

Immediate hypersensitivity reactions to food antigens

Detection of human IgE antibodies in vitro and exploration of immunologic mechanisms using the rat mast cell system

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Sera from 25 subjects with clear-cut clinical histories of immediate-type hypersensitivity reactions to foods (fish, peanut, chocolate, milk, egg, walnut, and shellfish) were studied with the use of the in vitro rat mast cell system in a series of controlled experiments. Significant rat mast cell alteration was produced by the sera in presence of either the suspected antigen or a preparation of monospecific anti-IgE. No degranulation occurred with unrelated food antigens or with anti-IgG, IgA, or IgM preparations in equivalent concentrations. The rat mast cell results correlated well with the clinical histories and the skin tests, except in 3 patients in whom significant degranulation occurred despite negative skin tests. In one patient no degranulation occurred despite a positive skin test. Specific degranulation was shown to occur as a result of either passive sensitization with reaginic serum or of the possible formation of antigen-antibody complexes. Complement seems to play no role in either mechanism. Heating the reaginic sera at 56° C. for 2 hours to abolish the cell-fixing properties of IgE resulted in the loss of the degranulating property of the sera in passive sensitization experiments. This test is proposed as a useful adjunct in the diagnosis of immediate hypersensitivity reactions to foods.

The diagnosis of immediate hypersensitivity reactions to foods usually depends on the clinical history, the relief of symptoms upon the elimination of foods, and aggravation upon reintroduction of a single food into the diet. The clinical history, however, is subjective, and the elimination and challenge procedures are often cumbersome for the patient. Moreover, immediate wheal and flare skin reactions to suspected food antigens are notorious for their unreliability. Thus, numerous in vitro procedures have been proposed as aids in the diagnosis of food allergy.^{7-13, 15-20} Most of these are poor indicators of immediate hypersensitivity in that they fail to detect IgE antibody. With the availability of an in vitro model to detect specific IgE antibody to penicillin and grass pollens in our laboratory²¹ and others,²⁵ we were prompted to evaluate a group of patients with immediate hypersensitivity reactions to a variety of common food antigens and to further explore the immunologic mechanisms involved in this in vitro system.

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TABLE I. Summary of findings in patients with immediate hypersensitivity reactions to foods—correlation with result of skin test and RMCD test

Antigen	No. subject	Symptoms	Skin test			RMCD test	
			+	—	N.D.	+	—
Milk	4	Urticaria, rhinitis, angioedema, asthma, shock	4	0		4	0
Egg	6	Urticaria, shock, eczema	5	0	1	6	0
Shellfish	8	Urticaria, angioedema	5	1	2	8	0
Fish	9	Urticaria, angioedema	6	1	2	9	0
Walnut	7	Urticaria, angioedema	7	0		7	0
Peanut	4	Urticaria, angioedema	3	1		4	0
Chocolate	3	Urticaria	3	0		2	1

N.D. = not done.

MATERIALS AND METHODS

Selection of patients

The study group consisted of 25 patients ranging in age from 13 months to 29 years. This group of patients had clear-cut clinical histories of immediate hypersensitivity reactions to a variety of food antigens either with or without positive skin tests.* As can be seen in Table I, there was a total of 41 reactions implicated among the 25 subjects. Clinical symptoms ranged from urticaria to anaphylactic shock. They were of an immediate or explosive nature, occurring within minutes of the food ingestion. Serum samples were drawn 4 to 20 weeks after the clinical reactions.

Rat mast cell degranulation technique (RMCD)

The RMCD technique as performed in this laboratory has been described in detail elsewhere.¹⁴ Briefly, mast cells collected from the peritoneal cavities of Wistar strain albino rats were suspended in medium 199 containing ethylenediaminetetraacetic acid (EDTA). After washing, the cells were placed in an ice bath at 4° C. Equal aliquots of subjects' sera, rat mast cells, and specific antigen (fish, peanut, chocolate, milk, egg, shellfish, walnut†) were mixed in a siliconized tube and incubated for 3 minutes at 37° C. The mixture was then placed on a siliconized slide and covered with a coverslip previously dyed with neutral red (Fig. 1), and 100 cells were counted by an unbiased observer. A positive test in this laboratory is one that produces more than 2.5 times the per cent degranulation seen in control situations.

