Leukemia may be considered as a generalized, purposeless, self-perpetuating abnormal proliferation (slow or rapid) of one of the leukocytic tissues, often associated with abnormal white cell counts, and eventually leading to anemia, thrombocytopenia, and death. In this statement there is no hint as to the cause of the proliferation; why should one of the leukocytic tissues suddenly begin to grow in an abnormal fashion? Like other infectious proliferation, is it possible that some agents (virus?) may be responsible for the proliferative process of leukemia? Although the viral etiology of leukemia requires to be proved, but this theory fails to explain the self-perpetuating mechanism inherent in the leukemic disease. Nevertheless, the self-perpetuating feature of the leukemic process, its tendency to recur even after reversal by a therapeutic agent, its "relentless" character terminating only with death of the individual—these features are identical with other neoplastic diseases. Thus the definition of leukemia as given above already includes the term "neoplastic," since the terms "purposeless," "self-perpetuating," and "abnormal" are in themselves sufficiently indicative of neoplastic proliferation. Thus leukemia seems to be more than a mere reactive phenomenon; it is an abnormal growth pattern which once begun, persists until its evil
effects have caused the death of its victim.

Again, leukemia (I) implies a condition of the blood characterized by a greatly increased leukocytic count. Although this is frequently the case, it is by no means a constant phenomenon. Furthermore, it is not in reality the blood that is fundamentally abnormal in leukemia, but rather the tissues - the several tissues that produced the blood cells and that are proliferating in an abnormal fashion; as well as the many tissues in which the proliferating and exfoliated cells settle and often accumulate. The blood is indeed of secondary importance in leukemia, it is merely a "traffic-stream" through which cells pass from their sites of origin in the blood forming organs to tissues all over the body and from these tissues to their sites of eventual destruction. Thus in the presence of a disturbance in one of the blood forming organs, characterized by rapid proliferation and "exfoliation" of cells the blood stream might show considerable number of leukocytes on their way to various tissues; however, should exfoliation and death of the cell be simultaneously very rapid, the blood itself might carry relatively few cells. Also, if the outlying tissues were particularly "avid" for the leukocytes just produced in the proliferating tissues, the blood leukocytes might be normal in number whereas those in the tissues might be greatly increased. It is conceivable too, that in some cases relatively little exfoliation of abnormal cells takes place and the process is one of
maturation-arrest in which primitive cells cannot mature beyond a certain point, perhaps, because of a metabolic defect. These possibilities are raised simply to emphasize that leukemia is not a "blood" disease, but more fundamentally an abnormal proliferation of one of the leukocytic tissues, in which the blood cells may or may not be involved. To be sure, the findings in the blood are usually a reflection of the status of the blood forming tissues.

In considering white blood cell counts, whether normally, in infections, or in leukemia, it is therefore essential to have in mind three distinct areas, as follows:

1. The blood forming organs.
2. The blood.
3. The tissues.

The blood is simply a moving stream interposed between the blood forming organs, on the one hand, and the tissues on the other; its leukocytes may be linked to "automobiles of different makes on their way from certain areas to other areas".

In the presence of a high leukocytic count, one cannot
be sure, furthermore, that a given leukocytic tissue is proliferating to an unusual degree; the high white cell count may be due to a delay in the removal of cells from the blood i.e., they may be accumulating there. Thus a high leukocyte count may be due to an excessive degree of proliferation with exfoliation or it may be accumulative. Finally, there are possibilities that in certain types of leukemia an actual lack of proliferation may be present, in which primitive cells pile up in large number because of an acquired metabolic (enzymatic ?) defect with resultant arrested maturation at a primitive level. Thus, what we call leukemia is probably represented by several different types of abnormal white cell growth.

