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DISTURBANCES IN GAMMA GLOBULIN SYNTHESIS AS "EXPERIMENTS OF NATURE"

E. Mead Johnson Award

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[In presenting the E. Mead Johnson Award to Dr. Good, Dr. Bost, President of the Academy, commented, "Robert A. Good is American Legion Memorial Heart Research Professor of Pediatrics at the University of Minnesota. Dr. Good was born in Crosby, Minnesota, on May 21, 1922; received his A.B. in 1944 from the University of Minnesota; M.D. in 1947, University of Minnesota Medical School; Ph.D. in anatomy and bacteriology in 1947, University of Minnesota Graduate School; intern and resident 1947 to 1949, University of Minnesota Hospitals; Rockefeller Institute Medical Research, New York City, 1949 to 1950, and on the staff of the University of Minnesota Medical School since 1950.

"The Awards Committee made the selection with the following notation:

"The nomination of Robert A. Good by the American Academy of Pediatrics for the 1955 first E. Mead Johnson Award for Research is based on his studies of agammaglobulinemia and particularly on his perception and demonstration of the fact that studies of this disorder could aid in an understanding of fundamental physiologic mechanisms. Of special importance are his observations in this disorder of the re-

lationship to the state and behavior of plasma cells and of the significance of a successful dermal homograft from an unrelated donor. The Committee on Awards was also aware of his studies on C-reactive protein in rheumatic fever and of his basic contributions to present understanding of the Shwartzman reaction."]

PROGRESS in basic science, when applied to clinical medicine, has often provided useful insight into the mechanisms of disease. One reflection of the impact of the basic sciences on clinical medicine is the relatively recent discovery and definition of several diseases featured by disturbances in protein synthesis. Advances resulting in the availability of effective methods for physical and chemical separation and analysis of the serum proteins, quantitative immunologic techniques, and qualitative and quantitative morphologic criteria have contributed to present understanding of the metabolic disturbances in patients with these diseases. In this field recent discov-

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eries suggest that only a beginning has been made and that much progress can be anticipated in the next few years as a result of the application of methods of protein chemistry and quantitative immunology to clinical medicine.

As first pointed out by Osler and clearly reiterated by McQuarrie,¹ the clinical investigator has at his disposal a powerful weapon with which to supplement tools available from the basic sciences. Amongst patients, particularly those with strange and unusual diseases, may be found "experiments of nature" which, properly considered, permit acquisition of new and useful knowledge applicable far beyond the patients and diseases studied. Advances to be anticipated from study of such patients concern organ, tissue or cellular function, physiologic interrelationships and the nature of pathologic processes. "Experiments of nature" are often unique in that they cannot be duplicated in the laboratory or reproduced at will in the clinic. One of the responsibilities of the clinical investigator is to recognize such "experiments" and to attempt their interpretation. Quantitative biologic methods increase the frequency with which experiments of nature may be recognized and often permit their precise definition and analysis. This happy combination often can be employed to gain useful new information or to establish an incisive point of view. A recent attempt to classify certain metabolic disturbances as hereditary "molecular" disorders² reflects an appreciation of this relationship.

The conviction that certain patients with disturbances of gamma globulin synthesis present such "experiments of nature" prompted the present investigation. We believe that from study of these patients important mechanisms concerning protein synthesis, protein metabolism, immunologic processes, mechanisms of disease and host reactivity may be elucidated. In studying patients with disturbances in globulin synthesis we have attempted to place our initial investigations on a broad base in an effort to gain insight into the nature of these

processes and hence into the nature of human diseases based upon disturbances in their execution.

To date we have had the opportunity to study patients representing a whole spectrum of diseases based on, or associated with, disturbances in gamma globulin synthesis. Among these are included:

Children having congenital agammaglobulinemia associated with generalized immunologic paralysis.

Adults having acquired agammaglobulinemia associated with generalized immunologic paresis or paralysis.

Children having transient hypogammaglobulinemia of infancy associated with an apparent delay in assumption of immunologic responsibility.

Children having marked hypergammaglobulinemia associated with a marked decrease in resistance to infection.

Preadolescent and adolescent females having *extreme* hypergammaglobulinemia associated with generalized inflammatory liver disease.

Children having generalized insufficiency of protein synthesis including hypogammaglobulinemia, featured clinically by the occurrence of nonproteinemic edema and profound hypoproteinemia.

Adults having multiple myeloma, featured by the excessive production of abnormal proteins (closely related to the gamma globulins). This disturbance is frequently associated with *deficient* production of normal gamma globulins and immunologic paralysis or paresis.

Because our study of the problem of agammaglobulinemia is most complete, my remarks this morning will be limited to our attempt to interpret the experiment of nature represented by this group of cases. It is our strong feeling, however, that comparable detailed investigation of representatives from each of the above named disorders of gamma globulin metabolism will reveal much of importance to immunologic theory and practice.

Agammaglobulinemia was first recognized by Bruton,³ who found that an 8-year-

old boy suffering recurrent bacterial infections possessed no gamma globulin peak on electrophoretic analysis of the serum proteins. Immunologic studies revealing failure of the immune response suggested that the clinical disorder is based on a deficiency in the immune mechanism. Subsequently studies by Bruton *et al.*⁴ and Janeway *et al.*⁵ showed that agammaglobulinemia is an isolated disturbance of protein metabolism in which absence of gamma globulin is the only demonstrable abnormality. The studies of the Boston group established that agammaglobulinemia is due to deficient production of gamma globulin and not to increased destruction of this compound. Parenterally administered gamma globulin had, in these patients, a survival time somewhat longer than that described by others⁶⁻⁹ for normal human subjects, and immunologic studies showed that these patients failed to produce antibodies against several different antigens. Thus, the pioneer work revealed that this was a new disease characterized by the following manifestations:

Increased susceptibility to bacterial infection.

Absence of gamma globulin from the serum.

Absence of antibody from the blood and tissues.

Failure of antibody production in response to antigenic stimulation.

Following the clinical and laboratory definition of agammaglobulinemia, electrophoretic study of the serum or plasma from patients suffering recurrent infection has turned up numerous cases of this disease.¹⁰⁻³⁰ Indeed the number of reports pouring into the medical journals establishes that agammaglobulinemia, although probably uncommon, is not rare. At the present writing 56 cases of isolated agammaglobulinemia have been reported.

Present evidence indicates that at least 2 forms of isolated agammaglobulinemia exist. In one form the disease appears to be congenital, sex-linked, and familial. Thus far this form of disease has been reported

only in boys and numerous instances of its development among male siblings have been observed.^{15,31} Its occurrence in male cousins born of sisters supports the concept that congenital agammaglobulinemia may be an inborn error of metabolism transmitted as a sex-linked recessive trait.³¹⁻³³ The recent discovery of a female child suffering from what appears to be "congenital" agammaglobulinemia indicates either that the syndrome may be based on a different hereditary pattern in some cases or that the acquired disease may begin in infancy.²⁵

The other clinical form of isolated agammaglobulinemia appears to be an acquired disease which occurs in either sex at any age. This disease has been found principally among adults but a few cases beginning in childhood have been discovered. The patients with acquired agammaglobulinemia, like the children with congenital agammaglobulinemia, are inordinately susceptible to infection. Following the development of the disease their lives become a succession of severe bacterial infections. Patients thus far reported have regularly suffered recurrent bouts of severe pulmonary disease often resulting in bronchiectasis.²⁹ Some of them have had recurrent bacterial meningitis,^{5, 14, 21} recurrent diarrhea,²¹ recurrent otitis, sinusitis and pharyngitis^{15, 21} and several have suffered from a sprue-like syndrome.^{23, 24, 29}

Immunologic studies reveal that patients with acquired agammaglobulinemia also have a marked immunologic deficit. They fail to form antibody in response to stimulation with even potent bacterial antigens.^{3, 35} Acquired agammaglobulinemia is a sporadic disease and studies of the members of the family do not reveal disturbances in gamma globulin metabolism in these cases.³⁶

Young *et al.*²⁸ have recently described in an adult male a case of agammaglobulinemia apparently dating back to an early age. Electrophoretic examination of the serum proteins revealed an increased gamma globulin concentration in the serum

of the patient's mother and older brother. The authors interpret this finding as indicating the existence of familial dysproteinemia, of which the agammaglobulinemia was an expression in the index case. Interesting in this regard is that electrophoretic study of the entire family of our most recent patient with agammaglobulinemia revealed that the mother had an abnormally high gamma globulin concentration for which no explanation was apparent. None of the other 4 family studies which we have carried out have turned up evidence of dysproteinemia among the relatives of the agammaglobulinemic patient.

DIAGNOSIS OF AGAMMAGLOBULINEMIA

Once the possibility of agammaglobulinemia is suspected, the diagnosis is readily made by relatively simple laboratory procedures. Either free or paper electrophoretic analysis of the serum or plasma proteins revealing normal concentrations of albumin, alpha and beta₁ globulin along with com-

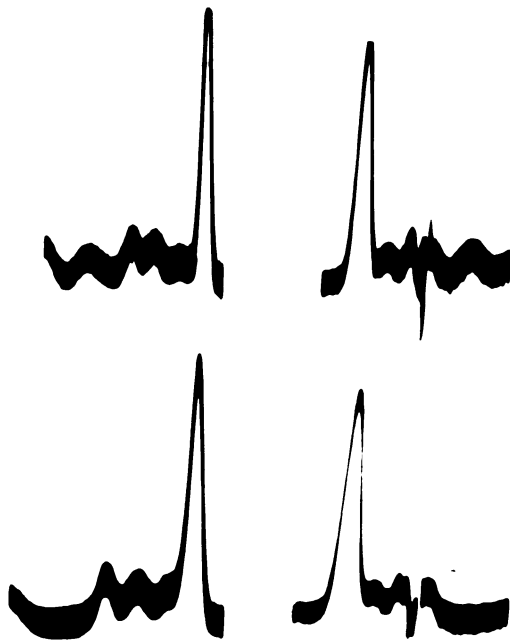


FIG. 1. Comparison of free electrophoretic patterns in normal persons (*upper*) and in patients with agammaglobulinemia (*lower*). Note the complete absence of gamma globulin as revealed in this way.

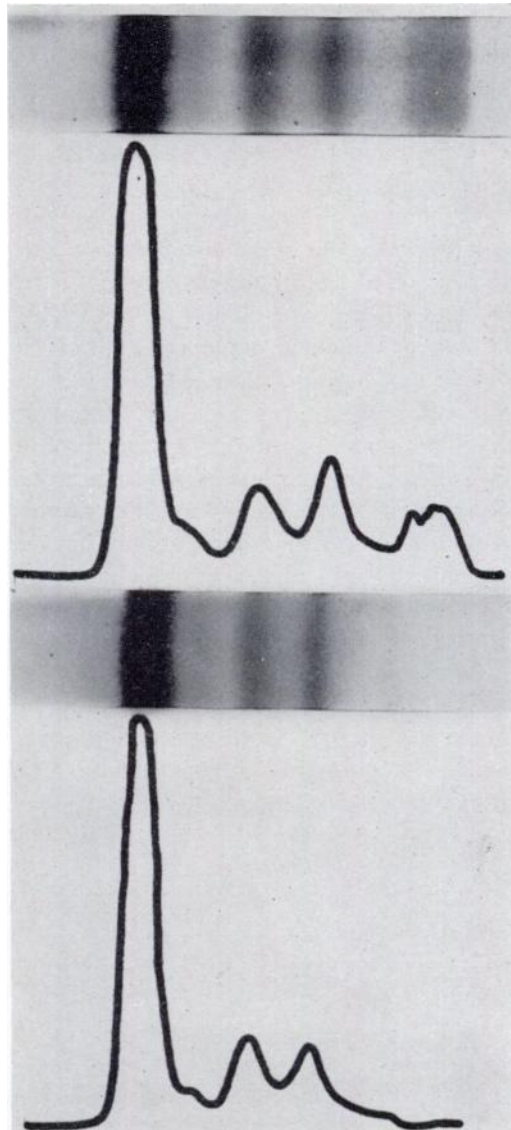


FIG. 2. Comparison of paper zone electrophoretic pattern in normal persons (*upper*) and in patients with agammaglobulinemia (*lower*). Note the diagnostic absence of gamma globulin revealed by this technique.

plete absence of gamma globulin and decreased beta₂ globulin establishes the diagnosis. Figure 1 shows a typical diagnostic free electrophoretic pattern from a patient with agammaglobulinemia together with a similar pattern from a normal child. Figure 2 compares the diagnostic appearance of the paper electrophoretic pattern and a typical normal fractionation obtained by the same method.

Screening Tests

For the laboratory which is not equipped to perform electrophoretic studies, several simple, relatively reliable screening tests are available:

FRACTIONATION OF THE SERUM PROTEINS BY THE HOWE METHOD: If the serum proteins are fractionated using a final concentration of 21 per cent sodium sulfate, a so-called globulin fraction is precipitated. This "globulin" fraction consists largely of beta globulin and the gamma globulin, albumin and alpha globulin being left in solution. A ratio between the "albumin" and "globulin" is the Howe A/G ratio.

$$\frac{\text{Albumin} + \alpha \text{ globulin}}{\text{Beta} + \gamma \text{ globulin}}$$

Beta + gamma globulin

Under ordinary circumstances this A/G ratio ranges between 2.0 and 3.5. In patients suffering from agammaglobulinemia extremely high Howe A/G ratios obtain. In 4 of our patients with agammaglobulinemia in whom this study was carried out, the Howe A/G ratio ranged from 9.0 to 13.1.

Kunkel's zinc turbidity test for gamma globulin,³⁷ usually available in the diagnostic clinical laboratory as a liver function test, depends on the precipitation of a protein from the serum in proportion to the gamma globulin concentration of the latter. Normal values in our laboratory have ranged from 2.5 to 13 turbidity units with a mean figure of 5 to 6 units. In patients with agammaglobulinemia this value is regularly extremely low giving either a zero value or insignificant readings up to 1 unit.

Low total protein together with low globulin and high A/G ratio with the commonly used 26 per cent sodium sulfate fractionation. Although less dramatic than the alteration shown on Howe fractionation even the methods of protein fractionation widely used at present reveal a low globulin concentration and a high A/G ratio in patients with agammaglobulinemia. This finding, distinctly unusual in patients who present as these often do with chronic or recurrent infectious disease, is a helpful screening test.

Absence of isoagglutinins in the serum:

If the patient suspected to have agammaglobulinemia is of blood group A, B or O, simple determination of antibody titer against the heterologous blood group cells provides a screening diagnostic test of great clinical usefulness. None of the patients with agammaglobulinemia studied thus far has had more than an insignificant amount of these antibodies in the serum, whereas immunologically normal persons have relatively high titers. Any blood bank or serologic laboratory can easily set up this simple test. This determination is probably the most generally available test which can be used for screening and has proved to be one of the best methods available for this purpose.

Schick test: Since almost all children who have had a series of injections of diphtheria toxoid are Schick negative, simple performance of the Schick test can be used as a diagnostic screening test for agammaglobulinemia. A positive reaction following immunization procedures which are otherwise highly effective is presumptive evidence of an immunologic deficit and hence compatible with the diagnosis of agammaglobulinemia.

Qualitative or semiquantitative precipitin test for gamma globulin: Gitlin¹⁵ has described a simple screening test for agammaglobulinemia which depends on precipitation of gamma globulin with antiserum prepared by injection of rabbits with purified human gamma globulin. Unfortunately this test, although diagnostic and extremely simple of execution, is not readily available since preparation of highly purified gamma globulin is still somewhat difficult. This test could be made generally available through the commercial production of specific anti-gamma globulin serum from a suitable laboratory animal.

Bone marrow biopsy: Study of smears obtained from the bone marrow of normal persons regularly shows a significant number of plasma cells. Such is not the case with bone marrow from patients with agammaglobulinemia. Complete or virtual-

ly complete absence of plasma cells from the bone marrow smear provides good presumptive evidence of isolated agammaglobulinemia. Since plasma cells are relatively infrequent in the marrow of normal persons (0.1 to 0.8 per cent of nucleated cells) the presumption must be made on the basis of an extreme scarcity of these cells or on the basis of their complete absence from the marrow smear.

Neuhauser³⁸ has called attention to a screening test based on almost complete absence of adenoid tissue in these patients. Roentgenograms of the nasopharynx in children with agammaglobulinemia reveal no evidence of adenoid tissue. Thus the nasopharynx appears similar to that of children previously subjected to adenoidec-tomy. Although not of great practical im-portance, the deficiency of lymphoid tissue thus revealed is of fundamental significance in this disease.

Since none of the presumptive diagnostic procedures nor any combination thereof provides conclusive proof of agammaglobulinemia, the final diagnosis depends on electrophoretic or immunochemical analy-sis of the serum proteins.