Experimental design

The basic design we have used in the RMCD experiments is as follows: (1) rat mast cells (RMC) + sensitive serum (SS) + antigen (Ag); (2) RMC + SS + anti-IgE, anti-IgG, anti-IgA, or anti-IgM; (3) RMC + SS; (4) RMC + Ag or anti-IgE, anti-IgG, anti-IgA, anti-IgM; (5) RMC + nonsensitive serum (NSS) ± Ag or anti-IgE, anti-IgG, anti-IgA, anti-IgM.

Situation 1 depicts the test as ordinarily performed with both the antigen and antibody present. In *Situation 2*, antihuman IgG, IgA, IgM,‡ or IgE§ may be substituted for the antigen. To eliminate inclusion of values for nonspecific degranulation caused by the subjects'

*Hollister-Steir antigens in concentration of 1:20 for scratch testing.

†Hollister-Steir antigens in concentrations of 10 µg per milliliter.

‡Hyland Laboratory, Costa Mesa, Calif.

§Kindly provided by Dr. Kimishige Ishizaka. The rabbit antiserum was obtained by immunization of rabbits with IgE myeloma protein. The antibody content was measured by precipitin reaction with the Fc fragments of the myeloma protein.

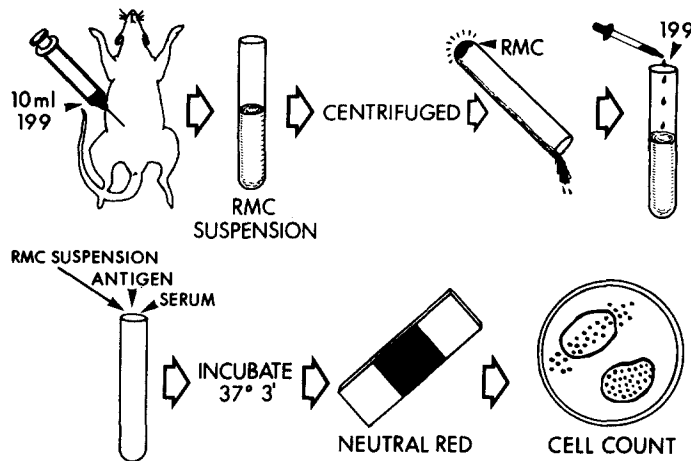


FIG. 1. Rat mast cell degranulation procedure.

sera alone or the antigen alone, controls are prepared in each experiment, as shown in *Situations 3 and 4*. As additional controls, sera of nonatopic, nonsensitive subjects (NSS), usually neonates, are included both with and without each antigen or antihuman IgG, IgA, IgM, or IgE (*Situation 5*).

Immunological mechanisms

In an attempt to elucidate the immunologic mechanisms involved in mast cell degranulation, the following experiments were performed:

Preformed antigen-antibody complexes. Sera from patients with sensitivity to shrimp and egg albumin were incubated with the specific antigen for 3 minutes at 37° C. After it was added to the rat mast cell suspension, the mixture was incubated an additional 3 minutes at 37° C. The cells were then examined in the usual manner.

In vitro passive sensitization of rat mast cells with reaginic serum. Aliquots of rat mast cell suspension were incubated with serum from a milk-sensitive subject or with serum from a nonatopic individual. Following three washings, the passively sensitized cells were challenged with milk antigen as well as anti-IgG, -IgA, -IgM, and -IgE.

Role of complement in rat mast cell degranulation. The RMCD technique was performed in the usual manner with serum from a shrimp-sensitive subject, which had been heated at 56° C. for 30 minutes to inactivate the complement content. A control experiment was then performed in which the heated sensitive serum (56° C. × 30 minutes) was mixed with an equal volume of a fresh, nonheated, nonatopic serum as a source of complement.* Furthermore, the possible participation of complement in the system was explored by preparing preformed antigen-antibody complexes consisting of heated sensitive serum (56° C. × 30 minutes) and specific antigen.

Role of reaginic antibody (IgE) in the RMCD system. An aliquot of serum from the same subject as used in the preceding experiments was heated at 56° C. for 120 minutes (to inactivate the IgE content). Rat mast cells were then incubated with this heated serum and challenged with the specific antigen or anti-IgE.

