

Serum immunoglobulins

III. Abnormalities associated with chronic urticaria in children

Rebecca H. Buckley, M.D., and Susan C. Dees, M.D., Durham, N. C.

Serum levels of immunoglobulins G, A, and M were determined in 16 children with chronic urticaria. The outstanding clinical feature of the group was recurrent upper respiratory infection. In 9 cases immunoglobulin levels were below the normal range for age for this laboratory. Four examples of multiple immunoglobulin deficiency and 5 examples of single immunoglobulin deficiency were noted, including a complete IgA deficiency. A statistical comparison of the entire urticaria group data with data from normal subjects revealed significantly ($p = < 0.01$) lower mean levels of IgG, IgA, and IgM in the urticaria group. We found evidence supporting functional immunologic impairment in the form of (a) low isoagglutinins in 4 patients and (b) failure to give delayed hypersensitivity reactions in 4 of 10 children tested. Abnormally low immunoglobulin levels were found in 6 immediate family members (representing 4 of 10 families studied), and there were frequent positive family histories of allergy, repeated infection, and collagen disease. These findings suggest that abnormal immunologic function with resultant repeated bacterial infection may be causally related to the development of urticaria in certain individuals, and that chronic urticaria may be the presenting clinical manifestation of an immunologic-deficiency state.

Although chronic urticaria is seen most commonly in women in the third and fourth decades of life,¹⁻³ its occurrence in childhood is not rare.⁴ The etiology of the condition can seldom be definitely determined in either age group. Numerous reviews bear this out,⁵⁻⁷ even though a variety of causes for urticaria are listed in the literature (including psychogenic and physical causes, food, drug, and inhalant allergens, and infectious agents).⁵ Chronic urticaria has also been described in association with collagen diseases, such as systemic lupus erythematosus and dermatomyositis, and with Hodgkin's disease and other malignancies.⁸

From the Division of Immunology, Department of Microbiology and Immunology and the Department of Pediatrics, Duke University Medical Center, Durham, N. C.

Supported by grants from the Duke University Council on Research.

Received for publication Sept. 5, 1967,

In our laboratory during the course of a study of immunoglobulin levels in various types of illnesses, we observed that several children with chronic urticaria had abnormally low levels of one or more of the immunoglobulins G, A, and M. This was in contrast to children with other allergic problems, whose levels did not differ significantly from those found in a group of 201 normal individuals (ranging in age from infancy to adulthood).⁹ Over the past 2 years we have performed serum immunoglobulin analyses on 16 children with whom chronic urticaria or angioneurotic edema was the major, if not the sole, clinical problem. This report presents the results of these studies, together with data from selected tests of immunologic function and pertinent clinical features found in these children.

MATERIALS AND METHODS

Patients

The children whose findings are the subject of this report are among patients seen in the Allergy Division of the Department of Pediatrics at the Duke University Medical Center during the years 1965 and 1966. They were brought to our attention because they presented with or were being followed for recurrent urticaria or angioedema as their predominant or sole clinical problem. The group was comprised of 16 Caucasian children, 9 girls and 7 boys. Ages of the patients ranged from 1 to 17 years, but the majority were from 10 to 14 years of age. The duration of their hives (not necessarily present at all times, but recurring intermittently for varying lengths of time) was from 6 months to 7 years. Two of the children had recurrent angioedema without significant urticaria.

Skin tests

Immediate skin tests were performed by standard scratch and intradermal techniques. Delayed hypersensitivity was assayed by intradermal skin tests with 0.1 ml. of each of the following nine antigens: mumps (Eli Lilly & Co., 1:1), *Candida albicans* (1:2,000), Trichophyton (Hollister-Stier Laboratories, 1:1,000), P.P.D. (Parke, Davis & Co., 0.002 mg. per milliliter), blastomycin (1:2,000), coccidioidin (1:2,000), histoplasmin (1:2,000), and BAC respiratory and BAC monilia (Hoffman Laboratories, 1:10). The *Candida*, blastomycin, coccidioidin, and histoplasmin antigens were heat-killed suspensions prepared in the Duke Mycology Laboratory. Reactions were recorded at 48 and 72 hours and those that gave reactions of erythema and induration 5 mm. or greater were considered positive (5 to 9 mm., 1⁺; 10 to 14 mm., 2⁺; 15 to 19 mm., 3⁺; and 20 mm. or greater, 4⁺).