True Gamma Globulin Concentration

Although present nomenclature based on electrophoretic analysis of the serum labels these patients as agammaglobulinemic, this concept is probably in error. Both free and paper electrophoretic methods fail to detect small amounts of gamma globulin. More precise analysis based upon extremely sensitive immunochemical studies^{15, 39} reveals that all of the patients studied possess minute amounts of gamma globulin in their serums. Listed in Table I are the results of immunochemical measurements carried out in 6 of our patients. Three of these had the acquired form of agammaglobulinemia and 3 had the congenital disease. These observations indicate that gamma globulin in measurable amount is present in each of these patients. Although it follows that extreme hypogammaglobulinemia is a more accurate term for this disease, popular us-

TABLE I
CONCENTRATION OF GAMMA GLOBULIN BY IMMUNOLOGIC
METHOD IN PATIENTS WITH AGAMMAGLOBULINEMIA

Patient Age and Sex	Date	Form of Disease	Conc. of Gamma Globulin mg./100 ml.
W.A. 6 yr. male	11-12-54	Congenital	4.4 4.0
E.S. 7 yr. male	2-17-55 3- 9-55 3-24-55 4- 4-55	Congenital	13.8 13.1 10.0 9.1
T.A. 1 yr. male	11-20-54 11-29-54	Congenital	13.1 14.1
L.L. 30 yr. female	9-22-54; 11- 5-54	Acquired	12.2 10.0
F.H. 58 yr. male		Acquired	40.0
M.O. adult female	5-27-54 6- 7-54	Acquired	40.6 28.1
Normal children			600-1300

age dictates that "agammaglobulinemia" be retained.

Survival of Gamma Globulin in Patients with Agammaglobulinemia

Gamma globulin tagged with I-131 when injected into normal persons has an appar-ent half-life of 14 to 20 days. Lang *et al.*,¹⁹ injecting gamma globulin tagged with I-131, found no significant deviation from the nor-mal decay time in patients with agamma-globulinemia. Gitlin *et al.*,¹⁵ using an im-munologic method to quantitate gamma globulin concentration, reported that the half-life of gamma globulin in agamma-globulinemic subjects ranged from 25 to 30 days.

In the patients with agammaglobulin-emia whom we have studied,³⁷ the half-life of unlabeled and presumably unaltered gamma globulin quantitated electrophore-tically, immunologically and turbidimetri-

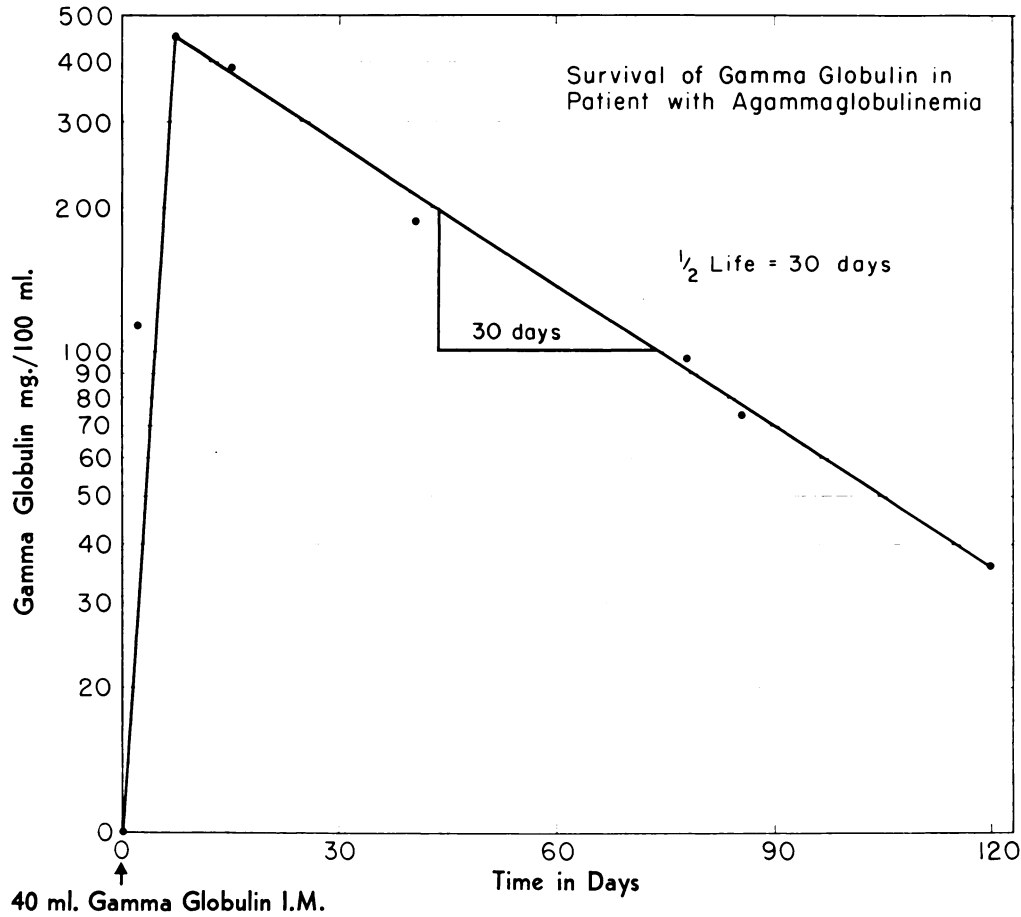


FIG. 3. Survival of gamma globulin in a patient with agammaglobulinemia injected intramuscularly with 4 ml./kg. of 15 per cent gamma globulin (Red Cross). The serums from serial bleedings were analyzed by paper zone electrophoresis.

cally has been found to be approximately 30 days. A typical experiment performed on a 16-month-old child is illustrated in Figure 3. T.A. was given an intramuscular injection of 50 ml. of concentrated solution of gamma globulin (Red Cross 15 per cent gamma globulin). Four days later the circulating gamma globulin concentration had risen from the base-line value of 12 mg./100 ml. to 460 mg./100 ml. After 120 days the gamma globulin concentration was found to be 38 mg./100 ml. The decay curve obtained by quantitative measurement of gamma globulin at weekly intervals described a logarithmic decline with half-life of 30 days. Similar findings have been obtained in 3 other cases. From these observations one can be certain that the

low levels of gamma globulin in the serum of these patients are due to failure of production of this protein and not to enhanced destruction or loss.

Other Diseases Featured by Hypogammaglobulinemia

Electrophoretic agammaglobulinemia or extreme hypogammaglobulinemia occur as concomitants of several human diseases other than the one associated with isolated failure of gamma globulin synthesis. Such instances have been described in patients failing in the general fabrication of serum protein, patients destroying the serum proteins with inordinate rapidity and in patients losing large amounts of protein in the urine. Krebs⁴⁰ described agammaglob-

ulinemia among the defects of protein metabolism in a severely malnourished patient. In the syndrome first described by McQuarrie *et al.*,⁴¹ studied by others⁴²⁻⁵¹ and defined electrophoretically by Fried and Henley,⁵² hypogammaglobulinemia occurs as a part of a disease featured by generalized failure of protein fabrication. This syndrome may be transient or permanent.

Pathologic study of McQuarrie's case provided evidence that the disturbance of protein synthesis was due to hepatic abnormality. Although the clinical problem in these patients is usually formation of edema due to the albumin deficit, increased susceptibility to infection and death from bronchopneumonia have been described.⁴¹ Ulstrom⁵³ has recently studied a case in which hypoalbuminemia, edema, hypogammaglobulinemia and anemia were all temporary, apparently interrelated defects of protein synthesis in an infant. Recently he has studied 3 additional patients with this syndrome.⁵⁴

In the nephrotic syndrome hypogammaglobulinemia may occur along with hypoalbuminemia as a reflection of the loss of these protein constituents in the urine.⁵⁵ Patients with nephrosis are not lacking in ability to synthesize serum protein; indeed, evidence^{56, 57} indicates that increased fabrication of both albumin and gamma globulin occurs in patients with this disease. That the extremely low concentrations of gamma globulin in patients with nephrosis may be responsible for their susceptibility to bacterial infection seems likely.

In a few patients⁵⁸⁻⁶¹ increased destruction of serum proteins may be responsible for gamma globulin deficit but thus far this disturbance too has been associated with deficiencies in the other serum proteins, and the gamma globulin defect is only one of the abnormalities demonstrable.

Hypogammaglobulinemia is a frequent concomitant of multiple myeloma^{28, 62-64} and in this disease the gamma globulin deficit may be associated with immunologic failure.^{65, 66} The deficiency of normally migrating gamma globulin in patients with multiple myeloma appears to be a price

paid for the synthesis of inordinate amounts of abnormal globulins by these patients.⁶⁷ The latter compounds, although migrating abnormally on electrophoretic analysis, are immunologic relatives of the normal gamma globulins.^{68, 69} Like the children and adults with isolated agammaglobulinemia, these patients may experience frequent, severe bacterial infections.

Perhaps the most frequent form of isolated agammaglobulinemia as determined electrophoretically is the transient form which occurs in infants between the second and sixth months of life. In these babies there is an apparent delay in assumption of immunologic responsibility. Normally newborn infants are born with a complement of gamma globulin in their serums at least equal to and usually greater than that of the maternal serum.⁷⁰⁻⁷³ During the first weeks of life a rapid decrease in gamma globulin concentration takes place which follows a logarithmic decay curve with a one-half survival time of approximately 20 days.⁷³ From these data and those to be presented later in this report it can be reasoned that for a variable period during the neonatal period the infant does not produce significant amounts of gamma globulin. If this nonproductive period is inordinately prolonged and production of gamma globulin begins late, the concentration of gamma globulin may decrease to levels that are pathologically low. Recently, Spain *et al.*⁷⁴ have attributed some cases of pneumonia resulting in crib death to this form of agammaglobulinemia. Several such cases have been studied in our clinic and laboratory. In each of these infants, overwhelming and sometimes recalcitrant pneumonia has been associated with the extremely low levels of gamma globulin. This disease may occur in either sex and appears to be self-limited as indicated by Kelley's study,⁷⁵ Martin's observations⁷⁶ and our own recent experiences.

During the past 2 years we have had the opportunity to study 10 patients with isolated agammaglobulinemia. Eight of these patients were children. All the children were males and in 3 families male siblings

were affected. In each of the children symptoms referable to the metabolic abnormality had been present dating back to the second half of the first year of life. Two of the patients were adults, one a male and the other a female. In both instances the history provided strong presumptive evidence that the agammaglobulinemia and immunologic abnormality had been acquired. In both the adults and the children the clinical disease was featured by recurrent bacterial infection due primarily to the pyogenic pathogens. Thus, recurrent attacks of meningitis, otitis media, bronchitis, pneumonia, and septicemia due principally to pneumococci, streptococci, staphylococci and Hemophilus influenzae were recorded. However, on several occasions infections with gram-negative organisms, e.g., Pseudomonas, Proteus, or Neisseria intracellularis occurred.

Eight of our patients have been subjected to intensive immunologic, biochemical and hematologic study. From analysis of the unique experiment of nature posed by these patients much that is new concerning immunologic mechanisms and immunologic, biochemical and hematologic interactions has been learned. It is our attempt to interpret this incisive experiment which follows.

The Immunologic Handicap in Agammaglobulinemia

The immunologic handicap in patients with agammaglobulinemia has been revealed in the following ways:

Search for immune response to ubiquitous antigens.

Search for natural agglutinins.

Study of the primary response.

Stimulation with potent bacterial antigens.

Stimulation with heterologous blood group antigens.

Stimulation with potent virus antigens.

Study of the secondary response to the antigens employed in stimulation with potent virus antigens.

In Table II is summarized the response of the patients with agammaglobulinemia

TABLE II
IMMUNE RESPONSE TO UBIQUITOUS ANTIGEN IN
PATIENTS WITH AGAMMAGLOBULINEMIA

Schick test positive	8/8
Dick test positive	5/5
ASO titer <1 u./ml.	6/8
ASO titer <1.6 u./ml.	2/8
Streptococcal antihyaluronidase present	0/8
Streptococcal antidesoxyribonuclease present	0/4
Mumps C.F. antibody present	0/8
Herpes neutralizing antibody present	0/7
Polio neutralizing antibody (all 3 types)	0/4

to antigens ubiquitous in the environment. These studies represent search for evidence of immunologic response to antigens which might be expected to produce an immunologic response in most persons in our culture during the course of their maturation. In none of the patients with agammaglobulinemia were significant amounts of these antibodies to be found in the serum. We interpret these observations as a strong indication that each of the patients with agammaglobulinemia is suffering from a profound immunologic handicap since the immunologic response anticipated in normal persons has not occurred.

Following completion of this study 6 of the patients with agammaglobulinemia were subjected to intensive stimulation with potent bacterial antigens. These results, summarized in Table III, establish the severity of the immunologic defect in these patients. The most intensive stimulation with polysaccharide antigens, protein polysaccharide complexes and protein antigens derived from bacterial sources were employed in the immunologic challenges. Failure of antibody production was the result in each instance.

The natural isoagglutinins against the heterologous blood groups were next studied in the patients with agammaglobulinemia. It has long been known that in normal patients of blood group O, A and B, isoagglutinins against the heterologous blood group substances are absent at birth and uniformly appear in the serum in appreciable concentration during the first

TABLE III
RESPONSE OF PATIENTS WITH AGAMMAGLOBULINEMIA TO POTENT BACTERIAL ANTIGENS

Group	Patient	Age (yr.)	Sex	Stimulation	Result
Congenital agammaglobulinemia	B.H.	5	M	Primary, secondary and tertiary stimulation with TAB, DPT,* pneumococcal polysaccharide type 1 and 2.	No response†
	E.S.	7	M	Primary, secondary and tertiary stimulation with TAB, DPT,* pneumococcal polysaccharide type 1 and 2.	No response†
	W.A.	6	M	Primary, secondary and tertiary stimulation with TAB, DPT,* pneumococcal polysaccharide type 1 and 2.	No response†
	T.A.	1	M	Primary, secondary and tertiary stimulation with TAB, DPT,* pneumococcal polysaccharide type 1 and 2.	No response†
Acquired agammaglobulinemia	F.H.	58	M	Primary, secondary and tertiary stimulation with TAB, DPT,* pneumococcal polysaccharide type 1 and 2.	No response†
	L.L.	30	F	Primary, secondary and tertiary stimulation with TAB, DPT,* pneumococcal polysaccharide type 1 and 2.	No response†

* Diphtheria, pertussis and tetanus antigens.

† Antibody response measured by agglutinin titer vs. TAB, Shick test and agglutinins vs. DPT and quantitative precipitin vs. pneumococcal polysaccharide.

year of life. Consequently in both normal children and normal adults the "natural" isoagglutinins against heterologous blood groups are readily demonstrable. In contradistinction, antibodies against the heterologous blood group substances were either completely absent or present in extremely low titer in the patients with agammaglobulinemia. The results are summarized in Table IV. Four of our patients with agammaglobulinemia were of blood group O. None of these possessed detectable antibodies against either A or B cells. One patient with congenital agammaglobulinemia was of blood group B and possessed no antibody against group A cells. One of the patients with acquired agammaglobulinemia was of blood group A as were 2 patients with the congenital form. Each possessed minute amounts of agglutinating antibody against group B cells. In no case did the antibody titer overlap that observed in a large group of normal children studied as controls. The results are summarized in Table IV.

After these observations were available, 6 of the patients with agammaglobulinemia were stimulated by the intravenous or intramuscular injection of varying amounts of mismatched cells. Whereas small

TABLE IV
BLOOD GROUP AND ISOAGGLUTININ TITERS IN PATIENTS WITH AGAMMAGLOBULINEMIA

Group	Patient Age and Sex	Blood Group	Isoagglutinin Titer	
			Anti A	Anti B
Congenital agammaglobulinemia	B.H. 5 yr. male	B	no titer	—
	E.S. 7 yr. male	O	no titer	no titer
	W.A. 6 yr. male	O	no titer	no titer
	T.A. 1 yr. male	O	no titer	no titer
	F.T. 2 yr. male	A	—	pos. in 1-5 dilution
	T.T. 2 yr. male	A	—	pos. in 1-5 dilution
	1 yr. male			
Acquired agammaglobulinemia	L.L. 30 yr. female	O	no titer	no titer
	F.H. 58 yr. male	B	—	pos. in 1-2 dilution
Normal	Normal 20 cases	O	1:343.0*	1:185.1*
	Normal 18 cases	A	—	1:179.0*
	Normal 15 cases	B	1:194.0*	—

* Geometric mean titer.

amounts (1 to 2 ml.) of such mismatched cells produced febrile reactions, fall in platelets, sudden decrease in polymorphonuclear leukocytes and malaise upon their intravenous injection into immunologically normal recipients; up to 20 ml. of packed mismatched cells were injected intravenously into the patients with agammaglobulinemia without reaction (Table V).

The intensive antigenic stimulation afforded by the intravenous or intramuscular injection of mismatched blood cells failed to induce antibody production in 4 of 5 cases in which it was tried. In 1 case, a patient with acquired agammaglobulinemia of blood group A who possessed a low titer of anti B antibody initially, intramuscular injection of 2 ml. mismatched blood cells (group B) resulted in feeble 1-tube antibody response. In 4 cases repeated injections of varying amounts of mismatched cells were given, employing the several different routes for administration of antigen. No response was obtained. The results are summarized in Table VI.

For comparison, a sharp 3-tube rise in anti-B antibody titer was obtained in a normal patient who was immunologically group A and who was given an intramuscular injection of 2 ml. of group B cells.