RESULTS

Figs. 2 and 3 show the results obtained when rat mast cells were incubated with sera of milk-, egg-, shellfish-, walnut-, fish-, peanut-, and chocolate-sensitive

*The total hemolytic complement activity was determined in this serum and found to be within normal limits.

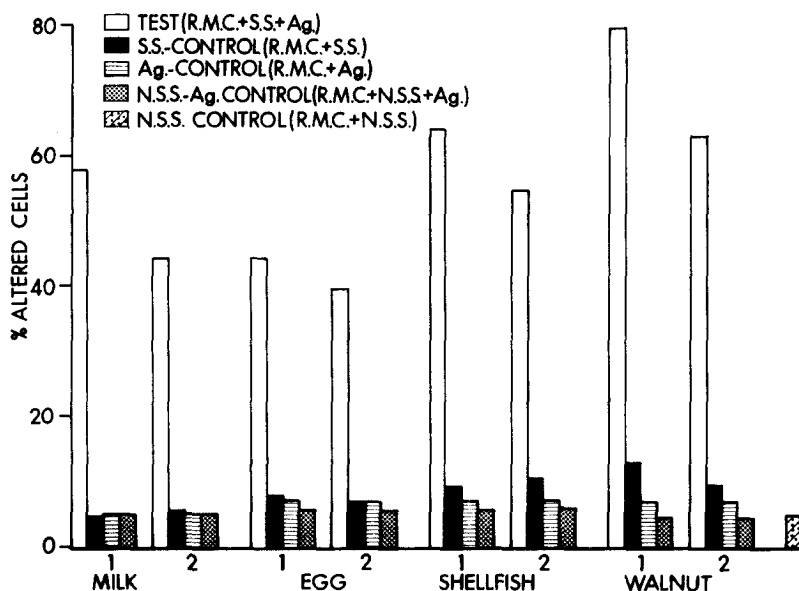


FIG. 2. Mast cells incubated with food-sensitive sera and challenged with specific antigens.

subjects and the specific antigens. The positive results obtained in the test situations are readily seen when compared to the values for the subject's own serum control, nonatopic controls, or the antigen control. No significant mast cell degranulation was observed when mast cells were incubated with sera of food-sensitive subjects and challenged with unrelated food antigens to which they were not sensitive by clinical history.

Fig. 4 demonstrates the class of immunoglobulins involved in mast cell degranulation; when mast cells were incubated with food sensitive sera and challenged with antihuman IgG, anti-IgA, anti-IgM, and anti-IgE, only the anti-IgE caused significant mast cell alteration, indicating that IgE antibodies are being detected by the technique. Similar results using anti-IgE have been obtained with a larger number of food-sensitive sera and not illustrated in Fig. 4.

Since sensitive serum, antigen, and mast cells are added simultaneously in the test procedure, it is possible that mast cell degranulation can be caused by either the action of IgE antibody-antigen complexes, by antigen combining with IgE fixed upon the mast cell membrane, or possibly as a result of both of these mechanisms. When antigen and reaginic serum were incubated at 37° C. for 3 minutes and then added to rat mast cells, results were almost identical to those obtained in the usual manner, i.e., rat mast cells, antigen, and sera simultaneously admixed. In addition, Fig. 5 illustrates the results of passive sensitization and washing experiments. It is apparent that a significant degree of mast cell alteration is caused after passive sensitization with milk-sensitive serum and washings followed by challenge with either milk or anti-IgE, while values for the nonatopic serum (NSS) remain at low levels for both milk and anti-IgE.

