Serum Protein Changes in Malignant Diseases.
I. The Acute Leukemias

By John L. Fahey and Dane R. Boggs

Among the profound effects of malignant neoplasia are changes of serum protein components, but the significance of these changes is poorly understood. The acute leukemias were selected for further study because the rapid course and the ability to induce complete remission in the disease seemed to offer opportunity for detailed observations. To delineate the effects due solely to acute leukemia in the 110 patients examined, a separate evaluation was made of lymphoblastic and myeloblastic forms, of uncomplicated leukemia and the effects of complications such as infection and hepatic disease, and of the effects of therapeutic agents. The value of certain of these distinctions has been noted in several reports. It was with particular interest that we observed differences between the serum protein patterns of uncomplicated cases of acute myeloblastic and lymphoblastic leukemia.

Materials and Methods

Serum protein analyses: Serum electrophoretic analyses were performed on 171 samples from 82 patients with acute lymphoblastic leukemia and 64 samples from 28 patients with acute myeloblastic leukemia. Twenty-two analyses in lymphoblastic leukemia and 15 in myeloblastic leukemia represent a single determination per patient. The rest of the analyses were determinations obtained at intervals of four weeks or more from the remaining patients.

Blood was obtained in the fasting state, allowed to clot for several hours at room temperature, and the serum separated and frozen for future analysis. Many sera were analyzed at the time of receipt, but in each patient with two or more samples, the analyses on all sera were performed together at a later date.

Total serum protein determinations were made by the biuret technic. Two paper electrophoretic procedures were employed during the course of these studies. The method initially used has been described in detail elsewhere and the leukemic sera analyzed by this method were compared with normal values obtained in 44 samples of serum from normal young adults, equally divided as to sex, age 18 to 35, in good health and without history of hepatic disease. Subsequently a modified procedure was employed differing from the first in the use of S&S-2043A paper strips, alcoholic bromphenol blue staining procedures and alcoholic rinsing steps. The mean normal values plus the standard deviation for this procedure which is now in use in this laboratory were based on analyses of serum from 20 young adults, selected as above, and are given in table 1.
Each electrophoretic component was first calculated in terms of the serum concentration in grams per cent. Subsequently this value was compared with the mean normal value for that component and analytic procedure, and the value was finally expressed as the percentage of the mean normal value. This has the advantage of making data directly comparable to other data obtained by procedures having different normal values. By this method of recording, an albumin level of 3.00 grams per cent would be (3.00 - 4.16) 72 per cent, and a gamma globulin concentration of 3.00 grams per cent would be (3.00 1.17) 256 per cent.

Several studies have shown that the values for the serum electrophoretic components, including the gamma globulins, are approximately the same in children 5 years of age and older as in normal young adults. Thus we have considered the young adult range of normal values employed here to be applicable to all of our disease categories. Only 10 patients with lymphoblastic leukemia representing 26 analyses were under 5 years of age.

Clinical Appraisal: Clinical evaluation of each patient for each serum sample date was done without knowledge of the serum protein findings. Morphologic classification was made by standard criteria.* Initial morphologic distinctions between acute lymphoblastic and acute myeloblastic leukemia were difficult in certain instances, but repeated examination yielded a specific diagnosis. Some cases of myeloblastic leukemia could be considered myelomonocytic leukemia, but no cases of monocytic (histiocytic?) leukemia were included.

The effect of age on the serum protein changes in acute lymphoblastic leukemia was evaluated by a comparison of data from 58 patients with lymphoblastic leukemia under age 15 and from 24 patients age 15 or over. No difference was found and observations in children and adults are considered together throughout this report. Only three patients with acute myeloblastic leukemia were under age 15 and their protein changes were similar to those in adults with myeloblastic leukemia.

Disease activity was graded as complete remission, partial remission, and active disease, according to published criteria. The active disease group was arbitrarily subdivided into “active” and “very active” disease, the latter group characterized by a platelet count of less than 30,000 per mm.3 and an absolute granulocyte count of less than 200 per mm.3 The total leukocyte count was recorded as were the number of transfusions given during the week prior to obtaining the sample. The type and duration of therapy were noted.

