

Immunochemical identification of the allergens in egg white

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The allergenic activities of egg white components were tested by radioimmuno-electrophoresis (RIEP) and RAST with highly purified proteins. Sera from 33 patients who reacted to egg ingestion were used for both methods. Ovalbumin and ovomucoid were shown to be strong allergens by both techniques. Patients varied in relative reactivity to these two allergens. Conalbumin was found to be an important allergen by RAST, but its activity was underestimated by RIEP. Lysozyme was only a weak allergen. Two sera reacted by RIEP with other neutral egg white components. Both ovalbumin and ovomucoid were found in hard- and soft-boiled egg white in immunologically recognizable form, and small amounts of conalbumin were also detectable in cooked egg. There was no significant association of pattern of reactivity with age, sex, or presence of eczema. The sera of patients who experienced gastrointestinal symptoms from ingestion of egg white were less reactive with ovomucoid than sera from patients with other symptoms. (J ALLERGY CLIN IMMUNOL 71:481, 1983.)

Egg white is a common cause of allergic reactions to foods in both adults and children.¹ Skin-test reactivity and clinical reactions to egg ingestion are particularly common in children with atopic eczema.^{2, 3} The specificities of IgE antibodies against egg white have been studied by skin tests^{1, 4, 5} and by the RAST.^{3, 6, 7} Using skin testing as a measure of allergenicity, Bleumink and Young⁵ reported that ovomucoid is the major skin-reactive allergen in hen's egg white and that other proteins, including ovalbumin, had much less skin reactivity. However, other investigators using skin testing¹ and RAST⁷ have found that ovalbumin gives stronger reactions than ovomucoid in the majority of egg white-allergic patients.

Egg white is known to contain six major proteins—ovalbumin, ovomucoid (trypsin inhibitor), conalbumin (ovotransferrin), ovomacroglobulin (line 18), ovomucin, and lysozyme—and several trace proteins, including avidin, ribonuclease, ovomucoid, flavoprotein, and catalase.⁸ All of the major components can be recognized by immunoelectrophoresis using suitable rabbit antisera against egg white. In this

study we used RIEP, incubating immunoelectrophoresis strips prepared with egg white and rabbit antiserum with sera from egg-allergic individuals and then with ¹²⁵I-labeled anti-human IgE to identify the specific allergens recognized by each patient. In addition, four highly purified egg white proteins were evaluated by RAST for IgE antibody binding activity.

MATERIALS AND METHODS

Materials

Egg white was obtained from fresh eggs bought locally. Hard-boiled eggs were cooked at 100° C for 20 min and soft-boiled eggs were cooked for 3 min. Component proteins from hen's eggs were purchased from Sigma Chemical Co., St. Louis, Mo. The radiolabeled anti-human IgE was identical to that used for RAST.⁹ Rabbit antisera were produced by immunization in Freund's complete adjuvant according to a schedule we have previously described.¹⁰ It was necessary to supplement the raw egg white immunizations with lysozyme and ovomucoid immunizations to obtain suitable antisera for immunoelectrophoresis. The antisera were tested by crossed immunoelectrophoresis against egg white. A pool was prepared that had high titers against all four major components; this pool was used in all experiments.

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Abbreviations used

RAST:	Radioallergosorbent test
RIEP:	Radioimmuno-electrophoresis
HPLC:	High-performance liquid chromatography