Response of the Patient with Agammaglobulinemia to Virus Infections and to Viral Antigens

A clinical paradox noted in patients with agammaglobulinemia concerns virus infections. Children with agammaglobulinemia have a surprising ability to cope successfully with most virus infections in spite of their immunologic handicap. Children with congenital agammaglobulinemia may develop virus diseases, express the usual symptoms and clinical course, recover from them, and even appear to resist recurrences. For example, patients with agammaglobulinemia whom we have studied have suffered from rubeola, rubella, varicella, the common cold, nonbacterial upper respiratory disease (ARD), mumps and polio. These infections have not been different

TABLE V

RESPONSE OF PATIENTS WITH AGAMMAGLOBULINEMIA TO THE INTRAVENOUS INJECTION OF MISMATCHED BLOOD

Patient	Age yr.	Sex	Diagnosis	Intravenous Injection	Pre-injection			2 Hours Post-injection		
					Temp. °C.	Neutrophils	Platelets	Temp. °C.	Neutrophils	Platelets
E.S.	7	M Blood Group O	Agammaglobulinemia, immunologic paralysis	20 ml. packed group A cells	37.4	3200	350,000	37.6	3000	365,000
W.A.	6	M Blood Group O	Agammaglobulinemia, immunologic paralysis	15 ml. packed group A cells	37.6	2900	420,000	37.6	3320	385,000
T.A.	1	M Blood Group O	Agammaglobulinemia, immunologic paralysis	10 ml. packed group A cells	37.8	2250	302,000	37.6	2640	410,000
L.L.	30	F Blood Group O	Agammaglobulinemia, immunologic paralysis	15 ml. packed AB cells	37.0	4240	315,000	37.0	4050	285,000
R.N.	5	M Blood Group O	Leukemia in remission	2 ml. group A blood cells	37.4	2250	225,000	38.6	550	115,000
B.H.	3	M Blood Group O	Brain tumor	2 ml. group A blood cells	37.6	4580	265,000	38.8	740	185,000
R.T.	4	M Blood Group O	Hydrocephalic infant	2 ml. group A blood cells	37.6	3950	212,000	37.9	1250	126,000

TABLE VI
EFFECT OF PARENTERAL ADMINISTRATION OF HETEROLOGOUS BLOOD GROUP CELLS ON THE ISOAGGLUTININ TITERS OF PATIENTS WITH AGAMMAGLOBULINEMIA

Patient	Blood Group	Primary Stimulation	Secondary Stimulation	Tertiary Stimulation	Initial Titer	Titer*		
						After 1	After 2	After 3
E.S. 7 years old, male Congenital agammaglobulinemia	O	20 ml. packed A cells i.v.	10 ml. packed A cells i.m.	1 ml. packed A cells i.d. 10 ml. packed A cells i.m.	no titer	no titer	no titer	no titer
W.A. 6 years old, male Congenital agammaglobulinemia		15 ml. packed A cells i.v.	10 ml. packed A cells i.m.	1 ml. packed A cells i.d. 5 ml. packed A cells i.m.	no titer	no titer	no titer	no titer
T.A. 1 year old, male Congenital agammaglobulinemia	O	10 ml. packed A cells i.v.	10 ml. packed A cells i.v.	1 ml. packed A cells i.d. 10 ml. packed A cells i.m.	no titer	no titer	no titer	no titer
L.L. 30 years old, female, acquired agammaglobulinemia	O	15 ml. packed B cells i.v.	20 ml. packed AB cells i.v.	10 ml. packed A cells i.m.	no titer	no titer	no titer	no titer
F.H. 53 years old, male Acquired agammaglobulinemia	A	2 ml. packed B cells i.m.	—	—	1/2	1/4		
R.G. 32 years old, male	A	2 ml. packed B cells i.m.	—	—	1/160	1/1280		

* Antibody titer measured 10 days after each antigenic stimulation.

from those occurring in normal persons. Although the observations of Keiden and McCarthy,⁷⁷ Kempe and others^{78, 79} that certain infants with agammaglobulinemia or hypogammaglobulinemia developed vaccinia gangrenosa kept us from vaccinating the children in our series, 5 of our patients had been vaccinated prior to consulting us. In 4 of these children the reaction to vaccination described was typical of the primary response. One of the children, however, failed to react and on 3 different occasions vaccination has produced no response in this child. In 2 of the others, repeated vaccination produced an accelerated response. These observations are similar to those of Janeway *et al.*⁵ Re-exposure to exanthematous infections indicates that appreciable clinical resistance is induced by a previously occurring virus infection. For example, a 7-year-old boy with agammaglobulinemia (E.S.) who had never been given gamma globulin, had typical rubeola when he was 5 years old. The illness was featured by coryza, cough, typical rash and fever up to 39.8°C. He recovered from this

infection after the usual stormy febrile course of 6 days duration. During his sixth and seventh years of life while he was being studied on our clinical service, he was re-exposed by intimate contact with infective cases of measles on 3 separate occasions. No gamma globulin was given. In spite of his known immunologic handicap he did not develop measles after any of these exposures. Similar exposure occurred in another 6-year-old boy with agammaglobulinemia (W.A.) who had not had measles. This boy developed typical rubeola 14 days after exposure to one of the same patients to whom E.S. had been exposed. His disease differed in no way from that of several other children who developed the infection after exposure to the same contact. Figure 4 shows this child during the exanthematous phase of measles. Subsequent intimate re-exposure of this second child to measles on 2 occasions did not produce disease. This same 6-year-old boy with agammaglobulinemia developed chicken pox at the age of 4 years. This illness was unusual in that he had a mild secondary



FIG. 4. Patient with agammaglobulinemia at the height of an attack of measles. Note the characteristic morbilliform rash.

outbreak of skin lesions 2 weeks after subsidence of the initial infection. Subsequent intimate re-exposure to chicken pox both in the hospital and at home, however, failed to produce a recurrence of this disease.

During the 2-year period of observation of 8 patients with agammaglobulinemia, susceptibility to virus infections has not been notable. Clinically these patients have not been inordinately susceptible to the common cold, or infections with the A.P.C. group of viruses. They have not developed atypical pneumonia, influenza or other recognizable virus infections. In contradistinction, during periods when gamma globulin is not being administered or antibiotics are not being given as a prophylactic measure, they begin again to experience frequent, life-threatening bacterial infections.

Like the children with the congenital disease, adults with acquired agammaglobulinemia tolerate certain virus infections and resist their recurrence. For example,

the 7-year-old daughter of a 30-year-old female with agammaglobulinemia developed both measles and chicken pox during the period when the patient was known to be agammaglobulinemic without transmitting the disease to her mother.

Some evidence suggests that generalization from these observations to all virus diseases may be erroneous. For example, repeated episodes of mumps have been reported in agammaglobulinemic patients.³³ Whether these be true recurrences of mumps is of course open to question because other forms of parotitis may have been confused with the epidemic disease, no virus studies have been done, and the immune response is not helpful in these patients. In addition, one of our patients with acquired agammaglobulinemia developed what appeared to be homologous serum hepatitis which took a fulminant course with death in hepatic coma 2 weeks after the onset of the jaundice.⁸⁰ Janeway⁸¹ also has observed the development in a child with agammaglobulinemia of hepatitis which became chronic and ultimately led to the death of the patient after a prolonged illness. It may be that some virus infections are handled well by these patients while others, in which circulating antibody or gamma globulin play a vital role in recovery, are handled with lesser facility.

In an attempt to unravel the paradox presented by these observations we have studied the immunologic response of patients with agammaglobulinemia to virus antigens.

Influenza vaccine, mumps vaccine, spotted fever, Q fever, typhus, western equine encephalitis antigens and all 3 types of poliomyelitis virus were injected repeatedly into each of 4 patients with agammaglobulinemia. In only 1 instance was there any evidence of antibody production. The 58-year-old man with acquired agammaglobulinemia produced antibody against type I poliomyelitis virus in 1:4 titer. This patient and the 3 others produced no neutralizing, complement fixing, or hemagglutination inhibiting antibodies against any of

the other antigens employed. The results are summarized in Table VII.

It must be concluded from these data that patients with agammaglobulinemia do not respond to virus antigens by antibody production any more satisfactorily than they do to bacterial antigens, simple protein antigens or blood cell antigens. In light of these observations it must now be reasoned either that minute amounts of anti-virus antibody which cannot be de-

tected by the most delicate *in-vitro* techniques can protect against virus infection or that mechanisms other than those involving antibody production operate in clinical expression of, recovery from, and resistance to certain virus diseases.

The Nature of Bacterial Type Hypersensitivity

Although most reports on agammaglobulinemia emphasize that repeated tuber-

TABLE VII

RESPONSE OF PATIENTS WITH AGAMMAGLOBULINEMIA TO STIMULATION WITH VIRUS AND RICKETTSIAL ANTIGENS

Antigen Used	Test Used to Detect Response	Patient and Antibody Response Obtained*				
		Congenital Agammaglobulinemia			Acquired Agammaglobulinemia	
		<i>E.S.</i> 7 years old, male	<i>W.A.</i> 6 years old, male	<i>T.A.</i> 1 year old, male	<i>F.H.</i> 58 years old, male	<i>L.L.</i> 30 years old, female
1. Influenza vaccine Lederle	3 injections of 1 ml. each, hemagglutination inhibition	No response	No response	No response	No response †	No response †
2. Mumps virus vaccine Lederle	3 injections of 1 ml. each, complement fixation	No response	No response	No response	No response	No response
3. Spotted fever group antigens	3 injections of 1 ml. each, complement fixation, hemagglutination inhibition	No response	No response	No response	No response	
4. Typhus vaccine	Same as 3	No response	No response	—	No response	
5. Q fever antigen	Same as 3	No response	No response	—	No response	—
6. Western equine encephalitis	3 injections of 1 ml. each, neutralization, complement fixation	No response	No response	—	No response	—
7. Polio virus types I, II, and III	1 ml. × 2 at weekly intervals, then 1 ml. 1 month later, neutralization of cytotoxic effect on HeLa cells	No response ‡	No response**	No response	1:4 titer to type I, no response to other antigens	No response

* Immune response to each antigen was obtained in simultaneous controls.

† Had previously had mumps.

** Had previously had clinical poliomyelitis which left residual paralysis in both lower extremities.

‡ This patient was injected with 1 cc. Salk polio vaccine on 5 separate instances without ever producing detectable antibody.

culin reactions have been negative in these patients, the problem of bacterial or delayed type hypersensitivity is brought into focus by 2 case reports. Zinneman *et al.*⁸² described an adult male with acquired agammaglobulinemia who was tuberculin positive and Seltzer *et al.*²⁷ studied a patient with the acquired disease who possessed a positive histoplasmin skin test in association with calcified pulmonary lesions. We were provoked to investigate this matter by the observation of a questionably positive tuberculin reaction in a 6-year-old boy with congenital agammaglobulinemia.¹⁴

In another instance we observed the development of an angry inflammatory skin lesion in the diaper area of an infant with agammaglobulinemia having an infection with monilia (Fig. 5). This lesion did not look like the usual ammoniacal diaper rash but rather like the periorificial rash seen in generalized moniliasis. That this skin lesion might reflect bacterial-type hypersensitivity to the fungus was considered likely. Two weeks following initiation of treatment of

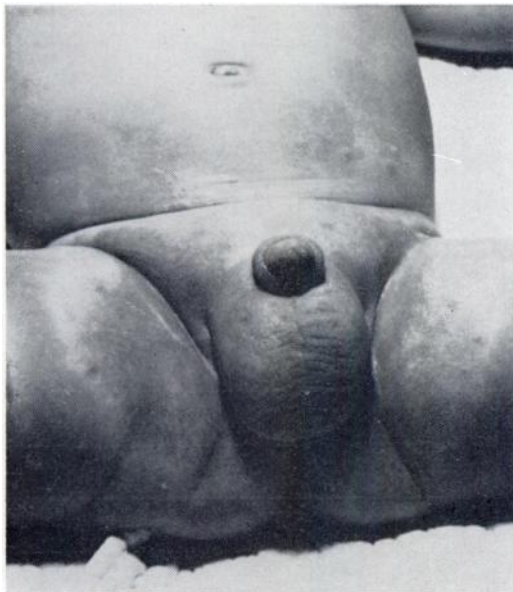


FIG. 5. Patient with agammaglobulinemia suffering from monilia infection. Note the erythematous, indurated, inflammatory reaction which we attributed to bacterial type hypersensitivity to the monilia. The reaction disappeared upon treatment with Mycostatin®.

the monilia infection with Mycostatin®, the skin lesion abated.

Our experimental approach to this question has been fourfold.

To study the delayed reactivity of patients with agammaglobulinemia to antigens which commonly produce bacterial type hypersensitivity in the population at large.

To attempt passively to transfer delayed hypersensitivity in these patients by injection of circulating leukocytes from a sensitive donor.

To attempt to induce the development of delayed type hypersensitivity in these patients by skin sensitization.

To attempt to transfer bacterial-type hypersensitivity from patients with agammaglobulinemia to normal persons.

Although results of the study appear to be somewhat contradictory they are presented because of their importance in understanding the relationship of the classical immune response to bacterial or delayed type hypersensitivity. In Table VIII are presented data showing the skin reaction to tuberculin, streptococcal products, pneumococcal and streptococcal vaccines in these patients.

As may be seen in this table, all except one of the patients were clearly tuberculin negative. This patient developed erythema without significant induration with each dose of tuberculin used. The erythema persisted for 3 days on each test. Simultaneous saline controls were negative. The lack of induration made it impossible to define this reaction as a positive tuberculin skin test. None of the patients developed significant delayed reaction to the streptokinase-streptodornase antigen, to the whole group A streptococcus vaccine,^{*} or to pneumococcus vaccine.

In Table IX the incidence of bacterial type hypersensitivity to the streptococcal

* Prepared from suspension of 8 different types of group A streptococci, this vaccine produced + delayed skin reactions in 40 to 50 per cent of normal adults.

TABLE VIII
 DELAYED (BACTERIAL-TYPE) HYPERSENSITIVITY IN PATIENTS WITH AGAMMAGLOBULINEMIA

Test Performed	Patient, Age, Sex							
	<i>B.H.</i> 5 yr. male	<i>E.S.</i> 7 yr. male	<i>W.A.</i> 6 yr. male	<i>T.A.</i> 1 yr. male	<i>T.T.</i> 15 mo. male	<i>F.T.</i> 20 mo. male	<i>L.L.</i> 30 yr. female	<i>F.H.</i> 58 yr. male
Tuberculin								
1:10,000	neg	neg	?+	neg			neg	neg
1:1,000	neg	neg	?+	neg	neg	neg	neg	neg
1:100	neg	neg	?+	neg	neg	neg	neg	neg
1:10		neg	—	neg			neg	neg
P.P.D.								
1st strength	neg	neg	neg	neg	neg		neg	neg
2nd strength	neg	neg	?+	neg	neg		neg	neg
SK-SD								
10 USK—2.5 USD	neg	neg	neg	neg	—	—	neg	neg
100 USK—25 USD	neg	neg	neg	neg	—	—	neg	neg
1000 USK—250 USD	neg	neg	neg	neg	—	—	neg	±
Whole group A streptococcus vaccine*	neg	neg	neg	neg	—	—	neg	neg
Whole pneumococcus vaccine†	neg	neg	neg	neg	—	—	neg	neg

* Prepared from a washed suspension of 8 different types of group A streptococci. This vaccine produced a delayed skin reaction in 40 per cent of hospitalized children more than 5 years of age and in 50 to 60 per cent of healthy adults.

† Prepared from a washed suspension of a rough strain of pneumococci. This vaccine produced a positive delayed skin reaction in approximately 25 per cent of healthy adults and older children.

TABLE IX
 BACTERIAL TYPE HYPERSENSITIVITY TO STREPTOCOCCAL PRODUCTS IN PATIENTS WITH AGAMMAGLOBULINEMIA AND NORMAL PERSONS*

Vaccine Injected Intradermally	Group	Number	Number Positive	Number Negative	Per Cent Positive
Streptokinase 10 units Streptodornase 2.5 units in physiologic saline	Agammaglobulinemia	6	0	6	0
	Hospitalized children 2-5 years	34	16	18	47.0
	Hospitalized children 7-16 years	44	40	4	90.9
	Healthy adults	16	14	2	87.5
Streptokinase 100 units Streptodornase 25 units in 0.1 ml. physiologic saline	Agammaglobulinemia	6	0	6	0
	Normal adults	16	14	2	87.5
	Normal children 2-16 years	78	60	18	76.9

* Test read 24, 48, 72 and 96 hours after injection of antigen.

antigens, S.K. and S.D., in the patient with agammaglobulinemia is compared to that of normal children and adults. It may be seen that the patients with agammaglobulinemia do not react to the intradermal injection of these antigens whereas most normal children and adults possessed delayed sensitivity to them. Assuming that the infections producing hypersensitivity to these antigens have occurred at least as frequently in the patient with agammaglobulinemia as in the normal person, it follows from these data that a deficiency in development of bacterial type hypersensitivity must also be a feature of this disease.

Passive Transfer of Delayed Type Hypersensitivity to Patients with Agammaglobulinemia

In an attempt to initiate immunologic responsiveness in patients with agammaglobulinemia, 3 of them were given subcutaneous injections of leukocytes from donors sensitive to the streptokinase-streptodornase antigen and one was given leukocytes from a patient sensitive to tuberculin. In this same experiment, to gain information concerning cellular basis of antibody production, these cells were taken from donors who had received 3 prior injections of typhoid-paratyphoid antigens.