Fever of undetermined etiology was defined as that occurring in the absence of demonstrable infection. Infection serious enough to be associated with fever was noted. Hepatic disease was considered to be present if the total serum bilirubin exceeded 1 mg. per cent or bromsulphalein retention at 45 minutes exceeded 5 per cent. These tests constituted the primary criteria of liver disease, for hepatic size may be altered in acute leukemia without evidence of functional deficit. The colloidal tests were considered to be unreliable indices of hepatic damage in these diseases because of their dependence on the balance of serum protein components. Although bilirubin elevations in the acute leukemias may occur secondary to hemolytic processes, elevations on this basis were rarely encountered in the absence of impaired liver function.

Statistical Methods: Two forms of comparison were used. In order to determine the significance of the number of determinations falling outside two standard deviations from the normal mean binomial confidence limits were applied. Chi square testing was done by comparing two groups of analyses and using the number of observations in each group above and below the combined mean of the two groups. The latter method has the disadvantage of giving equal importance to both mild and marked alterations. This, however, probably adds to the validity of positive results. The two methods agreed in all instances where both were applicable.

*We are indebted to Dr. George Brecher of the Clinical Pathology Service, Clinical Center, NIH, for examining the bone marrow material from these patients and helping to establish the morphologic diagnoses.
RESULTS

1. Uncomplicated Acute Leukemia

Significant abnormalities of the serum proteins remained when all samples obtained during fever, infection or liver disease were omitted. The results of 144 analyses in uncomplicated acute leukemia are presented graphically in figure 1. Median values and the range of values obtained for each electrophoretic component in uncomplicated acute myeloblastic and acute lymphoblastic leukemia are given in table 1.

Low serum albumin levels can be caused by myeloblastic or lymphoblastic leukemic processes. Complications such as infection or hepatic disease, while capable of causing further lowering of the serum albumin could not be considered primarily responsible for the low albumin values.

Alpha-1 globulins were most notable for the wide range of values encountered in both diseases (fig. 1). The median level, however, was not significantly altered. Values in myeloblastic leukemia ranged from 20 to 245 per cent of normal, and in lymphoblastic leukemia from 75 to 195 per cent. This range of alpha-1 globulin levels probably reflects differing changes in the many individual components which together comprise the alpha-1 globulin group. Variability of the alpha-1 globulin levels contrasted with the much smaller range of values obtained with the single protein, albumin.

Alpha-2 globulin levels were more often elevated in lymphoblastic leukemia (median value = 120 per cent of normal mean, p < 0.01) than in myeloblastic leukemia (mean value = 85 per cent). This electrophoretic category, a multicomponent group, was also quite variable, and the considerations noted above for alpha-1 globulins, also apply to the alpha-2 globulin group.

Beta globulin levels did not differ significantly from normal in either of the acute leukemias.

Gamma globulin levels were strikingly elevated in myeloblastic leukemia (median value = 140 per cent of normal). Values greater than two standard deviations above the normal mean were obtained in 43 per cent of the determinations. Review of clinical data indicated that this elevation usually occurred independently of preceding infection or hepatic disease. The median

<table>
<thead>
<tr>
<th>Normal Values (Gm. %)</th>
<th>Acute Lymphoblastic Leukemia</th>
<th>Acute Myeloblastic Leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ±1 S.D.</td>
<td>Median</td>
<td>Range of Values</td>
</tr>
<tr>
<td>Total Protein</td>
<td>7.1 (0.62)</td>
<td>80</td>
</tr>
<tr>
<td>Albumin</td>
<td>4.16 (0.32)</td>
<td>80*</td>
</tr>
<tr>
<td>Alpha-1</td>
<td>30 (0.05)</td>
<td>105</td>
</tr>
<tr>
<td>Alpha-2</td>
<td>6.8 (0.10)</td>
<td>120*</td>
</tr>
<tr>
<td>Beta</td>
<td>7.7 (0.10)</td>
<td>100</td>
</tr>
<tr>
<td>Gamma</td>
<td>1.17 (0.20)</td>
<td>90</td>
</tr>
</tbody>
</table>

*Statistically significant change by chi square test (see text).
gamma globulin level in myeloblastic leukemia was found to be significantly elevated above normal ($p < 0.01$).