Fig. 6 demonstrates that complement does not play a role in the mast cell degranulation process, as atopic serum heated at 56° C. for 30 minutes to in-

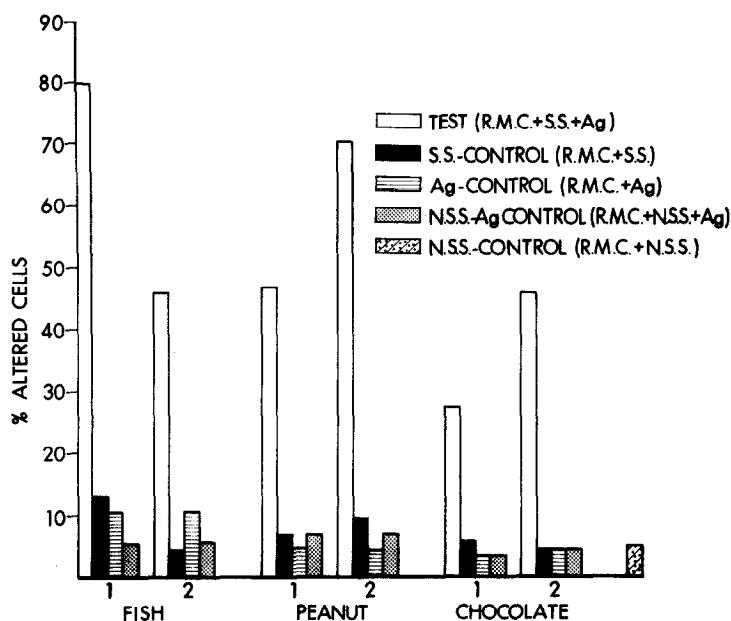


FIG. 3. Mast cells incubated with food-sensitive sera and challenged with specific antigens ($10 \mu\text{g}$ protein nitrogen per milliliter).

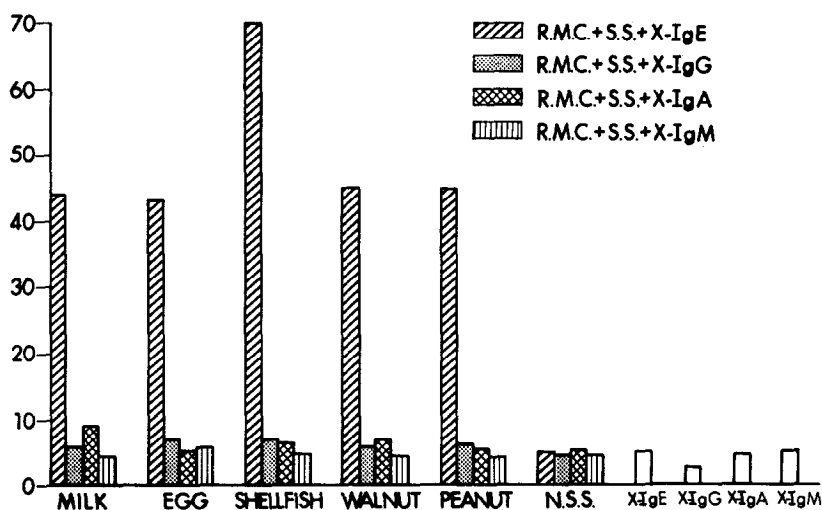


FIG. 4. Mast cells incubated with food-sensitive sera and challenged with anti-IgE, -G, -A, -M ($0.3 \mu\text{g}$ antibody nitrogen per milliliter).

activate the complement content does not lose its degranulating ability (*E, F, G, H*). Furthermore, when the same serum heated under the same conditions ($56^\circ \text{C.} \times 30$ minutes) is used in preformed antigen-antibody complexes, the latter also result in significant degranulation of the mast cells (*I, J*). However, the same serum when heated at 56°C. for 120 minutes to abolish the cell-