Tests for circulating antibodies

Isoagglutinin titers were determined by tube dilution. Schick tests (Eli Lilly & Co.) were performed according to standard technique. Antibodies to diphtheria and tetanus toxoids were measured by a modification of Boyden's tanned cell hemagglutination procedure.¹⁰ Human type O, Rh positive erythrocytes were washed three times in phosphate-buffered saline, pH 7.2 (PBS),

then resuspended in PBS to give a 2.5 per cent suspension. Equal volumes of 2.5 per cent red blood cells and 1:20,000 tannic acid in PBS were incubated for 10 minutes at 37° C. The cells were then washed three times in PBS (1,000 r.p.m. for 5 minutes) and resuspended in PBS to give a 1.25 per cent suspension. Concentrated diphtheria toxoid* (1,600 flocculation units per milliliter, lot NZ157P) or tetanus toxoid* (220 flocculation units per milliliter, lot LP224) were diluted 1:10 in PBS, mixed with an equal volume of tanned red blood cells (1.25 per cent suspension), and incubated 15 minutes at 37° C. The cells were then washed twice in 1:100 normal rabbit serum (NRS) in PBS and finally resuspended in 1:100 NRS to give a 1.25 per cent suspension. Aliquots of 0.05 ml. were added to 0.05 ml. aliquots of dilutions of patients' sera in 1:100 NRS. The tests were read after overnight incubation at 4° C.

Immunoglobulin and B₁C determinations

Immunoglobulins G, A, and M were quantitatively measured by a modification of Mancini's single radial diffusion method,¹¹ with specific antisera and primary reference standards prepared in our laboratory. Normal values for serum immunoglobulins G, A, and M in 201 normal individuals ranging in age from infancy to adulthood have also been determined for our laboratory. Details of the modification and reproducibility of the method, preparation of reagents, and statistical analysis of the normal values obtained are presented in another report.⁹ Levels of serum B₁C were determined on commercial antibody-agar plates.†

RESULTS

Clinical findings

The outstanding historical feature of the group was recurrent upper respiratory infection, reported by all but 1 patient. Nine of the 16 children had undergone tonsillectomy and adenoidectomy for this problem. Table I lists the various foci of infection and organisms isolated from the nasopharynx during examination of these children in our clinic. The most frequent type of infection was pharyngitis, found in 10 children, followed by sinusitis in 8 and otitis in 6. Sinusitis was documented by x-ray in 7. Coagulase-positive *Staphylococcus* was isolated from 10 individuals, beta hemolytic *Streptococcus* from 8, *Pneumococcus* from 4, *Candida albicans* from 4, and *Mycoplasma* from 3. *Staphylococci* and *streptococci* were frequently isolated on more than one occasion from the same individual, and ASO titers were elevated in 2 additional children from whom we were not able to culture streptococci. Remission of hives was frequently noted following antibiotic therapy of these children. Parasitic infestation was proved in 4 members of the group. Personal history was positive for other allergies in only 8 of the 16 children.

*Supplied by the Division of Biologic Laboratories, Massachusetts Department of Public Health, Boston, Mass.

†Immunoplates, Hyland Laboratories, Los Angeles, Calif.

Table I. Evidence of infection in 16 patients with chronic urticaria

	Number of patients
History of recurrent infection	15
Foci of infection	
Throat	10
Sinus	8
Ears	6
Nasopharyngeal cultures	
Staphylococcus aureus	10
β H. Streptococcus	8
Pneumococcus	4
C. albicans	4
Mycoplasma pn.	3

Table II. Skin test reactions in patients with chronic urticaria

Antigens	Reactions			
	Immediate*	Delayed		
		3 or more sites positive	1 site positive	All sites negative
Pollen, foods, or inhalants	5/15†	N.O.‡	N.O.‡	N.O.‡
Bacterial, viral, or fungal agents	10/10†	4/10†	2/10†	4/10†

*The reactions were 2 to 4+ positive.

†Number of subjects giving stated reaction out of number tested.

‡N.O. = not observed.

Skin testing

The results of immediate and delayed skin tests are presented in Table II. All but 1 of the children were tested for immediate hypersensitivity with a large number of pollen, food, and inhalant antigens, and only 5 gave 2 to 4+ reactions. Conversely, when a panel of bacterial, viral, and fungal antigens was tested in 10 of the 16 children, it was noted that all 10 (including 7 who failed to give significant immediate reactions to the other types of antigens) gave large immediate reactions to one or more of these agents. Four of these last 10 children failed, however, to give delayed reactions to any of these agents (which included such antigens as mumps, Trichophyton, and Candida), even in the presence of positive histories of infections with the organisms from which the antigens were derived.