TABLE X
TRANSFER OF BACTERIAL TYPE HYPERSENSITIVITY TO PATIENTS WITH AGAMMAGLOBULINEMIA

Patient	Skin Reaction to SK-SD Prior to Injection of Leukocytes	Skin Reaction to SK-SD 48 Hours After Injection of Leukocytes from Sensitive Donor	Skin Reaction 4 Months After Injection of Leukocytes	Skin Reaction 1 Year After Injection of Leukocytes
E.S. 7 years old male— congenital agammaglobulinemia	neg	++	+++	+++
W.A. 6 years old male— congenital agammaglobulinemia	neg	++	+	neg
F.H. 58 years old male— acquired agammaglobulinemia	neg	+++	--	--
T.A. 1 year old male— congenital agammaglobulinemia	<i>Not Given Injections of Leukocytes</i>			
	neg	neg	neg	neg
L.L. 30 years old female— acquired agammaglobulinemia	neg	neg	neg	neg

The leukocytes were separated from the erythrocytes and serum by a modification of the fibrinogen precipitation technique of Lawrence.⁸³ Between 2 and 4 ml. of packed leukocytes (the leukocytes from 1000 ml. of freshly drawn blood) were re-suspended in 20 ml. of saline and divided into 2 equal portions. One portion was injected intravenously and the other subcutaneously. With the method used, 90 to 95 per cent of the cells were viable when injected into the recipients. Although the donors of the white cells were shown to be producing antibody vigorously against typhoid H, O and B antigens at the time of the transfer, no circulating antibody or detectable gamma globulin was produced in the recipients following transfer of the leukocytes from the circulating blood. These data provide evidence that none of the leukocytes from the peripheral blood are capable of producing agglutinins in amounts detectable by the immunologic methods employed.

On Table X are summarized results showing that sensitivity to streptococcal antigens was readily transferred to the agammaglobulinemic patient by the injection of viable leukocytes. A similar experiment carried out with leukocytes from a tuberculin sensitive donor showed that delayed sensitivity to tuberculin could also be produced in patients with agammaglobuline-

mia in this way. The delayed sensitivity lasted between 3 months and 1½ years. In Figure 6 are shown pictures of the skin reaction to streptococcal products prior to the injection of leukocytes, 72 hours after the injection, and 1½ years after the "passive" sensitization.

Because it is inconceivable that the leukocytes originally injected could survive as long as 1½ years in the agammaglobulinemic recipient, it must be concluded from these observations that patients with agammaglobulinemia can, at least, sustain a state of hypersensitivity.

Development of Sensitivity to 2-4,dinitrofluorobenzine in the patient with Agammaglobulinemia

Skin sensitivity to 2-4,dinitrofluorobenzine (DFNB) is readily produced by application of this compound in vesicant doses to the skin of normal human subjects.⁸⁴ Studies in our laboratory indicate that similar skin sensitivity to DFNB is readily produced in patients with agammaglobulinemia. We have further observed that this reactivity can be transferred to a nonsensitive recipient by the subcutaneous injection of viable leukocytes from the actively sensitized child with agammaglobulinemia. On the other hand, sensitization to DFNB was not produced by the transfer of a large volume of serum from the sensi-

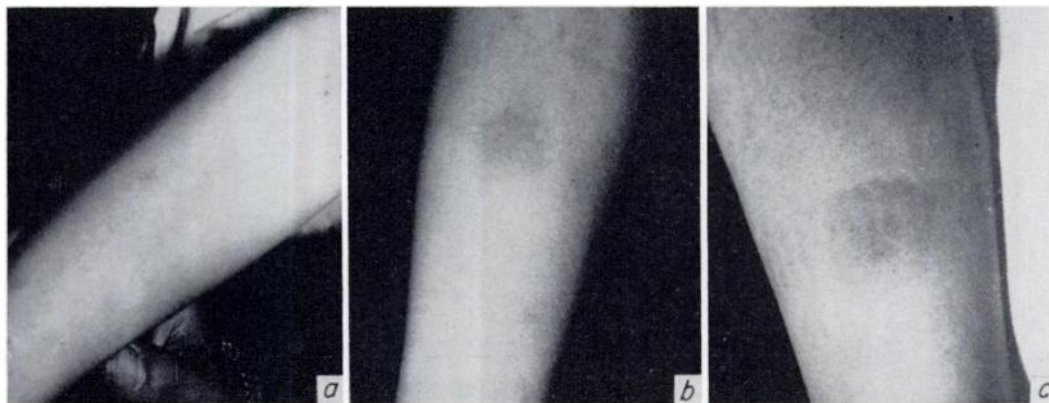


FIG. 6. Skin reactions to streptokinase-streptodornase (SK-SD) in a patient with agammaglobulinemia: (a) prior to injection of leukocytes; (b) 72 hours following subcutaneous and intravenous injection of 0.5 ml. of leukocytes from a donor sensitive to SK-SD; (c) 18 months following subcutaneous and intravenous injection of 0.5 ml. of leukocytes from donor sensitive to SK-SD.

tized agammaglobulinemic donor. These findings confirm the observations of Porter³³ that the properly stimulated patient with agammaglobulinemia can develop delayed skin sensitivity to chemicals in spite of his complete refractoriness to the formation of circulating antibodies in response to antigenic stimulation. Figure 7 illustrates the reactions produced before and after sensitization with DFNB in patients with agammaglobulinemia.

These results make it necessary to propose that development of skin sensitivity must either be independent of the synthesis of circulating antibody and gamma globulin or reflect a stage of the latter process proximal to the metabolic block responsible for agammaglobulinemia. If either be the case, then explanation of the infrequent occurrence of the naturally developing sensitivity to streptococcal products becomes difficult. Two possibilities seem to be compatible with the facts and may be suggested. It might be that with delayed hypersensitivity, as with gamma globulin and antibody synthesis, a quantitative but not qualitative deficiency of responsiveness exists in the patients with agammaglobulinemia which is expressed in the failure to develop hypersensitivity to streptococcal products in the course of natural experience with streptococcal infection. If this were

the case, a sufficiently intense natural or artificial sensitizing stimulus might be able to induce the hypersensitivity state whereas the naturally occurring stimulation might be ineffective.

Another possible explanation for this discrepancy could be that in patients with agammaglobulinemia, hypersensitivity to streptococcal and pneumococcal products fails to develop because the patients do not receive *adequate stimulation* from prolonged infection. A reason for failure of stimulation might be that the immunologic handicap demands effective antibiotic therapy and consequent elimination of infecting streptococci. Studies to clarify this relationship are in progress.

Since this study was completed, Waldenstrom *et al.*³⁰ have reported that vaccination with BCG produced bacterial type hypersensitivity in 2 of 3 patients with congenital agammaglobulinemia. Immunization with BCG in each case was completed long before any gamma globulin was administered to the patients.

In an effort to complete the immunological investigation on patients with agammaglobulinemia, 4 additional studies were performed:

Search for immediate type skin sensitivity.

Production of the Prausnitz-Küstner reaction in the agammaglobulinemic patient.

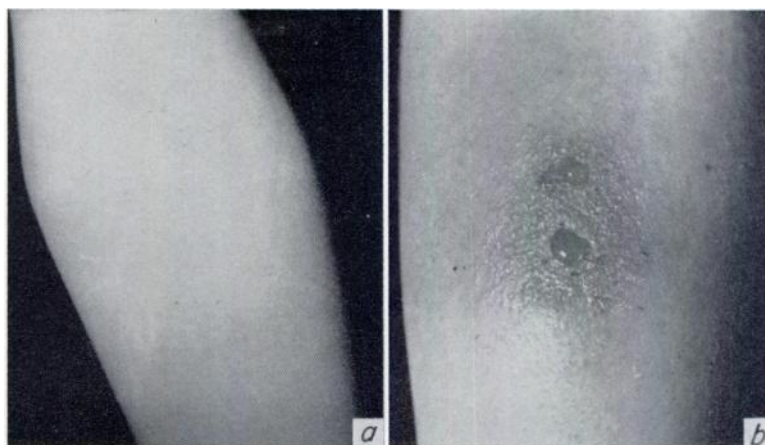


FIG. 7. Skin reaction to 2,4-dinitrofluorobenzene: (a) before stimulation with vesicant dose of 2,4-dinitrofluorobenzene; (b) 14 days following stimulation with vesicant dose of 2,4-dinitrofluorobenzene to the skin of the back.

Measurement of the serum complement concentration.

Attempt to inhibit the immune response by the intravenous injection of serum from a patient with agammaglobulinemia.

Because in several instances members of the families of patients with agammaglobulinemia gave histories suggestive or definitive of allergy and because the relation of atopic type hypersensitivity to the formation of other forms of circulating antibody has not been established, it seemed of interest to search for atopic sensitivity among patients with agammaglobulinemia. Skin testing with 114 different allergens including all of the food, pollen and inhalant antigens employed in our allergy clinic, were applied to the skin of each of 6 patients with agammaglobulinemia. In no instance did a significant immediate skin reaction develop. That this result could not be attributed to failure of responsiveness was established when immediate type wheal and flare reactivity to egg white and cotton seed was passively transferred from a child allergic to these antigens to the skin of a child with agammaglobulinemia by the classical Prausnitz-Küstner test. These studies do not signify that the patient with agammaglobulinemia is unable to develop spontaneously the immediate wheal and flare type hypersensitivity but so far as we know this phenomenon has not yet been demonstrated in patients with this disease.

Finally, using a modification of the method of Wedgwood and Janeway,⁸⁵ serum complement values were measured in 6 of the patients with agammaglobulinemia. In Table XI these values are compared to normal values obtained in our laboratory and the mean normal complement value obtained by Wedgwood and Janeway.⁸⁵ As may be seen from the tables, normal or slightly elevated complement concentrations were present in each of the patients with agammaglobulinemia studied.

Because it is conceivable that agammaglobulinemia is due to the production in the patient with agammaglobulinemia of a circulating substance which inhibits the

TABLE XI
SERUM COMPLEMENT CONCENTRATION IN PATIENTS
WITH AGAMMAGLOBULINEMIA

<i>Patient</i>	<i>Complement Concentration units/ml.</i>	<i>Classification of Disease</i>
E.S. 7 years old male	62.0	Congenital agammaglobulinemia
B.H. 5 years old male	84.0	Congenital agammaglobulinemia
W.A. 6 years old male	96.0	Congenital agammaglobulinemia
T.A. 1 year old male	80.0	Congenital agammaglobulinemia
L.L. 30 years old female	74.0	Acquired agammaglobulinemia
F.H. 58 years old male	80.0	Acquired agammaglobulinemia
Normal values	60-75 units	normals
Wedgwood and Janeway ⁸⁵ Normals	Mean 47.7 units	normals

formation of antibody and gamma globulin, it was deemed important to search for such a compound by administering agammaglobulinemic blood to an immunologically normal recipient.

An 8-year-old boy with congenital agammaglobulinemia was bled of 200 ml. of blood, the plasma removed and 75 ml. injected intravenously in a 3½-year-old child with irreversible hydrocephalus. Immediately following injection of the plasma the recipient child was injected with 0.5 ml. T.A.B. vaccine and 0.5 ml. D.P.T. vaccine. Prior to the injection of plasma the recipient child was skin tested and found to be Schick positive. Samples of blood were

taken from the recipient child prior to and at weekly intervals after the injections of vaccine. Serial studies of the gamma globulin concentration and determination of antibody titer to typhoid-paratyphoid vaccine revealed vigorous antibody response and maintenance of normal gamma globulin concentration over a 1-month period following injection of the plasma. The Schick test performed 1 month after injection of DPT antigen had converted from positive to negative.

Similar studies performed in an adult human volunteer who was the recipient of a subcutaneous injection of leukocytes from a child with agammaglobulinemia revealed no effect of the leukocytes on the antibody response or the gamma globulin concentration of the immunologically normal person.

Results of Antigenic Stimulation After Injection of Gamma Globulin

The association of agammaglobulinemia with generalized immunologic paralysis as demonstrated in this study focuses attention on the relationship between antibody and gamma globulin. Although small amounts of antibody occur in fractions other than the gamma globulin fraction, it has been well established that the bulk of serum antibody in man is contained in the gamma and beta₂ globulin fractions. These are the protein components of the serum which are lacking in patients with agammaglobulinemia. It has further been shown that proteins immunologically related to the gamma globulins exist throughout the globulin spectrum as it is defined electrophoretically. It thus is possible that all antibody is gamma globulin even though some is found with other electrophoretic components.

With respect to the relationship between gamma globulin and antibody, at least 3 possibilities exist:

All gamma globulin is antibody and hence failure in formation of one would be associated with failure of formation of the other.

Some gamma globulin is antibody under which hypothesis the synthesis of the components might be independent.

Antibody is formed by modification of existing gamma globulin under which circumstance failure of formation of gamma globulin would result in failure of formation of antibody because the essential precursor would be lacking.

If the latter were true and if the metabolic defect in agammaglobulinemia involved synthesis of the gamma globulin but not formation of antibody, one might anticipate that the block to antibody synthesis might be overcome by the administration of preformed gamma globulin from healthy persons. This possibility was suggested by the dramatic protection afforded patients with agammaglobulinemia by the administration of gamma globulin. That this is not the case was shown in the following experiment.

Two children with congenital agammaglobulinemia were given gamma globulin intramuscularly in large dosage (4 ml./kg). This injection resulted in a rise of serum gamma globulin concentration from less than 12 mg./100 ml. in both children to levels between 400 and 500 mg./100 ml. The latter concentration approaches the concentration of gamma globulin observed in immunologically normal children.

Following injection of the gamma globulin, each child was given a series of 3 injections of typhoid, paratyphoid vaccine, mumps vaccine and diphtheria toxoid. Bleedings were taken 5, 10, 15 and 25 days after the injection of the vaccine.

Just as had been the case prior to administration of gamma globulin, no detectable antibody was formed when the immunization was attempted while these patients had serum concentrations of gamma globulin which were close to the normal levels.

Reaction to Gram-Negative Bacterial Endotoxin

Parenteral injection of endotoxins derived from gram-negative bacteria produces in man and animals a stereotyped series of

TABLE XII
 REACTIONS OF PATIENTS WITH AGAMMAGLOBULINEMIA AND NORMAL PERSONS TO
 GRAM-NEGATIVE BACTERIAL ENDOTOXINS

Group	Patient Age Sex	Intradermal Injection				Intravenous Injection			
		0.2 ml. Typhoid Vaccine		2.5 µg. Piromen®		10 ⁷ Typhoid-para- typhoid Organisms		50 µg. Piromen®	
		Erythema	Induration	Erythema	Induration	Maximum Temp. °C.	Systemic Reaction	Maximum Temp. °C.	Systemic Reaction
Agammaglobulinemia	E.S. 7 yr. male	4+	3+	3+	2+	39.6	Chills, headache, nausea	39.2	Chilliness, malaise
	R.H. 58 yr. male	4+	4+	2+	2+	39.3	Chills, headache, weakness	39.0	—
	L.L. 30 yr. female	4+	4+	2+	2+	39.2	Chills, weakness	38.8	—
	W.A. 7 yr. male	3+	3+	2+	2+	40.9 104.8	Chills, headache, malaise	39.6	Chilliness
	T.A. 1 yr. male	3+	2+	2+	2+	40.2	—	39.7	Chills, malaise
	B.H. 6 yr. male	4+	3+	3+	2+	39.9	Chills, malaise	39.0	—
Normal persons	R.G. 32 yr. male	4+	4+	2+	2+	39.3	Chills, headache, weakness	38.7	Chilliness
	J.P. 12 yr. male	4+	3+	1+	1+	39.6	Chills, headache	39.1	Chilliness, malaise
	R.L. 9 yr. male	3+	3+	2+	1+	40.2	Chills, Headache	39.0	—
	R.A. 7 yr. female	3+	3+	2+	1+	40.1	Chills, malaise	39.6	Chills
	W.O. 7 yr. female	4+	4+	2+	2+	40.7 105.2	Chills, headache, malaise	39.0	—

responses.⁸⁶ The effects of endotoxin which have been most extensively studied in man are the pyrogenic effect, the local inflammatory reaction and the phenomenon of refractoriness. Although the relationship of these reactions to classical immune phenomena have been much discussed it must be stated that dissociation of the local and systemic reactions to gram negative bacterial endotoxins from classical immune phenomena and from bacterial type hypersensitivity cannot be made unequivocally on the basis of available evidence. It seemed of interest then to determine whether patients with agammaglobulinemia who were completely lacking in circulating antibodies and capacity for their production, would react in the usual way to gram-negative endotoxin.

In order to investigate the nature of reactions of human hosts to parenteral administration of endotoxin as well as to gain information concerning the reactivity of patients with agammaglobulinemia to bacterial products, preparations containing gram-negative bacterial endotoxins were injected into normal adults and children and into subjects with agammaglobulinemia. The results summarized in Table XII indicate that, just as normal persons, patients with agammaglobulinemia develop an intense delayed inflammatory reaction to intradermally injected endotoxin, and develop headache, chills, fever and malaise

in response to the intravenous injection of endotoxin. In Figure 8 are illustrated the reactions of a normal child and of a child with agammaglobulinemia to the intradermal injection of endotoxin from *Pseudomonas aeruginosa* (Piromen[®]). Additional experiments established that refractoriness to the pyrogenic actions and intoxicating effects of endotoxin develop equally well in the patient with agammaglobulinemia and the immunologically normal patient (Fig. 9).