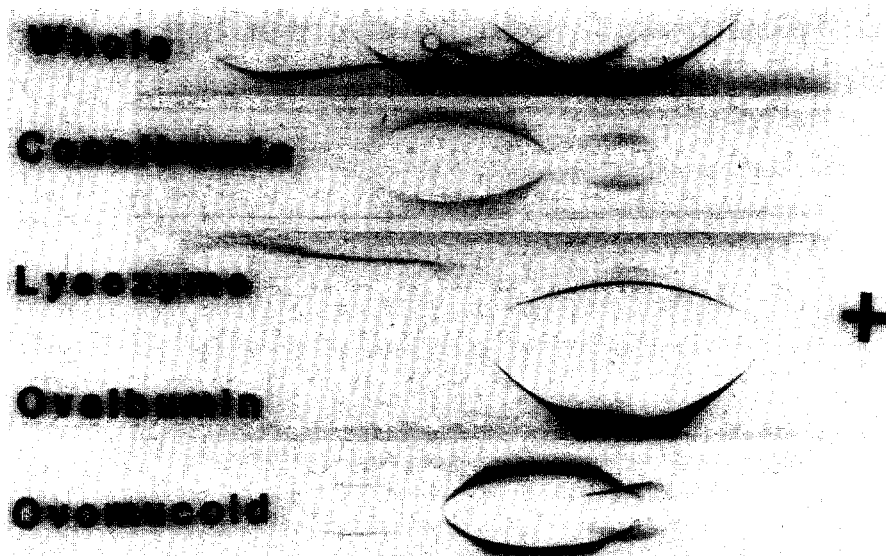


FIG. 1. Immunoelectrophoresis of whole raw egg white, conalbumin, lysozyme, ovalbumin, and ovomucoid against rabbit anti-egg white serum. The proteins are all commercial preparations before HPLC.

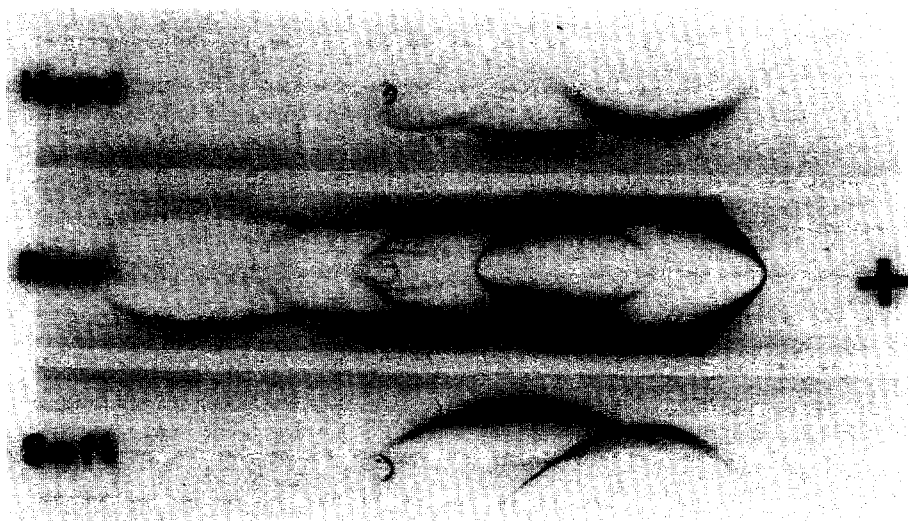


FIG. 2. Immunoelectrophoresis of extract of hard-boiled egg white, raw egg white, and extract of soft-boiled egg white against rabbit anti-egg white serum.

Patient sera

Sera from patients with known clinical histories of allergy to egg white were used after screening by RAST to egg white. In all cases, patients had experienced at least two similar reactions from ingestion of egg. All patients skin tested had positive reactions. Some of these sera were used in previous studies,^{2, 6, 7} and all sera studied had RAST binding to egg white of more than 2% of counts added after subtraction of nonspecific binding. Control sera were obtained from subjects without histories of food allergy and all were RAST-negative to egg white. All sera were stored frozen. The positive sera are described in Table I.

RIEP

Immunoelectrophoresis plates were prepared from 1% ME agarose (Marine Colloids, Rockland Me.) in a buffer consisting of 44.3 gm of tris(hydroxymethyl)aminomethane, 22.4 gm of barbital, 0.53 gm of calcium lactate, and 1 gm of sodium azide per 5 L. The same buffer was used in the electrode vessels. Approximately 3 μ l of a 1:10 dilution of egg white were pipetted into each well, and electrophoresis was performed for 45 min at 5 V/cm. Approximately 75 μ l of rabbit antiserum were added to each trough, and immunodiffusion was allowed to proceed overnight in a humid chamber. The plate was washed in 0.9% NaCl and