Circulating antibodies

Isoagglutinins were determined in each patient in the group and were found to be low¹² (1:8 to 1:16) in 4, 2 of whom had multiple immunoglobulin deficiencies and 2 of whom had a single immunoglobulin deficiency. Isoagglutinin titers were 1:8 in both parents of a child whose immunoglobulin abnor-

malities involved two fractions. The Schick test was positive in only 1 of 10 children tested, and diphtheria and tetanus tanned cell titers all rose above 10,000 following booster injections given 10 patients in the group (including the 1 with a positive Schick test). Normal titers for tetanus and diphtheria agglutinins are reported to range from 10,000 to 40,000 following stimulation.¹³

Serum immunoglobulins and B₁C

Serum immunoglobulin values on the 16 children are compared graphically in Fig. 1 with the geometric means and bounds for normal children of appropri-

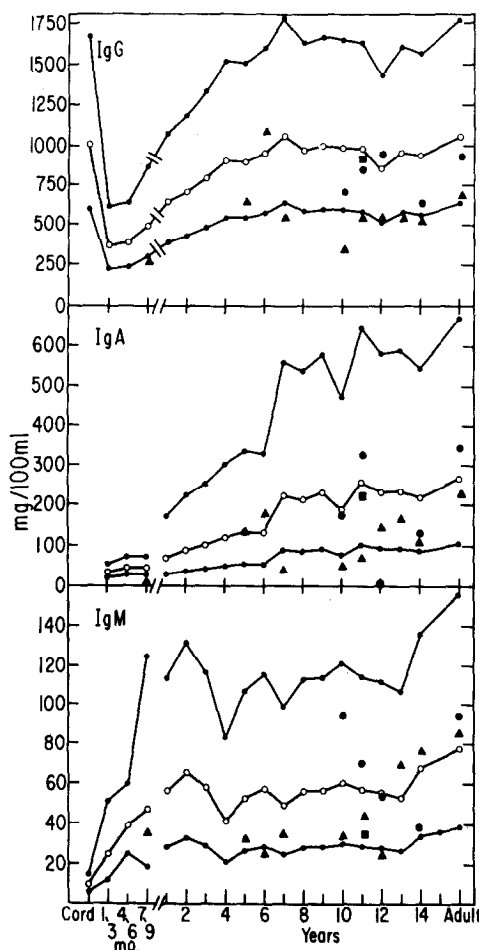


Fig. 1

Immunoglobulin levels in 16 children with chronic urticaria compared with the geometric means (open circles) and bounds (closed circles) for a group of 201 normal individuals ranging from infancy to adulthood. The normal bounds represent the antilogs of the mean $\log \pm$ twice the pooled standard deviations of the mean logs. Where values from more than one individual are plotted at the same age, the same symbol is used for all three of a given individual's immunoglobulin values.

ate age tested in our laboratory. The normal bounds represent the antilogs of the mean logarithms $\pm$ two pooled standard deviations of the mean logs. Although multiple samples were obtained from most of the 16 individuals in the present study over the 2 year period, values remained fairly constant when adjusted for age; hence, the data here represent values from only one analysis per patient. It can be seen that 6 values for IgG in the urticaria group fall below the normal bounds for age and that only 2 lie above the mean. Similarly, 5 values for IgA fall below the normal bounds, and only 3 lie above the mean. Two values for IgM fall below the normal range, but 6 lie above the mean. The data were also statistically compared to the normal data as follows. Since we had found a significant influence of race upon levels of IgG at several ages in our analysis of the normal data,⁹ we compared the IgG levels from the present group (entirely Caucasian) with IgG levels from only the white normal subjects. As no effect for race was found in levels of IgA and IgM,⁹ the levels of these immunoglobulins were compared with the normal data obtained from both Negro and Caucasian subjects. Adult levels for IgG are achieved by age six, for IgA by age seven, and for IgM by age one, as shown by our statistical analysis of the normal subject data which has been described previously.⁹ Immunoglobulin levels in the children from the present study were compared with levels in the normal subject group, by the Student's *t* test, but omitting children under age six (2 patients) when comparing IgG levels, and under age seven (3 patients) for the IgA comparisons. The results of these comparisons showed highly significant differences ($p < 0.005$) for IgG and IgA values and ($p < 0.01$) for IgM values. Levels of B₁C in the 16 patients were moderately elevated (mean, 186 mg. per 100 ml., as compared to a normal mean of 145 mg. per 100 ml.).

