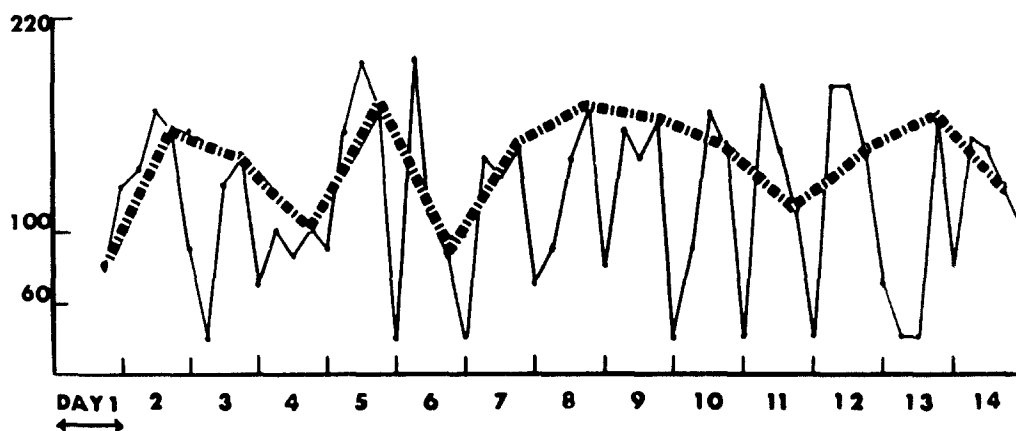


Fig. 2

The dashed line shows peak expiratory flow rate performed once daily.

of ever-changing events resulting from a highly variable exposure to inhaled allergens. As long as continuous objective measurement is technically impossible, we will have to depend on what the patient perceives to assess the disease. What is necessary is to sharpen the recording of the patient's perception to the point where it becomes a reliable tool. Admittedly, patients' perceptions are not ordinarily as accurate as objective measurements; but in order to gain insight into the individual's total experience with an ever-changing condition, one sacrifices great accuracy.

What is the best way to record the patients' perceptions of disease? Memory is, of course, highly fallible; and the recollection of experience must be caught while it is still fresh. After some trial we have settled on a twice-a-day recording of individuals' experiences. Patients object to more frequent recording, and once-a-day recording already begins to lose some of the immediacy hoped for. Individual symptoms must be recorded as a means of making the patients' perceptions as detailed as possible. In hay fever we have found it suitable to ask patients to record separately: (1) stuffy, runny nose, (2) sneezing, (3) itchy eyes, and (4) cough. Furthermore, one has to learn to live with the patients' desire to use medications when symptoms are present. We furnish our patients with antihistamine tablets in generous supply and ask them to record the number of pills taken, and this becomes an item in our evaluation.

Insofar as symptom reporting is concerned, it is desirable to attempt to assess duration rather than severity. What the patient interprets as severe to some extent represents long duration anyway. The result is a symptom diary which asks patients to check whether a symptom was entirely absent, lasted only up to $\frac{1}{2}$ hour, lasted $\frac{1}{2}$ to 2 hours, or lasted over 2 hours. A place is provided for the number of antihistamines taken as well as a listing for other medications, if any. This diary is adapted slightly from that developed by Dr. Marie Rhyne¹⁹ who spent many years trying diary forms, analyzing results for reliability, and working out these questions which would most accurately reflect hay fever.

An important aspect of obtaining good reporting is the selection of patients. Individuals with obvious personality defects must be eliminated and patients of good education and steady job history selected in order to achieve the best results.

Studies by Lowell and Franklin⁴ and Rhyne¹⁹ have shown that adequate methods of symptom reporting can be used to assess disease accurately. Data so obtained can be analyzed by standard statistical methods, and the method of assessment by detailed symptom reporting opens the way to making the unbiased clinical judgments required for correlations with laboratory studies.

As an example of how well symptom reporting correlates with actual exposure, Fig. 3 shows the relationship between a daily average of scores reported by 20 hay fever patients living in the Baltimore area with daily semiquantitative pollen counts obtained by a roto-slide sampler and demonstrates the agreement between ups and downs of pollen exposure and the ups and down of disease.

Another development which has aided in making quantifiable observations in hay fever has been the isolation of purified allergenic principles from pollens such as ragweed and grass. The best example is the potent allergen of ragweed pollen, antigen E, isolated by King in 1961. We were privileged to perform the activity testing which showed that its biological activity is extreme, $1 \mu\mu\text{g}$ (0.000000000001 Gm.) commonly being sufficient to elicit an allergic response in the form of a positive skin test. It is a simple protein of 38,000 molecular weight. Although it comprises only 0.5 per cent of the solids extractable from pollen and only 6 per cent of the extractable protein, it represents more than 90 per cent of the biological activity in pollen extract as measured by skin tests or histamine release in most subjects.^{13, 14}

