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Use of turbidity as screening procedures for agammaglobulinemia : establishment of normal values in childhood

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THE USE OF TURBIDITY TESTS AS SCREENING PROCEDURES FOR AGAMMAGLOBULINEMIA; ESTABLISHMENT OF NORMAL VALUES IN CHILDHOOD

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INTRODUCTION

There is little in the realm of medicine more intriguing than the uncovering of a new clinical entity. During the past few years medical literature has been replete with discussions of such an entity, termed variously agammaglobulinemia or hypogammaglobulinemia, and characterized by an absence or marked deficiency of circulating gamma globulin. Bruton (7) first described this disorder in 1952, and since that time cases have been reported in surprising numbers from all corners of the globe. Since antibodies reside for the most part in the gamma globulin fraction of the serum proteins, persons afflicted with this malady have an unusual susceptibility to infectious disease. Therefore, it becomes readily apparent that this diagnosis must be entertained in any patient with a history of recurrent infections. This becomes especially important when it is realized that these patients can be greatly benefited by prophylactic substitution therapy with gamma globulin.

At present, the diagnosis can be made with certainty only through relatively expensive methods available at research centers or large teaching institutions. This points up the need for a screening test which may be made available to the average physician. Such a test should be relatively accurate, inexpensive, simple to perform, and reasonably rapid. This paper concerns itself with the development of a test which fulfills the above qualifications.

In this project two such tests were evaluated. Both tests measure the serum gamma globulin concentration through turbidimetric methods. The project concerned itself with the establishment of normal levels of gamma globulin as elicited by each test. Since the serum gamma globulin concentration is known to vary with increasing age during childhood, not reaching adult levels until five to nine years of age, normal values were established for each year of age during childhood using methods of statistical analysis.

It is hoped that through the establishment of such a screening test, the financial barrier will be removed and greater expediency will be employed in bringing patients afflicted with this condition to proper therapy.

GAMMA GLOBULIN

1. The Nature of Gamma Globulin

Since the common denominator of this paper is gamma globulin, it seems advisable to dwell for a time on the nature of this portion of the plasma proteins.

Physical and Chemical Charecteristics. Interest in the character of the plasma proteins received its greatest impetus with the development of electrophoretic analysis of the plasma. By this procedure, proteins in solution at pH levels above and below their isoelectric points migrate in an electric field toward the oppositely charged pole. Protein molecules of the same kind move at the same rate and form sharp boundaries in the solution as detected by a special optical system. Cohn (9) originally separated the plasma proteins into six major fractions. Fraction II-III was known to contain beta and gamma globulins. Later, fraction II-III was subfractionated into fractions II, III-1, and III-2, with most of the gamma globulin being in II, and some in III-1.

The gamma globulin of human plasma is physically and chemically heterogeneous. Deutsch et. al. (15) separated a protein from normal human plasma with an electrophoretic mobility between that of normal serum gamma globulin and the beta globulins. Due to the similarity of this protein to normal serum gamma globulin, as regards its physical and chemical characteristics and its antibody functions, they arbitrarily named it gamma $_1$ globulin, assigning

the designation gamma $_2$ globulin to the normal serum gamma globulin.
Cohn (10) observed that the isoelectric pH of gamma $_1$ globulin is
5.7, while that of gamma $_2$ globulin is 7.3. He also observed that
gamma $_1$ globulin has a faster electrophoretic mobility. By immun-
ologic methods two other gamma globulins of isoelectric points be-
tween the above two were isolated by Cohn. These were designated
II-1,2 and II-3. The latter are also of intermediate electrophoretic
mobility. Because the electrophoretic patterns of II-1,2 and II-3
are relatively asymetrical, it is postulated that they are less
homogeneous than either the gamma $_1$ globulin or the gamma $_2$ glob-
ulin fractions.

The molecular weight of the gamma globulins vary from 150,000
to 300,000. Dixon (17) determined the half-life of human gamma
globulin by labeling with radio active iodine. He found that the
half-life in children, six months to eight years of age, averages
20.3 days, with the value declining as age increases. In adults
he found the average half-life to be 13.1 days. These determina-
tions have assumed importance in attempts to define the etiology
of agammaglobulinemia.

Immunologic Characteristics. By immunologic analysis Enders
(21) showed that the gamma globulin fraction of the plasma proteins
contains distinct antibodies against measles, influenza, diphtheria,
mumps, pertussis, poliomyelitis, scarlet fever, typhoid, vaccinia,

infectious hepatitis, and lymphocytic choriomeningitis. Deutsch
(15) found that the gamma $_1$ globulin, in contrast to gamma $_2$ glob-
ulin contains the major portions, if not all, of the typhoid "O"
agglutinin, and the isohemagglutinins. In addition, the hemophilus
pertussis agglutinin shows a higher concentration in the gamma $_1$
globulin.

There is some controversy over the true relation of gamma glob-
ulin and antibody. Kass (38) believes that since the human serum
gamma globulin contains almost all the antibody activity of pooled
plasmas, and since attempts to demonstrate antigenic and physical
differences between immune and non-immune globulins have been un-
successful, there is little doubt that antibodies are modified gamma
globulins. Boyd and Bernard (5) believe that all gamma globulin
may be antibody of one sort or another, whereas Kabat (37) has
reviewed their work and feels that there is little factual evidence
to support the suggestion. He points out that gamma globulin is
a considerable portion of the total serum protein whereas the amount
of circulating antibody, in terms of milligrams of protein in non-
immune individuals, is extremely small.

Site of Formation and Synthesis of Gamma Globulin. Despite
considerable investigation, there is still relatively little known
regarding either the site of formation or the synthesis of gamma
globulin.

For a long time the reticulo-endothelial system has been
looked upon as the site of antibody formation. The lymph nodes

and spleen are known to be important sources of antibodies, but
the specific cell type which forms antibodies has not been estab-
lished with certainty. The role of the liver in gamma globulin
synthesis is questionable. It is known that in parenchymal hepatic
disease there is an elevation of gamma globulin, but that in disease
or obstruction of the biliary ducts there is no such elevation.

In recent years the lymphocyte and the plasma cell have been
debated as the cells producing antibody. Ehrich (20) presents one
of the leading arguments in favor of the lymphocyte. He cannulated
the afferent and efferent lymphatics of a lymph node regional to
the site of injection of an antigen, and detected antibody in the
efferent lymph before its appearance in the blood stream or afferent
lymph. Dougherty, Chase, and White (19) noted a marked release of
antibody into the blood stream upon the dissolution of lymphocytes
with ACTH. By serologic methods, Kass (38) showed the presence of
gamma globulin in cell suspensions of lymphocytes.

European authors seem to be the leading proponents of the
theory that the plasma cell is the site of antibody formation.
Bjørnboe and co-workers (3) demonstrated very high antibody titers
in extracts of plasma cell infiltrations in hyperimmune animals.
Tissue culture methods have given further support to the plasma
cell theory. Fagraeus (22) cultivated splenic red pulp and Mal-
pighian corpuscles separately. Ultimately, he found that antibody
was produced only in the red pulp and chiefly by immature plasma
cells. Keuning and Van der Slikke (40) found that large, immature
lymphoid cells were capable of synthesizing antibody. They

hypothesize that these lymphoid cells, since they are concentrated in the red pulp, will ultimately develop into mature plasma cells. Good (26) noted a deficiency of plasma cells in hematopoietic centers and inflammatory exudates of children with agammaglobulinemia. He also observed, following three weeks of intensive antigenic stimulation, that whereas normal children developed bone marrow plasmacytosis, children with agammaglobulinemia did not do so. Craig (12) demonstrated that the lymph nodes of children with agammaglobulinemia failed to show a development of active germinal centers and plasmacytosis following antigenic stimulation, in contrast to the lymph nodes of normal children.

The mode of synthesis of gamma globulin is relatively unknown. It has been observed that in experimentally induced pyridoxine deficiency in animals, hypogammaglobulinemia occurs (78). The fact that conjugated enthanolamine was noted in the plasma and urine of a patient with agammaglobulinemia suggests further investigation regarding its role in gamma globulin synthesis (63). Conjugated ethanolamine has also been found in the urine of premature infants who are known to be deficient in antibody formation.

Perhaps the riddle of gamma globulin formation will be unravelled through studies on patients with agammaglobulinemia. Certainly these patients present hitherto unavailable opportunities for study in this field.

Normal Levels of Gamma Globulin. Numerous determinations have been made of the levels of serum gamma globulin, both in

health and in disease. Most of these studies have found that the
normal adult level of gamma globulin varies from 0.60 to 1.2 gm.
per 100 cc., with an average of about 0.75 gm. per 100 cc. (18, 49).
Studies in children have revealed that the concentration of gamma
globulin decreased from unusually high levels in the newborn to
unusually low values in infancy, then gradually increased with age
to reach the normal adult level in mid-childhood, or, as has been
variously reported, between two and nine years of age (41, 50, 74).
Orlandini (57) found that the mean gamma globulin level in newborn
infants was slightly higher than in their mothers, with values
corresponding to those for normal adults. In the first month of
life, the gamma globulin decreased to a level one-third of that
found at birth, the fastest decrease occuring in the first week.
Until three months of age there was little change. Then there was
a slow rise until at two to five years, adult values were approx-
imated. Orlandini also noted that there was no difference between
the levels in breast and artificially fed babies.

2. Gamma Globulin in Disease

Inasmuch as this paper concerns itself with one particular
abnormality of gamma globulin, it is well at this point to cite
other instances of disease states in which there is an abnormal
level of gamma globulin.

Since the greatest protion of humoral antibodies reside in
the gamma globulin fraction of the serum proteins, it seems

only reasonable to expect that a stimulation of antibody production would result in a variation of the gamma globulin level. Therefore, we find that the gamma globulin is elevated in numerous acute and chronic infections. The rise in acute infections occurs during the later stages and during convalescence. Elevated levels are also found in active tuberculosis, rheumatic fever, disseminated lupus erythematosus, cardiac failure, sarcoidosis, lymphogranuloma venereum, rheumatoid arthritis, leprosy, kala azar, and multiple myeloma (47, 37). Some of the highest gamma globulin levels are seen in typhus, where it is elevated by the fifth day after onset, before the rash appears or the Weil-Felix test becomes positive (54).

In parenchymal hepatic disease there is an elevation of the gamma globulin. However, obstructive disease of the biliary ducts, unless associated with hepatic metastases or secondary hepatitis, causes no significant alteration (54). Gamma globulin also rises in response to immunization, the rise being quantitatively equal to the antibody rise (71). There is often an elevation of the gamma globulin level in Hodgkin's disease and in the lymphomas. The reason for the elevation in this instance is disputed, some feeling it is due to lymph node hyperplasia, and others believing it to be secondary to hepatic replacement (1).

Decreased gamma globulin levels are seen in numerous states. One might surmise that malnutrition would commonly produce this change. However, Bigler (2) observed that the gamma globulin fraction was the most refractory to variation with malnutrition of

any of the serum protein fractions. It is only in markedly severe malnutrition that hypogammaglobulinemia occurs (42). Abnormally rapid loss of gamma globulin from the plasma is the commonset cause of hypogammaglobulinemia. In the nephrotic syndrome there is excessively rapid transcapillary loss of gamma globulin, with a parallel loss of albumin, in the urine (78). Because of this, replacement therapy with gamma globulin, to protect nephrotic children from infection, has proved unsatisfactory.

Other theoretic causes for an increased rate of gamma globulin removal include fixation of the protein in antigen-antibody reactions and an increased rate of protein catabolism, as in Cushing's syndrome. In the latter condition, the increased adrenal cortical activity causes a release of gamma globulin into the serum, presumably through dissolution of the precursor cells, either the lymphocyte or the plasma cell. Following adrenalectomy, the gamma globulin level returns to normal (48).