TABLE I. RIEP analysis of sera from patients allergic to egg white

Patient	Age (yr)	Sex	Symptoms on egg ingestion	Ovalbumin	Ovomucoid	Conalbumin
9620	2½	M	Anaphylaxis	+	+	+
9281	2	M	Wheeze	+	+	0
9278	1½	M	Angioedema, wheeze	+	2+	0
9256	1	M	Urticaria, angioedema, respiratory distress	2+	+	0
8876	1	M	Anaphylaxis, eczema	2+	0	+
8823	4	M	Hyper-IgE syndrome	5+	2+	3+
8621	1	F	Vomiting	+	0	0
8423	2	F	GI, refuses egg	+	+	0
8164	35	F	Vomiting wheeze	+	0	0
8161	1	M	Eczema	+	+	0
8076	1	M	Choking, hoarseness	+	2+	±
7714	2	F	Vomiting, eczema	2+	0	0
7019	2	M	Angioedema, urticaria	+	2+	+
6901	1	M	Urticaria	+	2+	0
6808	1½	M	Anaphylaxis	3+	4+	2+
6782	3	F	Angioedema, wheeze, vomiting	4+	2+	4+
6743	2	M	Wheeze, diarrhea	+	+	0
6624	5	M	Anaphylaxis	+	3+	0
6621	1½	M	GI bleed	+	0	0
6609	2½	M	Urticaria	2+	±	+
6596	15	M	Anaphylaxis	2+	+	2+
938	Child	F	Eczema	+	0	±
887	2½	M	Eczema	3+	4+	0
842	22	M	Anaphylaxis	+	+	+
772	15	M	Urticaria, angioedema, eczema	5+	5+	3+
707	16	F	Eczema, flare	0	2+	0
626	1	M	Urticaria, angioedema	5+	0	0
576	2	M	Urticaria, angioedema	2+	4+	+
488	15	M	Wheeze	+	±	2+
345	2	M	Eczema, flare	3+	0	0
334	5	M	Eczema, flare	5+	3+	3+
301	14	F	Urticaria, angioedema	0	3+	0
13	6 mo.	M	Anaphylaxis	2+	0	0

press-dried for three cycles and then air dried. At this time the patterns were cut apart and at least one from each plate was stained in 0.1% Serva violet 49 dissolved in 40% methanol/10% acetic acid. Single unstained patterns were placed in 11 by 75 mm test tubes and rocked overnight with 0.2 ml of patient serum and 4.3 ml of buffer consisting of 10.3 gm of boric acid and 7.8 gm of NaCl per liter adjusted to pH 8.0 with NaOH. Each strip was then washed three times with borate buffer by rocking for 30 min. The tracer antibody (200,000 counts per minute) dissolved in borate buffer was added to each tube and rocking was continued overnight. After three saline washes and one water wash, the strips were evaluated for radioactivity in a gamma counter, taped to cardboard, and exposed to Kodak X-Omat AR film (XAR-5) in X-ray holders for 2, 4, and 10 days. After developing, the autoradiographs were aligned with the immunoelectrophoresis strips, and the arcs visible on the film were scored from +, for a barely visible but definite arc, to

5+, for the strongest arc. Calibration of the scale was performed by comparing autoradiographs from the three exposure times and radioactivity as measured in the gamma counter. All of the radioactive arcs observed corresponded to precipitin arcs visible on the original stained strips.

Egg proteins

Ovalbumin, ovomucoid, conalbumin, and lysozyme were purchased from Sigma. Each protein was purified by ion-exchange HPLC on a Synchronpak AX-300 (Synchron, Inc.) column, 4 by 250 mm, with a gradient of 0 to 0.5M sodium acetate in 0.02M Tris acetate buffer, pH 8.0.¹¹ Non-overlapping fractions were pooled and concentrated by ultrafiltration. Each protein was demonstrated to be free of the others by analytical chromatography on Synchronpak AX-300 and by immunoelectrophoresis with 15 µl of 20 mg/ml solution. These techniques did not detect any cross-contamination.