These observations must be taken as substantial support for the concept that both local and systemic reactions to gram-negative bacterial endotoxins are independent of mechanisms based on anaphylactic type hypersensitivity. It can be further concluded that refractoriness to the febrile and intoxicating effect of endotoxins may occur in the complete absence of circulating antibody.

Acute Phase Reactants

Study of the patient with agammaglobulinemia has provided evidence concerning the relationship between acute phase reactions and antibody production. During the acute phase of many unrelated diseases, changes occur in the circulating blood which in the aggregate have been referred to as the acute phase phenomena. The sedimentation rate is the acute phase reaction most widely used in clinical medicine and is the prototype of this group of reactions.

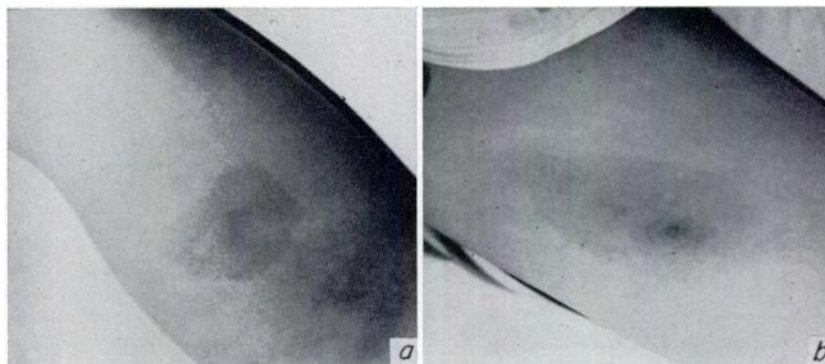


FIG. 8. Comparison of reactions of patient with agammaglobulinemia and normal child to intradermal injection of gram-negative bacterial endotoxin. (a) Child with agammaglobulinemia 36 hours following intradermal injection of 2.5 µg. Piromen[®]. (b) Normal child 36 hours following intradermal injection of 2.5 µg. Piromen[®].

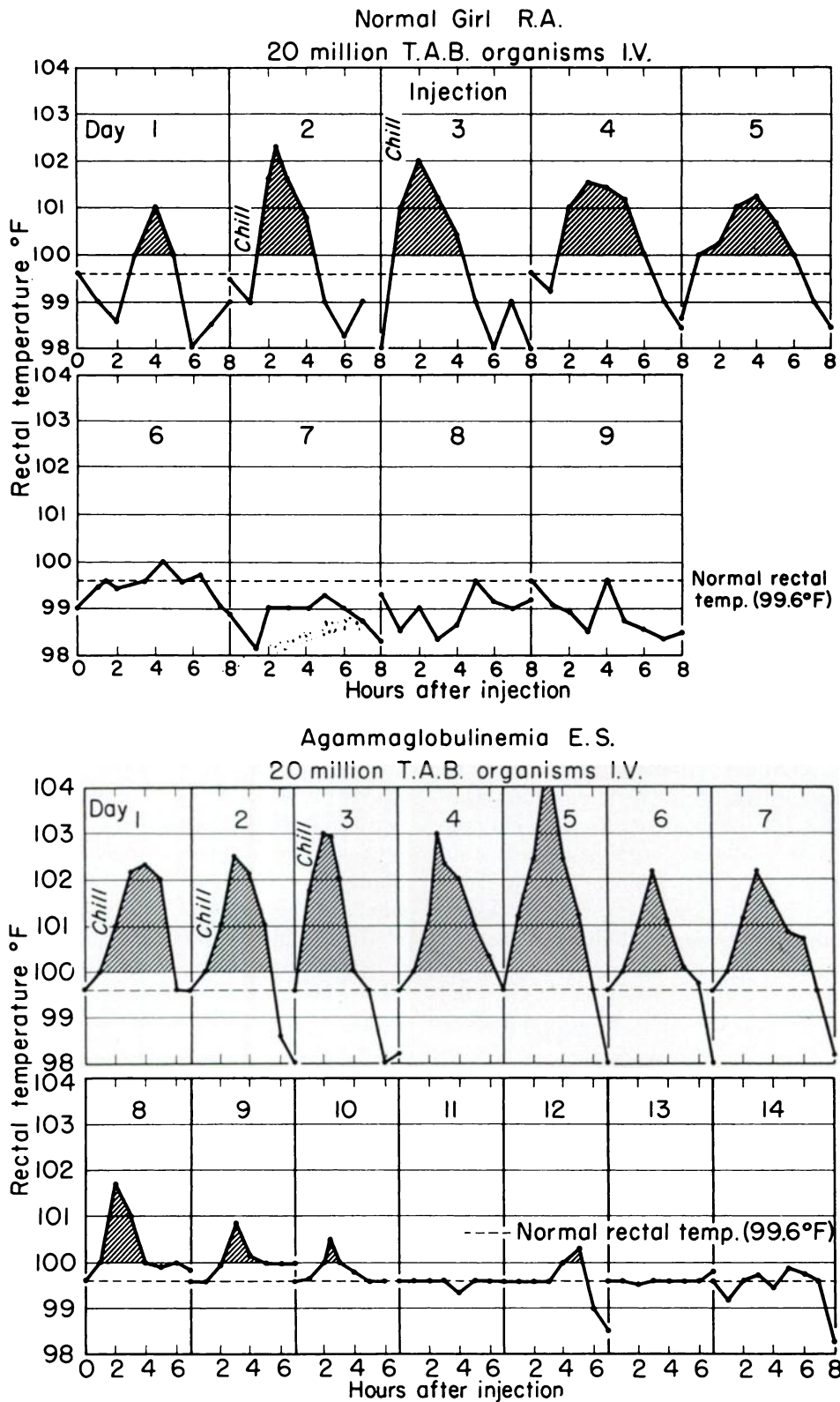


FIG. 9. Development of refractoriness to endotoxin in normal child and in child with agammaglobulinemia. (a) (Upper) Serial daily temperature responses to intravenous injection of 20,000,000 typhoid-paratyphoid organisms in a child with agammaglobulinemia. (b) (Lower) Serial daily temperature responses identical stimulation in immunologically normal child. Refractoriness to febrile and intoxicating effects of endotoxin developed in the absence of immune response in the patient with agammaglobulinemia.

Also included among the acute phase reactions are the appearance in the blood of the C-reactive protein,⁸⁷ increase in serum concentration of mucoproteins and alpha globulins^{88,89} and increase in plasma concentration of fibrinogen and heparin precipitable factor of Thomas *et al.*^{90,91} At one time or another, each of these acute phase reactions has been related to the immune response. For example, it has been proposed that C-reactive protein represents a kind of natural antibody.^{92,93} Further, evidence has been presented indicating that production of antibody is correlated with the prior appearance of C-reactive protein in the serum,^{94,95} and these observations have been interpreted as indicating that the appearance of C-reactive protein in the serum reflects an essential step in the formation of antibody. Consequently, we postulated that should any of the acute phases reactions reflect an essential event in the formation or release of antibody, the patients with agammaglobulinemia with their immunologic paralysis might show abnormalities in the formation or liberation of these reactants.

Our studies of acute phase phenomena in agammaglobulinemia have included the following:

Observations of acute phase reactions during steady state of good health in patients with agammaglobulinemia.

Observation of acute phase reactions during acute bacterial infections in patients with agammaglobulinemia.

Observations of response of some of the acute phase reactions to intradermal, intramuscular and intravenous injections of typhoid paratyphoid vaccine and polysaccharide pyrogen from *Pseudomonas* (Piro-men®).*

Serial observation of C-reactive protein concentration during the development of refractoriness to endotoxin of gram-negative bacteria.

* Generously supplied for these studies by Dr. Harry L. Fevold of the Baxter Laboratories, Morton Grove, Illinois.

In patients with agammaglobulinemia who are free of infection, the acute phase reactions did not differ from those observed in a large group of normal persons (Table XIII). For example, the sedimentation rate varied from 3 to 13 mm. in 60 minutes (Westergren), C-reactive protein was absent from the serum, the serum concentration of mucoprotein and alpha globulins and the plasma concentration of fibrinogen and heparin-precipitable fraction were within the normal range.

As in normal persons, acute disease in the patient with agammaglobulinemia produced a rise in sedimentation rate, and in the concentrations of mucoprotein, fibrino-

TABLE XIII
ACUTE PHASE REACTANTS IN PATIENTS WITH
AGAMMAGLOBULINEMIA FREE OF DISEASE

Patient Age Sex	Sedimen- tation Rate mm./hr.	C- Reactive Protein	Muco- protein Tyrosine mg./100 ml.	Fibrin- ogen mg./100 ml.	HPP* mg./100 ml.
E.S. 7 yr. male	7	neg	2.89	253	130
B.H. 6 yr. male	6	neg	2.90	240	220
T.A. 1 yr. male	10	neg	3.00	322	—
W.A. 6 yr. male	8	neg	3.20	320	150
L.L. 30 yr. female	13	neg	3.30	320	—
F.H. 58 yr. male	4	neg	2.70	262	—
Normal	0-15	neg	2.10- 3.50	250- 350	40-200

* Heparin precipitable protein of Thomas, Smith & Von Korff.

TABLE XIV

ACUTE PHASE REACTIONS IN PATIENTS WITH AGAMMAGLOBULINEMIA DURING ACUTE DISEASE

<i>Patient Age, Sex</i>	<i>Clinical Disease</i>	<i>C-Reactive Protein 0-4+</i>	<i>Sedimentation Rate mm./hr.</i>	<i>Mucoprotein Tyrosine mg./100 ml.</i>
E.S. 7 yr. Male	Congenital agammaglobulinemia, Pneumococcus pneumonia	3+	42	8.7
W.A. 6 yr. Male	Congenital agammaglobulinemia, Staphylococcus pneumonia and otitis media	4+	38	9.2
F.T. 23 mo. Male	Congenital agammaglobulinemia, bacterial pneumonia	4+	94	10.8
T.T. 15 mo. Male	Congenital agammaglobulinemia, Streptococcus pharyngitis, otitis and cervical adenitis	2+	34	6.7
F.H. 58 yr. Male	Acquired agammaglobulinemia, Staphylococcus septicemia	4+	28	8.8
L.L. 30 yr. Female	Acquired agammaglobulinemia, bacterial lobar pneumonia	3+	22	7.2
Normal 1-60	—	0	0-15	2.5 ± 0.3

gen, heparin precipitable protein, and alpha globulins (electrophoresis) in the serum (Table XIV). In addition, C-reactive protein regularly appeared in the serum during acute infection in these patients.

Similarly, intradermal, subcutaneous or intravenous injections of typhoid-paratyphoid vaccine and Piromen® resulted in the appearance of C-reactive protein in the serum of both patients with agammaglobulinemia and normal persons. Finally, as refractoriness to the effects of endotoxin developed in both normal persons and in patients with agammaglobulinemia, the C-reactive protein disappeared from the serum even though a stimulus was being used which previously resulted in the appearance of this protein in the serum. Thus from these studies it was established that with respect to the behavior of the acute phase phenomena, the patients with agam-

maglobulinemia did not differ from the normal persons. Conversely, it could be reasoned that the acute phase reactions studied probably did not represent antibody production nor did they reflect the operation of mechanisms resulting in the actual formation and release of the antibody and gamma globulin.

The possibility still exists that any of the acute phase reactions, e.g., C-reactive protein formation, might reflect an essential process in preparation for antibody synthesis and still lack relationship to the elaboration or liberation of the antibody itself which appears to be deficient in patients with agammaglobulinemia.

Sufficient, however, for present consideration is the observation that all of the acute phase reactions thus far studied are entirely normal in patients completely lacking in ability to synthesize gamma globulin

and circulating antibody. During the course of these studies it was also observed that 3 of 4 patients with agammaglobulinemia had normal concentrations of properdin in their serums.* One of the patients with agammaglobulinemia lacked properdin on 2 occasions. This relationship is being investigated further.

The Function of the Adrenal Gland in Agammaglobulinemia

Treatment of animals or humans with cortisone or compound F results in lymphopenia,⁹⁶ eosinopenia,⁹⁷ inhibition of antibody production,⁹⁸ decreased gamma globulin concentration⁹⁹ and enhanced susceptibility to infection.¹⁰⁰ Agammaglobulinemia as a clinical entity has been associated with each of these phenomena. For example, Young and Wolfson,²⁸ Keiden and McCarthy⁷⁷ and Rhon *et al.*²² reported reduced numbers of lymphocytes as a characteristic feature in their cases of agammaglobulinemia. One of our patients showed complete absence of eosinophils from the peripheral blood and virtually complete absence of these cells from the bone marrow. The enhanced susceptibility to infection, absence of antibody response and deficient gamma globulin level represents the essence of the disease. On the basis of these observations it seemed pertinent to determine so far as possible the status of adrenal-pituitary function in patients with agammaglobulinemia. Consequently, in cooperation with Kelley,¹⁰¹ we studied adrenal function in 5 cases of agammaglobulinemia. The methods used to assay the function of this system included serum concentrations of 17-hydroxycorticosteroids obtained in the fasting state and total eosinophil counts, response of serum concentrations of 17-hydroxycorticosteroids to intramuscular injections of 25 mg. of adrenocorticotrophic hormone (ACTH), per cent fall in absolute eosinophil count following intramuscular

* The authors are indebted to Dr. Louis Pillemer of the Institute of Pathology, Western Reserve University, Cleveland, Ohio, for the performance of the properdin determinations.

injection of ACTH, and diurnal variation of eosinophil concentration.

In 4 of 5 cases studied, the total absolute eosinophil count was normal and in 4 of 4 cases studied the concentration of 17-hydroxycorticosteroids in serum was found to be well within the normal range. Each of the patients with agammaglobulinemia tested showed a sharp rise in serum concentration of 17-hydroxycorticosteroids and a sharp fall in total eosinophil count following stimulation with ACTH.

The results are summarized in Table XV. It was further observed that 4 of the patients with agammaglobulinemia had normal diurnal variations in eosinophil counts, significantly higher absolute counts being

TABLE XV
ADRENAL FUNCTION IN PATIENTS WITH
AGAMMAGLOBULINEMIA

Patient Age Sex	Eosinophil % Depletion 4 Hours After ACTH*	Increase in 17-OHCS (mg./100 ml.) After ACTH†
F.H. 58 yr. male	No eosinophils in the blood or bone marrow.	7.0
E.S. 7 yr. male	Test 1 82 Test 2 79	— 27.2
W.A. 6 yr. male	Test 1 77 Test 2 96	27.6 14.6
T.A. 1 yr. male	81	30.2
B.H. 5 yr. male	74	—
Mean Normal	81.5 <50	21.7 17.8 (mean)

* Total eosinophil count done prior to, 2 hours and 4 hours after injection of 25 mg. ACTH intramuscularly.

† Blood for 17-hydroxycorticosteroid determination was collected prior to and 2 hours after injection of 25 mg. ACTH intramuscularly.

observed at 6 A.M. than at 12 noon on each instance.

These observations must be interpreted as indicating that pituitary-adrenal cortical function is regularly normal in patients with agammaglobulinemia and that the hematologic and immunologic abnormalities observed in these patients are based on other mechanisms.

Liver Function Studies and Hemostatic Mechanisms

Although much evidence indicates that the production of gamma globulin and antibodies is extrahepatic,¹⁰² previous studies by McQuarrie *et al.*⁴⁵ suggest that

certain patients with deficient production of both albumin and globulin have an abnormality of hepatic structure. Consequently, it seemed prudent to study thoroughly the hepatic function in patients with isolated agammaglobulinemia. Summarized in Table XVI are the results of a battery of liver function tests on 8 patients with agammaglobulinemia. Six were patients with congenital agammaglobulinemia and 2 suffered from the acquired form of the disease. It is clear from these data that hepatic function in agammaglobulinemia is entirely normal. Even the most delicate tests of liver function available, e.g., the content of urobilinogen in a 24-hour speci-

TABLE XVI
LIVER FUNCTION IN AGAMMAGLOBULINEMIA

Patient Age Sex	Serum Bilirubin mg./100 ml.		Cholesterol Flocculation	Thymol Turbidity Unit	Cholesterol mg./100 ml.	Cholesterol Esters mg./100 ml.	Urobilinogen in Urine mg./d.	Cholinesterase ΔpH/hr.	Bromsulfalein % Retention
	1 min.	Total							
Congenital Agammaglobulinemia									
B.H. 5 yr. Male	0.1	0.8	0	1	212	155	0.3	1.07	>1%
E.S. 7 yr. Male	0.1	0.4	0	0	108	70	0.8	0.74	1%
T.A. 1 yr. Male	0.3	0.4	0	1	77	57	trace	0.81	1%
W.A. 7 yr. Male	0.1	0.3	0	0	175	122	0.4	0.45	1%
F.T. 23 mo. Male	0.1	0.3	0	0	—	—	1.0	—	0%
T.T. 15 mo. Male	0.2	0.5	0	0	—	—	0.8	—	1%
Acquired Agammaglobulinemia									
F.H. 58 yr. Male	0.1	0.4	0	0	212	160	0.3	0.61	0%
L.L. 30 yr. Female	0.1	0.4	0	0	252	189	0.4	0.86	0%
Control									
Normal	0.0-0.2	0.2-1.0	0-2+	1-4	150-250	68-76%	0-3	0.52-1.08	>5%

men of urine and bromsulfalein retention, fell within the normal range. This observation is supported by histologic studies of the liver tissue from 2 patients with agammaglobulinemia. Sections of liver were obtained at biopsy in one and at post-mortem examination in the other. Both of the biopsies showed normal morphology of the hepatic cells, normal organization of the liver tissue and normal staining characteristics of the histologic components of the liver. These observations, supported by the finding that all tests of hemostatic function are normal in patients with agammaglobulinemia and that all proteins other than gamma globulin are present in normal concentration in the serums of these patients, must be taken as evidence that isolated failure of gamma globulin synthesis is not attributable to generalized hepatic malfunction. These observations are likewise consistent with the inference that gamma globulin and antibody formation are both dependent on extrahepatic mechanisms.