Isolated cases of hypogammaglobulinemia have been noted in association with various diseases. Such examples have been reported in malignant lymphomas (1), pyoderma gangrenosum (53), and multiple myeloma (78).

There is also a broad group of idiopathic hypogammaglobulinemias, including congenital and acquired agammaglobulinemia. Myers and Taylor (56) were the first to report such a case. Their patient had had hypoproteinemia with intermittent edema all his life. Renal and hepatic function tests were all reported as normal. Thompson (72) reported a similar case in a two year old girl

who had had anasarca since birth. This patient subsequently suc-
cumbed to broncho-pneumonia. For eleven years Schick (67) obser-
ved a female patient who had intermittent edema and hypoproteinemia.
Marked hypogammaglobulinemia was noted on electrophoresis of serum,
although she had no unusual susceptibility to infection and Schick
and Dick tests were negative. Wyngaarden (75) and Fried (23) re-
ported similar cases in male children who had edema, hypoprotein-
emia without proteinuria, and hypogammaglobulinemia. These latter
patients had no increased susceptibility to bacterial infection
even though attempts to stimulate antibody formation by injection
of diphtheria toxoid and typhoid and pertussis vaccines failed.
In both there was some question of hepatic dysfunction. Homberger
(32) described an entity called Familial Idiopathic Dysproteinemia,
characterized by alterations of various components of the plasma
proteins in members of a family. In the member of the family with
hypogammaglobulinemia, he noted that the injected gamma globulin
disappeared from the plasma at an excessively rapid rate. The
common factor in all of the above cases is that of unknown etiology.
This leads, appropriately, to a discussion of agammaglobulinemia,
the most recently described of these entities, and the major topic
of this paper.

AGAMMAGLOBULINEMIA

1. General Considerations

The first case of agammaglobulinemia was reported in June, 1952, by Bruton (7) of the Walter Reed Army Medical Center. His case was an eight year old boy who had been seen for nineteen episodes of clinical sepsis during a four year period. In general he responded to antibiotics, was well nourished and of normal growth. Since the same type of pneumococcus was isolated on ten different instances from his serum, attempts were made to immunize the boy against this bacterium. However, all attempts to demonstrate an antibody titer to the pneumococcocal vaccines failed. Subsequently it was noted that his Schick test was positive although the patient had had a diphtheria toxoid series and boosters. Finally, a Tiselius electrophoretic analysis of the serum was done, revealing an absence of gamma globulin, although all other protein fractions were normal. The patient was then given monthly injections of immune serum globulin and was rendered completely free of infections for the succeeding 30 months.

Within the next few months Bruton (6) reported three more cases of this entity in boys with histories of multiple infections since infancy or early childhood. He postulated that the entity had not previously been recognized because specific methods for the detection of the serum gamma globulins were lacking and because the severe infections were usually fatal prior to the era of antibiotic therapy. Burton defined agammaglobulinemia as an

entity characterized by an absence of serum gamma globulin in the face of a normal total serum protein concentration and normal albumin/globulin ratio (8). Janeway (35) then reported sixteen cases of agammaglobulinemia, all in male children. He noted that there were several instances in which brothers of the mothers of these patients, or male siblings in previous generations, had succumbed fairly early in life to severe infection, but that all females had been unaffected. In reviewing the literature, the only case of agammaglobulinemia in childhood that has affected a female was reported by Keidan (39). The latter was a case of fatal generalized vaccinia occurring in a seven weeks old child. However, since the serum gamma globulin is normally at a very low level at that age, the diagnosis must be questioned.

The first case of agammaglobulinemia in an adult was reported by Sturgis (34) in a 36 year old male. This patient had been well until age 27, but had had recurrent infections since that time. The serum gamma globulin level was at the lower limits of detecta - bility by immunochemical analysis. He coined the term "acquired agammaglobulinemia" for this entity in contradistinction to the childhood disease. Subsequent cases of agammaglobulinemia in adults of both sexes, and characterized by onset of recurrent sepsis relatively late in life, were reported by Zinneman (79), Sanford (64), Seltzer (68), and Wall (73). Gitlin (25) concluded that the adult disease was similar in many respects to that seen in children. However, he noted that it differed from the childhood disease in that 1) the concentration of the serum gamma globulin tends to be

higher, 2) either males or females may be affected, and 3) the disease does not become manifest until adulthood. Because of the occurrence of the childhood disease solely in males, it was proposed that the defect in the gamma globulin was genetically determined, with inheritance as a sex-linked recessive trait, analagous to that of hemophilia (24).

It is of interest to note at this point that Janeway (36) has discovered five children with histories of recurrent sepsis, suggestive of agammaglobulinemia. However, in all of these patients the serum gamma globulin level was elevated, in one instance as high as 4.5 gm. per 100 cc. He believes that this condition may be due to a complete loss of the bactericidal property of the serum with excessive concentrations of antibody, due to a state equivalent to a prozone reaction.

2. Etiology

The true etiology of agammaglobulinemia is unknown. However, considerable effort has been expended in an attempt to accurately define the cause of the condition. A lower than normal level of any specific blood protein or blood cell may be due to a diminished rate of formation or delivery to the circulating blood, to an increased rate of utilization, destruction, or loss from the circulation, or to a combination of these processes. Hence, the mechanism of such an abnormality as agammaglobulinemia cannot be assumed to be due to a diminished rate of formation in any given case unless data on this or on the rate of disappearance of injected tracer

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doses can be obtained. With the use of radioactive isotopes and
purified protein fractions this can be done quite readily on a
research basis. Both Janeway (34), and Rosecan (63) have observed
that the half-life of passively transferred gamma globulin in
patients with agammaglobulinemia is normal. Therefore, it is assu-
med that the primary defect is deficient synthesis of the protein
fraction.

In an attempt to discover some specific defect of amino acid
metabolism in this condition, Rosecan (63) carried out paper chrom-
atography of the amino acids. However, a normal spectrum was ob-
tained. Nevertheless, he did observe abnormal amounts of conjugated
ethanolamine in the plasma and urine of one patient. This same
substance has also been found in the urine of premature infants who
are known to be deficient in antibody formation.

In view of the decrease in gamma globulin and increased sus-
ceptibility to bacterial infections in hyperadrenalism, and the
suppression of antibody formation by ACTH and cortisone, studies
of adrenal function in seven patients with agammaglobulinemia
were carried out by Good (27). He found normal adrenal function
in these patients, as determined by urinary 17-hydroxy-cortico-
sterone levels following ACTH administration.

Martin (55) suggests that the condition may be due to a
persistence of the fetal pattern of gamma globulin. He found,
through studies on premature infants, that there was an almost
total absence of gamma globulin, by electrophoresis, at or before
the 26th week of life.

3. Morbid Anatomy

The data obtained from a complete hematological analysis of
patients with agammaglobulinemia permits the conclusion that these
patients have a profound disturbance of the hematopoietic reticulum.
Good (26) has found that these patients regularly exhibit a defic-
ency of plasma cells in their hematopoietic centers and in their
inflammatory exudates, amounting to a virtual absence of plasma
cells. He also noted that these patients fail to respond to even
the most intensive antigenic stimulation with either antibody pro-
duction or plasma cell formation. Both Good (29), and Craig (12)
observed that the lymph nodes and bone marrows of these patients
fail to develop plasma cells and secondary follicles in response
to antigenic stimulation. Good (26) also noted that these patients
may respond to bacterial infection with extreme leukocytosis and
may develop spontaneously episodes of transient neutropenia, per-
sistent neutropenia, or may even develop cyclic neutropenia.

Two autopsied cases of agammaglobulinemia with bronchiectasis
were reported by Collins (11), in which lymphoid structures showed
no germinal centers, and no plasma cells could be demonstrated in
any tissue. The lymph nodes removed from these patients showed
rather narrow cortices, small, ill-defined follicles, and absence
of germinal centers and of pre-plasma cells and plasma cells. The
reticuloendothelial cells of the peripheral and central sinusoids
in these nodes were swollen. Aside from the changes in the lymph
nodes, similar decreased plasma cell activity was observed in the
spleen and bone marrow. Special stains failed to reveal the pre-

sence of plasma cells in any area of infection. Generally speaking,

other important organs, such as liver, kidney, and endocrine glands,

revealed no significant anatomical or histological changes, other

than changes due to secondary bacterial invasion.

To add some confusion to the situation, Young and Wolfson (77)

have reported three cases of agammaglobulinemia in adult males,

each of which exhibited deficiency of lymphocytes in the tissues

and peripheral blood. No mention is made of the status of the

plasma cells in this report.

4. Clinical Picture

As may be expected, the clinical picture in agammaglobulinemia

is that of recurring infections, manifested by intractable diarrhea,

pyoderma, purulent conjunctivitis, otitis media, purulent sinusitis,

bronchitis, bronchopneumonia, meningitis, or septic arthritis. It

has been observed repeatedly that the sequence of clinical diseases

which threaten the lives of these patients have their basis in ba-

cterial infection. Strangely enough, virus infections appear to

be handled efficiently and normally, and are not unusually severe

(26). This may be interpreted as evidence that recovery from virus

infection may be accomplished without the aid of antibody. Janeway

(35) has observed that in congenital agammaglobulinemia the problem

has been intractable diarrhea in the early months of life, and

pyogenic infections at a later date. Characteristically, each

infection responds to antibiotics, but the child soon succumbs to

another attack with the same or a different organism, and per-

manent damage, such as hydrocephalus following meningitis, or bronchiectasis following bronchopheumonia, may ensue.

The symptomatology in the adult form of agammaglobulinemia is similar to the above but the patients are relatively free of infection for the first few decades of life. Typical of these cases is that of a girl who was well until age 19, but then had 35 attacks of pneumonia in the next seven years (79). Another girl was well until age 15 at which time she had a second case of pertussis. In the subsequent four years she had seven attacks of pneumonia and several other febrile illnesses (31).

Agammaglobulinemia often coexists with profound hematologic disease. Cyclic neutropenia, persistent neutropenia, eosinopenia, extreme leukocytosis, thymic tumor, lymph node enlargement, and splenomegaly have all been noted (28, 45).

5. Diagnosis

Janeway (35) has established the following criteria for making a positive diagnosis of agammaglobulinemia: 1) history of multiple serious septic infections, 2) absence of isohemagglutinins or other naturally occurring antibodies which would be expected in the blood, including antibodies to those infections against which the patient has been immunized 3) absence of gamma globulin by electrophoretic analysis in the face of an otherwise relatively normal serum protein distribution, and 4) absence of gamma globulin, or at least a very low level, perferably below 100 mg. per 100 ml. as determined by a relatively specific method such as the immunochemical.

The importance of an accurate history cannot be overemphasized. Good (30) claims that a presumptive diagnosis of agammaglobulinemia was made on five of his seven patients by the admitting physician on a basis of history alone.

Patients with agammaglobulinemia show evidence of an immunological paralysis. They react positively to skin testing with Schick and Dick toxins, indicating an absence of tissue antibodies against these antigens. There are insignificant amounts of antibody against streptolysin, or streptococcal hyaluronidase, or desoxyribonuclease. In addition, Forssman antibody, cold agglutinins, and complement fixing and virus neutralizing antibodies, commonly found in the serum of normal patients, are lacking. There is an absence or marked deficiency of normally inherited plasma isohemagglutinins in these patients. The latter may be used as a simple laboratory screening test for the disease in patients of blood groups O, A, or B. Obviously, it could not be used for patients of type AB. It has also been noted in these patients that attempts to stimulate antibody production through immunization are unsuccessful (26).

In agammaglobulinemia normal results are seen in laboratory tests which depend upon abnormalities in serum gamma globulin. The thymol turbidity, cephalin-cholesterol flocculation, and colloidal gold flocculation tests are normal, although the patient's status suggests that they should be abnormal. These patients do have erythrocyte sedimentation rates consistent with their state

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of health or illness because fibrinogen, the chief plasma protein determinant of the sed rate, shows its usual increase in response to illness or injury (78). Also, the C-reactive protein and serum mucoprotein tests are unaffected by the disease, suggesting that these tests are dissociated from antibody production (26).