In leukemia there may be involvement of any one of the white-cell forming tissues, the bone marrow, the lymphoid tissue, the reticuloendothelial system or the system of plasmocytes. Fortunately, not all leukocytic proliferations are leukemic; in fact benign proliferations of the white cells represent one of the commonest forms of normal bodily reactions. Polymorphonuclear leukocytosis occurs not only in certain type of bacterial infections but also in psychologic stress, excitement, or even in exercise where bone marrow granulocytes proliferate. The actual infective organism in generalized proliferative disorder of the lymphoid tissues resulting from infectious mononucleosis has not been discovered. In the later case, in a few weeks, the lymph nodes regress, the
violent proliferation within them returns to normal, and the blood picture again reverts to its normal status, with perhaps a few abnormal lymphocytes present to indicate the previous disease. In certain conditions such as tuberculosis, malaria and kala-azar (where the relentless proliferations occur) in some of the white cell forming tissues. But these abnormal manifestations are not what we know leukemia.

Towards the middle of the nineteenth century the time became ripe for the recognition of leukemia as a distinct entity and we are now in a position to detect it to some extent by haematological means and when detected, attempt may be made to revert the blood picture to the normal pattern. But if we look a century back we find that there are records of only some stray incidents which could be connected with leukemia only after the application of clinical and microscopic evidence. Priority is not easy to allocate, since observations in some cases preceded publications by a number of years, while some early observers did not appreciate the significance of their discovery.

Leukemia was first described almost simultaneously by two brilliant young men who after applying their great gifts to a meticulous exploration of its features in the living and the dead, themselves engaged at once in a tussle over the honour of having been the first to identify this fatal disease.

It was Velpeau (2) who first described accurately a
case of leukemia in the year 1827. He described a patient with enlargement of the spleen and a rather similar naked-eye appearance of the blood, 'like the lees of wine'. About 1827, a number of workers such as Barth (3), in 1839, Bonne (4), devoted themselves to find out the hidden cause of this disease. But it should be realized that at the time of its discovery very little was known about the composition, the origins, and the function of normal blood, nor were there any good methods available for investigating them. Each step forward had to be preceded by the exploration of the normal. Much of the literature of the first hundred years echoes with the clash of controversy, lacking a basis of substantial facts. It is regrettable but true, that even to day we do not possess the answer to many of the fundamental questions about the mechanism of normal hematopoiesis on the regulation of the blood elements.

Inspite of these and other early observations, leukemia was not recognized as a definite entity until its description in 1845 by Bennett (5) in Scotland and by Virchow (6) in Germany. The independent publication, within one month of each other, of two cases was less remarkable than the fact that each observation came from the pen of a man who was to become a leader in his own field - Bennett in physiology and Virchow in pathology. In each of the two cases it was the post mortem appearance of the blood which first gave the hint that an unusual condition was present. In Virchow's patient the blood vessels contained a "yellowish-white almost greenish
mass. Microscopically it consisted "besides very few red blood corpuscles ...... of the same colourless or white bodies which also occur in normal blood, namely small, not quite regular protein molecules, larger, granular, fat containing non-nucleated corpuscles and granular cells with one rounded, horse-shoe shaped or trefoil-like having several hollowed-out distinct nuclei". The relation between red and colourless corpuscles was about the reverse of the normal one, so that Virchow coined the term "white blood" (Weisses Blut) to describe the condition. Later Bennett and Virchow described the disease as "suppuration of the blood" and "pyemic", respectively. Having reconsidered not only his own case but also those published by Bennett (5), Craigie (7) and Fuller (8), Virchow a few months later (1846) took a much more definite attitude against the pyemic theory of leukemia, pointing out that there was no evidence of local suppuration which could have spread to the blood that "pus" corpuscles were identical with the colourless bodies normally occurring in the blood, and that in leukemia (white blood) there was merely an increase in the normal member of these latter cells, such an increase was also shown by Fuller (8) who examined the blood three times during life in his patient and found on each occasion, "in addition to the natural blood corpuscles, a very large proportion of abnormal, granular, colourless globules". In the year 1847, Virchow first introduced the term "leukemia" and also stated that colourless corpuscles are always present
in normal blood and are increased after digestion, pregnancy, etc. Such an increase is not by itself a disease and must be distinguished from leukemia which is a definite pathologic state characterized not only by an increase in colourless corpuscles but also by a decrease in the number of red cells and dependent on changes in certain organs. The general view on the origin of the red blood cells was still that put forward by Hewson in the eighteenth century and that red cells are formed from the colourless corpuscles in the blood itself. Thus Bennett (1852) (9) suggested that the red cell was the "liberated nucleus of the colorless cell". Virchow himself, like many others, had at first accepted the transformation of colourless into colored corpuscles in the circulating blood, and had explained leukemia as a retardation in this process, with the production of increased numbers of white and of decreased quantities of red cells. By 1856, however, he had abandoned this view and now regarded the white corpuscles as "simple, non-specific cells" which are not transformed into red corpuscles once they have left the sites at which they themselves are produced. The transformation of lymph corpuscles into red cells does however, take place in the spleen and lymph nodes; but once they have reached the blood stream their specific metamorphosis into colored corpuscles becomes impossible". They circulate for a brief while and then perish.