Absolute values for the immunoglobulins G, A, and M in the 16 patients

Table III. *Serum immunoglobulin levels in 16 Caucasian children with chronic urticaria*

<i>Patient</i>	<i>Age (years)</i>	<i>Sex</i>	<i>IgG (mg./100 ml.)</i>	<i>IgA (mg./100 ml.)</i>	<i>IgM (mg./100 ml.)</i>
1	1	F	290*	14*	35
2	5	F	650	133	33
3	6	M	1,080	175	26*
4	7	F	560*	38*	37
5	10	F	355*	45*	34
6	10	M	720	181	95
7	11	M	550*	70*	45
8	11	F	940	209	34
9	11	F	860	333	70
10	12	M	960	0*	54
11	12	M	550	149	24*
12	13	F	570*	187	70
13	14	M	540*	103	77
14	14	M	655	111	39
15	16	F	950	337	95
16	17	F	675	238	85

*Values below normal range for age for this laboratory.

are presented in Table III. Four of the children (Patients 1, 4, 5, and 7) had levels of two immunoglobulins which were below the normal range for age for this laboratory, and 5 (Patients 3, 10, 11, 12, and 13) had levels of one immunoglobulin below the normal range. Five others (Patients 2, 6, 8, 14, and 16) had concentrations of one or more immunoglobulins which were in the lower range of normal (Fig. 1). Only 2 patients (Patients 9 and 15) had levels of all three immunoglobulins which were close to or above the normal geometric means.

Patients 1, 4, 5, and 7 had low levels of immunoglobulins G and A. Isoagglutinins were low in Patients 1 and 5 and delayed skin tests were all negative in Patients 4 and 5. Patient 7 had a positive Schick test and also had negative reactions to all delayed antigens tested (including mumps and Candida, in spite of having had severe thrush in the neonatal period and mumps during later childhood). Low values for only IgG were found in Patients 12 and 13. Patient 12 was the fourth child in the group failing to respond to any of the delayed hypersensitivity screening antigens. A complete IgA deficiency was found in Patient 10. He had a history of recurrent tonsillitis and sinusitis and had had hives longer than any other member of the group. He responded with immediate reactions to six of the nine bacterial, viral, and fungal antigens

Table IV. *Immunoglobulin levels and disease history of 27 immediate family members of 10 urticaria patients*

<i>Patient</i>	<i>Immunoglobulin findings</i>	<i>Clinical symptoms</i>
1	No abnormalities in parents	Mother: rheumatic heart disease
2	Mean IgG level in parents is 725 mg./100 ml.*	Mother: recurrent urticaria
3	No abnormalities	Negative for allergy or repeated infection
5	Father and sister have low IgM Mean IgG level in parents and sibs is 763 mg./100 ml.* Isoagglutinins 1:8 in both parents	Sister: recurrent otitis media and sinusitis
7	Mean IgG level in parents is 597 mg./100 ml.* Elevated IgM in father	Father: rheumatoid arthritis
8	IgG level low in mother Father and brother are normal	Brother: recurrent otitis media
10	Mean IgG level in parents and sister is 763 mg./100 ml.*	Mother: allergic rhinitis, Father: sinusitis, Sister: allergic to penicillin
11	Brother and sister have low IgM	Father, brother, and sister: recurrent sinusitis. Allergic rhinitis and asthma in several members
12	No abnormalities in father and brother	Negative for allergy or repeated infection
13	No abnormalities in parents	Negative for allergy or repeated infection

*Normal geometric mean and range for IgG (adult Caucasian) of this laboratory: 982 (600 to 1,606).

in the delayed hypersensitivity screening panel and with delayed reactions to three of the nine agents. Low levels of IgM were detected in Patients 3 and 11, and isoagglutinins were also found to be low in these children. In the 2 patients who had levels of all three immunoglobulins which were close to or above the normal range, there were fairly clear histories of drug-induced (Patient 9) and temperature change-induced (Patient 15) hives. Patient 15 was the only member of the group who had a negative history for recurrent infection.