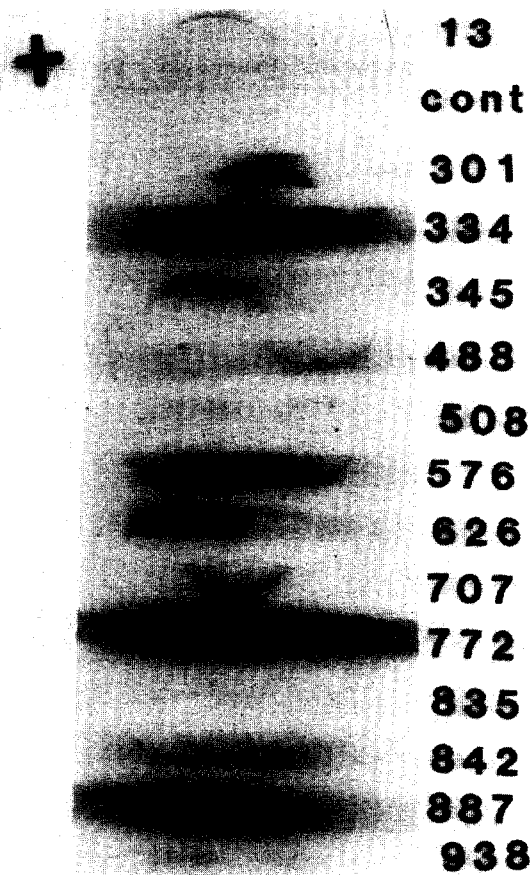


FIG. 3. RIEP of patient and control sera tested against raw egg white. The numbers correspond to the patient numbers in Table I. *cont*, Control serum. Patients 508 and 835 had eczema without clinical egg allergy.

RAST

RAST reagents were prepared and tests performed by the methods previously described.^{9, 10}

RESULTS

All 33 sera of egg white-allergic patients gave interpretable RIEP patterns. All negative control sera showed no arcs on the autoradiographs and no uptake of radioactivity when the strips were counted. The rabbit antiserum against egg white detected at least eight components by immunoelectrophoresis and at least 14 components by crossed immunoelectrophoresis. The immunoelectrophoretic analysis of the serum pool and identification of the major arcs (ovalbumin, ovomucoid, conalbumin, and lysozyme) are shown in Fig. 1. The immunoelectrophoretic pattern produced by the serum pool was similar to that reported by Miller,⁸ except that our pool reacted strongly with ovomucoid. The heat stability of the egg proteins was tested by cooking eggs, preparing a borate buffer extract of the white, and testing it by im-

TABLE II. RAST results for sera from egg white-allergic individuals to four highly purified egg proteins*

Serum	Ovalbumin	Ovomucoid	Conalbumin	Lysozyme
9620	11.3	6.4	8.0	2.4
9281	5.6	7.3	3.0	1.0
9278	7.5	8.4	2.3	4.2
9256	8.2	2.9	0.9	1.6
8876	14.2	7.1	14.3	5.0
8823	38.2	15.2	38.1	1.1
8621	5.9	1.2	0.7	0.2
8423	9.6	11.9	14.9	0.1
8164	3.3	2.8	1.8	0.5
8161	7.9	16.5	0.4	0.2
8076	16.1	29.3	13.5	0.8
7714	13.5	9.4	8.0	0.5
7019	9.7	15.4	8.3	0.6
6901	12.0	35.5	1.0	0.5
6808	17.0	31.1	34.7	1.2
6782	13.7	15.3	38.8	0.8
6743	1.5	0.8	1.2	0.1
6624	16.4	25.7	22.7	0.7
6621	5.4	0.7	2.3	0.3
6609	5.9	5.0	9.0	0.2
6596	7.1	6.4	23.1	1.0
938	5.0	2.9	13.5	0.5
887	18.9	30.0	6.2	2.9
842	8.1	4.9	6.5	1.4
772	14.3	19.5	16.4	3.0
707	4.3	10.5	2.6	4.8
626	32.4	5.0	3.4	6.4
576	29.8	40.0	23.4	12.7
488	3.3	4.4	4.4	1.1
345	15.9	5.3	1.2	1.3
334	20.3	22.8	35.9	2.4
301	8.6	12.9	3.6	0.7
13	7.2	0.5	0.5	0.9

*Results are expressed as percent binding after subtraction of non-specific binding. Values of 0.7% and greater are scored as positive. Nonspecific binding ranges from 0.3% to 0.5% for the four proteins tested.

muno-electrophoresis. The results are shown in Fig. 2. Both hard- and soft-boiled eggs contained immunologically recognizable ovalbumin, ovomucoid, and a third relatively neutral protein, possibly one of the forms of conalbumin.