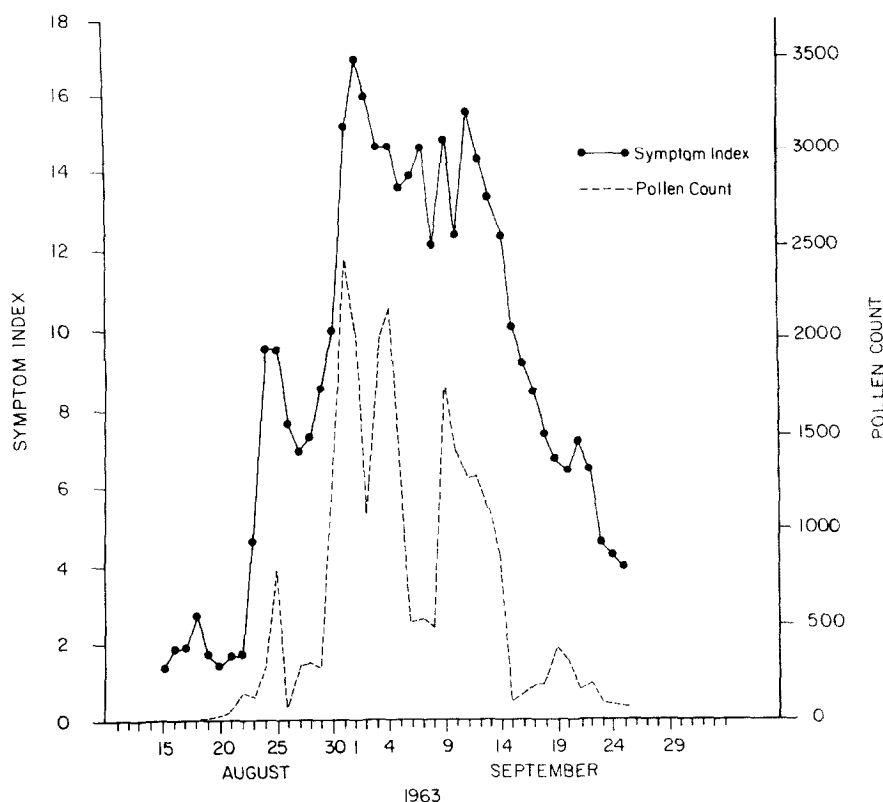


Fig. 3

Average daily symptom indexes of 20 untreated ragweed patients and semiquantitative ragweed pollen counts.

Whereas it was relatively easy to demonstrate that antigen E is responsible for a major portion of the biological activity of pollen in sensitive people, it was also important and less easy to determine whether it is responsible for the ability of pollen extracts to accomplish desensitization.

W. L. Winkenwerder and I set out some years ago to explore two questions:
(1) Is antigen E the material responsible for the efficacy of these inoculations?
(2) To what extent is the efficacy of prophylactic injections dependent on dose?

We therefore organized a series of double-blind studies carried out annually over a number of years exploring the efficacy of increasing doses of whole ragweed extract and of purified antigen E furnished by The Committee on Standardization of Allergens of the National Institutes of Health. Evaluation of results was by the symptom diaries just described. Careful precautions were taken to avoid bias by blinding both patients and evaluating physicians to the materials given. Table I shows the increasing doses given preseasonally to groups of about 20 matched hay fever patients who received either placebos, antigen E, or whole extract. In 1964, we began with what now seems to be an exceptionally modest cumulative preseasonal dose; i.e., 830 protein nitrogen units (PNU) of

Table I. Immunotherapy of hay fever

Year	Number of injections	Dose of antigen E	Dose of whole extract
1964	14	32 μ g	830 PNU
1965	15	252 μ g	2,440 PNU
1966	15	743 μ g	9,480 PNU
1967	8	1,340 μ g	9,640 PNU

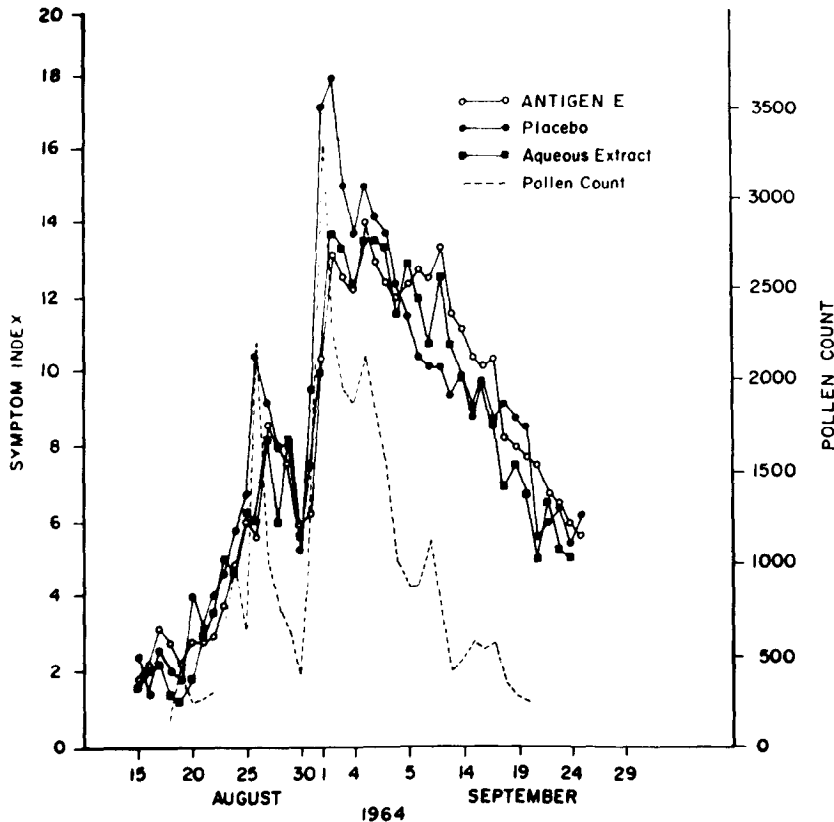


Fig. 4

Effect of low doses of pollen extract or antigen E on average daily symptom indexes. (J. ALLERGY 42: 93, 1968.²³)

whole extract and 32 μ g of antigen E. Yet a number of clinicians were using such doses of whole extract to the satisfaction of themselves and their patients (Fig. 4). Although there was a small favorable difference between the treated and placebo patients, this difference is not significant; and these rather small doses can be regarded as essentially noneffective. In 1965, in many of the same patients, the dose was raised about tenfold for antigen E to 300 μ g and threefold for whole extract to about 2,500 PNU. Fig. 5 shows that there was considerable difference in symptom scores between treated and control groups,