Gamma globulin levels in lymphoblastic leukemia, on the other hand, were usually normal or low. The median value for all uncomplicated analyses was 90 per cent of the normal mean. Patients who had no bacterial infection from the onset of acute lymphoblastic leukemia had significantly lower gamma globulin values than those patients with preceding infection ($p < 0.05$), and the mean gamma globulin level of the former group was 85 per cent of the normal while that of the latter was 105 per cent.

2. Disease Activity

Clinical and hematologic remission in acute lymphoblastic leukemia was frequently associated with a persistent alpha-2 globulin elevation and albumin depression. Although serial observations in 12 patients before and after hematologic improvement indicated that albumin and alpha globulin values tended to return toward normal with remission (table 2), the electrophoretic patterns during remission could not be shown statistically to differ from those of active lymphoblastic leukemia (fig. 2). The low remission rate in myeloblastic leukemia prevented meaningful comparisons in this disease.
Fig. 2.—Serum electrophoretic component values in acute lymphoblastic leukemia during active, uncomplicated disease and during hematologic remission. The data are plotted as in figure 1.

The level of circulating leukocytes in acute leukemia may not correlate with other parameters of disease activity and it was of interest to determine if electrophoretic results related in any way to this aspect of the disease. Comparison of electrophoretic values with leukocyte counts of (a) less than 5,000, (b) 5,000 to 10,000, (c) 10,000 to 50,000, and (d) greater than 50,000 failed to indicate any correlation between the level of circulating leukocytes and the serum electrophoretic components.

3. Fever and Infection

Fever, in the absence of demonstrable bacterial infection, is a frequent finding in the acute leukemias. Analysis of our data revealed that alpha-1 globulin elevation occurred with greater frequency (p = <0.05) in this group of febrile patients than in afebrile patients with otherwise equivalent leukemic disease.

Table 2.—Serum Protein Changes with Development of Hematologic Remission in Acute Lymphoblastic Leukemia

<table>
<thead>
<tr>
<th>Direction of change with remission* (12 patients)</th>
<th>Toward Normal</th>
<th>No Change</th>
<th>More Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>5</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Alpha-1 globulin</td>
<td>8</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Alpha-2 globulin</td>
<td>8</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Beta globulin</td>
<td>6</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Gamma globulin</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

*Change was considered to have occurred when the remission value differed by more than 10 per cent from the pre-remission value.
activity (fig. 3). Patients with myeloblastic and lymphoblastic leukemia experienced similar changes.

Bacterial infection and fever were associated with a similar rise of alpha-1 globulins (p < 0.001) and further decrease in albumin (p < 0.05) in both myeloblastic and lymphoblastic leukemia (fig. 3). No changes were noted in the alpha-2 globulins, beta globulins or gamma globulins. These infections were largely of an acute nature.

Profound depression of the gamma globulins was unusual in lymphoblastic leukemia, only 5 per cent of values were more than two standard deviations below the normal mean. The frequency of infection did not correlate with low gamma globulin levels. Evaluation of gamma globulin levels in children with lymphoblastic leukemia and no preceding bacterial infection revealed no statistically significant difference between those who subsequently did and did not develop infection. A rise in gamma globulin level was often noted following bacterial infection.

4. Therapeutic Agents

No significant change in the electrophoretic components could be attributed to therapy. Fifty-four analyses were performed during antimetabolite therapy (6-mercaptopurine, methotrexate and, rarely, 5-fluorouracil or 6-azauracil), 30 analyses during adrenocorticosteroid administration, and 56 analyses of samples from the same or similar patients obtained prior to therapy, or after

Fig. 3.—Serum electrophoretic changes associated with fever and with fever and infection in acute lymphoblastic leukemia. The data are plotted as in figure 1.
therapeutic attempts had been exhausted. Comparison of protein values in patients who had received no therapy, therapy for less than three weeks or therapy for more than three weeks, and examination of serial samples from individual patients during therapy failed to reveal changes attributable directly to the therapeutic agents.