Hematologic Disturbances

With the observations of Young and Wolfson,²⁸ Keiden *et al.*⁷⁷ and Rhon *et al.*²² that agammaglobulinemia may be associated with lymphopenia of the peripheral blood it became necessary to make a systematic study of the hematologic aspects of this disease. In the course of our investigation it was discovered that hematologic disease is a regular accompaniment of agammaglobulinemia. Listed in Table XVII is a summary of the hematologic abnormalities observed in our patients. Four of the nine studied had episodic neutropenia which had been observed by reliable physicians or were observed in the course of our studies. In the 3 whom we studied during episodes of transient neutropenia the hematologic disturbance was featured by the virtual disappearance of neutrophils from the peripheral blood. Bone marrow examination revealed a marked shift to the left and hypoplasia of the precursor cells in the neutrophilic series. No circulating leukocyte agglutinins could be found in

TABLE XVII
HEMATOLOGIC ABNORMALITIES IN PATIENTS
WITH AGAMMAGLOBULINEMIA

<i>Hematologic Disorder</i>	<i>Incidence</i>
<i>Our Cases</i>	
Transient aregenerative neutropenia recurrent	3/8
Persistent aregenerative neutropenia	2/8
Cyclic aregenerative neutropenia	1/8
Aregenerative eosinopenia	1/8
Thymic tumor	1/8
Benign diffuse proliferation of reticulum (mesenchyme)	1/8
Hemolytic anemia, negative Coombs' test	1/8
Deficient plasma cells in bone marrow and lymph nodes	8/8
Failure of plasma cell response to antigenic stimulation	6/6
Lymphopenia	0/8
<i>From experience of others</i>	
Cyclic neutropenia	
Profound aregenerative lymphopenia	
Thymic tumor	
Aregenerative anemia and neutropenia	
Hypersplenism	
Deficiency of plasma cells	

any instance. On the basis of these findings the neutropenia was classified as aregenerative in type. According to the history provided by the referring physician the neutropenia in several instances, although transient, tended to be recurrent. When these episodes were first discovered, occurring as they did in these patients who were subject to recurring, sometimes overwhelming, bacterial infection, we attributed the hematologic disease to the infections or the associated antibiotic or chemotherapy. However, in 2 instances we observed the development of the transient neutropenia in patients having no overt signs or symptoms of infection. One of these patients was being treated with only small amounts of penicillin orally, the other was being given no antibiotics at the time the neutropenia occurred.

As we have studied these patients we have become more and more inclined to the view that the transient episodes of neutropenia reflect a significant underlying

disturbance of hematopoietic function which is often associated with agammaglobulinemia.

Support for this concept has come from study of several other cases. For example, 2 male siblings with agammaglobulinemia each had a persistent blood dyscrasia involving the polymorphonuclear leukocytes. In 1 instance extreme neutropenia persisted at levels often approaching zero and regularly below 1000 leukocytes/mm.³ except for a brief period after splenectomy at 11 months of age following which a significant leukocytosis occurred. Within 1 month after splenectomy, however, the neutrophil count dropped to the low levels present prior to surgery where it persisted until the child's death 10 months later. The persistent neutropenia in this patient was associated with apparent arrest in development of the neutrophilic precursors in the bone marrow.

Both members of another sibling pair from a different family suffered from combined neutropenia and agammaglobulinemia. In 1 instance the neutropenia was transient and was associated with and attributed to the severe recurrent bacterial infections and the necessary intensive antibiotic therapy. However, when we discovered that the 3-month-old sibling also had agammaglobulinemia we began to study his blood count regularly 2 times each week. After a period of observation of approximately 1 month this child developed a profound neutropenia lasting ap-

proximately 60 days. The neutropenia, dismissed when it occurred in the older child as a possible reflection of bacterial or viral trauma to the hematopoietic tissues, developed in this instance in the apparent absence of infection. The extreme neutropenia in both children appeared to result from the failure of the bone marrow to produce neutrophils. Figure 10 illustrates the cyclic neutropenia, persistent neutropenia and prolonged transient neutropenia observed in patients with agammaglobulinemia.

Recently other authors have described cases of agammaglobulinemia associated with disturbances in production of neutrophils.^{16, 18, 103}

F. H., a 58-year-old male with acquired agammaglobulinemia, possessed almost no eosinophils in either his peripheral blood or bone marrow. In this instance the failure to form eosinophils was apparently analogous to the neutrophilic dyscrasia described above. The bone marrow revealed almost complete failure of maturation along eosinophilic lines and very few of the earliest stages in neutrophilic development could be found.

This same patient was discovered to have a huge thymoma at the time he developed the recurrent respiratory disease associated with agammaglobulinemia. A thymus tumor weighing 540 gm. removed at surgery was described pathologically as a tumor of the thymus due primarily to a benign pro-

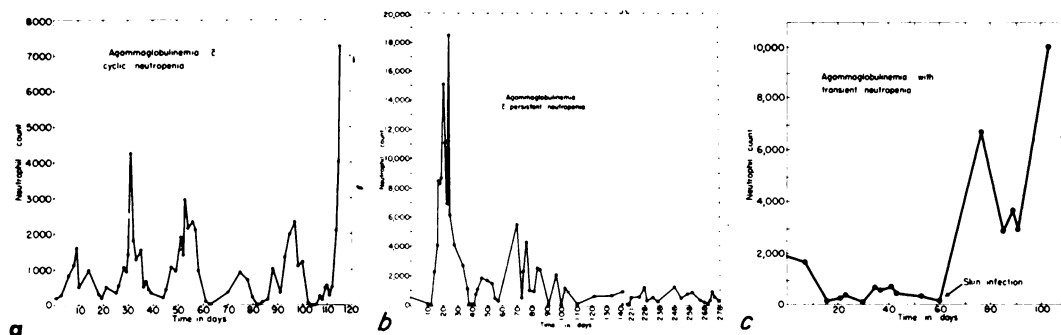


FIG. 10. Serial neutrophil counts on 3 patients with agammaglobulinemia. (a) Cyclic neutropenia with agammaglobulinemia. (b) Persistent neutropenia with agammaglobulinemia. (c) Prolonged transient neutropenia with agammaglobulinemia.

liferation of the thymic reticulum. Recently another patient having acquired agammaglobulinemia, blood dyscrasia and large benign thymoma has been discovered.¹⁰³

In Loeb's case persistent agranulocytosis and aregenerative anemia were the blood dyscrasias associated with the thymoma and agammaglobulinemia while in our case a persistent aregenerative eosinopenia was observed.

Finally, the adult female with acquired agammaglobulinemia whom we have studied has still another hematopoietic dyscrasia. Three years prior to our study and 4 years following the apparent onset of agammaglobulinemia she developed hemolytic anemia with a negative Coombs test and associated with a large spleen. At this time she also had leukopenia and thrombocytopenia. A diagnosis of hypersplenism was made and splenectomy was performed. This operation cured the hemolytic anemia, leukopenia and thrombopenia. On morphologic study the splenomegaly was found to be due to a pronounced benign hypertrophy and proliferation of the reticulum. Histologic study suggested that the reticular proliferation might be associated with a granulomatous process but none could be clearly defined. Similarly, recent study of the liver, bone marrow and lymph nodes from this patient reveal that the hematologic abnormality is widespread

since in each of these organs a generalized hyperplasia of the mesenchymal cells is to be seen. In Figure 11 are illustrated the proliferation of the splenic and lymph node reticulum which featured the hematologic abnormality in this case.

Response of Hematopoietic Tissue to Antigenic Stimulation

In extensive hematologic studies reported elsewhere¹⁰⁴ we have compared the bone marrow and the lymph nodes of patients with agammaglobulinemia to those of normal children and adults. Whereas normal numbers of lymphocytes were to be found in the peripheral blood and bone marrow of our patients with agammaglobulinemia, a clearly defined deficiency in the numbers of plasma cells was discovered in the bone marrows of these patients. In the lymph nodes of the patients with agammaglobulinemia, morphologic abnormalities were regularly present. In the children with congenital agammaglobulinemia the lymph nodes were smaller than normal and microscopic study revealed a relatively thin cortex with few primary follicles and no secondary follicles. In Figure 12 lymph nodes from agammaglobulinemic and normal children are compared.

Antigenic stimulation using potent bacterial antigens administered subcutaneously and intravenously regularly produced sig-

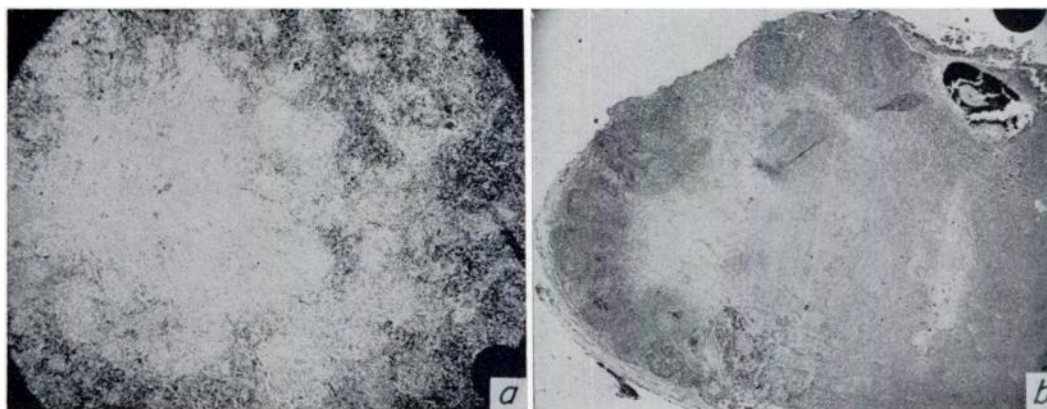


FIG. 11. Benign proliferation of the reticulum in an adult female with acquired agammaglobulinemia. (a) View of spleen. ($\times 50$, reduced $\frac{1}{4}$.) (b) Low-power view of the lymph node. ($\times 8$, reduced $\frac{1}{4}$.) Note proliferation of reticulum in both areas.

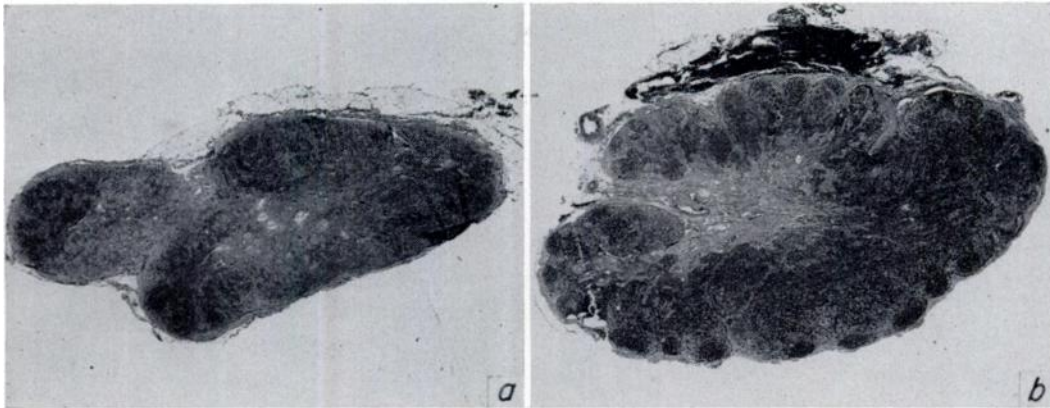


FIG. 12. Comparison of low power views of the regional lymph nodes, stimulated by intradermal injection, from normal child and from child with agammaglobulinemia. Note the deficiency of lymphoid follicles, thinness of the cortex and the relative acellularity of the node from the patient with agammaglobulinemia (a) as compared to the node from the normal child (b). ($\times 10$, reduced $\frac{1}{4}$.)

nificant increases in number of plasma cells in the bone marrows of normal children. However identical antigenic stimulation of the patient with agammaglobulinemia did not result in formation of plasma cells. In Figure 13, bone marrow from a normal child following antigenic stimulation is compared to bone marrow from a child with agammaglobulinemia. The proliferation of plasma cells following antigenic stimulation featuring the bone marrow response of the normal patient is to be noted.

Similarly, when the response of the lymph nodes to antigenic stimulation was studied, striking differences were found be-

tween normal children and children with agammaglobulinemia. Four to six days after injection of antigen into the skin and subcutaneous tissues of the medial aspect of the thigh, the inguinal lymph nodes of the normal child revealed proliferation of lymphatic tissue reflected in an increased thickness of the cortex and the formation of secondary follicles.

In addition, the sections and imprints from the node revealed proliferation of the reticulum (mesenchyme), budding of the lymphocytes, plasma cell proliferation, and maturation especially marked in the medullary portion of the nodes. In the

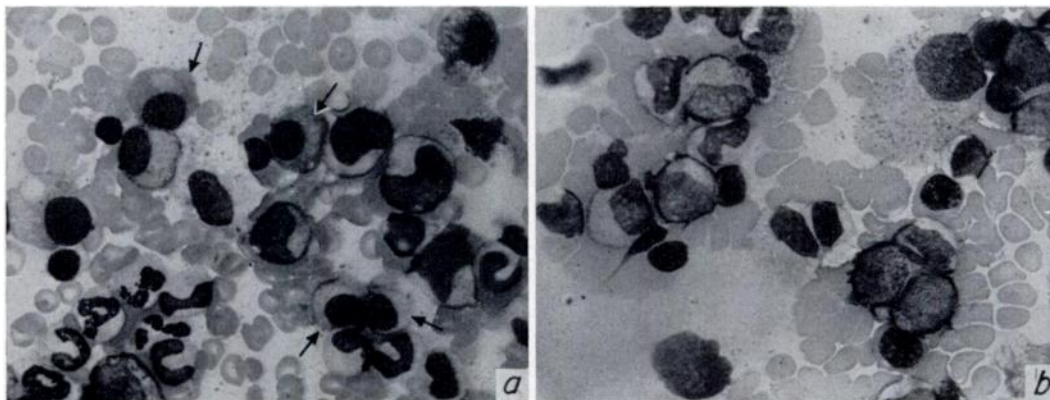


FIG. 13. Comparison of bone marrow from immunologically normal child (a) and from patient with agammaglobulinemia (b) 8 days following a series of 5 intravenous injections of T.A.B. antigen. Note accumulation of plasma cells in the bone marrow of the normal child (arrows) and their absence from the marrow of the patient with agammaglobulinemia.

nodes taken from the patients with agammaglobulinemia provided identical stimulation, similar changes had taken place. Enlargement of the node, increased numbers of lymphocytes and developing lymphocytes, proliferation of the reticulum and budding of the lymphocytes, all could be readily identified as the result of the antigenic stimulation. In 2 particulars, however, the lymph nodes of the patients with

agammaglobulinemia failed to respond, namely, the formation and proliferation of plasma cells did not occur and secondary follicles did not develop in response to antigenic stimulation. These differences were all the more striking as the secondary, tertiary and quaternary responses to antigen were studied. In Figure 14 high power views of sections and imprints from the medullary portion of the lymph nodes of the

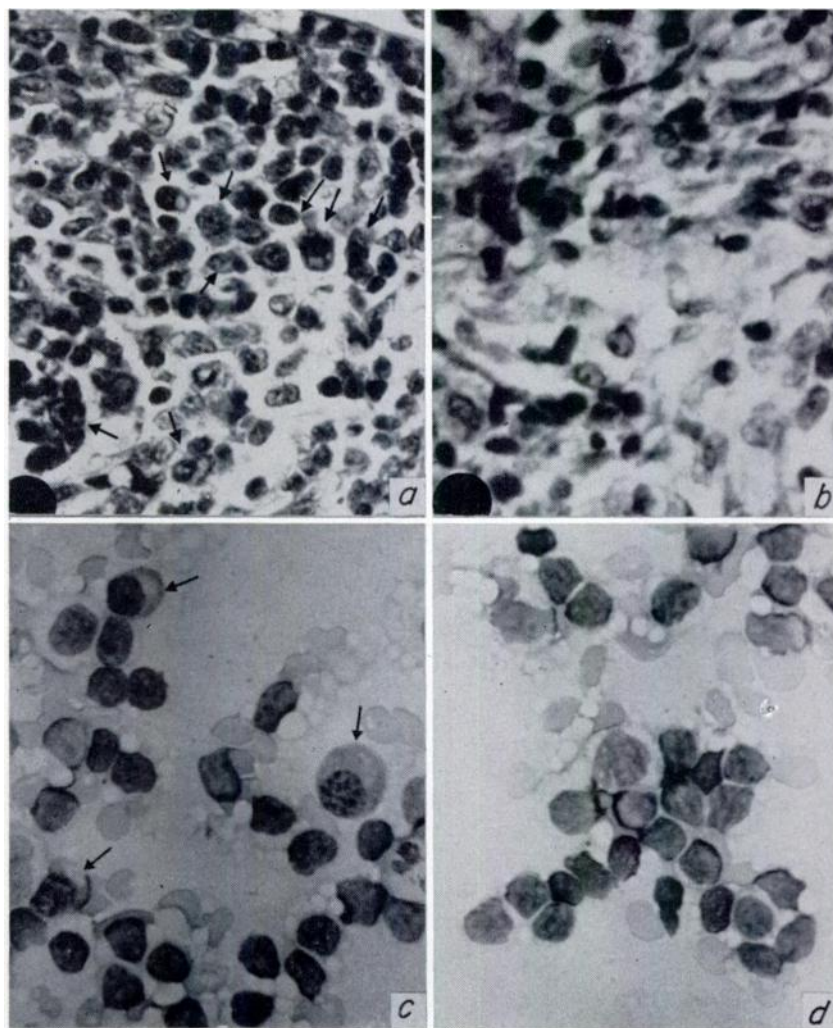


FIG. 14. Comparison of the response of lymph nodes from an immunologically normal child and from a child with agammaglobulinemia following antigenic stimulation. (a) Section from node removed from the inguinal region of a normal child 4 days after injection of typhoid-paratyphoid antigen into the skin and subcutaneous tissues of the medial aspects of the thigh. Note proliferation of plasma cells in the medullary cords. ($\times 400$, reduced $\frac{1}{4}$.) (b) Comparable area from node of a child with agammaglobulinemia stimulated in the same way. ($\times 400$, reduced $\frac{1}{4}$.) Note absence of plasma cells. (c) Imprint from the lymph node of a normal child 6 days after antigenic stimulation. Plasma cell accumulation is clearly shown. ($\times 800$, reduced $\frac{1}{4}$.) (d) Imprint from the lymph node of a child with agammaglobulinemia 6 days after injection of antigen. Plasma cells are absent.

normal child 4 days after antigenic stimulation are compared to the sections and imprints from the medullary portion of the lymph node of the patient with agammaglobulinemia after comparable stimulation.