As was stated above, the serum isohemagglutinin level may be used as a simple screening test for agammaglobulinemia, as may the Schick test if the patient has been previously immunized against diphtheria. However, neither of these is specific for the gamma globulin fraction of the serum proteins, but only detections of a single antibody component. As a result, Good (30) feels that tests such as the zinc turbidity test of Kunkel or the gamma globulin turbidity test of De la Huerga are more satisfactory, not only from the standpoint of accuracy and simplicity, but also from that of time required to perform the test. Gitlin (25) has developed a simple procedure using horse antiserum against human gamma globulin incorporated in agar and placed in a long thin tube. The serum of the patient is simply placed over the antiserum-agar in the tube and the tube is allowed to remain at room temperature for 24 hours. The presence of a band of precipitate in the agar indicates the presence of gamma globulin in the patient's serum and the distance the band has migrated from the interface indicates the concentration of gamma globulin.

As was mentioned previously, the positive diagnosis of agammaglobulinemia is made on the basis of Tiselius electrophoretic patterns of the serum proteins and immunochemical analysis

of the serum. It must be realized that electrophoresis is not accurate in revealing small amounts of protein, so that at gamma globulin levels below 150-200 mg. per 100 ml., the immunochemical methods are more specific (6). It must also be realized that at levels of gamma globulin above the aforementioned, the electrophoretic method is more accurate. This may explain the discrepancy in a recently reported case in which the electrophoretic analysis of the serum proteins revealed a gamma globulin concentration of 300 mg. per 100 ml., while immunochemical methods resulted in a concentration of 76 mg. per 100 ml. (46). It should also be emphasized that cyclic changes of the gamma globulin level occur in some patients. Therefore, single determinations of the level are often not sufficient (76).

There is one form of hypogammaglobulinemia that occurs in infancy, between four and fifteen weeks of age, which is a variant of the normal physiologic decline of gamma globulin due to the gamma globulin of maternal origin being metabolized. Levels as low as 75 mg. per 100 ml. may be observed at this age. However, since the condition is transient and lasts only for a short period, the patient usually encounters no difficulty. The existence of such a state makes a definite diagnosis of agammaglobulinemia very difficult in a patient under six months of age (25).

6. Treatment

Early recognition of the presence of agammaglobulinemia is essential so that treatment may be instituted before irreversible structural damage to the various organs has occurred. Although

definitive therapy is not yet at hand, these patients are greatly
helped by intramuscular injections of gamma globulin and by pro-
viding prophylactic antibiotic therapy against the infections. It
has been recognized (35) that giving the gamma globulin when the
child is suffering from an acute infection does very little good.
Antibiotics must be used in this instance, with the gamma globulin
therapy reserved for keeping the patient free from further infections.
It is apparently neither necessary nor practical to achieve normal
blood levels of gamma globulin to prevent infection. Levels of
only 100-150 mg. per 100 cc. are effective. The usual dosage given
to produce this level is 0.1 mg. per kg. body weight every two to
three weeks (35, 62, 64). It will be realized that this dosage,
using the commercial 15 per cent solution, approaches 15-25 ml.
The material cannot be administered intravenously because of severe
hypotensive reactions, and intramuscular injections of over 20
ml. are prone to cause chills, fever, myalgia, and faintness.
Since the virus of homologous serum jaundice is not associated with
the gamma globulin fraction, there is little danger of serum
hepatitis, even with prolonged administration (78).

Another aspect of the therapeutic problem was brought out by
Spain et. al. (70) who believe that since there is an unusual in-
cidence of sudden death due to overwhelming sepsis from three to
twenty weeks of age, and since low levels of gamma globulin usually
occur at this age, routine injections of small amounts of gamma glob-
ulin might be advisable.

Since hypogamma globulinemia has occurred in experimentally
induced pyridoxine deficiency in animals, large doses of pyridoxine

(100 mg. per day) with and without whole vitamin B complex, were administered to two patients, but with no effect. Likewise, treatment with ACTH and cortisone has been unsuccessful (78).

It will be seen that the above therapy still leaves these patients with a handicap of immunologic unresponsiveness. The next step is to induce in these patients a capacity to react to antigenic material. Good (30) believes that the homotransplantation of lymph nodes to these people may alleviate this defect.

7. Prospectus

Studies of cases of agammaglobulinemia, besides being interesting in themselves, have opened a new vista in the study of immunological phenomena. From the above-mentioned findings, it will be seen that much needs to be learned concerning the basis of resistance to and recovery from virus infections, the relationship of anaphylactic antibody production to bacterial type hypersensitivity, and the significance of the basis for acute phase reactions such as the sedimentation rate and the C-reactive protein. Another outgrowth of studies on these patients has been the finding of Good and Varco (26) that they will accept split thickness and full thickness skin homografts with facility. Whereas such grafts usually necrose and slough within a period of one month, the grafts on Good's patient have taken, grown, and are surviving without showing any reaction 17 months after application (30).

THE MEASUREMENT OF THE SERUM GAMMA GLOBULIN
CONCENTRATION WITH TURBIDITY TESTS

Among the available means of measuring the serum gamma globulin
concentration prior to 1947 were electrophoretic, immunochemical,
and quantitative salt-precepitation techniques. Since all of these
methods require an excessive amount of time, specially trained
personnel, and expensive equipment, other tests have been recently
devised which may be used as routine screening procedures. The
latter tests utilize turbidimetric principles for the assessment
of the serum gamma globulin concentration. This paper is concerned
with the establishment of these methods as screening procedures in
the diagnosis of agammaglobulinemia.

The two tests that will be evaluated in this paper are the
zinc sulphate turbidity test of Kunkel, developed in 1947, and the
gamma globulin turbidity test of De la Huerga and Popper, developed
in 1949. The zinc sulphate turbidity test is based on the fact
that in solutions alkaline to the isoelectric pH of the protein
molecule, the protein ion, which is negatively charged, combines
with a positively charged metal ion to give an insoluble precipit-
ate of metal proteinate. It is evident that the pH of the pro-
tein solution is of primary importance in this method (44). The
gamma globulin turbidity test has its basis in the fact that when
increasingly large quantities of very soluble salts, such as amm-
onium sulphate, are added to protein solutions, the solubility of
the protein decreases. In this method, precipitation of the pro-
tein by salting out is most complete at or near its isoelectric

pH (33). In both tests the turbidity values are expressed in arb-
itrary units, based on the comparative turbidity of a standard
suspension of barium sulphate, similar to that employed for the
estimation of thymol turbidity.

Kunkel (43) analyzed the precipitate of the zinc sulphate
turbidity test and found that the chief component was gamma glob-
ulin with small amounts of other protein fractions which were
carried down with the gamma globulin. Numerous electrophoretic
patterns **showed** that the elevation of the gamma globulin compon-
ent correlated well with the intensity of the reaction. Elevat-
ions of the beta globulins were not reflected by the turbidity
reaction. De la Huerga et. al. (14) analyzed the precipitate of
the gamma globulin turbidity test and observed that approximately
75% was gamma $_2$ globulin, 15% lay between the gamma $_1$ globulin
and the beta globulin, and less than 10% was composed of other
serum protein fractions. They also found that only traces of
gamma globulin remained in the supernatent. Ricketts (61) noted
that the zinc sulphate turbidity test correlated more closely
with electrophoresis than did the gamma globulin turbidity test
in seven normal sera.

Kunkel (43) found that the normal range for his test was
between 2 and 8 units. He arrived at this conclusion after per-
forming approximately 1000 determinations. However, other in-
vestigators have cited 16 units to be the upper limit of normal
for this test (51, 66), and 3 units to be the lower limit of no-
rmal (58). De la Huerga and Popper (13) performed the gamma

globulin turbidity test on 52 normal adults and found a range of
2.3 to 5.6 units, with a mean of 4.0 units. There is no record
in the literature that normal values for these tests have ever
been ascertained for the early years of life when there is known
to be a fluctuation of the gamma globulin level.

The zinc sulphate turbidity test is depressed by an elevat-
ion of the serum albumin and also by biliary substances in the
serum (60). The biliary substances which depress the test are
believed to be of lipid character, such as lecithins (59). It
has also been observed that a high lipid intake will depress the
test (14). On the other hand, the gamma globulin turbidity test
is said to be uninfluenced by variations in the albumin concent-
ration of the serum (14). Marked lipemia or excess jaundice will
cause some increase in the readings of the latter test, but can
easily be corrected for by dissolving the precipitate with water
and re-calculation of the initial reading (13). There is a tend-
ency for both tests to be much lower, as expressed in units, in
the normal and low ranges than the gamma globulin concentration
would indicate. This tendency is an expression of a relatively
much smaller precipitate in the lower ranges (59).

The above turbidity tests have won considerable favor as
routine methods for the determination of the serum gamma globulin
concentration. Discombe et. al. (16) believe that since filter
paper electrophoresis is time-consuming and gives an objective
measurement of the protein components only with laborious methods,
the zinc sulphate turbidity test can dispense with any need for

electrophoretic methods for routine purposes. This same group noted that although agreement between laboratories on the same sample of serum is usually close, it is not exact. Therefore, each laboratory should reconstruct normal standards for each test. Utilization of these tests has permitted more widespread evaluation of the gamma globulin levels in various disease states than would have been possible with more cumbersome methods (47,52,58, 65).

MATERIALS AND METHODS

Samples of serum from 372 patients were subjected to both the zinc sulphate turbidity test and to the gamma globulin turbidity test by the author. As will be noted in Appendix I, the patients were obtained from the hospital of the University of Nebraska College of Medicine (UNH), the dispensary of the University of Nebraska College of Medicine (UND), Children's Memorial Hospital (CMH), and the Child Saving Institute (CSI), all located in Omaha, Nebraska. A small number of patients were referred by private physicians (PP). 306 of the patients had no history of an infectious process for at least one month prior to obtaining the blood sample, and had no condition that is known to produce an elevation of the serum gamma globulin level. The remainder of the patients failed to meet these criteria and were not used in the calculation of normal values for the turbidimetric tests. An attempt was made to obtain a fairly equal distribution of patients in each age group. However, it will be noted that there is a disproportionately large number of five year old children. The latter is explained by the large number of children of this age attending the dispensary of the University of Nebraska College of Medicine for pre-school physical examinations during the interval this study was being conducted.

Blood was obtained by venipuncture from a femoral vein in the younger children and from an arm vein in the older ones. The femoral bloods were obtained by the author, and the antecubital

venipunctures were performed by the author or by medical tech-
nologists of the University of Nebraska College of Medicine.
Care was taken to minimize hemolysis of the blood sample by using
no smaller calibre needle than 20-guage for the femoral punctures,
and no smaller than 22-guage for the antecubital punctures.

As was mentioned above, the zinc sulphate turbidity and
the gamma globulin turbidity tests were performed on all serum
specimens. On three patients in whom inordinately low values
were found on both of the aforementioned tests, on four normal
children selected at random, and on one patient with nephrosis
a variable number of the following determinations were performed:
complete blood count, urinalysis, serology, sedimentation rate,
Schick test, thymol turbidity test, cephalin-cholesterol flocc-
ulation test, serum cholesterol level, total serum protein level
and albumin/globulin ratio (Biuret method), isohemagglutinin
titres, and agglutination titres against Brucella abortus and
Typhoid "O" and "H" antigens. These tests, together with filter
paper electrophoretic studies were done by medical technologists
of the hospital of the University of Nebraska College of Medicine.
Free electrophoresis, after the method of Tiselius, was performed
by Dr. Violet Wilder of the Department of Biochemistry of the
University of Nebraska College of Medicine.