Immunoglobulin levels and disease history in family members

Of the entire group of 16 families, there was a positive history of allergy in 12, recurrent infection in 6, collagen disease in 2, and malignancy in 2. Serum samples were obtained from 27 members of the immediate families of 10 of the children. Table IV lists the instances where abnormalities were detected, as well as the family history of allergic disease, collagen disease, or recurrent infection in these 10 families. Abnormally low values for one or more immunoglobulins were found in 6 relatives, representing 4 of the 10 families. Mean IgG levels were low (or in the low normal range) in 4 of the families.

DISCUSSION

The observations described in this group of children with chronic urticaria strongly suggest that the presence of recurrent bacterial infection is in some way related to their clinical manifestation of urticaria. The presence of immunoglobulin abnormalities in these same children further suggests that an underlying immunological deficiency may be responsible for their undue susceptibility to infection. The incidence of associated recurrent bacterial infection seen in this group of children certainly appears to be more frequent than the literature indicates.¹⁴

We can only speculate about the actual mechanism by which infections could trigger the urticarial reactions. The presence of immediate hypersensitivity to a number of bacterial, viral, and fungal antigens, associated with a low incidence of positive immediate reactions to other types of antigens among the group, strongly implicates bacterial allergy as an etiologic factor. Also, a number of these children reportedly improved after bacterial vaccine therapy was begun, and eradicating bacteria by antibiotic therapy frequently caused remission of the urticaria.

As noted elsewhere, assessment of normality of immunoglobulin levels in children is dependent upon having appropriate age-related normal values for comparison.⁹ The highly significant differences between the immunoglobulin levels in the urticaria patients and the normal group indicate that these children are different from the normal children with respect to this parameter of their immunity. The significance of this finding in regard to functional aspects of immunity is not yet known. However, the finding of low isoagglutinins in 4 members of the group and a lack of delayed hypersensitivity response in 4 of 10 subjects evaluated for this mechanism are in support of the existence of functional immunologic deficiency among these children.

In view of the fact that in adults chronic urticaria is seen most commonly in women near menopause,¹⁻³ it is interesting to note that most of the children in this study were at or near puberty. The role of endocrinological factors in the pathogenesis of urticaria has long been questioned.^{15, 16}

The coexistence of immediate hypersensitivity reactions and immunologic abnormality is not unprecedented. Allergic phenomena have been described in association with acquired and congenital hypogammaglobulinemia,¹⁷⁻¹⁹ the Wiskott-Aldrich syndrome,²⁰ and more recently in a young child with thymic aplasia, lymphoma, and agammaglobulinemia.²¹ In the last case, there was a history of urticaria and angioedema in the mother and in 2 other relatives, and an over-all high incidence of allergic disease in the family. Immunoglobulin abnormalities and a high incidence of allergy, repeated infection, and collagen disease, as found in members of the immediate families of the patients in the present report, have also been found in families of patients with acquired hypogammaglobulinemia.²²⁻²⁴ Chronic urticaria has occasionally been described in collagen disease.⁸ Such associations suggest a complex interrelationship between these diseases and immunologic abnormality. Our studies indicate that chronic urticaria may be a presenting manifestation of immunologic deficiency and that there may be a hereditary predisposition to this deficiency in some cases.

Children with chronic urticaria who have a history of recurrent bacterial infection should be carefully evaluated for an underlying immunologic alteration. Likewise, a thorough search should be conducted for a hidden focus of infection, and adequate and appropriate antibiotic therapy given. Arbitrary use of gamma globulin injections should be avoided since a number of these children may be capable of making antibodies to gamma globulin.²⁵ Rather, it is recommended that these individuals be tested to determine the level of impairment of immunologic function before a decision is made to treat them with passive immunotherapy.