In nodes from persons immunologically normal, repeated exposure to antigenic stimulation (anamnesic response) leads to striking secondary follicle formation in the cortex and extraordinary plasma cell proliferation in the medullary cords, whereas the patients with agammaglobulinemia having been subjected to the same stimulation still lack secondary follicles and medullary plasmacytosis.

The absence of plasma cells and failure of plasma cells to develop in the bone marrow and lymph nodes in response to antigenic stimulation was present in each of the patients with agammaglobulinemia studied. Thus, unlike the other hematologic disturbances described above, failure of secondary follicle formation and failure of plasma cell development are common to all the patients with agammaglobulinemia.

On the basis of these studies elucidating the hematologic disturbances which may be observed in patients with agammaglobulinemia, it is attractive to attempt to define the disease itself in terms of a cellular deficit. For example, it seems reasonable to postulate that in agammaglobulinemia we are dealing with an abnormality of reticular

cell (multipotent, mesenchymal cell) function. This disturbance *may* be reflected in failure of heteroplastic metamorphosis of the reticulum toward mature neutrophils, mature eosinophils, mature lymphocytes or even erythrocytes in some patients. However, the deficit gains its ultimate and uniform expression as failure of antibody and gamma globulin synthesis as a consequence of failure of the reticulum to mature along the plasma cell line in response to antigenic stimulation in all of these patients.

Support for this hypothesis was gained from 2 additional studies. One of the children with agammaglobulinemia was subjected to pulmonary lobectomy following the development of bronchiectasis. Because lobectomy was performed after a long period of uncontrolled infection, the tissue obtained for study was considered to be representative of the response of a patient with agammaglobulinemia to a chronic suppurative process. Comparably diseased tissue from an immunologically normal child was obtained for comparison. The difference in appearance of the exudate in the 2 children was striking. The accumulation of numerous plasma cells featured the chronic inflammatory exudate of the bronchiectatic process of the immunologically normal child whereas no plasma cells were to be found on extensive search of the inflammatory exudate of the patient with

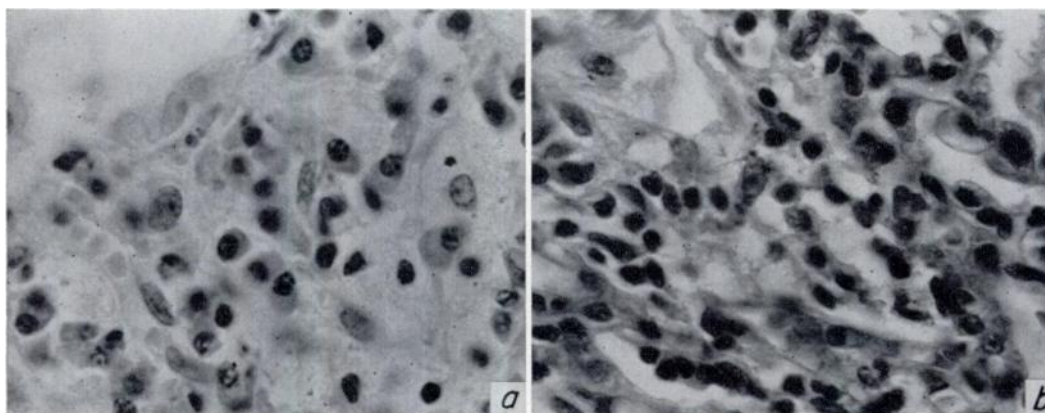


Fig. 15. Comparison of the exudate from chronic inflammatory process (bronchiectasis) in the immunologically normal person (a) and in a patient with agammaglobulinemia (b). Note the abundance of plasma cells in the exudate from the immunologically normal person and their complete absence from a comparable process in the patient with agammaglobulinemia.

agammaglobulinemia. In all other respects the 2 exudative processes were comparable. This observation is illustrated in Figure 15 where the plasma cell-poor exudate from the bronchiectatic process of the patient with agammaglobulinemia is compared to the plasma-cell rich exudate from the bronchiectatic process of the immunologically normal person. The exudate of the bronchiectatic process of the patients with agammaglobulinemia contained lymphocytes, monocytes, macrophages and some epithelioid cells but contained no plasma cells whatsoever.

Similarly, the interstitial tissue of the appendix of adults and older children always contains an abundance of plasma cells. Study of the appendix from the 58-year-old man with acquired agammaglobulinemia who died of hepatitis showed that in this location, as in the other areas, plasma cells are completely lacking in patients with agammaglobulinemia. In Figure 16 the appendix from a patient with normal immunologic capacity (a) and one with agammaglobulinemia (b) are illustrated.

Numerous other studies designed to interpret the incisive "experiment of nature" represented by the agammaglobulinemic state have been carried out, but space does

not permit their detailed documentation here. Brief mention of 3 of these, however, seems in order.

Transplantation of Skin to Patients with Agammaglobulinemia

Recent evidence indicates that homotransplantation failure has an immunologic basis.¹⁰⁵⁻¹¹⁴ Transplantation of skin from unrelated donors to 2 children with congenital agammaglobulinemia resulted in what now appears to be permanent survival of the homotransplant. In 1 child the transplanted skin has persisted 21 months while in the other the transplant has remained in place 1 year. In contradistinction, skin from the children with agammaglobulinemia transplanted to immunologically normal persons was accepted initially and rejected in the usual fashion between 12 and 37 days after application. In an adult with acquired agammaglobulinemia and associated immunologic paresis rather than paralysis, prolonged survival of the graft occurred. However, ultimately the transplant sloughed between 11 and 16 weeks after its application. In contrast, this patient's own skin was rejected by an immunologically normal person beginning 14 days after application and completed 10

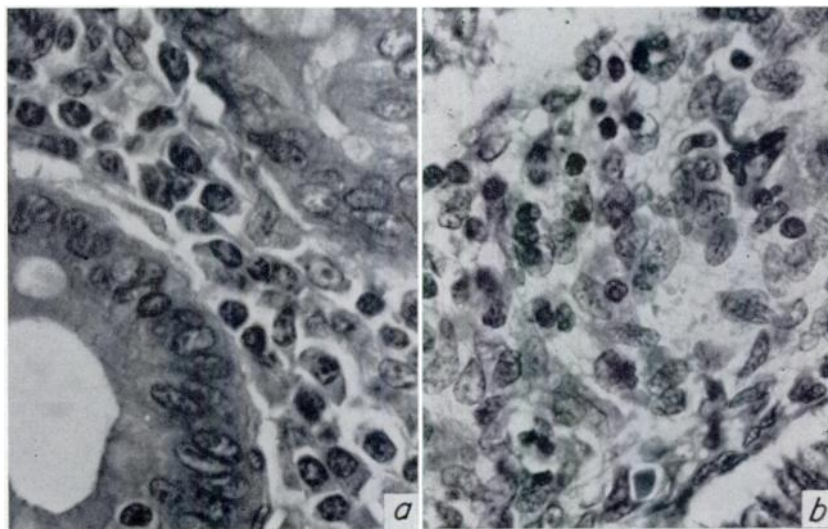


FIG. 16. Comparison of the appendix from an immunologically normal person (a) and from a patient with agammaglobulinemia (b). Note the accumulation of plasma cells in the normal appendix and their complete absence from the appendix of the patient with agammaglobulinemia.



FIG. 17. Homotransplant of skin on the thigh of a child with agammaglobulinemia 1 year following transplantation. The distal tattooed portion is full thickness and the proximal portion is split thickness. This graft appears to have grown with the patient during the 2 years following transplantation.

days later. In Figure 17 the successful transplantation of skin to a child with agammaglobulinemia is illustrated. The transplant shown had been in place 6 months at the time the photograph was taken.

Occurrence of Pregnancy in a Female with Agammaglobulinemia

A 30-year-old female, with acquired agammaglobulinemia of 8 years' duration, became pregnant during the period of our study. Electrophoretic analysis of the serum proteins, immunochemical determinations of the gamma globulin concentration, and studies of the immunologic response were carried out prior to pregnancy, during the first trimester, during the third trimester and

following delivery. The patient was given no gamma globulin or blood during the pregnancy but instead was kept free of infection by continuous prophylaxis with oxytetracycline. The concentration of gamma globulin remained relatively constant between 10 and 15 mg./100 ml. during the entire period of pregnancy and in the neonatal period. No antibody was produced in response to multiple injections of typhoid-paratyphoid vaccine or diphtheria, pertussis and tetanus vaccine prior to pregnancy, or to typhoid-paratyphoid vaccine given during the first and second trimester. During the third trimester, however, injections of typhoid-paratyphoid antigen resulted in production of a small but significant amount of antibody against H, O and B antigens. Following delivery of the baby and placenta, the concentration of antibody in the serum declined rapidly and immunologic responsiveness disappeared completely. Studies of the mother's bone marrow and regional lymph nodes after each antigenic stimulation revealed no evidence of plasma cell proliferation, and hence, according to our view, no morphologic basis for the immunologic response was observed.

Further, the baby possessed no demonstrable serum antibodies against any of the antigens administered and lymph nodes and bone marrow of the baby contained no plasma cells. Study of the placenta, however, was of great interest. Suspensions of placenta showed antibody to H, O and B antigen even after dilution to 1:40. Finally, morphologic study of the placenta from this patient revealed plasma cells to be present in the interstitial tissue.

Consequently, we have inferred from this study that a patient with agammaglobulinemia becomes capable of antibody production during the third trimester and that the production of antibody is not a function of the fetus but probably can be attributed to local antibody production within the placenta itself.

Using immunologic, immunochemical, electrophoretic and morphologic methods, study of the baby born of this pregnancy

provided information to support this concept. For example, the baby was agammaglobulinemic in the neonatal period and failed to respond to stimulation with several different antigens. This condition of immunologic unresponsiveness persisted for approximately 50 days. During the second neonatal month, however, the baby began to form both gamma globulin and antibody. Now at 1 year of age this child is in every respect biochemically and immunologically normal. Extensive study of the hematopoietic system revealed through bone marrow and lymph node biopsies that the hematopoietic tissues do not respond to antigenic stimulation with plasma cell formation in the immediate neonatal period or during the first neonatal month. In contradistinction, when the baby was 3 months of age, the development of plasmacytosis both in bone marrow and in regional lymph nodes draining the site of antigenic injection revealed the immunologic responsiveness acquired through maturation.

SUMMARY AND CONCLUSIONS

The experiment of nature presented by patients with agammaglobulinemia is discussed and related to other syndromes associated with disturbance in gamma globulin metabolism.

Measurement of serum gamma globulin concentration by an immunologic method revealed minute amounts of gamma globulin to be present in the serum of each of the patients with agammaglobulinemia. Measurement of the survival time of intramuscularly injected gamma globulin indicated that in patients with agammaglobulinemia this protein has a half-life of approximately 30 days.

The immunologic handicap in patients with agammaglobulinemia is defined in terms of response to ubiquitous antigen, the presence of "natural antibodies" and the primary, secondary, and tertiary responses to bacterial antigens, heterologous blood group antigens and virus antigens. The clinical paradox posed by the apparently satisfactory resistance of patients with

agammaglobulinemia to certain virus infections and the failure of their response to virus antigen is discussed. The capacity of patients with agammaglobulinemia to develop bacterial-type hypersensitivity is documented. This observation dissociates bacterial type hypersensitivity from the classical immune response which results in accumulation of antibody in the circulating blood.

The development of immediate-type sensitivity in patients with agammaglobulinemia after intradermal injection of serum from a patient known to be atopic is described. Serum complement, acute phase reactants and properdin are present in normal concentrations in the serums of patients with agammaglobulinemia. The acute phase responses to infection and intoxication are normal in patients with agammaglobulinemia. The persistence of immunologic paralysis after the administration of gamma globulin to patients with agammaglobulinemia is documented.

Skin reactions, febrile and toxic response of patients with agammaglobulinemia to gram-negative endotoxin do not differ from those of immunologically normal persons. Refractoriness to the toxic and pyrogenic effects of gram-negative endotoxin develops in patients with agammaglobulinemia just as in normal persons.

The normal function of the pituitary-adrenal system in patients with agammaglobulinemia is established.

Multiple hematologic disturbances observed in patients with agammaglobulinemia are described. These include transient neutropenia, cyclic neutropenia, persistent neutropenia, eosinopenia, lymphopenia, aregenerative anemia, benign proliferation of the hematopoietic reticulum and thymoma. Virtual absence of plasma cells from the hematopoietic tissues and inflammatory exudates of patients with agammaglobulinemia is described. Failure of plasma cell formation in response to antigenic stimulation is reported as a constant characteristic of the patient with agammaglobulinemia. That all the hema-

tologic abnormalities in agammaglobulinemia have a common basis in a functional abnormality of the hematopoietic reticulum is proposed.

Successful homotransplantation of skin in the patient with agammaglobulinemia is described. Transplantation of skin from 4 patients with agammaglobulinemia to 4 immunologically normal children resulted in the expected homotransplantation failure.

Immunologic, biochemical and hematologic studies of a woman with agammaglobulinemia during pregnancy are briefly described.

Immunologic, biochemical, and hematologic investigations of a child born of a mother with agammaglobulinemia are mentioned.