**DISCUSSION**

Although acute myeloblastic and lymphoblastic leukemia are often distinguishable on the basis of morphology, characteristic age of onset, response to therapy, etc., a distinction between serum protein changes in the two diseases is not generally made. In several studies the number of individual cases of acute leukemia was small or the studies were concerned with only one form of leukemia but a compilation and comparison of data from previous reports (table 3) was found to agree rather well with our findings. Wall examined 110 cases of acute leukemia and emphasized the hypogammaglobulinemic tendency in acute lymphoblastic leukemia and also noted that gamma globulins may be diffusely increased in acute myeloblastic leukemia.4

The significant increase of total alpha-2 globulin level in lymphoblastic leukemia emphasizes that changes within this interesting group of proteins can be caused solely by the leukemic process, and the wide range of alpha-1 globulin values indicates profound alteration of individual proteins within this group. Detailed study of individual alpha globulin changes will be of interest because many otherwise distinct proteins are grouped together in the alpha globulin electrophoretic components and large changes in individual proteins cannot be fully appreciated by paper electrophoresis.

The gamma globulin differences between acute myeloblastic and acute lymphoblastic leukemia were particularly interesting, especially when it is noted that the chronic leukemias of corresponding morphology are characterized by similar differences. The gamma globulin levels in chronic myelocytic leukemia are often elevated. In chronic lymphocytic leukemia, on the other hand, markedly decreased gamma globulins have been found in about half of the cases and elevated levels are uncommon.

The gamma globulin increases occurring in acute myeloblastic leukemia are not attributable to the older age distribution of these patients, although gamma globulin levels tend to increase slightly with age. In 37 normal males, aged 65 to 85, examined concurrently with this study, the mean gamma globulin levels are

<table>
<thead>
<tr>
<th>Diagnosis (No. of observations)</th>
<th>Albumin</th>
<th>Alpha-1</th>
<th>Alpha-2</th>
<th>Beta</th>
<th>Gamma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Myeloblastic (160)</td>
<td>56</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Acute Lymphoblastic (80)</td>
<td>75</td>
<td>24</td>
<td>1</td>
<td>6</td>
<td>45</td>
</tr>
</tbody>
</table>

*Decreased or increased values were those outside the normal limits given by each author.*

L = Low, N = Normal, H = High.
lin level was only slightly higher (113 per cent) than the young normal value. While the gamma globulin changes are not found in every case of malignant leukocyte diseases, normal or low gamma globulin levels are characteristic of the lymphocytic leukemias and elevated gamma globulins are characteristic of the myelocytic leukemias.

A suggestion that the serum protein changes in acute leukemia are “non-specific” is somewhat misleading. The serum protein alterations reported here for the acute leukemias appear to be specifically due to the presence of the leukemic processes and are not attributable to any complicating diseases. The multiple and opposite alterations among the serum proteins (decreased albumin and increased alpha-2 or gamma globulin) can be viewed as indices of the profound effects of leukemic neoplasms in the human host and, in particular, upon the metabolic mechanisms involved in the control and maintenance of individual proteins. The full significance of the serum protein changes in acute leukemia is still not known, as is emphasized by the poor correlation between the stage of disease and serum protein changes in acute lymphoblastic leukemia. The persistence of serum protein changes despite complete hematologic remission was noted also by Olmer and the poor correlation between progression of disease and progression of serum protein abnormalities has been found by others. Malignant tissue activity, however, is hard to quantitate. In many instances disease activity is appraised indirectly as in terms of anemia, blood platelet or neutrophil levels, body weight losses, etc., which might not correlate closely with serum protein changes. Whether the persistence of serum protein changes during remission, as is seen in acute leukemia but not in Hodgkin’s disease, represents persistence of otherwise undetectable disease activity or has some other implication remains to be seen.

**SUMMARY**

A study of the serum protein changes and clinical events in acute myeloblastic and acute lymphoblastic leukemia was undertaken as a part of investigations on the effects of malignancies in man. In order to appraise the effects of the leukemic processes, an evaluation of the effects of disease type, activity, complications and therapy was also undertaken. Over a five-year period, clinical appraisal and electrophoretic serum protein analyses were compared 171 times in 82 patients with acute lymphoblastic leukemia and 64 times in 28 patients with acute myeloblastic leukemia.