The results of RIEP and patient data are presented in Table I, and a representative selection of the autoradiographs are shown in Fig. 3. Of 33 sera of egg white-allergic patients, 31 contained IgE antibodies reacting with ovalbumin, while 22 reacted with ovomucoid, 14 reacted with conalbumin, and two reacted with other proteins. No reactions with lysozyme were seen, even with the strongest sera. Oval-

bumin produced the strongest reaction in 16 cases and ovomucoid produced the strongest reaction in 10 cases. One serum reacted most strongly with conalbumin. Eight sera were positive to ovalbumin and negative to ovomucoid, while only two were positive to ovomucoid and negative to ovalbumin. The pattern observed was independent of the strength of reaction. Some very strong sera appeared to be specific for one allergen.

The RAST results obtained with the sera used for RIEP are presented in Table II. All 33 patient sera were positive to ovalbumin, 32 were positive to ovomucoid, 31 to conalbumin, and 22 to lysozyme. The binding to lysozyme was always much weaker than that observed for the other proteins. Thirteen sera bound more to ovalbumin than to ovomucoid and 12 bound more to ovomucoid than to ovalbumin. The strongest binding was to conalbumin six cases. Only a single serum was RAST positive to ovalbumin and negative to ovomucoid. No sera were positive only to ovomucoid. The RAST results indicate the presence of IgE antibodies that bind to all three of the major proteins of egg white in 30 of 33 allergic sera tested. Immunochemical analysis and HPLC of the purified antigens indicated less than 0.25% cross-contamination.

The patterns observed by RIEP and RAST are compared in Table III. Both techniques gave similar results for ovalbumin and agreement is relatively good for ovomucoid, although 10 sera were ovomucoid negative by RIEP but positive by RAST. RIEP was poor at detecting conalbumin reactivity even for some sera with over 20% binding by RAST. It is possible that rabbit antibodies could block the allergenic sites in some cases. RAST showed that fewer of the sera were highly specific than RIEP and also was more sensitive than RIEP. RAST should detect lower-affinity antibodies, since the serum is diluted 1:6 rather than 1:21.5 as in RIEP.

There were no statistically significant correlations of pattern of reactivity with age, sex, or symptoms from eating egg, although ovomucoid reactivity was much weaker than ovalbumin and conalbumin reactivity in sera from patients with gastrointestinal symptoms. There was also no significant correlation of pattern of reactivity with the presence of eczema. These data show that ovalbumin is a strong allergen and is present in cooked egg white; ovomucoid is a weaker but still important allergen and is also found in cooked egg. Conalbumin is also an allergen and appears to be less heat stable. There are also several minor allergens found in only two of 33 sera by RIEP. Lysozyme appears to be much less allergenic, possibly because it is destroyed by cooking.

TABLE III. Summary of reactivities to egg allergens of sera from patients allergic to egg white

	RIEP	RAST
Allergic sera positive	33	33
Control sera positive	0	0
Ovalbumin positive	31	33
Ovomucoid positive	22	32
Conalbumin positive	13	31
Other component positive	2	22 (Lysozyme)
Ovalbumin stronger than ovomucoid	16	13
Ovomucoid stronger than ovalbumin	10	12
Ovalbumin = ovomucoid	7	8
Conalbumin strongest	1	6
Conalbumin = strongest of ovalbumin and ovomucoid	4	6
Ovalbumin positive, ovomucoid negative	8	1
Ovalbumin negative, ovomucoid positive	2	0

DISCUSSION

The results obtained in this study show that ovalbumin and ovomucoid are both strong allergens in egg white, with both percent of egg-allergic patients who react to it and strength of reaction as criteria. Conalbumin is also an important allergen and there are at least three other minor allergens present. Lysozyme is a weak allergen. Both ovalbumin and ovomucoid are present in immunologically recognizable form after boiling an egg for over 20 min, showing that they both can be clinically relevant allergens.

These results are at variance with those reported by Bleumink and Young,⁵ who claim that by skin testing, ovomucoid was many times more reactive than ovalbumin. This claim has been disputed by others using both skin testing¹ and RAST⁷ for analyzing allergen specificities. Part of the reason for the discrepancy may be the patient population. Bleumink and Young⁵ used 13 patients with a "positive intracutaneous immediate reaction to egg-white." The patients were further described: "All of these persons had atopic dermatitis, some of them (4 of 13) also asthma and/or rhinitis. The majority of the atopic patients showed urticaria, dermatitis and angioedema after eating (minute amounts) of egg."