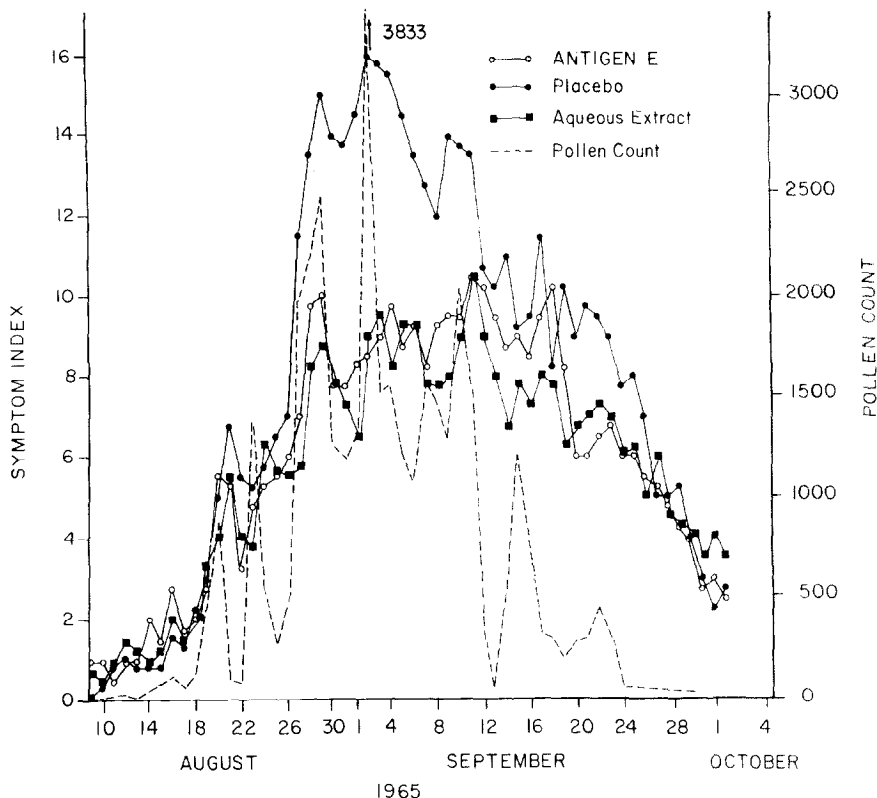


Fig. 5

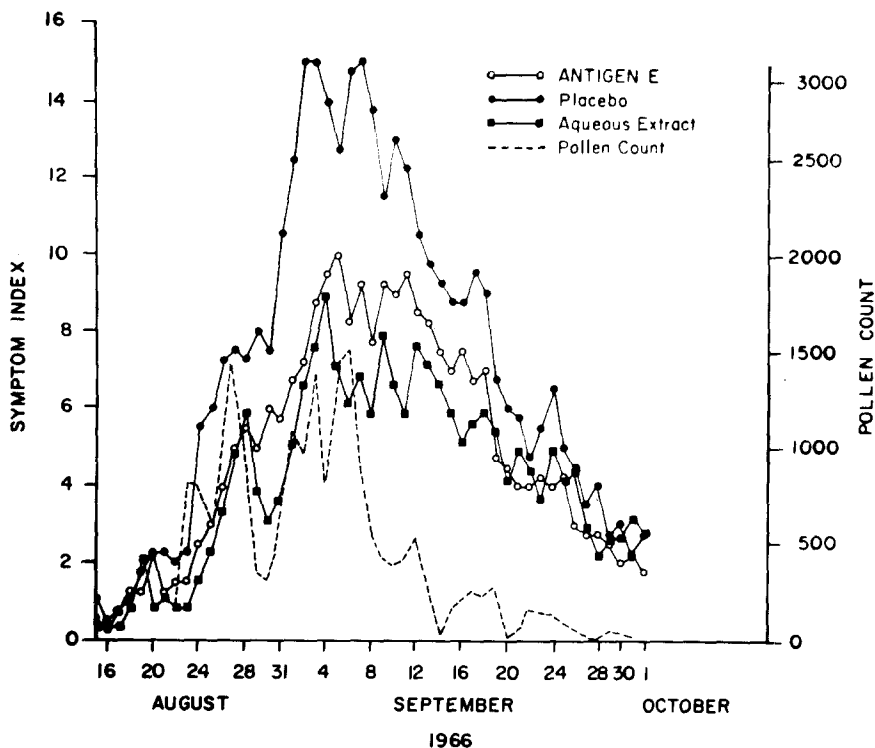
Effect of *moderate* doses of pollen extract (about 2,500 PNU) or antigen E (about 250 μ g) on average daily symptom indexes. (J. ALLERGY 42: 93, 1968.²³)

particularly when pollen exposure was heavy. This result was significant by statistical test at the 2 per cent level.

In 1966, further increase in dosage was attempted, but frequent local and occasional systemic reactions represented a limitation of dosage in the 15 weekly injections planned so that only about a threefold further increase in dosage was achieved, 750 μ g of antigen E and 9,500 PNU of whole extract. Fig. 6 shows that, while results continued to be significant from a statistical standpoint, evidence for further clinical improvement from this modest increase in dosage was not forthcoming.

Fig. 7 shows a scattergram of individual patients' average scores in each of the groups which shows that there is considerable overlap between groups and that, for many patients, this degree of relief is far from a cure.²⁰

In 1967, a related study was performed independently, by Dr. Sadan in our laboratory and a group in the department of pediatrics, comparing whole extract and placebos in children. A larger number of injections was given, and doses in the treated group averaged 22,000 PNU. Fig. 8 shows that these patients experienced an apparently greater degree of relief, such that all but 5

**Fig. 6**

Effect of *higher* doses of pollen extract (about 9,500 PNU) or antigen E (about 750 μ g) on average daily symptom indexes. (J. ALLERGY 42: 93, 1968.²³)

of the children had less severe symptoms than any of the control patients. Whether this result depends on dose or on a greater responsiveness to treatment in children is not clear and will require further study.²¹

One other question raised by these studies was whether the annual repetition of inoculations began, after several years, to excite an anamnestic response and thus provide a degree of improvement not to be accomplished in the first year. In 1968, an entire group of newly selected patients never before treated was divided into matched groups, and in the first season of treatment they were given doses of the sort previously arrived at after 3 years of treatment. These patients received 1 mg. of antigen E or 9,000 PNU of whole extract, and Fig. 9 shows that they did as well as our earlier patients after 3 years' treatment.