Following are the methods employed in the performance of
the zinc sulphate turbidity and gamma globulin turbidity tests:
Zinc Sulphate Turbidity Test: Estimation of the turbidity was

made by the method of Kunkel (26). To 6 cc. of the reagent*, 0.1 cc of serum was added. The mixture was allowed to stand for 30 minutes, at 25° C., then was shaken, and finally the turbidity was read in a Coleman Jr. spectrophotometer at a wave length setting of 650 mu. Values obtained on the spectrophotometer were converted to units, as advocated by Kunkel (27), using the standard curve described by Shank and Hoagland for the thymol turbidity test (69). This curve is constructed using a standard of 3 cc. of barium chloride solution (containing 1.15 gm. $BaCl_2 \cdot H_2O$ per 100 ml.) made to 100 cc. with 0.2N H_2SO_4. This suspension gives a turbidity equivalent to 20 units, and other values are derived using various dilutions of this suspension with distilled water.

Gamma Globulin Turbidity Test: The method of De la Huerga and Popper (29) was used for estimation of the turbidity. To 5 cc. of the reagent **, 0.1 cc. of serum was added. After allowing the mixture to stand for 30 minutes at 25° C., the test tube or cuvette was inverted twice without shaking and the turbidity was read in a Coleman Jr. spectrophotometer using a wave length of 650 mu. The transmission was translated into units using the standard curve constructed for the zinc sulphate turbidity test.

```
*Reagent:    ZnSO4·7H2O   - 24 mg.
             Barbital      - 280 mg.
             Sodium Barbital  - 210 mg.
             Dissolve in distilled H2O to 1 L.
             pH - 7.6

**Reagent:   (NH4)2SO4    - 189 gm.
             NaCl          - 29.3 Gm.
             Dissolve in distilled H2O to 1 L.
```

RESULTS OF THE STUDY

Since the objective of this project was to attempt to define normal values of the aforementioned gamma globulin turbidity tests so that they may be used as screening procedures for agammaglobulinemia, a statistical analysis was done on the results of the tests in the 306 normal children. Tables I and II, below, summarize the results of this analysis, which is presented in complete form in Appendix I.

So that it might be more clearly seen at which age of childhood the gamma globulin levels, as expressed by these tests, reached adult values, graphic representation of the variation of

Table 1. Summary of Results of the Zinc Sulphate Turbidity Test

Age	No. of Cases	Range	Mean	Standard Deviation	Standard Error of the Mean
1. Newborn (to one week)	19	4.1-14.0	8.55	2.54	0.57
2. To 15 weeks	18	1.5-5.5	3.16	1.04	0.27
3. To 6 months	11	0.7-5.0	2.66	1.20	0.37
4. To One year	15	1.6-7.2	4.15	1.42	0.37
5. To Two years	29	2.6-11.5	5.77	2.15	0.40
6. To Three years	16	2.7-11.7	5.21	2.03	0.52
7. To Four years	24	3.0-7.7	5.57	1.48	0.30
8. To Five years	21	2.2-13.2	5.78	2.81	0.64
9. To Six years	69	1.0-9.2	5.33	1.63	0.19
10. To Seven years	15	3.5-9.5	6.37	1.52	0.40
11. To Eight years	19	2.7-10.7	6.78	2.30	0.54
12. To Nine years	16	4.5-9.2	6.42	1.40	0.36
13. To Ten years	8	2.7-11.5	6.40	1.89	0.70
14. Over Ten years	26	2.2-12.0	6.58	1.89	0.38

Table II. Summary of Results of the Gamma
Globulin Turbidity Test

Age	No. of Cases	Range	Mean	Standard Deviation	Standard Error of the Mean
1. Newborn (to one week)	19	2.2-7.7	4.60	1.53	0.35
2. To 15 weeks	18	0.5-2.5	1.37	0.58	0.14
3. To 6 months	11	0.7-2.0	1.31	0.37	0.11
4. To One year	15	0.5-2.7	1.59	0.58	0.16
5. To Two years	29	1.0-4.2	2.30	0.93	0.17
6. To Three years	16	1.0-3.7	2.13	0.66	0.16
7. To Four years	24	1.5-4.0	2.55	0.68	0.14
8. To Five years	21	1.1-4.5	2.48	1.06	0.24
9. To Six years	69	0.5-4.5	2.49	0.81	0.09
10. To Seven years	15	1.7-3.7	2.82	0.51	0.13
11. To Eight years	19	1.1-4.7	2.98	0.88	0.21
12. To Nine years	16	1.6-4.2	2.80	0.61	0.15
13. To Ten years	8	1.7-4.0	3.40	0.71	0.27
14. Over Ten years	26	2.5-5.0	3.40	0.71	0.14

the mean levels of these tests with age was constructed in Fig.
1. Figs. 2 and 3 were constructed to reveal the range of tur-
bidity at each age which encompassed two standard deviations
from the mean.

In the course of the study, two supposedly normal patients
(Nos. 25 and 149) were found to have markedly low levels of gamma
globulin by both turbidity tests. To determine the etiology of
such low levels, and also to ascertain whether these represented
cases of "latent" idiopathic hypogammaglobulinemia, an evaluation
of renal and hepatic function and of antibody formation, together
with electrophoretic analysis of the serum was carried out. Re-
sults of this analysis, and also of determinations made on sev-
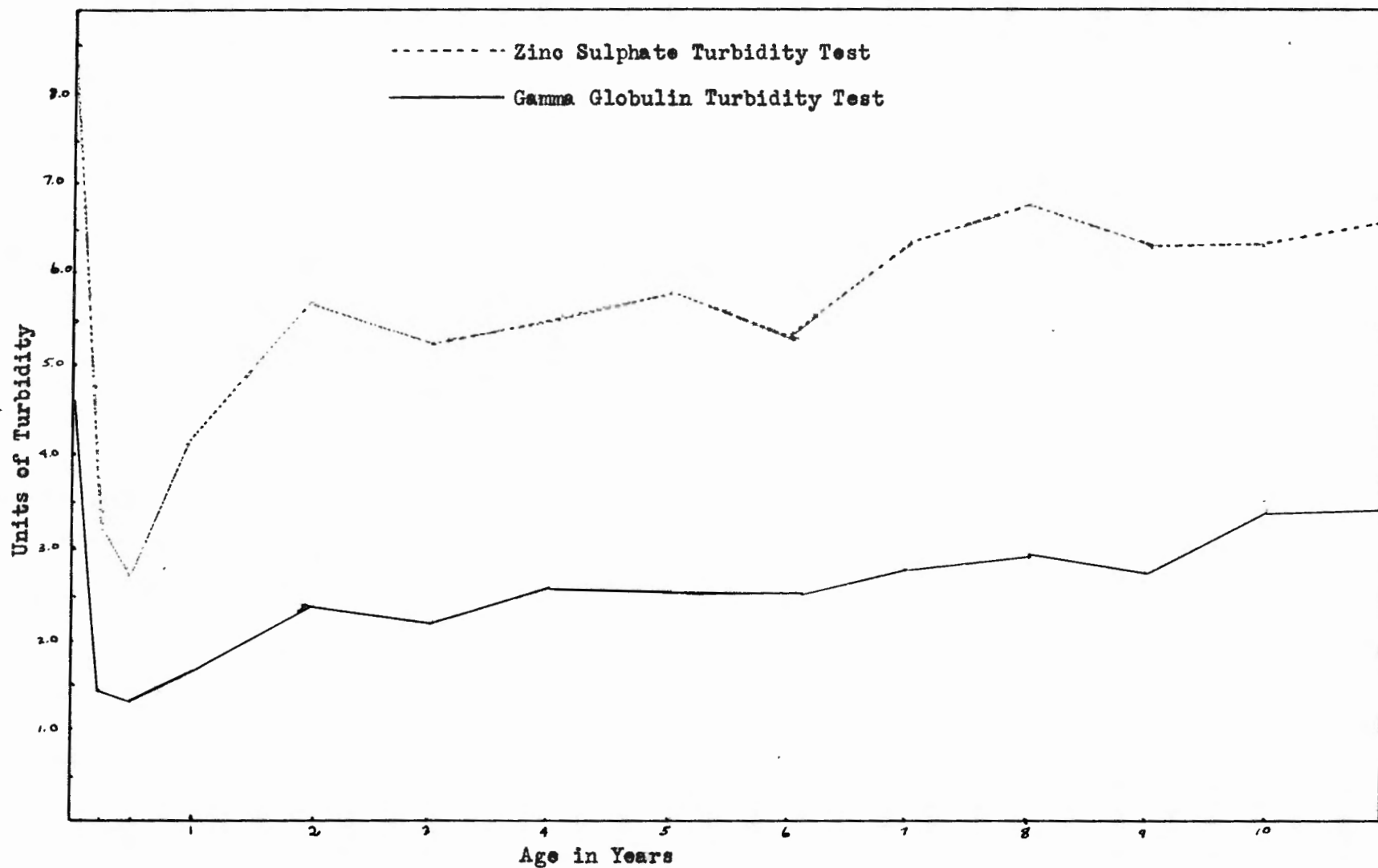
Fig. 1. Variation of the mean levels of the Zinc Sulphate Turbidity
and Gamma Globulin Turbidity tests with age.
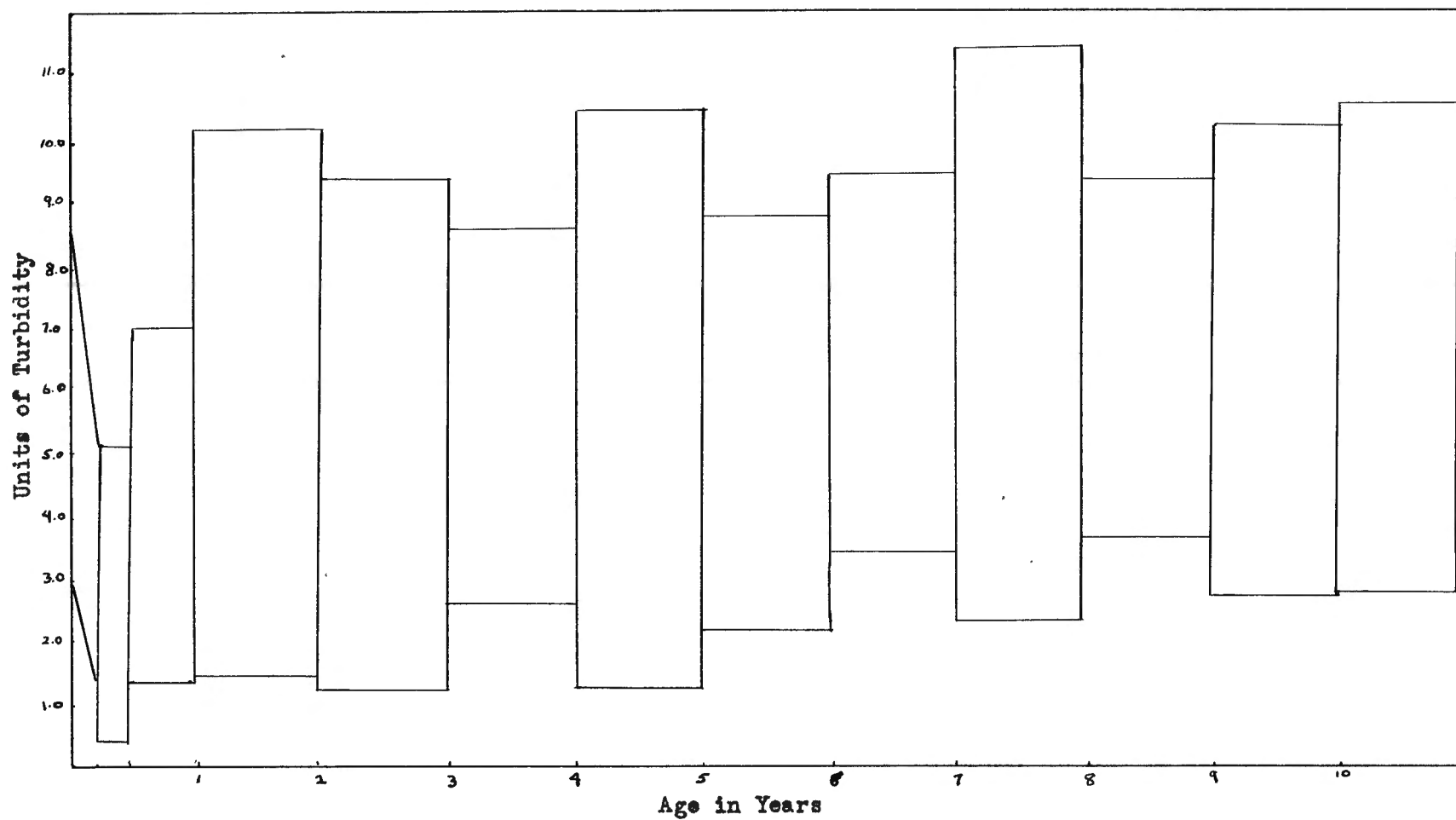
Fig. 2. Variation of two standard deviations from the mean turbidity
level of the Zinc Sulphate Turbidity test with age.
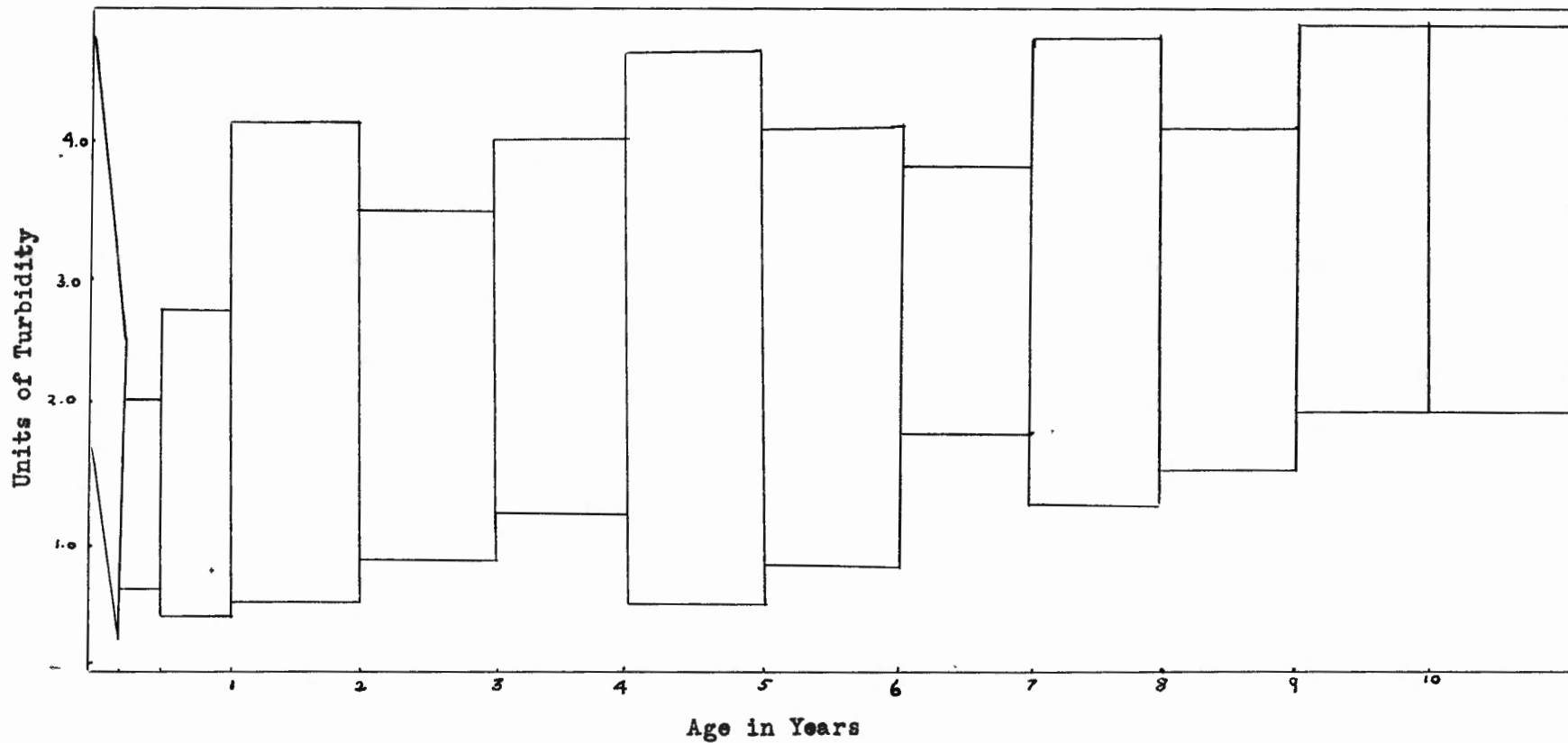
Fig. 3. Variation of two standard deviations from the mean of the
Gamma Globulin Turbidity test with age.