Serum electrophoretic evaluation showed characteristic patterns in the two types of acute leukemia studied. Analyses conducted in the absence of fever, infection or liver disease typically revealed elevation of the gamma globulins in myeloblastic but not in lymphoblastic leukemia. Alpha-2 globulin elevation, however, was representative of active lymphoblastic leukemia. Serum albumin was significantly lowered, and the beta globulins component remained essentially normal in both myeloblastic and lymphoblastic leukemia. Alpha-1 and alpha-2 globulin values were notable for the wide range of values obtained.
Hematologic remission in some patients with acute lymphoblastic leukemia was associated with a return of albumin and alpha globulin values toward normal, but the total experience indicated a general persistence during remission of the abnormalities seen in active diseases.

Fever, in the absence of infection, was associated with elevation of the alpha-1 globulin component. Bacterial infection was associated with similar elevation of the alpha-1 globulin fraction and, in addition, a further fall in serum albumin levels.

Marked depression of the gamma globulins was unusual. The mild decreases encountered in lymphoblastic leukemia could not be related to the frequency of bacterial infection. Administration of antimetabolites or adrenal corticosteroids could not be shown to produce any direct effect on the serum electrophoretic components.

**Summary in Interlingua**

Un studio del alterationes de proteina seral in correlation con le eventos clinic in acute leucemia myeloblastic e in acute leucemia lymphoblastic esseva effectuate como parte del investigation del effectos de malignitates in le homine. Pro evalutar le effectos del processos leucemia, etiam le rolo del typo de morbo, del activitate, del complicationes, e del therapia esseva studiate. In le curso de un quinquennne periodo, le evaluacion clinic e le analyse electrophoretic del proteinas del sero esseva comparate 171 vices in 82 patientes con acute leucemia lymphoblastic e 64 vices in 28 patientes con acute leucemia myeloblastic.

Le evaluacion electrophoretic del seros revelava certe configurationes characteristic occurrente in ambe typos de leucemia studiate. Analyses conducite in le absentia de febre, infection, o morbo hepatic monstrava tipicamente un elevation del globulinas gamma in leucemia myeloblastic sed non in leucemia lymphoblastic. Del altere latere, elevation de globulina alpha-2 esseva characteristic de de active leucemia lymphoblastic. Le albumina del sero esseva significativamente reducite, e le componente de globulinas beta remaneva essentialmente normal in leucemia myeloblastic e lymphoblastic. Le valores de globulina alpha-1 e alpha-2 esseva notable per lor extense variabilitate.

Remission hematologic in certe patientes con acute leucemia lymphoblastic esseva associate con un retorno del valores pro albumina e pro globulina alpha verso le norma, sed le experientia total indicava un persistentia general, durante le remission, del anormalitates vidite durante le phases active.

Febre, in le absentia de infection, esseva associate con elevationes del componente de globulina alpha-1. Infection bacterial esseva similemente associate con un elevation del globulina alpha-1 sed additionalmente con un declina ancoras plus marcate del nivellos de albumina seral.

Un marcate depression del globulinas gamma esseva inusual. Le leve declinos incontrate in leucemia lymphoblastic non poteva esser correlationate con le frequentia del infectiones bacterial. Il non esseva possibile demonstrar
que le administration de antimetabolitos o de corticosteroides adrenal produceva un efecto directe super le componentes electrophoretic del sero.

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FAHEY AND BOGGS


Erythropoiesis in rats has been assessed by the reticulocytosis and the incorporation of radio-iron into the red cells. In anemias induced by red cell antibodies, marrow activity was diminished as compared with the response in post-hemorrhagic anemia. The authors deduce that anti-red cell serum may have an effect on erythropoiesis.—G. M.


A case report of the third of four siblings to die in infancy of a condition characterised by pancytopenia with atypical mononuclear cells in blood and marrow, hepatosplenomegaly and proliferation of histiocytes throughout the reticuloendothelial system, with phagocytosis of erythrocytes and leucocytes. The red cell survival was markedly shortened, and the bone marrow showed erythroid hyperplasia. The Coombs test was negative, but a platelet agglutinin was present in the child's serum. Splenectomy and prednisolone were without effect. The survival of both his own and donor red cells in the father's circulation was slightly shorter than normal; it is suggested that this is a recessive condition, and that the father and the one surviving child, who had a mild anaemia with atypical mononuclears in the blood during infancy, are heterozygotes. [This condition was first described by J. W. Farquhar and A. E. Claireaux (Arch.dis.Child. 27:519, 1952) in the first two children of this family.]—R. M. H,
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