REFERENCES

1. Fink, A. I., and Gay, L. N.: A Critical Review of 170 Cases of Urticaria and Angio-neurotic Edema Followed for a Period of From Two to Ten Years, *J. ALLERGY* **5**: 615, 1934. (Abst.)
2. Eisenberg, B. C.: Management of Chronic Urticaria, *J. A. M. A.* **169**: 14, 1959.
3. Steinhardt, M. J.: Urticaria and Angioedema. Statistical Survey of Five Hundred Cases, *Ann. Allergy* **12**: 659, 1954.
4. Ratner, B., Collins-Williams, C., and Untracht, S.: Allergic Dermal-Respiratory Syndrome in Children, *Am. J. Dis. Child.* **82**: 666, 1951.
5. Beall, G. N.: Urticaria: A Review of Laboratory and Clinical Observations, *Medicine* **43**: 131, 1964.
6. Green, G. R., Koelsche, G. A., and Kierland, R. R.: Etiology and Pathogenesis of Chronic Urticaria, *Ann. Allergy* **23**: 30, 1965.
7. Shelley, W. B.: Case Report: An Analysis of a Case of Chronic Urticaria, *Ann. Allergy* **24**: 421, 1966.
8. Sheldon, J. M., Matthews, K. P., and Lovell, R. G.: The Vexing Urticaria Problem: Present Concepts of Etiology and Management, *J. ALLERGY* **25**: 525, 1954.
9. Buckley, R. H., and Dees, S. C.: Serum Immunoglobulins. I. Levels in Normal Children and in Uncomplicated Childhood Allergy. To be published in *Pediatrics*.
10. Boyden, S. V.: The Adsorption of Proteins on Erythrocytes Treated With Tannic Acid and Subsequent Hemagglutination by Antiprotein Sera, *J. Exper. Med.* **93**: 107, 1951.
11. Mancini, G., Carbonara, A. O., and Heremans, J. F.: Immunochemical Quantitation of Antigens by Single Radial Immunodiffusion, *Internat. J. Immunochem.* **2**: 235, 1965.

12. Thomsen, O., and Kettel, K.: The Titers of Human Isoagglutinins and Corresponding Red Cell Receptors at Different Ages, *Ztschr. f. Immunitätsforsch. u. exper. therap.* **63**: 67, 1929.
13. Cline, M. J., and Fudenberg, H. H.: Defective RNA Synthesis in Lymphocytes From Patients With Primary Agammaglobulinemia, *Science* **150**: 1311, 1965.
14. Sherman, W. B., and Kessler, W. R.: *Allergy in Pediatric Practice*, St. Louis, 1957, The C. V. Mosby Company, p. 209.
15. Zondek, B., and Bromberg, Y. M.: Endocrine Allergy. I. Allergic Sensitivity to Endogenous Hormones, *J. ALLERGY* **16**: 1, 1945.
16. Zondek, B., and Bromberg, Y. M.: Endocrine Allergy. II. Clinical Reactions of Allergy to Endogenous Hormones and Their Treatment, *J. Obst. & Gynec.* **54**: 1, 1947.
17. Painter, F. S., and Korst, D. R.: Studies on Acquired Hypogammaglobulinemia. Evidence of Antibody Formation and Report of a Case With Bronchial Asthma, *New England J. Med.* **260**: 15, 1959.
18. Goodman, D. H.: Hypogammaglobulinemia With Respiratory Allergy, *J. ALLERGY* **31**: 298, 1960.
19. Peterson, R. D. A., Page, A. R., and Good, R. A.: Wheal and Erythema Allergy in Patients with Agammaglobulinemia, *J. ALLERGY* **33**: 406, 1962.
20. Huntley, C. C., and Dees, S. C.: Eczema Associated With Thrombocytopenic Purpura and Purulent Otitis Media. Reports of Five Fatal Cases, *Pediatrics* **19**: 351, 1957.
21. Goldman, A. S., et al.: Thymic A lymphoplasia, Lymphoma, and Dys-gamma-globulinemia, Hyper-gamma A-, Normo-gamma M-, Hypo-gamma G-, A-gamma D-, and Gamma E-globulinemia, Plasmacytosis, Normal Delayed Hypersensitivity, Severe Allergic Reactions, and Coombs' Positive Anemia, *Pediatrics* **39**: 348, 1967.
22. Goldfarb, A. A., and Fudenberg, H.: Hypogammaglobulinemia and Its Relationship to the Allergic Diathesis, *New York J. Med.* **61**: 2721, 1961.
23. Wolf, J. K.: Primary Acquired Agammaglobulinemia, With a Family History of Collagen Disease and Hematologic Disorders, *New England J. Med.* **266**: 473, 1962.
24. Wolheim, F. A., and Williams, R. C.: Immunoglobulin Studies in Six Kindreds of Patients With Adult Hypogammaglobulinemia, *J. Lab. & Clin. Med.* **66**: 433, 1965.
25. Steihl, E. R., and Fudenberg, H. H.: Antibodies to Gammaglobulin in Infants and Children Exposed to Isologous Gamma Globulin, *Pediatrics* **35**: 229, 1965.