eral normal sera, are summarized in table III. For comparison, free
and filter paper electrophoretic analysis was performed on several
sera with normal gamma globulin levels by turbidity testing, as well
as on serum from a patient with nephrosis (No. 351), in which a pro-
found hypogammaglobulinemia is known to occur. Table IV contains
the results of the calculation of serum protein fractions from the
electrophoretic patterns in Appendix II. One patient (No. 160)
who had a moderately low level of gamma globulin by turbidity test-
ing in the presence of a scalp infection could not be evaluated
beyond performance of filter paper electrophoresis, since she lived
at a considerable distance from Omaha.

It will be seen that the two patients with markedly low
values of the turbidity tests were found to have gamma globulin
levels, as denoted by free electrophoresis, which were well above
those diagnostic of agammaglobulinemia. Nevertheless, it is ob-
vious that their levels were considerably below those of the three
patients with normal values of zinc turbidity and gamma globulin
turbidity. It is interesting to note that the level in one patient
(No. 149), although above those cited by Janeway (45) to be diagno-
stic of agammaglobulinemia, was equivalent to those reported for a
patient with "hypogammaglobulinemia" (85). Chemical and immuno-
logic analysis failed to offer an explanation for the low values in
these patients; the reduced isohemagglutinin titers and slight ele-
vation of the serum chelesterol being the only tests with abnormal
results.

Table III

Summary of Chemical and Immunologic Studies on Selected Patients with Low and Normal Turbidity Levels

Case and Serum No.	Hemogram		U. A.		Serology	Sed Rate	TSP (gm%)	Total Serum		ZST	GGT	Schick	Thymol Turbidity	Cephalin-Chol. Floccul.	Serum Cholester. (mg %)	Isohemagglut Titer	Brucella agglut.	Typhoid agglut.
	Hb	WBC	Alb	Sug				Alb (%)	Glob (%)									
25.	13.6	5300	0	0	neg.	15	6.9	4.5	2.4	1.1	0.5	0	3.8	24h---0 48h---0	279	anti-A 1:16 anti-B 1:8	0	o
28.	12.2	9200	0	00	neg.	24	7.3	4.3	3.0	5.2	3.0							
149.	14.2	5200	0	0	neg.		6.6	4.5	2.1	1.0	0.7	0	2.2	24h---1 48h---2	283	anti-B 1:64	0	o
160.	12.8	9300	0	0	neg.					2.2	1.0							
199.	16.3	8000	00	0	neg.	13	7.8	4.8	3.0	5.2	2.6							
207.	12.8	9600	0	0	neg.		8.5	5.2	2.3	6.5	3.7							
263.	13.2	6700	0	0	neg.	18	7.1	4.1	3.0	7.0	3.7							
351.	11.5	18100	4+	0	neg.		3.1	2.3	0.8	0.7	0.5				236			

TSP - Total Serum Protein

ZST - Zinc Sulphate Turbidity test (in units)

GGT - Gamma Globulin Turbidity test (in units)

Table IV

Calculated Serum Protein Fractions from Free

Electrophoretic Patterns

Case and Serum No.	TSP (gm %)	ZST	GGT	Albumin (% of TSP)	Globulins (% of TSP)			
					α_1	α_2	β	γ
25.	6.9	1.1	0.5	65.6	4.2	9.9	11.5	8.9
149.	6.6	1.0	0.7	62.8	5.1	11.2	13.0	7.9
199.	7.8	5.2	2.6	55.5	7.7	11.4	13.0	12.5
207.	8.5	6.5	3.7	57.7	4.9	11.1	11.5	14.8
263.	7.1	7.0	3.7	58.5	4.5	9.5	13.0	14.5

TSP - Total Serum Proteins

ZST - Zinc Sulphate Turbidity test (in units)

GGT - Gamma Globulin Turbidity test (in units)

DISCUSSION

As has previously been stated, the prime concern of this study was to establish normal levels for gamma globulin turbidity tests during childhood.

B oth the zinc turbidity and gamma globulin turbidity tests exhibited the expected drop in turbidity levels in the early weeks of life, reaching lowest levels between the 15th and 24th weeks. Thereupon the mean levels of both tests increased rapidly with age so that relative stability was reached by the second year. From that point the turbidity levels were observed to increase very gradually, and adult levels were reached generally by the seventh to eighth year of life. It was interesting to note that the fluctuation of the mean levels of each test with age followed relatively the same curve. In general, these observations agree with those that have been observed in studies of gamma globulin levels using electrophoresis and quantitative salt precipitation techniques (41, 50, 57, 74).

Due to the small sampling in the various age groups studied, and also to the fact that several of the groups demonstrate a fair amount of skewness, it is invalid to define normal levels soley on the basis of the calculated standard deviation from the mean. It is much more satisfactory to combine the calculated results, using two standard deviations from the mean, with a careful appraisal of the range of results at each age before

reaching any conclusions as to normal levels for each test.
In so doing, we may arbitrarily assign the following normal
limits for each test during childhood:

Age	ZST	GGT
To 6 Mos.	0.5-5.0	0.5-2.5
6 Mos.-1 Yr.	1.5-7.0	0.5-2.5
1 Yr.-6 Yrs.	2.0-9.0	1.0-4.5
6 Yrs.-Adult	2.0-10.0	1.5-5.0

Since there is a tendency for the results of both tests to
be lower in the low normal ranges than the gamma globulin con-
centration would indicate (32), a turbidity level of 1.5 units,
or less, with the zinc turbidity test, or of 0.5 units, or less,
by the gamma globulin turbidity test, should be obtained before
the diagnosis of agammaglobulinemia is seriously considered, and
more time-consuming and expensive tests are employed. As has
been previously noted, it is extremely difficult to diagnose ag-
ammaglobulinemia below six months of age, due to the physiologic
hypogammaglobulinemia at this stage of life (83).

In serving as screening precedures for agammaglobulinemia,
these tests are most effective when used together; in precip-
itating the gamma globulin by different methods, they serve to
complement one another. It is difficult to recommend one test
over the other. However, the gamma globulin turbidity test
seems to have greater consistency of results than its counter-

part. It possesses the disadvantage of having its normal levels fall in a lower spectrum of turbidity units than the zinc turbidity results, thus facilitating the appraisal of low results of the latter test. Although the reagent for the zinc turbidity test is slightly more tedious to prepare, requiring attention to the pH of the solution, the tests are equally simple to perform and require the same amount of time, technique, and equipment. In those patients in whom electrophoresis was performed, the relative turbidity levels correlated well with the electrophoretic measurement of the serum gamma globulin. Patients with diseases known to produce an elevation of the gamma globulin were generally found to have high turbidity levels (Appendix I).