Such results indicate that the efficacy of inoculation treatment does depend on dose, at least within broad limits, and that doses under 250 μ g of antigen E or 2,500 PNU of whole extract are essentially ineffective. It appears quite possible that better and better results will be achieved the higher doses go. Patterson and Pruzansky³³ have reported 4 patients administered doses of 40,000 to 210,000 PNU annually with good clinical and immunological results. Van Metre and associates²² have explored the clinical and immunological consequences, in a small number of patients, of annual doses in the neighborhood of

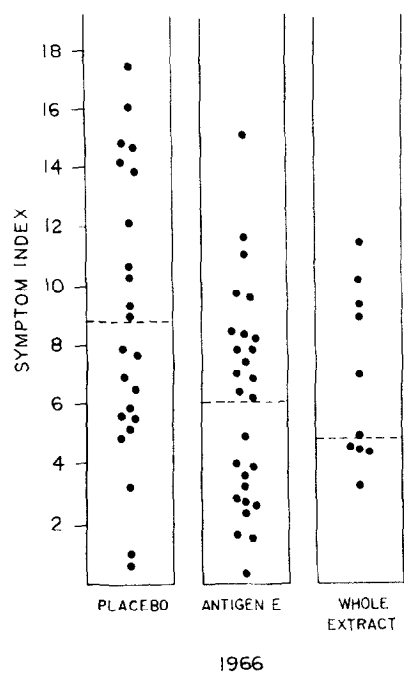


Fig. 7
Seasonal average symptom scores for individual patients. (*Adapted from J. ALLERGY* 42: 93, 1968.²³)

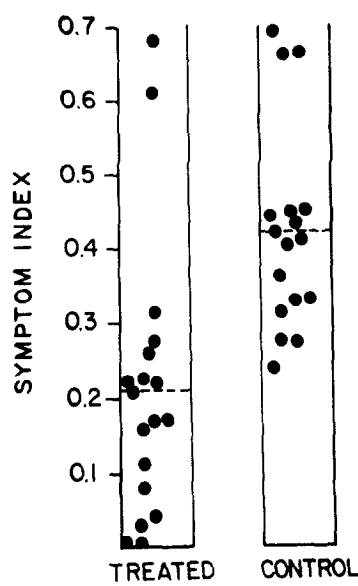
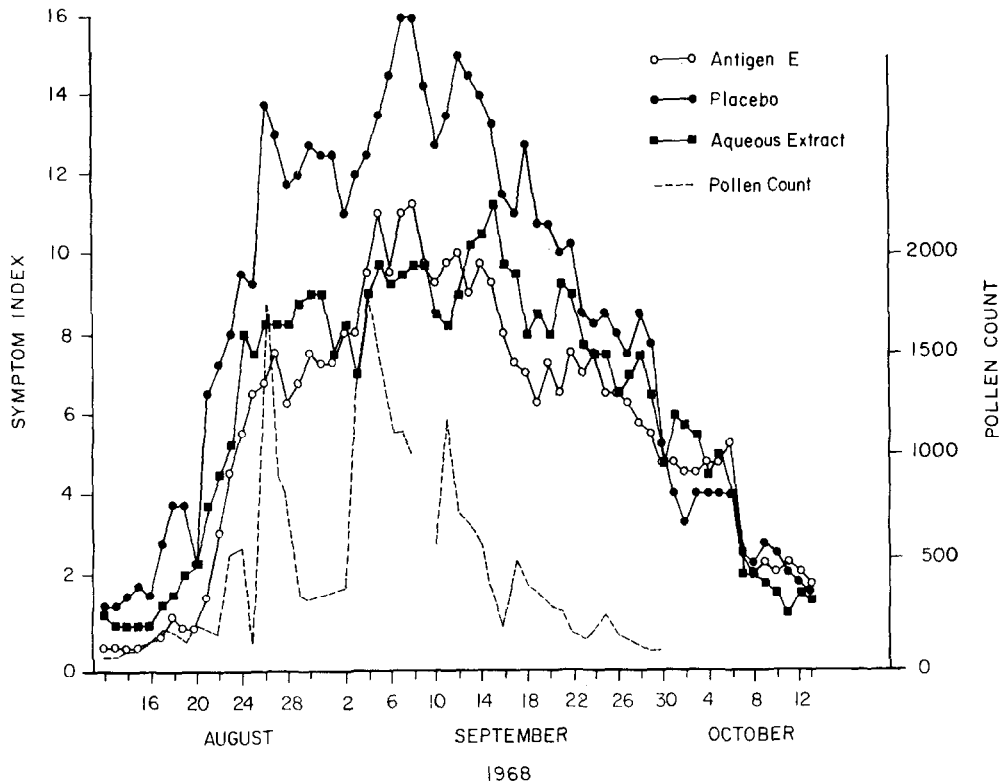


Fig. 8
Adjusted seasonal symptom indexes of children with hay fever. (Sadan, N., et al., New England J. Med. 280: 623, 1969.²¹)

**Fig. 9**

Effect on *one* season desensitization with moderately high dose of pollen extract (about 9,000 PNU) or antigen E (about 1.0 mg.) on average daily symptom indexes.

500,000 PNU and found preliminary evidence of almost complete amelioration of symptoms during ragweed pollen exposure.

Our ability to obtain results with antigen E equivalent to those obtained with whole unmodified extract of ragweed strongly suggests that antigen E is primarily responsible for the therapeutic effect of whole extract. It is unfortunate that our data on dosage with antigen E are not as precise as they may appear. In its purified form, this antigen is quite labile during storage and in early years probably underwent significant deterioration before being administered to patients. By 1967, by storing the antigen in 50 per cent glycerol when in a concentrated solution and making dilutions containing human serum albumin as a stabilizing macromolecule, the stability problem was solved; and dosage data with antigen E became more accurate.