One notable demonstration of Neumann's observation that the bone-marrow was an important site for the formation
myeloblasts.

Thus there was now chronic lymphocytic and chronic granulocytic (myelogenous) leukemia and acute lymphocytic, granulocytic (myeloblastic) and monocytic leukemia.

In classifying leukemias references may be made to the clinical acuteness of the disease, the number of leukocytes circulating in the peripheral blood and the presence of abnormal forms, the identity of the predominating cells and their stage of maturity, and the site of origin of the proliferating leukocytes.

### Classification of Leukemia

<table>
<thead>
<tr>
<th>General grouping according to site of origin</th>
<th>Degree of clinical acuteness and average survival untreated</th>
<th>Usual predominating cell type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloid or Myelogenous</td>
<td>(Acute (less than 6 months))</td>
<td>Myeloblastic</td>
</tr>
<tr>
<td></td>
<td>Subacute</td>
<td>Promyelocytic-myelocytic</td>
</tr>
<tr>
<td></td>
<td>(6-12 months)</td>
<td>Granulocytic</td>
</tr>
<tr>
<td></td>
<td>Chronic (more than 12 months)</td>
<td>myelocytic and polymorphonuclear</td>
</tr>
<tr>
<td></td>
<td>(Acute (less than 6 months))</td>
<td>Lymphoblastic</td>
</tr>
<tr>
<td>Lymphoid or lymphogenous</td>
<td>Subacute</td>
<td>Prolymphocytic</td>
</tr>
<tr>
<td></td>
<td>(6-12 months)</td>
<td></td>
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<th>Degree of clinical acuteness and average survival untreated</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>Undifferentiated stem cells</td>
</tr>
<tr>
<td>Not generally or clearly separable into acute or chronic form</td>
<td>Monoblastic-monocytic</td>
</tr>
<tr>
<td>Of variable, multiple or uncertain origin</td>
<td>Plasmoblastic-plasmocytic</td>
</tr>
</tbody>
</table>

A general distinction can be made on clinical grounds between acute and chronic leukemias. In the former the onset is commonly rapid and the course short and severe, if the disease is untreated, death usually occurs within 3 months of the onset. In chronic leukemia on the other hand the date of onset is often uncertain; the condition progresses relatively slowly and may remain mild for a long period with few manifestations of disease other than painless splenomegaly or lymphatic glandular enlargement. Patients with chronic leukemias survive commonly for 3 to 5 years from the time of first symptoms. The distinction between acute and chronic is also accepted, but only in a clinical sense, since there is no well-defined anatomic criterion to differentiate acute from chronic leukemia. Experimental studies indicate that acute leukemia is not basically different from chronic leukemia, it is only a question of rate of proliferation and degree of differentiation. It has been stated (15) that acute and chronic leukemias are different diseases, the acute
consisting of proliferation of abnormal and the chronic of normal leukocyte and that one cannot be transformed into the other. This is contrary to experience with transplanted leukemias, in which successive transformation from the chronic to the highly acute form is common. Although it is true that the human disease is either chronic or acute from the start, progressive changes in types are difficult to evaluate, since alterations in the organs cannot easily be followed.
low in blood cells as compared with that of plasma. This observation supports the findings of Erickson et al. (28) and Brun (29).