These turbidity tests are superior to filter paper electrophoresis as screening procedures for agammaglobulinemia, not only from the obvious saving in time, expense, and equipment, but also because they offer a more objective delineation of gamma globulin levels. Due to the procedure of the filter paper electrophoretic analysis, there is a definite tendency for a greater percentage of false negative results, whereas the turbidity tests would tend toward false positive results in diagnosing agammaglobulinemia. The latter is certainly the evil to be preferred. The serum isohemagglutinin level and the Schick test have also been used as screening procedures, but possess the disadvantage of measuring only a single antibody component, rather than the gamma globulin fraction.

SUMMARY AND CONCLUSIONS

The primary objective of this study was to evaluate two turbidity tests, the zinc sulphate turbidity test of Kunkel and the gamma globulin turbidity test of De la Huerga and Popper, as screening tests for agammaglobulinemia. Prefacing this project, a review of the literature concerning the nature of gamma globulin, agammaglobulinemia, and the turbidimetric measurement of serum gamma globulin was presented.

The above turbidity tests were applied to sera from 372 children, 306 of whom were in good health. From the results in the latter children, normal levels for the tests were ascertained. It is believed that this is the first time such levels have been determined in childhood for these tests.

As an ancillary study, renal and hepatic function tests together with electrophoresis and an evaluation of antibody status were carried out in several children with low levels and in others with normal levels by the turbidity tests.

It is concluded that these turbidity tests are useful tools in screening patients for the diagnosis of agammaglobulinemia. They seem superior to filter paper electrophoresis, isohemagglutinin titers, or performance of the Schick test in this respect.

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It is fitting at this time to thank all those people whose
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tion of the statistical data.

APPENDIX I

Abbreviations used in subsequent tables:

UNH — Hospital of the University of
    Nebraska College of Medicine
UND — Dispensary of the University of
    Nebraska College of Medicine
CMH — Children's Memorial Hospital
CSI — Child Saving Institute
PP   — Patients referred by private
    physicians

ZST — Zinc Sulphate Turbidity test
GGT — Gamma Globulin Turbidity test

I. Newborn (to one week of age)

Case and Serum No.	Age (days)	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
38.	1	M	UNH	14.0	7.5	
328.	1	M	UNH	5.5	3.2	
329.	1	M	UNH	7.7	4.0	
3.	2	F	UNH	8.2	5.0	
4.	2	M	UNH	11.5	7.7	
13.	2	F	UNH	5.0	2.7	
331.	2	M	UNH	6.5	3.2	
332.	2	M	UNH	9.7	5.7	
334.	2	M	UNH	4.1	2.2	
1.	3	M	UNH	10.0	6.1	
2.	3	F	UNH	10.2	5.5	
10.	3	M	UNH	7.7	4.2	
14.	3	F	UNH	8.5	4.2	
37.	3	F	UNH	9.0	4.2	
39.	3	F	UNH	10.2	3.7	
333.	3	M	UNH	6.7	4.2	
11.	4	F	UNH	12.5	6.2	
12.	7	F	UNH	9.5	4.7	
330.	7	M	UNH	6.0	2.7	

Total Cases - 19
  Range of ZST - 4.1-14.0
  Range of GGT - 2.2-7.7

	ZST	GGT
Mean Levels:	8.55	4.66
Standard Deviation:	2.54	1.53
Standard Error of the Mean:	0.56	0.34

II. To 15 Weeks

Case and Serum No.	Age (wks.)	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
84.	2	F	CSI	4.0	1.0	
131.	4	F	CSI	2.0	1.0	
35.	5	F	UND	3.7	1.2	
58.	7	F	CSI	4.5	1.2	
75.	7	F	CSI	3.7	1.7	
83.	7	F	CSI	4.0	2.0	
261.	7	M	UNH	4.7	2.2	harelip
130.	8	M	CSI	3.8	1.5	
95.	9	F	CSI	2.2	0.5	
54.	11	F	CSI	2.5	0.7	
93.	11	M	CSI	3.2	1.0	
56.	12	F	CSI	5.5	2.5	
60.	12	M	CSI	1.5	1.2	
59.	14	F	CSI	2.5	1.7	
52.	15	F	CSI	2.2	2.0	
53.	15	M	CSI	1.5	0.5	
55.	15	F	CSI	3.7	2.0	
86.	15	M	CSI	1.7	0.7	

Total Cases — 18
  Range of ZST — 1.5-5.5
  Range of GGT — 0.5-2.5

	ZST	GGT
Mean Levels:	3.16	1.37
Standard Deviation:	1.04	0.58
Standard Error of the Mean:	0.26	0.13

71.	4	F	UND	3.7	2.2	pharyngitis
162.	5	F	UNH	1.5	1.2	pneumonia
228.	7	F	UNH	4.5	2.2	diarrhea
73.	8	M	UNH	6.5	2.0	Gaucher's disease
158.	12	F	UNH	6.2	1.2	pneumonia
275.	14	F	UNH	3.5	1.6	acute otitis media

III. To Six Months

Case and Serum No.	Age (wks.)	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
89.	16	M	CSI	3.7	1.5	
110.	16	F	UND	2.0	1.0	
118.	16	M	UND	0.7	0.7	
344.	16	F	UNH	2.0	1.1	clubfoot
88.	17	F	CSI	2.5	2.0	
85.	18	M	CSI	1.7	1.5	
51.	19	M	CSI	2.5	1.7	
87.	19	M	CSI	5.0	1.7	
90.	22	M	CSI	3.5	1.2	
230.	22	M	UND	1.7	1.0	
94.	23	F	CSI	4.0	1.0	

Total Cases - 11
  Range of ZST - 0.7-5.0
  Range of GGT - 0.7-2.0

	ZST	GGT
Mean Levels:	2.66	1.31
Standard Deviation	1.20	0.37
Standard Error of the Mean:	0.36	0.11

IV. To One Year

Case and Serum No.	Age (mos.)	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
77.	6	F	CSI	4.2	1.7	
91.	6	F	CSI	5.5	1.5	
92.	6	M	CSI	3.7	0.7	
96.	6	F	CSI	4.5	1.5	
260.	6	F	UNH	3.7	1.6	hemangioma
302.	7	F	UND	3.7	2.1	
366.	7	F	UND	2.2	1.1	
42.	9	F	UND	7.2	2.2	
70.	9	F	UND	5.5	2.2	
121.	9	F	CSI	3.5	1.2	
148.	9	M	UND	6.0	2.7	
251.	9	M	UND	4.7	2.2	
303.	10	M	UND	1.6	1.1	
76.	11	F	CSI	3.5	0.5	
122.	11	M	CSI	2.7	1.5	

Total Cases — 15
   Range of ZST — 1.6-7.2
   Range of GGT — 0.5-2.7

	ZST	GGT
Mean Levels:	4.15	1.59
Standard Deviation:	1.42	0.58
Standard Error of the Mean:	0.36	0.15

8.	9	F	UNH	27.2	12.5	mucoviscidosis with pulmonary complications

V. To Two Years

Case and Serum No.	Age (mos.)	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
79.	12	F	CSI	7.0	3.7	
109.	12	F	UND	4.7	1.5	
123.	12	M	CSI	3.0	1.0	
124.	12	F	CSI	4.2	1.2	
229.	12	F	UNH	5.2	2.0	cerebral concussion
288.	13	M	UND	2.6	1.1	
293.	13	F	UND	7.7	2.2	
297.	13	F	UND	6.5	2.5	
343.	13	M	UND	3.7	2.0	
145.	15	M	UND	5.7	3.0	
257.	15	F	UND	10.0	4.0	iron-defic. anemia
82.	16	M	CSI	11.5	3.2	
125.	16	M	CSI	6.5	2.2	
151.	16	M	UND	4.2	1.2	
210.	18	M	UNH	3.7	1.5	inguinal hernia
211.	18	F	UND	7.2	2.7	
356.	18	F	UND	6.5	3.5	
358.	18	M	UND	6.0	2.6	
78.	19	F	CSI	6.0	1.7	
80.	19	M	CSI	6.2	3.0	
150.	19	F	UND	3.0	1.1	
167.	19	F	UND	4.2	3.2	
231.	19	F	UND	4.0	1.5	
245.	19	F	UND	7.7	3.1	
355.	20	M	UND	9.0	4.2	
134.	21	M	UND	3.2	1.2	
81.	22	F	CSI	8.0	2.6	
105.	22	M	UND	4.5	1.2	
141.	22	M	UND	5.7	2.7	

Total Cases — 29
    Range of ZST — 2.6—11.5
    Range of GGT — 1.0—4.2

				ZST	GGT	
Mean Levels:				5.77	2.30	
Standard Deviation:				2.15	0.93	
Standard Error of the Mean:				0.40	0.17	

27.	18	F	UNH	10.5	2.2	nasopharyngitis
165.	18	M	UND	7.0	3.7	nasopharyngitis
198.	19	M	UND	2.2	1.2	pertussis
350.	14	F	UND	4.2	2.0	pertussis
357.	18	M	UNH	8.7	5.7	foot abscess
367.	20	M	CMH	3.7	2.1	furunculosis

VI. To Three Years

Case and Serum No.	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
40.	M	UND	6.7	2.5	
44.	F	UND	6.5	1.7	
47.	M	UND	11.7	3.7	
104.	M	UND	5.5	2.0	
116.	F	UND	5.0	2.2	
119.	M	UND	4.0	1.7	
142.	F	UND	4.0	2.2	
152.	M	UNH	2.7	1.0	medulloblastoma
156.	F	UNH	6.7	3.0	pre-auricular sinus
202.	M	UND	4.0	2.0	
209.	M	UND	4.0	1.5	
254.	M	UND	5.0	2.6	
259.	M	UND	4.7	2.1	
273.	F	UND	5.5	2.7	
284.	F	UNH	4.2	2.0	strabismus
292.	M	UND	3.1	1.2	

Total Cases - 16
  Range of ZST - 2.7-11.7
  Range of GGT - 1.0-3.7

	ZST	GGT
Mean Levels:	5.21	2.13
Standard Deviation:	2.03	0.66
Standard Error of the Mean:	0.51	0.16

160.	F	UND	2.2	1.0	pustular scalp infection

VII. To Four Years

Case and Serum No.	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
24.	M	UND	7.7	3.0	
43.	F	UND	7.5	3.2	
107.	F	UND	7.7	3.5	
115.	F	UND	6.5	2.2	
127.	M	CSI	6.2	3.2	
128.	M	CSI	5.0	2.0	
129.	F	CSI	7.2	3.0	
140.	F	UND	4.5	2.5	
143.	F	UND	6.5	2.7	
166.	M	UND	6.2	4.0	
188.	F	UND	3.2	1.7	
193.	F	UND	3.0	1.5	
194.	M	UND	5.2	2.0	
212.	F	UND	7.7	3.0	
221.	F	UND	5.7	3.2	
226.	F	UND	5.0	2.2	
237.	M	UNH	5.0	2.0	simple fracture
253.	F	UND	6.2	2.8	
270.	M	UND	7.2	3.0	
291.	M	UND	4.0	2.6	
311.	F	UND	3.7	1.7	
316.	M	UND	5.5	3.0	
365.	M	UND	3.7	1.7	
368.	M	PP	3.5	1.6	

Total Cases - 24
   Range of ZST - 3.0-7.7
   Range of GGT - 1.5-4.0

	ZST	GGT
Mean Levels:	5.57	2.55
Standard Deviation:	1.48	0.68
Standard Error of the Mean:	0.30	0.14

26.	F	UNH	10.2	4.2	suppurative arthritis
46.	F	UND	12.2	6.7	inf. mononucleosis
48.	F	UND	10.7	5.0	nasopharyngitis
50.	F	UND	9.0	5.7	tonsillitis
174.	M	UNH	5.5	3.0	viral pneumonia