Several investigators^{2, 3} have reported the noncorrelation of skin test and RAST in patients with atopic eczema. These studies have shown that skin reactivity is unreliable in many patients with eczema. In addition, Bleumink and Young made no distinction between those patients clinically allergic and those who

just gave a positive skin test without symptoms from egg ingestion.

It would be surprising if ovalbumin were not a strong allergen in man, since it is routinely used as a model allergen in mice, rats, and guinea pigs for the study of food allergy, and low doses are known to induce IgE antibody formation in responder strains of all these species.¹²⁻¹⁴ On the other hand, it is difficult to induce antibody production to ovomucoid. Only one of four rabbits we used produced a significant titer against ovomucoid. Both Bleumink and Young⁵ and Miller⁸ report similar experiences.

Egg white is particularly interesting as a natural allergen system, since it is relatively well characterized and there are only a few major proteins present. Of these only two are relatively heat stable and a third is marginally heat stable. It is necessary to study only the response to a maximum of three allergens to use this system as a model for human food allergy. All three of these proteins are commercially available, albeit in impure form, but can be readily purified by ion-exchange chromatography. The availability of food allergen model systems such as this should allow the performance of *in vitro* and *in vivo* studies using characterized allergens and patients, which help to establish the scientific basis of food allergy.

Note added in proof. In his recent paper, *A clinical and immunologic study of allergy to hen's egg white. III. Allergens in hen's egg white studied by crossed radio-immunoelectrophoresis (CRIE)* (Allergy 37:521, 1982), Tor Langeland reached similar conclusions to those stated in this paper about the relative importance of the allergens in egg white.

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REFERENCES

1. Perlman F: Food allergens, in Catsimpoolas N, editor: Immunological aspects of foods. Westport, Conn., 1977, The AVI Publishing Co., Inc., p. 279.
2. Hoffman DR, Yamamoto FY, Geller B, Haddad Z: Specific IgE antibodies in atopic eczema. J ALLERGY CLIN IMMUNOL 55:256, 1975.
3. Schur S, Hyde JS, Wypych JJ: Egg-white sensitivity and atopic eczema. J ALLERGY CLIN IMMUNOL 54:174, 1974.
4. Bleumink E, Young E: Studies on the atopic allergen in hen's egg. I. Identification of the skin reactive fraction in egg-white. Int Arch Allergy 35:1, 1969.
5. Bleumink E, Young E: Studies on the atopic allergen in hen's egg. II. Further characterization of the skin-reactive fraction in egg-white: immuno-electrophoretic studies. Int Arch Allergy 40:72, 1971.
6. Hoffman DR, Haddad ZH: Diagnosis of IgE-mediated reactions to food antigens by radioimmunoassay. J ALLERGY CLIN IMMUNOL 54:165, 1974.
7. Hoffman DR: Food allergy in children: RAST studies with milk and egg, in Evans R, editor: Advances in diagnosis of allergy: RAST. Miami, 1975, Symposia Specialists, p. 165.
8. Miller HT: Immunochemistry of avian egg white proteins, in Catsimpoolas N, editor: Immunological aspects of foods. Westport, Conn., 1977, The AVI Publishing Co., Inc., p. 152.
9. Hoffman DR: The use and interpretation of RAST to stinging insect venoms. Ann Allergy 42:224, 1979.
10. Hoffman DR, Shipman WH, Babin D: Allergens in bee venom. II. Two new high molecular weight allergenic specificities. J ALLERGY CLIN IMMUNOL 59:147, 1977.
11. Regnier FE, Gooding KM: High-performance liquid chromatography of proteins. Anal Biochem 103:1, 1980.
12. Hicks R, Okpako DT: The influence of the site of the sensitizing dose of antigen on the development and duration of anaphylactic hypersensitivity in the guinea pig. Int Arch Allergy Appl Immunol 33:131, 1968.
13. Takatsu K, Ishizaka K: Reaginic antibody formation in the mouse. VI. Suppression of IgE and IgG antibody response to ovalbumin following the administration of high dose urea-denatured antigen. Cell Immunol 20:276, 1975.
14. Bazin H, Platteau B: Production of circulating reaginic (IgE) antibodies by oral administration of ovalbumin to rats. Immunology 30:679, 1976.