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REFERENCES

1. McQuarrie, I.: Experiments of Nature, and Other Essays. Lawrence, Kansas, Univ. Kansas Exten. Div., 1944.
2. Hsia, D. Y., and Knox, W. E.: The heterozygous state in the hereditary molecular disorders (abstract). *Am. J. Dis. Child.*, **90**:523, 1955.
3. Bruton, O. C.: Agammaglobulinemia. *PEDIATRICS*, **9**:722, 1952.
4. Bruton, O. C., Apt, L., Gitlin, D., and Janeway, C. A.: Absence of serum gamma globulins (abstract). *Am. J. Dis. Child.*, **84**:632, 1952.
5. Janeway, C. A., Apt, L., and Gitlin, D.: "Agammaglobulinemia." *Tr. A. Am. Physicians*, **66**:200, 1953.
6. Dixon, F. J., Talmage, D. W., Maurer, P. H. and Deichmiller, M.: The half-life of homologous gamma globulin (antibody) in several species. *J. Exper. Med.*, **96**:313, 1952.
7. Orlandini T., O., Sass-Kortsak, A., and Ebbs, J. H.: Serum gamma globulin levels in infancy (abstract). *Am. J. Dis. Child.*, **84**:632, 1952.
8. Slater, R. J.: Investigation of an infant born of a mother suffering from cirrhosis of the liver. *PEDIATRICS*, **13**:308, 1954.
9. Armstrong, S. H., Kukral, J., Hershman, J., McLeod, K., and Wolter, J.: Comparison of the persistence in the blood of gamma globulins labeled with S_{35} and I_{131} in the same subjects (abstract). *J. Lab. & Clin. Med.*, **44**:762, 1954.
10. Jean, R.: Hypo- ou agamma-globulinémie isolée chez l'enfant. *Presse méd.*, **61**:828, 1953.
11. Hayles, A. B., Stickler, G. B., and McKenzie, B. F.: Decrease in serum gamma globulin (agammaglobulinemia): report of 3 cases. *PEDIATRICS*, **14**:449, 1954.
12. Fischer, C. C.: Variations in serum gamma globulin. *Postgrad. Med.*, **17**:406, 1955.
13. Tanis, A.: Agammaglobulinemia. Case reports. *Children's Memorial Hosp., Chicago*, **13**:3599, 1955.
14. Good, R. A., and Varco, R. L.: A clinical and experimental study of agammaglobulinemia. *Journal-Lancet*, **75**:245, 1955.
15. Gitlin, D.: Low resistance to infection: relationship to abnormalities in gamma globulin. *Bull. New York Acad. Med.*, **31**:359, 1955.
16. Laski, B., Sass-Kortsak, A., and Hillman, D. A.: Cyclic neutropenia and agammaglobulinemia. *Am. J. Dis. Child.*, **88**:820, 1954.
17. Arends, T., Coonrad, E. V., and Rundles, R. W.: Serum proteins in Hodgkin's disease and malignant lymphoma. *Am. J. Med.*, **16**:833, 1954.
18. Grant, G. H., and Wallace, W. D.: Agammaglobulinaemia. *Lancet*, **2**:671, 1954.
19. Lang, N., Schettler, G., and Wildhack, R.: Über einen Fall von Agammaglobulinämie und das Verhalten parenteral zugeführten radioaktiv markierten Gammaglobulins im Serum. *Klin. Wchnschr.*, **32**:856, 1954.
20. Moncke, C.: Essentielle Agammaglobulinämie. *Schweiz. med. Wchnschr.*, **84**:1033, 1954.
21. Prasad, A. S., and Koza, D. W.: Agammaglobulinemia. *Ann. Int. Med.*, **41**:629, 1954.
22. Rohn, R. J., Behnke, R. H., and Bond, W. H.: Acquired agammaglobulinemia with hypersplenism (abstract). *J. Lab. & Clin. Med.*, **44**:918, 1954.
23. Saslaw, S., and Wall, R. L.: Adult agammaglobulinemia (abstract). *J. Lab. & Clin. Med.*, **44**:925, 1954.
24. Sanford, J. B., Favour, C. B., and Tribeman, M. S.: Absence of serum gamma

- globulins in an adult. *New England J. Med.*, **250**:1077, 1954.
25. Zinneman, H. H., Hall, W. H., and Heller, B. I.: Acquired agammaglobulinemia. *J.A.M.A.*, **156**:1390, 1954.
 26. Collins, H. D., and Dudley, H. R.: Agammaglobulinemia and bronchiectasis: A report of two cases in adults with autopsy findings. *New England J. Med.*, **252**:255, 1955.
 27. Seltzer, S., Baron, S., and Toporek, M.: Idiopathic hypogammaglobulinemia and agammaglobulinemia. *New England J. Med.*, **252**:252, 1955.
 28. Young, I. I., Wolfson, W. Q., and Cohn, C.: Studies in serum proteins: agammaglobulinemia in the adult. *Am. J. Med.*, **19**:222, 1955.
 29. Rosecan, M., Trobaugh, F. E., Jr., and Danforth, W. H.: Agammaglobulinemia in the adult. *Am. J. Med.*, **19**:303, 1955.
 30. Kulneff, V. N., Pedersen, K. O., and Waldenström, J.: Drei Fälle von Agammaglobulinämie; ein klinischer, genetischer, und physikalisch-chemischer Beitrag zur Kenntnis des Proteinstoffwechsels. *Schweiz. med. Wchnschr.*, **85**:363, 1955.
 31. Good, R. A.: Agammaglobulinemia—a provocative experiment of nature. *Bull. Univ. Minn. Hosp. & Minn. Med. Found.*, **26**:1, 1954.
 32. Janeway, C. A.: Personal communication.
 33. Porter, H.: Congenital agammaglobulinemia: a sex-linked genetic trait and demonstration of delayed skin sensitivity (abstract). *Am. J. Dis. Child.*, **90**:617, 1955.
 34. Bowen, R.: Personal communication.
 35. Good, R. A., and Mazzitello, W. F.: Chest disease in patients with agammaglobulinemia. *Dis. Chest*, **29**:9, 1956.
 36. Good, R. A.: Electrophoretic studies of the families of patients with acquired agammaglobulinemia. Unpublished data.
 37. Kunkel, H. G.: Estimation of alterations of serum gamma globulin by a turbidimetric technique. *Proc. Soc. Exper. Biol. & Med.*, **66**:217, 1947.
 38. Neuhauser, H.: Personal communication.
 39. Good, R. A., and Zak, S. J.: Unpublished data.
 40. Krebs, E. G.: Depression of gamma globulin in hypoproteinemia due to malnutrition. *J. Lab. & Clin. Med.*, **31**:85, 1946.
 41. Thompson, W. H., Ziegler, M. R., and McQuarrie, I.: Comparative study of the inorganic metabolism in nephrosis and in edema of undetermined origin. *Am. J. Dis. Child.*, **44**:650, 1932.
 42. Keith, N. M.: General edema of indeterminate origin (abstract). *J. Clin. Investigation*, **11**:832, 1932.
 43. Meyers, W. K., and Taylor, F. H. L.: Hypoproteinemia probably due to deficient formation of plasma proteins: study of 1 case. *J.A.M.A.*, **101**:198, 1933.
 44. Cope, C. L., and Goadby, H. K.: Study of a case of idiopathic hypoproteinaemia. *Lancet*, **1**:1038, 1935.
 45. Thompson, W. H., McQuarrie, I., and Bell, E. T.: Edema associated with hypogenesis of serum proteins and atrophic changes in the liver; with studies of water and mineral exchanges. *J. Pediat.*, **9**:604, 1936.
 46. Binger, M. W., and Keith, N. M.: General edema of indeterminate etiology; report of 3 cases. *J.A.M.A.*, **109**:1, 1937.
 47. Rytand, D.: Edema with unexplained hypoproteinemia; syndrome of defective formation of serum proteins in absence of "loss and lack" of protein and demonstrable hepatic disease. *Arch. Int. Med.*, **69**:251, 1942.
 48. Schick, B., and Greenbaum, J. W.: Edema with hypoproteinemia due to congenital defect in protein formation. *J. Pediat.*, **27**:241, 1945.
 49. Messinger, M. J.: Generalized oedema associated with hypoproteinaemia. *Canad. M.A.J.*, **55**:381, 1946.
 50. Hertzog, F. V., and Faust, O. A.: Edema associated with temporary idiopathic hypoproteinemia. *J. Pediat.*, **36**:641, 1950.
 51. Illingworth, R. S., and Finch, E.: Chronic generalized oedema and hypoproteinaemia of obscure origin. *Arch. Dis. Childhood*, **29**:507, 1954.
 52. Fried, C. T., and Henley, W. L.: Deficiency of gamma globulin with edema and hypoproteinemia. *PEDIATRICS*, **14**:59, 1954.
 53. Ulstrom, R. A., Smith, N. J., and Zeldis, L.: Multiple plasma protein defects in an infant (abstract, privately printed). *Proc. West. Soc. Ped. Res.*, 1954, p. 12.
 54. Ulstrom, R. A.: Personal communication.
 55. Barnett, H. L., Forman, C. W., and Lauson, H. D.: The nephrotic syndrome, in children, in *Advances in Pediatrics*, vol. 5. Chicago, Yr. Bk. Pub., 1952, p. 53.
 56. Kelley, V. C., Ziegler, M., Doeden, D.,

- and McQuarrie, I.: Labeled methionine as an indicator of protein formation in children with lipoid nephrosis. *Proc. Soc. Exper. Biol. & Med.*, **75**:153, 1950.
57. Kunkel, H. G., and Ward, S. M.: Plasma esterase activity in patients with liver disease and nephrotic syndrome. *J. Exper. Med.*, **86**:325, 1947.
 58. Dixon, F. D.: Personal communication.
 59. Albright, F., Bartter, F. C., and Forbes, A.P.: The fate of human serum albumin administered intravenously to a patient with idiopathic hypoalbuminemia and hypoglobulinemia. *Tr. A. Am. Physicians*, **62**:204, 1949.
 60. Homburger, F., and Petermann, M. L.: Studies on hypoproteinemia. II. Familial idiopathic dysproteinemia. *Blood*, **4**:1085, 1949.
 61. Wyngaarden, J. B., Crawford, J. D., Chamberlin, H. R., and Lever, W. F.: Idiopathic hypoproteinemia; report of a case of transient edema, depression of plasma albumin and gamma globulin and eosinophilia. *PEDIATRICS*, **9**:729, 1952.
 62. Rundles, R. W., Coonrad, E. V., and Arends, T.: Serum proteins in leukemia. *Am. J. Med.*, **16**:842, 1954.
 63. Putnam, F. W.: Personal communication.
 64. Good, R. A.: Unpublished data.
 65. Zinneman, H. H., and Hall, W. H.: Recurrent pneumonia in multiple myeloma and some observations on immunological response. *Ann. Int. Med.*, **41**:1152, 1954.
 66. Lawson, H. A., Stuart, C. A., Paull, A. M., Phillips, A. M., and Phillips, R. W.: Observations on the antibody content of the blood in patients with multiple myeloma. *New England J. Med.*, **252**:13, 1955.
 67. Putnam, F. W.: Abnormal human serum globulins. *Science*, **122**:275, 1955.
 68. Kunkel, H. G., Slater, R. J., and Good, R. A.: Relation between certain myeloma proteins and normal gamma globulin. *Proc. Soc. Exper. Biol. & Med.*, **76**:190, 1951.
 69. Slater, R. J., Ward, S. M., and Kunkel, H. G.: Immunological relationships among the myeloma proteins. *J. Exper. Med.*, **101**:85, 1955.
 70. Longworth, L. G., Curtis, R. M., and Pembroke, R. H., Jr.: The electrophoretic analysis of maternal and fetal plasmas and sera. *J. Clin. Investigation*, **24**:46, 1945.
 71. Moore, D. H., Du Pan, R. M., and Buxton, C. L.: An electrophoretic study of maternal, fetal, and infant sera. *Am. J. Obst. & Gynec.*, **57**:312, 1949.
 72. Knapp, E. L., and Routh, J. I.: Electrophoretic studies of plasma proteins in normal children. *PEDIATRICS*, **4**:508, 1949.
 73. Orlandini T., O., Sass-Kortsak, A., and Ebbs, J. H.: Serum gamma globulin levels in normal infants. *PEDIATRICS*, **16**:575, 1955.
 74. Spain, D. M., Bradess, V. A., and Greenblatt, I. J.: Possible factor in sudden and unexpected death during infancy. *J.A.M.A.*, **156**:246, 1954.
 75. Kelley, V. C.: Personal communication.
 76. Martin, N. H.: Agammaglobulinaemia—a congenital defect. *Lancet*, **2**:1094, 1954.
 77. Keiden, S. E., McCarthy, K., and Haworth, J. C.: Fatal generalized vaccinia with failure of antibody production and absence of serum gamma globulin. *Arch. Dis. Childhood*, **28**:110, 1953.
 78. Kempe, C. H.: Faulty immune mechanism in serious complications of smallpox vaccination in children; consideration as an etiologic factor (abstract, private printing). *Proc. West. Soc. Ped. Res.*, 1954, p. 3.
 79. Kozinn, P. J., Sigel, M. M., and Gorrie, R.: Progressive vaccinia associated with agammaglobulinemia and defects in immune mechanism. *PEDIATRICS*, **16**:600, 1955.
 80. Good, R. A.: Fatal hepatitis in a patient with acquired agammaglobulinemia. Unpublished data.
 81. Janeway, C. A.: Personal communication.
 82. Zinneman, H. H.: Agammaglobulinemia. *Medical Grand Rounds, Veterans Hospital, Minneapolis*, **11**:12, 1953.
 83. Lawrence, H. S.: The transfer in humans of delayed skin sensitivity to streptococcal M substance and to tuberculin with disrupted leukocytes. *J. Clin. Investigation*, **34**:219, 1955.
 84. Good, R. A., and Zak, S. J.: Unpublished observations.
 85. Wedgwood, R. J. P., and Janeway, C. A.: Serum complement in children with "collagen diseases." *PEDIATRICS*, **11**:569, 1953.
 86. Thomas, L.: The physiological disturbances produced by endotoxins. *Ann. Rev. Physiol.*, **16**:467, 1954.
 87. Anderson, H. C., and McCarty, M.: Determination of C-reactive protein in the blood as a measure of the activity of the disease process in acute rheumatic

- fever. *Am. J. Med.*, **8**:445, 1950.
88. Kelley, V. C., Good, R. A., and McQuarrie, I.: Serum mucoproteins in children in health and disease with special reference to rheumatic fever. *PEDIATRICS*, **5**:824, 1950.
 89. Longworth, L. G., Shedlovsky, T., and MacInnes, D. A.: Electrophoretic patterns of normal and pathological human blood serum and plasma. *J. Exper. Med.*, **70**:399, 1939.
 90. Ham, T. H., and Curtis, F. C.: Sedimentation rate of erythrocytes. *Medicine*, **17**:447, 1938.
 91. Smith, R. T., Vaubel, C., and Thomas, L.: Experimental and clinical studies of fibrinogen and heparin precipitable fibrinogen, particularly as related to fibrinoid disease. *Bull. Univ. Minn. Hosp. & Minn. Med. Found.*, **26**:562, 1955.
 92. Francis, T., Jr., and Abernethy, T. J.: Cutaneous reactions in pneumonia to the somatic "C" polysaccharide of pneumococcus (abstract). *J. Clin. Investigation*, **13**:692, 1934.
 93. Löfstrom, G.: Nonspecific capsular swelling in pneumococci; a serological and clinical study. *Acta med. scandinav.*, Suppl. 141, 1943.
 94. Wood, H. F.: The relationship between the acute phase response and antibody production in the rabbit. I. Correlation between the early appearance of Cx-reactive protein and subsequent antibody production. *J. Exper. Med.*, **98**:311, 1953.
 95. Wood, H. F.: The relationship between the acute phase response and antibody production in the rabbit. II. The stimulation of Cx-reactive protein response by certain adjuvants and the relation of this response to enhancement of antibody formation. *J. Exper. Med.*, **98**:321, 1953.
 96. White, A.: Influence of endocrine secretions on structure and function of lymphoid tissue. *The Harvey Lectures (1947-48)*, Series 43, 1950, pp. 43-70.
 97. Sayers, G.: The adrenal cortex and homeostasis. *Physiol. Rev.*, **30**:241, 1950.
 98. Germuth, F. G., Jr., and Ottinger, B.: Effect of 17-hydroxy-11-dehydrocorticosterone (compound E) and of ACTH on Arthus reaction and antibody formation in the rabbit. *Proc. Soc. Exper. Biol. & Med.*, **74**:815, 1950.
 99. Bjørneboe, M., Fischel, E. E., and Stoerk, H. C.: Effect of cortisone and adrenocorticotrophic hormone on concentration of circulating antibody. *J. Exper. Med.*, **93**:37, 1951.
 100. Thomas, L.: Cortisone and infection. *Ann. New York Acad. Sc.*, **56**:799, 1953.
 101. Good, R. A., and Kelley, V. C.: V. Adrenal cortical function in patients with agammaglobulinemia. *Proc. Soc. Exper. Biol. & Med.*, **88**:99, 1955.
 102. McMaster, P. D.: The sites of antibody formation, in *The Nature and Significance of the Antibody Response*, ed. by A. M. Pappenheimer. New York, Columbia Univ. Press, 1953, pp. 14-15.
 103. Loeb, V.: Personal communications.
 104. Good, R. A.: Studies on agammaglobulinemia. II. Failure of plasma cell formation in the bone marrow and lymph nodes of patients with agammaglobulinemia. *J. Lab. & Clin. Med.*, **46**:167, 1955.
 105. Medawar, P. B.: The behaviour and fate of skin autografts and skin homografts in rabbits. *J. Anat.*, **78**:176, 1944.
 106. Medawar, P. B.: A second study of the behaviour and fate of skin homografts in rabbits. *J. Anat.*, **79**:157, 1945.
 107. Medawar, P. B.: Immunity to homologous grafted skin. III. The fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. *Brit. J. Exper. Path.*, **29**:58, 1948.
 108. Gibson, T., and Medawar, P. B.: The fate of skin homografts in man. *J. Anat.*, **77**:299, 1943.
 109. Morgan, J. A.: The influence of cortisone on the survival of homografts of skin in the rabbit. *Surgery*, **30**:506, 1951.
 110. Billingham, R. E., Krohn, P. L., and Medawar, P. B.: The effect of locally applied cortisone acetate on survival of skin homografts in rabbits. *Brit. M. J.*, **2**:1049, 1951.
 111. Allen, H. L., Williams, R. D., Lovingood, C. G., and Ellison, E. H.: The effect of donor skin desensitization and ACTH on survival of skin homografts in rabbits. *Ann. Surg.*, **135**:239, 1952.
 112. Amos, D. B., Gorer, P. A., Mikulska, B. M., Billingham, R. E., and Sparrow, E. M.: An antibody response to skin homografts in mice. *Brit. J. Exper. Path.*, **35**:203, 1954.
 113. Billingham, R. E., Brent, L., and Medawar, P. B.: Actively acquired tolerance of foreign cells. *Nature*, **172**:603, 1953.
 114. Mitchison, N. A.: Passive transfer of transplantation immunity. *Proc. Roy. Soc., London, s. B.*, **142**:72, 1954.

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Robert A. Good and Solomon J. Zak

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