VIII. To Five Years

Case and Serum No.	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
36.	F	UNH	3.7	1.5	strabismus
41.	M	UND	8.5	2.6	
97.	F	UND	13.2	4.0	
103.	M	UND	9.0	4.0	
126.	M	CSI	10.5	4.5	
139.	F	UND	6.5	2.5	
144.	F	UND	8.7	4.5	
153.	M	UND	2.7	1.5	
176.	M	UND	4.0	1.5	
191.	F	UND	4.2	2.2	
232.	F	UND	2.7	2.7	
234.	F	UND	5.5	2.7	
240.	M	UND	3.7	1.2	
246.	F	UND	4.7	1.7	
255.	F	UND	2.2	1.2	
277.	F	UND	5.5	2.2	
324.	M	UND	2.7	1.1	urticaria
325.	F	UND	5.7	3.0	
335.	F	UND	7.7	3.2	
364.	M	UND	4.2	1.6	
371.	M	UND	5.7	2.7	

Total Cases - 21
  Range of ZST - 2.2-13.2
  Range of GGT - 1.1-4.5

	ZST	GGT
Mean Levels:	5.78	2.48
Standard Deviation:	2.81	1.06
Standard Error of the Mean:	0.62	0.23

Case and Serum No.	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
6.	M	UNH	12.0	7.5	inf. mononucleosis
137.	F	UND	11.5	6.0	nasopharyngitis
307.	M	UND	12.5	5.7	inf. mononucleosis
339.	M	UND	12.5	5.7	inf. mononucleosis
351.	M	CMH	0.7	0.5	nephrosis - cellulitis

IX. To Six Years

Case and Serum No.	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
25.	M	UNH	1.1	0.5	speech defect
29.	F	UNH	5.5	2.2	appendectomy
30.	F	UNH	4.5	2.5	strabismus
67.	M	UND	4.2	2.2	
69.	M	UND	7.0	2.2	
108.	F	UND	7.7	2.2	
113.	F	UND	6.5	2.5	
114.	M	UND	4.0	1.5	
132.	F	UND	4.0	2.0	
149.	F	UNH	1.0	0.7	congenital ptosis
155.	M	UND	5.7	3.7	
161.	F	UND	3.5	1.5	
163.	F	UND	7.5	3.5	
164.	M	UND	3.5	2.0	
170.	F	UND	5.0	3.2	
171.	F	UND	3.5	1.7	
172.	F	UND	6.0	3.2	
175.	F	UND	4.5	2.5	
177.	F	UND	5.5	3.2	
178.	M	UND	4.5	2.5	
183.	M	PP	3.5	2.0	
185.	M	UND	4.7	4.0	
186.	M	UND	5.2	2.0	
189.	M	UND	5.7	1.7	
190.	M	UND	5.0	2.5	
192.	M	UND	4.5	3.0	
197.	F	UND	5.0	2.5	
200.	M	UNH	5.5	2.6	petit mal epilepsy
201.	F	UND	6.0	2.2	
213.	M	UND	4.7	2.1	
214.	M	UND	5.5	2.6	
215.	F	UND	5.5	2.7	
217.	F	UND	4.0	2.1	
219.	F	UND	5.0	3.5	
220.	M	UND	5.2	2.5	
225.	F	UND	6.2	2.2	
136.	M	UNH	9.0	3.5	inguinal hernia
238.	F	UND	7.0	2.7	
239.	M	UND	5.2	2.0	
241.	M	UND	8.0	3.2	
242.	F	UND	3.7	1.2	
243.	M	UND	5.7	3.2	
269.	F	UND	7.7	3.0	

Case and Serum No.	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
274.	F	UND	4.0	1.1	
278.	M	UND	2.8	1.3	
279.	M	UND	6.2	2.7	
281.	F	UND	4.0	2.2	
282.	F	UND	4.0	2.0	
283.	F	UND	5.5	2.2	
284.	M	UND	5.7	3.7	
295.	M	UND	8.5	4.5	
296.	F	UND	7.5	3.2	
298.	M	UND	4.7	1.6	
300.	F	PP	7.5	3.5	
312.	F	UND	4.7	2.7	
313.	F	UND	4.5	1.8	
317.	F	UND	7.5	3.7	
318.	F	UND	7.5	2.7	
320.	M	UND	5.7	2.2	
321.	M	UND	6.0	2.2	
322.	F	UND	4.7	2.2	
340.	M	UND	4.0	2.7	
346.	F	UND	5.2	2.7	
347.	M	UND	6.5	2.2	
348.	M	UND	5.2	2.6	
349.	M	UND	2.7	1.5	
369.	M	UND	9.2	4.2	
372.	M	UND	4.1	2.2	
373.	F	UND	7.5	4.0	

Total Cases - 69
  Range of ZST - 1.0-9.2
  Range of GGT - 0.5-4.5

			ZST	GGT
Mean Levels:			5.33	2.49
Standard Deviation:			1.63	0.81
Standard Error of the Mean:			0.19	0.09

Case	Sex	Source	ZST	GGT	Health Status
23.	M	UND	8.2	4.7	acute otitis media
49.	F	UND	10.7	4.7	tonsillitis
74.	M	UNH	10.2	5.7	meningitis
138.	F	UND	19.7	8.2	acute otitis media
218.	F	UND	10.7	4.7	tonsillitis
271.	F	UND	9.0	3.2	pustular skin rash
352.	M	CMH	0.7	0.5	nephrosis-otitis media
361.	M	UND	8.5	4.7	muscular dystrophy
363.	F	UND	9.7	4.2	nasopharyngitis

X. To Seven Years

Case and Serum No.	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
66.	M	UND	6.0	3.0	
72.	M	UNH	6.0	2.7	hydrocele
98.	F	UNH	6.0	1.7	strabismus
106.	M	UND	7.7	3.3	
111.	F	UND	6.2	2.5	
120.	M	UND	9.5	3.0	
159.	F	UND	3.5	2.2	
236.	F	UND	7.0	3.0	
249.	M	UND	5.5	2.6	
272.	M	UND	9.0	3.7	
285.	F	UND	7.0	3.5	
305.	F	UND	5.5	2.2	
337.	F	UND	4.5	3.0	
338.	M	UND	6.7	3.2	
370.	M	UND	5.5	2.7	

Total Cases - 15
   Range of ZST - 3.5-9.5
   Range of GGT - 1.7-3.7

	ZST	GGT
Mean Levels:	6.37	2.82
Standard Deviation:	1.52	0.51
Standard Error of the Mean:	0.39	0.13

102.	M	UND	11.5	4.7	inf. mononucleosis
184.	M	UND	8.0	4.0	nasopharyngitis
223.	F	UNH	9.7	4.5	submental absoess
233.	F	UND	8.5	4.5	heel abscess
247.	M	UNH	9.5	4.2	suppurative arthritis
248.	F	UND	10.5	7.5	pustular skin rash
258.	M	UND	7.0	4.7	acute bronchitis
354.	M	UND	8.5	4.1	nasopharyngitis

XI. To Eight Years

Case and Serum No.	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
22.	F	UND	10.7	3.0	
62.	M	UND	10.5	3.2	
64.	F	UND	10.5	3.7	
65.	F	UND	5.7	3.2	
100.	M	UND	7.2	2.7	
117.	F	UND	4.7	2.2	
169.	F	UND	5.5	2.7	
179.	M	UND	6.7	3.0	
206.	F	UND	2.7	1.1	
216.	F	UND	8.7	4.7	
244.	F	UND	4.5	2.2	
256.	F	UND	6.2	3.5	
287.	F	UNH	5.2	2.7	skin graft
294.	M	UND	5.0	2.2	
299.	M	UND	9.0	4.5	
308.	F	UND	7.5	2.7	
315.	M	UND	7.0	3.5	
326.	F	UND	7.5	3.7	
341.	M	UND	4.2	2.0	

Total Cases — 19
  Range of ZST — 2.7-10.7
  Range of GGT — 1.1-4.7

	ZST	GGT
Mean Levels:	6.78	2.98
Standard Deviation:	2.30	0.88
Standard Error of the Mean:	0.53	0.20

19.	F	UND	11.5	5.7	inf. mononucleosis
206.	F	UNH	4.7	2.1	furuncle of scalp
276.	F	UND	7.0	4.2	infected cut
301.	M	UNH	12.5	4.7	nasopharyngitis
323.	F	UNH	11.5	5.7	acute rheumatic fever

XII. To Nine Years

Case and Serum No.	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
21.	M	UND	8.7	2.7	
28.	F	UNH	5.2	3.0	keloids
99.	M	UND	7.5	2.7	
101.	F	UND	7.0	2.7	
154.	F	UND	6.5	2.2	
173.	M	UND	5.2	2.5	
227.	M	UND	6.7	2.8	
250.	F	UND	4.5	1.6	
290.	M	UND	5.2	3.5	
306.	M	UND	9.2	2.6	
310.	F	UND	4.5	2.2	
314.	F	UND	7.1	3.5	
336.	F	UND	5.7	3.2	
342.	F	UND	6.0	3.2	
345.	F	UND	8.2	4.2	
360.	M	UND	6.5	2.2	

Total Cases — 16
  Range of ZST — 4.5-9.2
  Range of GGT — 1.6-4.2

	ZST	GGT
Mean Levels:	6.42	2.80
Standard Deviation:	1.40	0.61
Standard Error of the Mean:	0.35	0.15

112.	M	UND	11.7	5.5	tonsillitis
208.	F	UND	9.7	4.5	infectious hepatitis
262.	F	UNH	4.7	3.7	pyelonephritis
286.	F	UND	6.2	4.7	infectious hepatitis

XIII. To Ten Years

Case and Serum No.	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
16.	F	UND	8.2	3.0	
168.	F	UND	4.7	3.2	
182.	F	PP	7.0	3.5	
199.	M	UNH	5.2	2.6	petit mal epilepsy
203.	M	UND	2.7	1.7	
224.	F	UND	11.5	4.0	
304.	M	UND	7.7	3.5	
327.	F	UND	4.2	2.7	

Total Cases - 8
   Range of ZST - 2.7-11.5
   Range of GGT - 1.7-4.0

	ZST	GGT
Mean Levels:	6.40	3.40
Standard Deviations:	1.89	0.71
Standard Error of the Means:	0.65	0.25

7.	F	UNH	8.5	5.0	tonsillitis
20.	M	UND	18.5	10.7	tonsillitis
235.	F	UND	9.0	4.0	pyelonephritis
319.	F	UND	13.7	4.7	acute glomerulonephritis

Case and Serum No.	Age (yrs.)	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
5.	10	M	UND	6.0	3.8	
57.	10	M	UND	12.0	5.0	
63.	10	M	UND	6.2	2.5	
180.	10	M	UND	5.2	3.7	
187.	10	M	UND	7.2	4.2	
263.	10	M	UNH	7.0	3.7	mental deficiency
268.	10	M	UND	4.2	2.7	
18.	11	M	UND	7.0	2.7	
133.	11	M	UND	6.5	4.0	
207.	11	M	UNH	6.5	3.7	cerebral concussion
252.	11	F	UND	7.0	4.2	
280.	11	F	UND	7.0	3.5	
309.	11	M	UND	8.0	3.1	
17.	12	M	UND	7.5	3.0	
32.	12	F	UND	10.0	3.5	
33.	12	F	UND	7.2	3.7	
34.	12	F	UND	5.0	2.5	
157.	12	M	UNH	2.2	2.5	megacolon
204.	12	F	UND	7.1	3.2	
205.	12	F	UND	7.0	3.7	
266.	12	M	UND	3.5	2.5	
267.	12	F	UND	7.5	3.7	
45.	14	M	UND	5.2	2.5	
359.	14	M	UND	5.0	2.7	
147.	15	M	UND	6.5	4.0	
146.	17	M	UND	7.7	4.2	