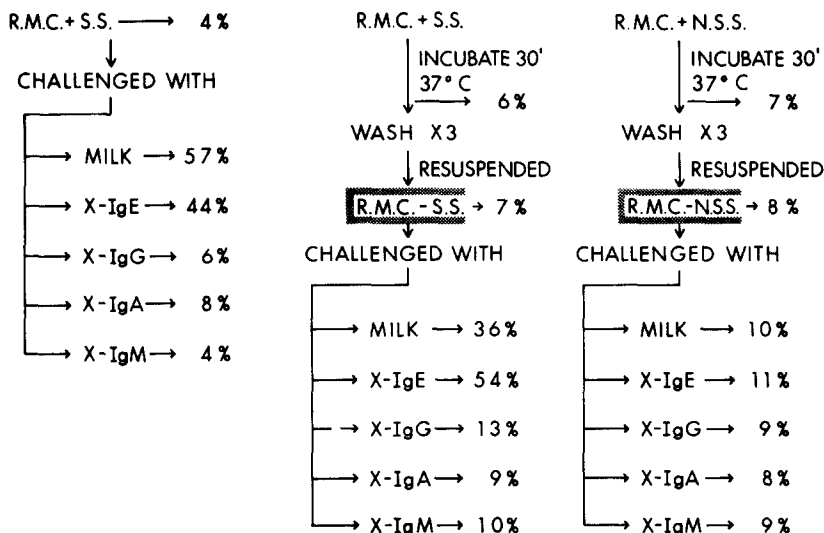


FIG. 5. Mast cells passively sensitized with antibody and challenged with specific antigen (10 μ g per milliliter) or anti-IgE, -G, -A, -M (0.3 μ g antibody nitrogen per milliliter).

fixing properties of IgE does lose its degranulating ability (*K*, *L*). The addition of fresh, unheated, complement-containing, nonatopic serum to sensitive serum that had been heat inactivated for 2 hours does not restore the degranulating properties (*M*). This would be expected, since the IgE-fixing properties of the sensitive serum had been lost by such heating. This observation provides further support for the capacity of IgE to fix to rat mast cells.

DISCUSSION

Numerous in vivo procedures for the diagnosis of food allergy have been proposed: elimination and challenge with suspected antigen,¹ skin tests and passive transfer, accelerated pulse test,² leukopenic index,³ counting of mast cells in nasal secretions,⁴ inhalation challenge with suspected food antigen,⁵ and the skin window technique.⁶ The shortcomings of these procedures have been reviewed in detail.⁷ Several investigators have applied a variety of in vitro immunologic techniques to the diagnosis and elucidation of the mechanisms involved in food allergy. Techniques such as tanned red cell hemagglutination,^{8, 9} bis-diazotized benzidine (BDB) hemagglutination,¹⁰ complement fixation,^{1, 11} gel diffusion,¹² and the Farr test¹³ have been proposed, but they all are poor indices of immediate hypersensitivity, in as much as they fail to detect IgE antibody to the suspected food antigens.

The human basophil leukocyte histamine release system, with the use of whole extracts of food antigens, has been found inadequate in detecting immediate hypersensitivity to foods.⁷ The indirect rabbit basophil degranulation test, an in vitro technique, was described by Shelley¹⁵ in 1962 and since then has been used by other workers such as Schwartz and associates,¹⁶ Dreyfuss and associates,¹⁷ and Katz and co-workers¹⁸ in the detection of immediate hypersensitivity

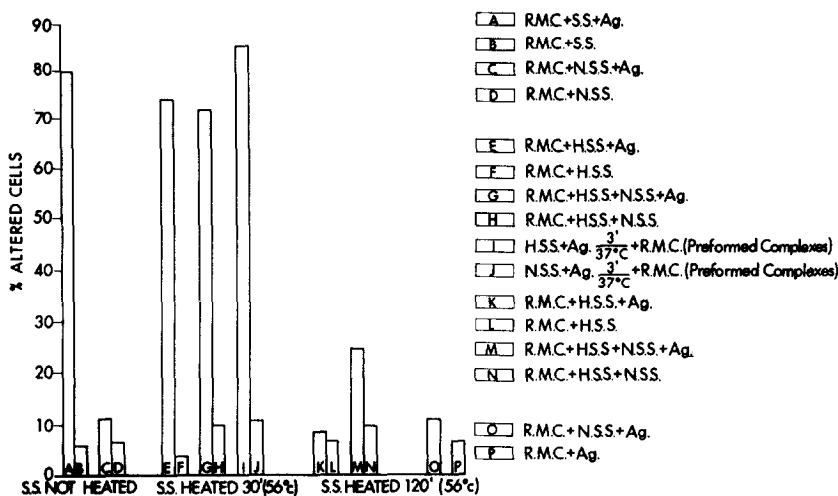


FIG. 6. Mast cells incubated with shrimp-sensitive serum heated at 56°C . for 30 and 120 minutes and challenged with shrimp antigen ($10\text{ }\mu\text{g}$ per milliliter).

reactions to a variety of drugs, pollens, and foods. In 1965, Kravis¹⁹ reported successful use of the rabbit basophil technique in detection of immediate hypersensitivity to egg in a small group of sensitive subjects but failed to obtain positive results in milk-sensitive subjects with immediate wheal and flare reaction to milk antigens.