Although the foregoing data provide strong evidence that immunotherapy depends on dose, the practitioner will have a difficult, if not impossible, task to use our data as a guide to dose. This problem revolves around the difficulties of standardization of allergenic extracts. Baer and associates²³ have developed data that show commercial ragweed extracts with the same PNU label vary as much as a hundredfold in both biological activity and in antigen E content. It is

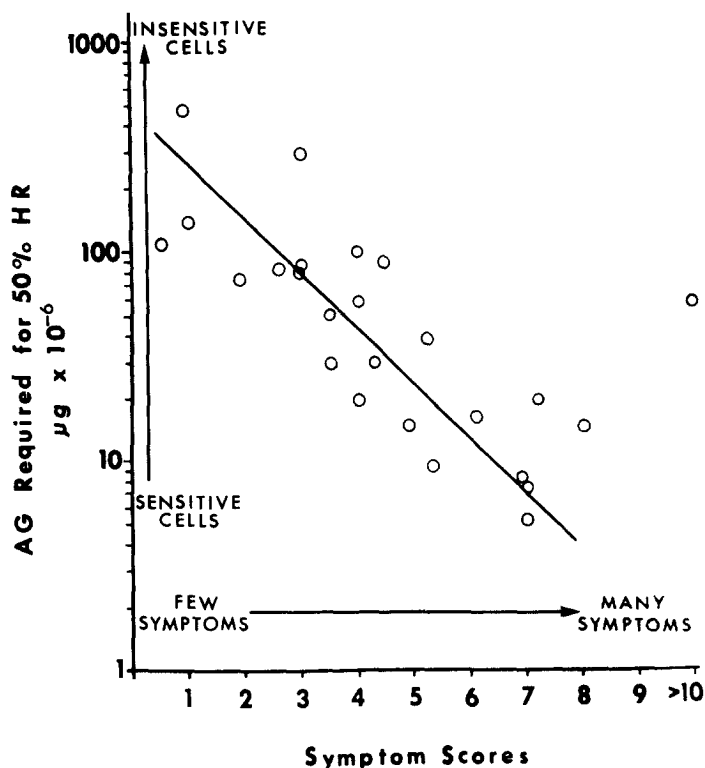


Fig. 10

Correlation between seasonal symptom indexes and cell sensitivity (antigen E required for 50 per cent histamine release). (J. Clin. Invest. 45: 1126, 1966.¹⁶)

clear from such data that PNU determinations have little to do with activity; whereas antigen E content determined by double diffusion in gel does reflect rather accurately the activity of ragweed extracts. I believe that a rational approach to dose will require labeling that gives information that can be related to biological activity. Determination of the content of specific allergens by double diffusion in gel may achieve this goal.

It is clear that a program aimed at settling all the problems of dosage, timing of injections, and therapeutic efficacy of adjuvants or modified allergens by repeated clinical trial will be incredibly laborious and time-consuming work. A variety of considerations, however, point to desensitization being an immunological phenomenon. Hence, a description of those immunological changes which lead to symptom relief might allow one to learn, with relatively few observations in a few patients, whether one procedure or another is likely to produce the desired effect.

Some 5 years ago we began a collaboration with L. M. Lichtenstein, who at that time, with A. G. Osler, had developed improved methods for studying the allergic reaction in vitro by observing allergen-induced histamine release from isolated washed leukocytes from hay fever patients.²⁴ The histamine-release

reaction is mediated by reaginic antibodies fixed to granulocytes containing histamine.²⁵ As the immunologically mediated release of histamine in the tissues of the respiratory mucosa appears to be the principal pathogenetic mechanism producing the symptoms of hay fever, this method allows the *in vitro* observation of the allergic reaction with the use of human materials.

While we have been studying our patients clinically, Lichtenstein has been studying their cellular and immunologic reactions with results that may be important clues to mechanism. These findings have been published elsewhere so I will not review them in detail but will try to give you an idea of the extent to which they explain the mechanism of immunotherapy.

It was quickly established that histamine release *in vitro* could be used to define the severity of the clinical sensitivity. Individual patients vary from one another by as much as 10,000-fold in their cellular sensitivity to a specific allergen. With the use of antigen E for the study of ragweed hay fever, we found that the amount of allergen required to release 50 per cent of the cellular histamine was a stable unchanging parameter from year to year in most untreated patients and could be referred to as cellular sensitivity. Cell sensitivity measurements would predict, with surprising accuracy, the severity of disease a patient suffers during natural exposure. Fig. 10 shows the correlation between measurements of cellular sensitivity and symptom indexes in a group of hay fever patients all living in the Baltimore area. The relationship between cell sensitivity and eventual symptoms has been invaluable in selecting patients for therapeutic

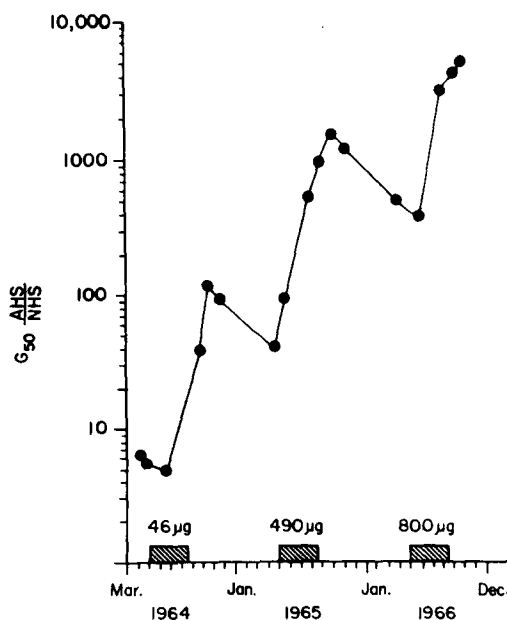


Fig. 11

Changes in "blocking" antibody titer with successive preseasonal courses of immunotherapy with increasing doses of antigen E. (*Am. J. Med.* 44: 514, 1968.¹⁷)

study, in that it is possible to match patients in various treatment groups with a reasonable assurance that, if not treated, they would have similar degrees of disease.¹⁶