The erythrocyte total lipids, cholesterol and phospholipids were studied with acute leukosis, with a view to elucidating the intrinsic defects in red cells observed in cases of leukemia (30). It was found that phosphorus-to-total lipid index was lower in myeloid and lymphatic case than the normal. The P-to-total lipid, cholesterol-to-total lipids and P-to-cholesterol ratios did not differ significantly whether the cells vols were below 90 μ3 or above.

Studies on the red cells of various animal species suggested that the phospholipid composition and their fatty acid pattern may be related to the permeability properties of the red cell membrane (31, 32).

Phospholipid analysis on abnormal red cells were carried out by a number of workers (33-40). Ways et al. (37) and Phillips (39) noted a decreased in the relative amount of lecithin and an increase in sphingomyelin in the red cells of patients with acanthocytosis. Allison et al. (36) studied two patients with hereditary spicerocytosis; they concluded that there was a partial block in the biosynthesis of phosphatidyl ethanolamine, resulting in a decreased amount of this phospholipid and an increased level of lysophosphatidyl ethanolamine. However, De Gier et al. (32) found a normal phospholipid
composition in spherocytic cells. The red-cell lipids in paroxysmal nocturnal haemoglobinuria were found by Harris et al. (33) to be markedly deficient in phosphatidylcholine, whereas Formijne et al. (34), Barry (35) and Phillips and Roome (38) recorded a normal composition.

Contradictory opinions have been expressed concerning the unsaturated fatty acid patterns of the red cells lipids. Munn and Crosby (41) found an excess of arachidonic acid and pentanoic acids and a relative deficiency of oleic acid in paroxysmal nocturnal haemoglobinuria (PNH) red cells. Lovelock and Prankerd confirmed this observation (42), but Leibetseder and Ahrens (43) found no clear-cut differences between the fatty acid patterns of normal and PNH red cells. Munn and Crosby (41) studied the fatty acids in various abnormalities of the human red cells and noted a number of deviations which seemed to be a consequence of immaturity rather than of any fault in red cell structure.

Lipid Incorporation

It has been demonstrated that lipid synthesis proceeds in reticulocytes and, perhaps, young circulating erythrocytes, but not in mature red cells. It is noteworthy that as red cells age in vivo they lose the capacity to synthesize lipids. This may be correlated with the loss of tricarboxylic acid cycle activity (44) and diminished capacity for reduced
pyridine nucleotide generation (45, 46). It has also been reported (47) that the lipid content of red cells decreases with aging. The diminution in lipid content may reflect the inability of erythrocytes to replace the lipid of the cell membrane.

It has been known for some years that mammalian cells can synthesize lipids from acetate and phosphate (48) and Altman (49) found that radioactive acetate is incorporated into the lipids of rabbit erythrocyte stroma.

Lipid synthesis by erythrocytes in vitro has been recently reported by several investigators (50-53).

Altman and Swisher (54), James et al. (55) and Rowe (52) obtained evidence which they interpreted as indicating that nucleated red cells actively synthesize lipids. These findings are attributable to the fact that in these earlier studies, the contribution to lipid synthesis by leukocytes and platelets contaminating the erythrocyte sample was not adequately evaluated.

Erythrocytes appear not to be able to metabolize acetate, which is in agreement with their known lack of Krebs cycle enzymes (25) respiratory enzymes (56) or lipase (57); the synthesis of lipid by reticulocytes (58) is not demonstrated or disproved by the findings, since all blood used had a normal low reticulocyte count and these cells were discarded.
in the leukocytes and erythrocyte separation.

The rapid metabolic rate of leukocytes is well-known; they have $Q_{\text{O}_2} = 32.6 \mu l$ of $\text{O}_2$/mg dry wt/hr as compared with $Q_{\text{O}_2}$ of liver, $= 11.6 \mu l$ (59) and all metabolic functions studied have confirmed this (60). The results reported here show that leukocytes are also very active in lipid synthesis.