Total Cases - 26
  Range of ZST - 2.2-12.0
  Range of GGT - 2.5-5.0

	ZST	GGT
Mean Levels:	6.58	3.40
Standard Deviation:	1.89	0.71
Standard Error of the Mean:	0.37	0.14

4.	12	M	UNH	20.0	11.0	cellulitis of foot
15.	12	F	UND	8.0	4.2	acute sinusitis
31.	10	F	UND	10.7	5.2	lactobacillus vaginal discharge
61.	12	M	UND	26.7	10.2	adrenal dysfunction-- hypertension, gyneco- mastia

XIV. Over Ten Years (cont.)

| Case and Serum No.	Age (yrs.)	Sex	Source	ZST (units)	GGT (units)	Health Status (if other than normal)
68.	11	M	UNH	7.7	4.5	acute rheumatic fever
135.	13	F	UND	7.5	4.5	acute otitis media
181.	11	F	CMH	6.5	3.2	ulcerative colitis with carcinoma of the colon
195.	11	M	UND	16.5	8.2	acute otitis media
222.	11	M	UNH	7.7	5.2	acute rheumatic fever
265.	13	M	UND	9.7	4.2	gastroenteritis
353.	10	F	UND	14.2	5.2	inf. mononucleosis
362.	12	F	UND	9.7	6.0	pyelonephritis

APPENDIX II

D. SMITH (Normal) 9-1-55
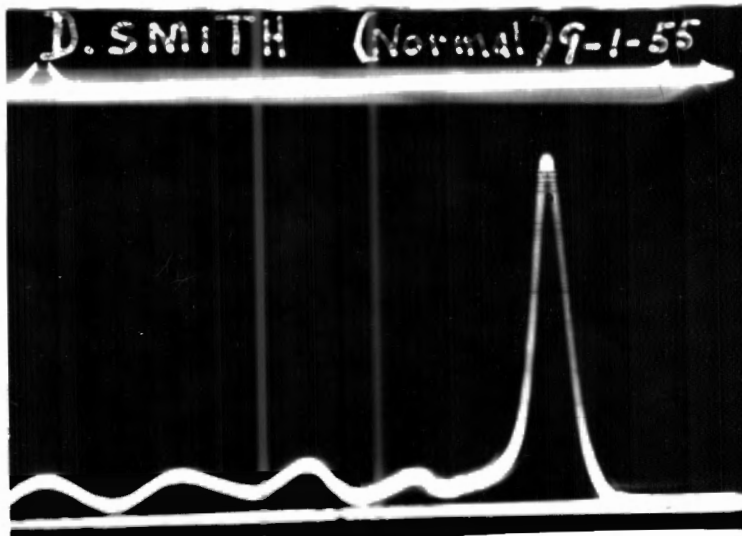
Case #263   Age: 10 years   Health Status: Normal

Total Serum Proteins - 7.1 gm. per 100 cc.
Zinc Sulphate Turbidity - 7.0 units
Gamma Globulin Turbidity - 3.7 units

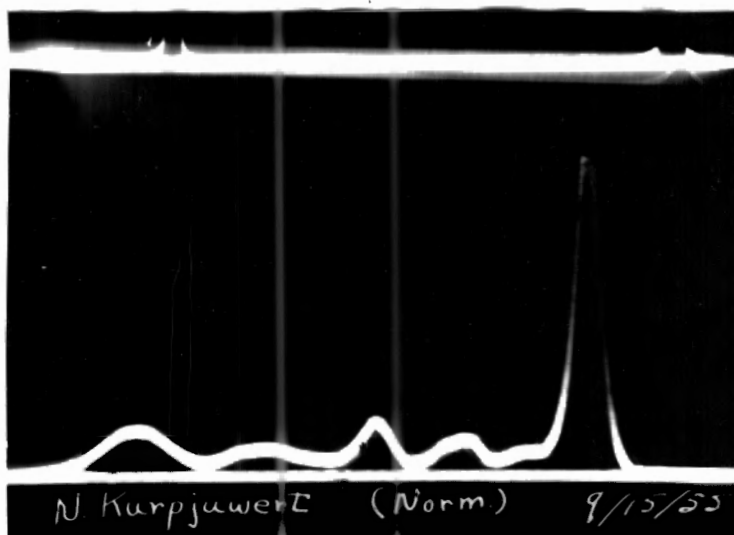Calculated Protein Fractions from Free Electrophoretic Pattern (% TSP)

alb - 58.5
α_1 - 4.5
α_2 - 9.5
β - 13.0
γ - 14.5

Averett (Norm) 8/12/55
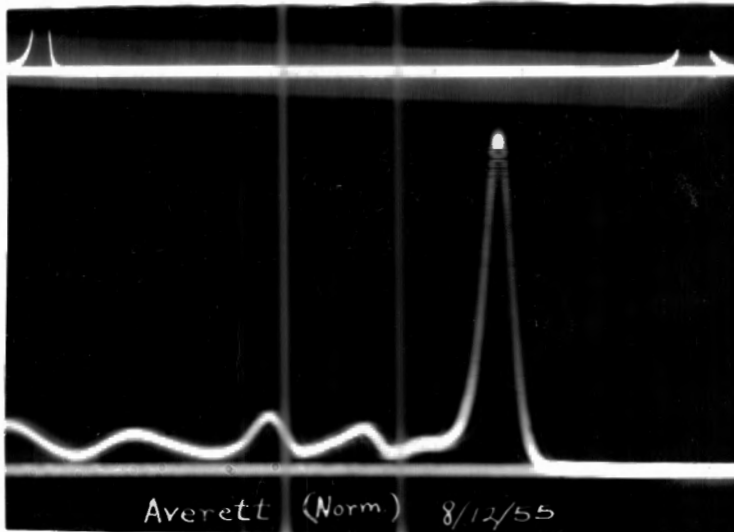
Case #207   Age: 11 years   Health Status: Cerebral Concussion

              Total Serum Proteins - 8.5 gm. per 100 cc.
              Zinc Sulphate Turbidity - 6.5 units
              Gamma Globulin Turbidity - 3.7 units

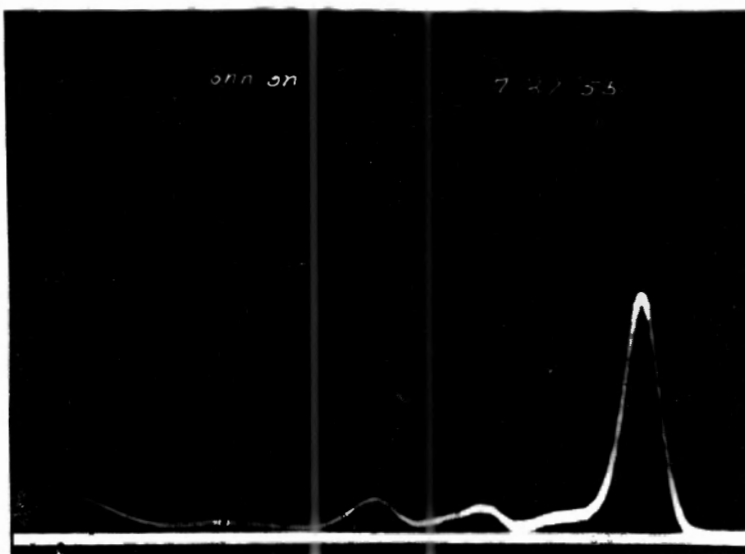Calculated Protein Fractions from Free Electrophoretic Pattern (% TSP)

                    alb - 57.5
                    α_1 - 4.9
                    α_2 - 11.1
                    β - 11.5
                    γ - 14.8

N Kurpjuwert (Norm.) 9/15/55

Case #199   Age: 9 years   Health Status: Petit Mal Epilepsy

Total Serum Proteins - 7.8 gm. per 100 cc.
Zinc Sulphate Turbidity - 5.2 units
Gamma Globulin Turbidity - 2.6 units

Calculated Protein Fractions from Free Electrophoretic Pattern (% TSP)

alb - 55.5
α_1 - 7.7
α_2 - 11.4
β - 13.0
γ - 12.5

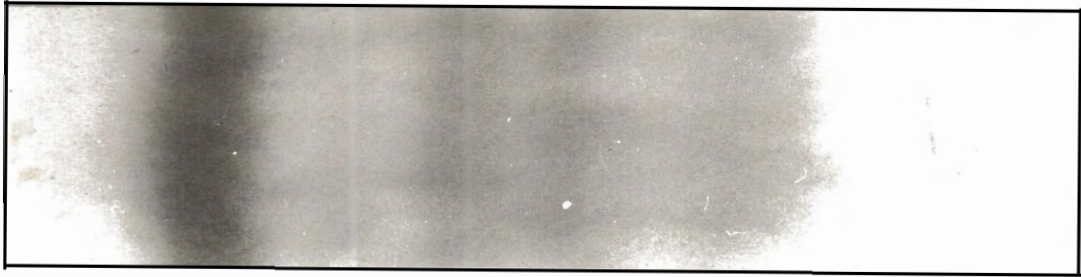Case #149   Age: 5 years   Health Status: Normal except for
                                                Congenital Ptosis

Total Serum Proteins - 6.6 gm. per 100 cc.
Zinc Sulphate Turbidity - 1.0 units
Gamma Globulin Turbidity - 0.7 unit

Calculated Protein Fractions from Free Electrophoretic Pattern (% TSP)

alb - 62.8
α_1 - 5.1
α_2 - 11.2
β - 13.0
γ - 7.9

Case #25   Age: 5 years   Health Status: Normal except for
                                                   Speech defect

                Total Serum Proteins – 6.9 gm. per 100 cc.
                Zinc Sulphate Turbidity – 1.1 units
                Gamma Globulin Turbidity – 0.5 unit

Calculated Protein Fractions from Free Electrophoretic Pattern (% TSP)

                        alb – 65.6
                        α_1 – 4.2
                        α_2 – 9.9
                        β – 11.5
                        γ – 8.9

Case #28   Age: 8 years   Health Status: Normal

Total Serum Proteins - 7.3 gm. per 100 cc.
Albumin - 4.3 gm. per 100 cc.
Globulin - 3.0 gm. per 100 cc.
Zinc Sulphate Turbidity - 5.2 units Gamma
Globulin Turbidity - 3.0 units
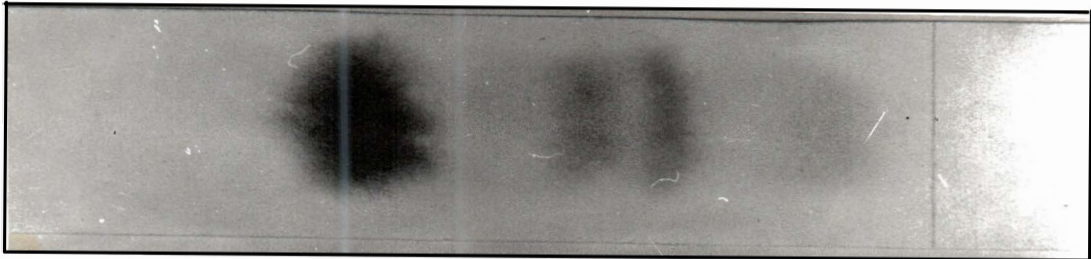
Case # 160   Age: 2 years   Health Status: Pyogenic
.Scalp Infection

Zinc Sulphate Turbidity - 2.2 units

Gamma Globulin Turbidit - 1.0 units

Case #351   Age: 4 years   Health Status: Nephrosis

Total Serum Proteins - 3.1 gm. per 100 cc.
Albumin - 0.8 gm. per 100 cc.
Globulin - 2.3 gm. per 100 cc.
Zinc Sulphate Turbidity - 0.75 units Gamma
Globulin Turbidity - 0.50 units

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