Lichtenstein's and Osler's²⁶ method may also be used to measure the development of antibodies after prophylactic inoculations by ability of such antibodies to block the histamine-release reaction. It seems likely that blocking antibodies measured by their ability to block passive transfers in skin are the same as those measured in vitro by blocking cellular histamine release, although it has not been formally proved. The in vitro method, however, is many times more accurate and carries no dangers of transmission of hepatitis. Antibodies so measured are normal IgG antibodies.²⁷ Serial determinations of blocking antibody (Fig. 11) show a rise during and after a preseasonal series of injections followed by a slow decline after a peak has been reached. This relationship in an individual treated with increasing doses over several years further shows that the peak is dose related and that there is a slow decline when injections are discontinued. As with blocking antibodies measured by other methods, peak titers do not bear a simple relationship to the results of treatment. Nevertheless, patients who received the highest doses of extract had the highest titers of antibody and, in general, a better symptomatic response.²⁸ This relationship is explored in Table II which segregates $\frac{1}{3}$ of the patients with the highest antibody responses and $\frac{1}{3}$ of the patients with lowest antibody responses. Those with high titers more regularly have low symptom scores and those with low titers more often have high scores, and the trend meets statistical tests of significance if large enough numbers of patients are examined. The relationship, however, is not direct enough to hope to use antibody titer alone as a guide to dosage. We conclude, as others have, that blocking antibodies measured in vitro will not account for all the results of desensitization although they probably play a role.

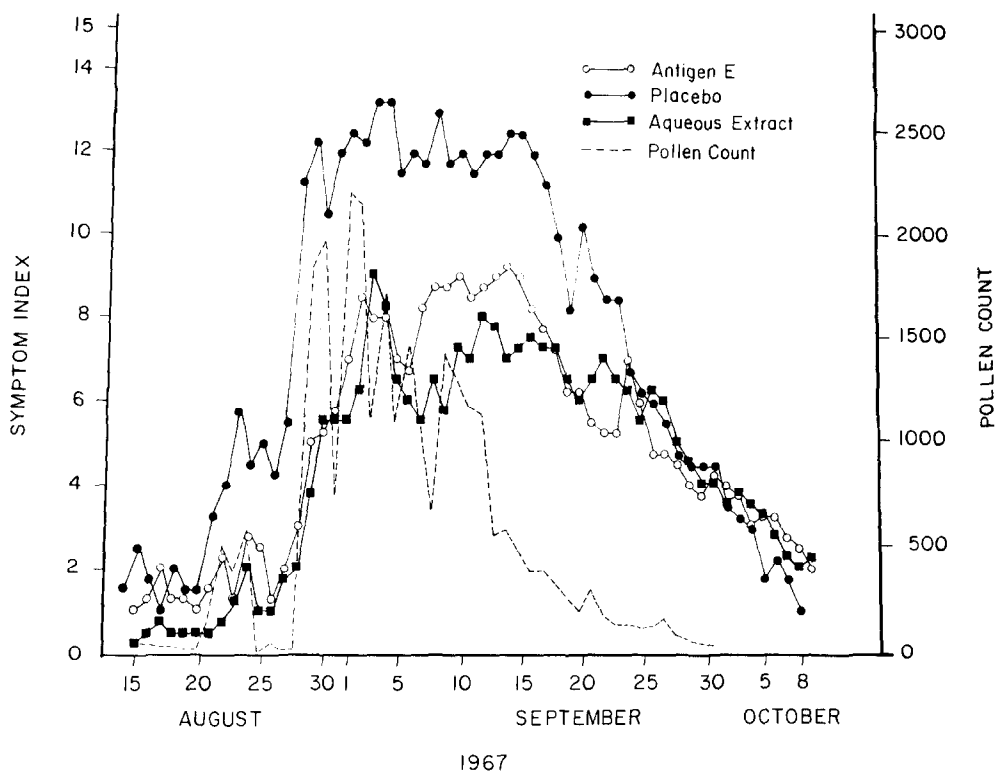
One practical result arises from demonstrating the slow decline of antibody levels following the final preseasonal injection. It seems likely that perennial booster injections could be given at relatively infrequent intervals. W. L. Winkewerder and I²⁹ have been following a group of patients previously treated successfully with preseasonal courses of weekly inoculations once preseasonal treatment was accomplished. Boosters have been given at 6 weekly intervals with only 8 injections annually. Antibody levels have been maintained with freedom from untoward reactions, and Fig. 12 shows that clinical results are good.

Other immunologic parameters have also been under study. Reagins may be measured in vitro by their ability to transfer to normal human leukocytes.

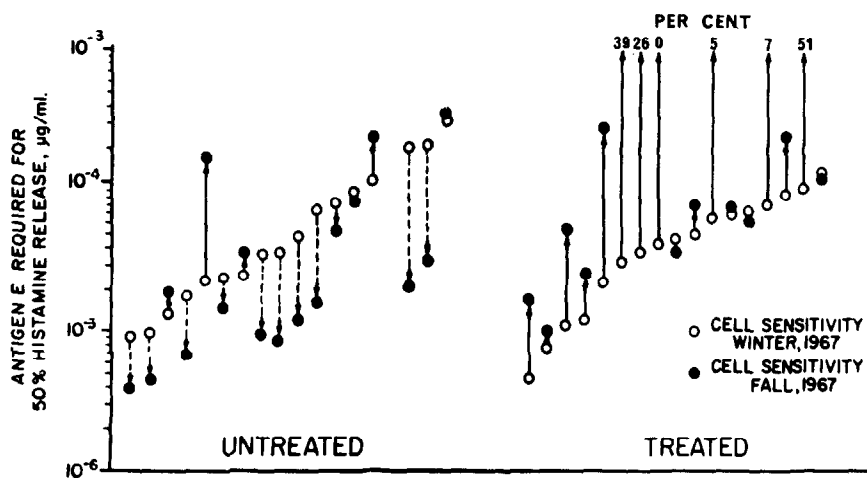
Table II. *Relationship of symptom score and blocking antibody level*

<i>Score</i>	<i>High (> 1,840) antibody</i>	<i>Low (< 130) antibody</i>
> 10	0*	3
5-10	3	3
< 5	4	1
Average score	5.1	8.1

*Number of patients.