Other authors²⁰ have not found the basophil degranulation test to be a satisfactory method for the diagnosis of food allergy. In 1965, Schwartz and co-workers,¹⁶ noting the wide variation in the suitability of individual rabbit basophils using the Shelley procedure, introduced an indirect rat mast cell degranulation technique and reported favorable results in a small number of patients with hypersensitivity to drugs, pollen, and foods.

We²¹ have previously reported on a modification of the rat mast cell degranulation technique in the detection of immediate hypersensitivity reactions to penicillin and grass pollen. We also established that these reactions are mediated by human IgE, as evidenced by specific degranulation of the mast cells by either antigen or a monospecific rabbit anti-IgE. Our results using foods as antigens in this in vitro system corroborate our earlier results with pollen,¹⁴ and penicillin.²⁶ It is interesting to note that all patients with clinical immediate-type hypersensitivity reactions to food and positive wheal and flare skin reactions to food antigens had a positive RMCD, with the exception of one patient with a clinical reaction and a positive skin test to chocolate antigen who gave a negative RMCD. This patient is a 13-month-old infant who had urticaria shortly following the "first ingestion" of chocolate. On the other hand, 3 patients with clinical histories of immediate hypersensitivity reactions following ingestion of a food (one shellfish, one peanut, and one fish) and a negative skin test to the food antigen had a positive RMCD, suggesting that this in vitro system may be a better indicator of immediate hypersensitivity than the skin test in some cases. That patients with a clinical reaction and positive skin tests to one food antigen

had negative RMCD tests with unrelated antigens gives evidence of the antigenic specificity of the in vitro procedure. It is interesting to mention in this respect that cross-reactivity was demonstrated between fish and shellfish with the use of the rat mast cell test,* as has been shown with other systems (skin test and clinically).

The incubation and washing experiment provides evidence for passive sensitization of rat mast cells by human reaginic IgE antibody, as shown by degranulation when either antigen or anti-IgE were subsequently added. However, this seems not to be the sole mechanism of degranulation, since mixtures of reaginic serum and specific antigen could lead to the degranulation of subsequently added rat mast cells. This observation suggests that antigen-antibody complexes effect rat mast cell degranulation under these circumstances, but it is difficult to exclude sensitization of the cells by dissociated or unreacted antibody. The role of complement in degranulation by either passive sensitization or antigen-antibody complexes seems to have been reasonably ruled out as shown by the heating experiment at 56° C. for 30 minutes. This is in accord with other in vivo and in vitro systems involving human IgE antibody, in which complement has been shown not to be implicated.²²

The negative results using serum heated for 2 hours at 56° C. provide further support for the fixation of human IgE on rat mast cells. Other evidence is the recent demonstration of a human myeloma E preparation on rat mast cells with the use of direct immunofluorescence²³ and radioactively labeled IgE.²⁴

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*Unpublished observation.