**Fig. 12**

Effect of 8 booster doses annually of pollen extract or antigen E (see Table I) on average daily symptom indexes.

**Fig. 13**

Pre- and posttreatment cell sensitivity of children treated with whole ragweed extract (about 22,000 PNU). (Sadan, N., et al., *New England J. Med.* 280: 623, 1969.²¹)

Levy and Osler,³⁰ studying some of our patients, found that there was an annual increase in reagin titer during natural exposure to ragweed pollen. Patients under treatment had less of a rise than untreated patients, and this may account for the eventual decline of reagins noted by Connell and Sherman³¹ after many years of treatment. Nevertheless, changes in reagin titer do not correlate well with the results of treatment in the first year or two, and serum titers of reagin correlate less well with symptomatology than do cell-sensitivity measurements. Our patients continue to have good titers of reaginic antibody, even those with the most gratifying relief of symptoms.

Although VanArsdel and Middleton³² and Pruzansky and Patterson³³ had noted reductions in cell sensitivity in some patients treated for grass or ragweed hay fever, our early studies indicated that cell sensitivity was not changed by the moderate doses employed at that time. However, the study of Sadan and associates in children employed an average dose of about 22,000 PNU, and a considerable number of patients developed a lessening of cell sensitivity (Fig. 13); i.e., greater concentrations of antigen were required for histamine release. A few of these patients had cells which no longer released histamine at any concentration of antigen.²¹

Subsequently, we have observed reduction or loss of cell sensitivity in adults with increasing frequency as we have employed larger doses. Nevertheless, a number of patients have shown evidence of improvement in the form of reduced symptom scores without changes in cell sensitivity. On the other hand, those patients who have considerable reductions in, or complete loss of, cell sensitivity are usually the patients with the lowest symptom scores even though there are inevitably a few exceptions to this rule.²⁹ It is a paradox that reduction or loss of the cells' ability to release histamine occurs even though reagins capable of sensitizing normal cells are still present in the serum with unchanged titer. Loss of cell sensitivity is specific, the cells will no longer react to antigen E of ragweed; but if the patient is sensitive to grass pollen and has not been treated for grass, the cells still react to grass antigen. Reductions in cell sensitivity are not particularly correlated with high titers of blocking antibody, and, of course, these measurements of cell sensitivity are performed on cells washed free of the bulk of serum antibodies.

To summarize, three types of immunological changes are now recognized as a result of injections:

1. Blocking antibodies rise with the peak titers being dependent on total dose. These antibodies decline slowly when inoculations are discontinued.

2. Moderate doses of extract cause little change in the sensitivity of histamine-containing cells, but larger doses frequently cause a specific reduction or loss of cell sensitivity. Cell sensitivity may also return when inoculations are stopped.

3. Reagins are not increased except perhaps transiently by inoculations but tend to decline slowly over months or years during inoculations. This is perhaps because annual mucosal stimulation no longer produces rises in titers.

No one of these results singly accounts for the results of inoculations. As noted in a variety of animal models, close observation of the pathogenesis of

hypersensitivity reactions often reveals the operation of multiple processes. It is hardly surprising, therefore, that immunologic management sets in motion more than one immunologic process. We consider it possible that not all of these processes are yet described, and we would encourage a further look for other immunological consequences of prophylactic injections.

It does seem that desensitization should not be referred to as desensitization. The term was originally used by experimental immunologists to describe the temporary protection achieved in anaphylactically sensitized animals by sublethal injections of the antigen to which they are sensitive. Such protection appears rapidly and involves the exhaustion of mediators or antibodies which confer the reaction. Sensitivity rapidly returns when inoculations are stopped. Prophylactic inoculations in man achieve protection only after months or years; improvement is achieved without exhaustion of mediators or antibodies; and protection is relatively long lasting. Since such protection is almost certainly immunological, we prefer the term "immunotherapy" to describe the method.

Because better methods of symptom reporting make the protection resulting from immunotherapy an observable and quantifiable event and purified allergens provide methods for standardizing the materials used for immunotherapy, it now looks possible to develop a more rational approach to the technique. Newer immunologic methods offer the promise of providing laboratory control for immunotherapy so that treatment can be tailored to the individual's needs.

REFERENCES

1. Noon, L.: Prophylactic inoculation for hay fever, *Lancet* 1: 1572, 1911.
2. Freeman, J.: Further observations on the treatment of hay fever by hypodermic inoculations of pollen vaccine, *Lancet* 2: 814, 1911.
3. Frankland, A. W.: Seasonal hay fever and asthma treated with pollen extracts, *Internat. Arch. Allergy* 6: 45, 1955.
4. Lowell, F. C., and Franklin, W. A.: "Double-blind" study of treatment with aqueous allergenic extracts in cases of allergic rhinitis, *J. ALLERGY* 34: 165, 1963.
5. Lowell, F. C., and Franklin, W. A.: "Double-blind" study of the effectiveness and specificity of injection therapy in ragweed hay fever, *New England J. Med.* 273: 675, 1965.
6. Frankland, A. W., Hughes, W. H., and Gorrill, R. H.: Autogenous bacterial vaccine in treatment of asthma, *Brit. M. J.* 2: 941, 1955.
7. Helander, E.: Bacterial vaccines in treatment of bronchial asthma, *Acta allergol.* 13: 47, 1959.
8. Johnstone, D. E.: Study of value of bacterial vaccines in treatment of bronchial asthma associated with respiratory infections, *Pediatrics* 24: 427, 1959.
9. Barr, S. E., Brown, H., Fuchs, M., Orvis, H., Connor, A., Murray, F. J., and Seltzer, A.: A double-blind study of the effects of bacterial vaccine on infective asthma, *J. ALLERGY* 36: 47, 1965.
10. Aas, K., Berdal, P., Henriksen, S. D., and Gardborg, O.: Bacterial allergy in childhood asthma and the effect of vaccine treatment, *Acta paediat.* 52: 338, 1963.
11. Fontana, V. J., Salanitro, A. S., Wolfe, A. I., and Moreno, F.: Bacterial vaccine and infectious asthma, *J. A. M. A.* 193: 895, 1965.
12. Norman, P. S., Rhyne, M. B., and Mellits, E. D.: The evaluation of agents for the treatment of seasonal respiratory allergies, in Lasagna, L., editor: *International Encyclopedia of Pharmacology and Therapeutics*, Vol. II, Section 6, Oxford, 1966, Pergamon Press, Inc., p. 639.
13. King, T. P., and Norman, P. S.: Isolation studies of allergens from ragweed pollen, *Biochemistry* 1: 709, 1962.
14. King, T. P., Norman, P. S., and Connell, J. T.: Isolation and characterization of allergens from ragweed pollen. II, *Biochemistry* 3: 458, 1964.
15. King, T. P., Norman, P. S., and Lichtenstein, L. M.: Isolation and characterization of allergens from ragweed pollen. IV, *Biochemistry* 6: 1992, 1967.
16. Lichtenstein, L. M., Norman, P. S., Winklenwerder, W. L., and Osler, A. G.: In vitro studies of human ragweed allergy: Changes in cellular and humoral activity associated with specific desensitization, *J. Clin. Invest.* 45: 1126, 1966.

17. Lichtenstein, L. M., Norman, P. S., and Winkenwerder, W. L.: Clinical and in vitro studies on the role of immunotherapy in ragweed hay fever, *Am. J. Med.* **44**: 514, 1968.
18. Chai, H., Purcell, K., Brady, K., and Falliers, C. J.: Therapeutic and investigational evaluation of asthmatic children, *J. ALLERGY* **41**: 23, 1968.
19. Rhyne, M. B.: Specific immunotherapy: Clinical aspects of measurement of response by controlled studies, *in* Rhyne, M. B., editor: *Childhood hay fever*, Springfield, Ill., Charles C Thomas, Publisher. In press.
20. Norman, P. S., Winkenwerder, W. L., and Lichtenstein, L. M.: Immunotherapy of hay fever with ragweed antigen E: Comparisons with whole pollen extract and placebo, *J. ALLERGY* **42**: 93, 1968.
21. Sadan, N., Rhyne, M. B., Mellits, E. D., Goldstein, E. A., Levy, D. A., and Lichtenstein, L. M.: Immunotherapy of pollinosis in children. Investigation of the immunological basis of clinical improvement, *New England J. Med.* **280**: 623, 1969.
22. Van Metre, Jr., T. E., Lichtenstein, L. M., and Turk, A.: Hay fever symptoms, blocking antibody levels, and leukocyte histamine release in patients receiving very high dosage immunotherapy with ragweed pollen extract, *J. ALLERGY* **43**: 180, 1969. (Abst.)
23. Baer, H., Godfrey, H., Maloney, C. J., Norman, P. S., and Lichtenstein, L. M.: The potency and antigen E content of commercially prepared ragweed extracts, *J. ALLERGY* **43**: 181, 1969. (Abst.)
24. Lichtenstein, L. M., and Osler, A. G.: Comparative studies of histamine release and potassium efflux from human leukocytes, *Proc. Soc. Exper. Biol. & Med.* **121**: 808, 1966.
25. Levy, D. A., and Osler, A. G.: Studies on the mechanisms of hypersensitivity phenomena. XIV. Passive sensitization in vitro of human leukocytes to ragweed pollen antigen, *J. Immunol.* **97**: 203, 1966.
26. Lichtenstein, L. M., and Osler, A. G.: Studies on the mechanisms of hypersensitivity phenomena. XII. An in vitro study of the reaction between ragweed pollen antigen, allergic human serum and ragweed-sensitive human leukocytes, *J. Immunol.* **96**: 169, 1966.
27. Lichtenstein, L. M., Holtzman, N. A., and Burnett, L. S.: A quantitative in vitro study of the chromatographic distribution and immunoglobulin characteristics of human blocking antibody, *J. Immunol.* **101**: 317, 1968.
28. Lichtenstein, L. M., Norman, P. S., and Winkenwerder, W. L.: Clinical and in vitro studies on the role of immunotherapy in ragweed hay fever, *Am. J. Med.* **44**: 514, 1968.
29. Norman, P. S., Winkenwerder, W. L., and Lichtenstein, L. M.: Unpublished data.
30. Levy, D. A., and Osler, A. G.: Studies on the mechanism of hypersensitivity phenomena. XVI. In vitro assays of reaginic activity in human sera: Effect of therapeutic immunization on seasonal titer changes, *J. Immunol.* **99**: 1068, 1967.
31. Connell, J. T., and Sherman, W. B.: Skin-sensitizing antibody titer. III. Relationship of the skin-sensitizing antibody titer to the intracutaneous skin test, to the tolerance of injections of antigens, and to the effects of prolonged treatment with antigen, *J. ALLERGY* **35**: 169, 1964.
32. VanArsdel, P. P., and Middleton, D.: The effect of hyposensitization on the in vitro histamine release by specific antigen, *J. ALLERGY* **32**: 348, 1961.
33. Pruzansky, J. J., and Patterson, R.: Histamine release from leukocytes of hypersensitive individuals. II. Reduced sensitivity of leukocytes after injection therapy, *J. ALLERGY* **39**: 44, 1967.