REFERENCES

- 1 Rowe, A. H., Rowe, A. H., Jr.: *Bronchial asthma, its diagnosis and treatment*, Springfield, Ill., 1963, Charles C Thomas, Publisher, p. 44.
- 2 Coca, A. F.: *Familial non-reaginic food allergy*, Springfield, Ill., 1943, Charles C Thomas, Publisher, p. 96.
- 3 Vaughn, W. T.: Leukopenic index as a diagnostic method in the study of food allergy, *J. Lab. Clin. Med.* **21**: 1278, 1936.
- 4 Shioda, H., and Mishima, T.: The significance of mast cells in nasal smears from patients with food allergy, *J. ALLERGY* **37**: 321, 1966.
- 5 Popa, V., Teculescu, D., Stănescu, D., and Gavrilescu, N.: The value of inhalation tests in perennial bronchial asthma, *J. ALLERGY* **42**: 130, 1968.
- 6 Bullock, J. D., Bodenbender, M. T., Kontras, S. B., and Miller, C. E.: Skin window as a diagnostic tool in food allergy, *Ann. Allergy* **26**: 177, 1968.
- 7 Dockhorn, R. J.: Histamine release from the leukocytes of food-allergic individuals using whole food extract antigens, *Ann. Allergy* **27**: 409, 1969.
- 8 Boyden, S. V.: Absorption of protein on erythrocytes treated with tannic acid and subsequent hemmagglutination by antiprotein sera, *J. Exp. Med.* **93**: 107, 1951.
- 9 Sheldon, J. M., Lovell, R. G., and Mathews, K. P.: *A manual of clinical allergy*, Philadelphia, 1967, W. B. Saunders Company, p. 216.
- 10 Stavitsky, A. B., and Arquilla, E. R.: Micro methods for study of proteins and antibodies, *Immunology* **74**: 306, 1955.
- 11 Mathews, T. S., and Soothill, J. F.: Complement activation after milk feeding in children with cow's milk allergy, *Lancet* **2**: 893, 1970.
- 12 Peterson, R. D. A., and Good, R. A.: Antibodies to cow's milk protein—their presence and significance, *Pediatrics* **31**: 209, 1963.

- 13 Barrick, R. H., and Farr, R. S.: The increased incidence of circulating anti-beef albumin in the sera of allergic persons and some comments regarding the possible significance of the occurrence, *J. ALLERGY* **36**: 374, 1965.
- 14 Korotzer, J. L., Haddad, Z. H., and Lopapa, A.: Detection of human IgE antibody by a modified rat mast cell degranulation technique, *Immunology* **20**: 545, 1971.
- 15 Shelley, W. B.: New serological test for allergy in man, *Nature* **195**: 1181, 1962.
- 16 Schwartz, J., Klopstock, A., Skert-Duvdevani, P., and Honig, S.: Detection of hypersensitivity by indirect rat mast cell degranulation, *Int. Arch. Allergy* **26**: 333, 1965.
- 17 Dreyfuss, F., Hellman, M., Klopstock, A., Schwartz, J., and Vardinan, N.: The basophil degranulation test in patients hypersensitive to drugs, *Acta Allergol.* **21**: 17, 1965.
- 18 Katz, H., Gill, K., Baxter, D., and Maschella, S.: Indirect basophil degranulation test in penicillin allergy, *J. A. M. A.* **188**: 351, 1964.
- 19 Kravis, L. P.: Observations on the basophils of the blood in eczema, *in* Holt, L. E. Jr., editor: Conference on infantile eczema, *J. Pediatr.* **66**: (No. 1, part 2) 218, 1965.
- 20 Reynolds, R. D., and Smith, R. E.: The indirect basophil degranulation test, *Ann. Allergy* **25**: 318, 1967.
- 21 Korotzer, J. L., and Haddad, Z. H.: In vitro detection of human IgE-mediated immediate hypersensitivity reactions to pollens and penicillin(s) by a modified rat mast cell degranulation (RMCD) technique, *J. ALLERGY* **45**: 126, 1970. (Abst.)
- 22 Ishizaka, K., and Ishizaka, T.: The significance of immunoglobulin E in reagenic hypersensitivity, *Ann. Allergy* **28**: 189, 1970.
- 23 Haddad, Z. H., Lopapa, A. F., and Korotzer, J.: Direct demonstration of human immunoglobulin E (IgE) on rat mast cells, after passive sensitization in vitro, *J. ALLERGY* **47**: 88, 1971. (Abst.)
- 24 Perelmutter, L., Liakopoulou, A., Larose, C., and Phills, J. A.: A study on the sensitization of rat mast cells with human IgE-type reagins, *J. ALLERGY* **47**: 88, 1971. (Abst.)
- 25 Perelmutter, L., and Khera, K.: A study on the detection of human reagins with rat peritoneal mast cells, *Int. Arch. Allergy Appl. Immunol.* **39**: 27, 1970.
- 26 Haddad, Z. H., and Korotzer, J. L.: In vitro studies on the mechanism of penicillin and ampicillin drug reactions, *Int. Arch. Allergy Appl. Immunol.* **40**: 72, 1971.