The normal leukocytes which actively synthesize lipids, is in accord with several recent communications (61-63) and also incorporate *in vitro* acetate (55, 64) and phosphate (65) into their lipids. Synthesis of lipids by human leukocytes *in vitro* has been studied, the amount of synthesis is sufficient to account for all the lipid synthesized by whole blood (25). It is probable that earlier reports of lipid synthesis by erythrocytes were due to leukocyte contamination. O'Donnell et al. (66) reported that the leukemic leukocytes incorporate more than normal in lipids.

The *in vitro* incorporation of $^{14}\text{C}$-acetate into the leukocyte lipid was reported by Rowe et al. (67, 68) and Bhattacharjee (69) in 1960. The leukocytes were responsible for all the detectable incorporation into the unsaponifiable lipids and most of that into the phospholipids fatty acids. There was considerable incorporation into the neutral saponifiable lipid fatty acids of both leukocytes and red cells. The incorporation into the unsaponifiable lipid was proportional to the number of leukocytes in the fraction.
The incorporation into the fatty acids of the phospholipids showed some scatter, while the incorporation of acetate into the fatty acids of neutral saponifiable lipid was associated with a high leukocyte count and leukocytes were 100-1000 times as active as erythrocytes in lipid synthesis (63).

Marks et al. (70) reported that lipid synthesis in human whole blood occurs in leukocytes and platelets. The rate of lipid synthesis on a cell basis is approx. 70-fold greater in leukocytes than in platelet. However, owing to the greater number of platelets in a volume of whole blood, 25 to 30% of the total lipid synthesis in whole blood may be attributable to platelets.

According to Malamos (71) leukemic leukocytes incorporated the highest percentage of radioactivity into the phospholipids fractions whereas normal leukocytes incorporated the highest percentage into the glyceride fractions. There is no conclusive evidence that the differential white cell count had an influence on these observations. The difference may be related to a more active lipid metabolism in leukemic cell.

Miras (72) demonstrated a higher uptake of radioactivity into the phospholipid fraction of the blood of leukemic patients compared with the blood of normal.

Costello et al. (73) showed that the uptake of $^{32}$P in
the phospholipids of squamous cell carcinoma in experimental animals was more than twice that of normal epidermis.

Whole blood or separated leukocytes when incubated with palmitate-1-\(^{14}\)C and fractionated, maximum uptake was noticed in the glycerides of normal and phospholipids of leukemic subjects (74).

Buchanan (25) reported that labeled lipid in plasma in which the leukocytes were incubated could be either due to lipids being released from living leukocytes into the plasma, or to lysis of leukocytes during incubation after they have synthesized labeled lipid. The former is the more likely explanation.

James et al. (50) reported that lipid synthesized by whole blood becomes attached to the plasma proteins mainly in the \(\alpha\)-globulins.

Leukocytes remain viable \textit{in vitro} for up to four days (75). If living leukocytes can lose lipid in the plasma, this may be an important source of the plasma lipid.

Isotope incorporated into lipids of leukocytes and platelets appeared rapidly in plasma lipids. This finding is in general agreement with that of James et al. (76), who demonstrated that plasma lipids rapidly become labeled during incubation of whole blood with acetate-1-\(^{14}\)C.

When whole blood (human) is incubated \textit{in vitro} with
radioactive acetate, radioactivity is incorporated into the phospholipids (50, 65). Moreover, there is an exchange of the radioactive phospholipids between the cells and the plasma (64). Fractionated phospholipids of human blood cells was found to contain ethanolamine containing cephalins, serine containing cephalins, phosphatidyl choline, sphingomyelin and other phospholipids (65). The fatty acids of the fractionated phospholipids were analysed by vapor phase chromatography and differential distributions of fatty acids for each fraction were observed. Two ethanol-containing phospholipid fractions were isolated, these differed in the rate of phosphate and acetate incorporation and in fatty acid composition (65, 77).

Lovelock et al. (64) reported the incorporation in vitro of radioactivity from acetate-2-14C into the lipids of whole blood. Lipids from the cells, plasma β-lipoprotein and the α-lipoproteins were fractionated into unsaponifiable lipid, saponifiable lipid and phospholipid. Exchanges of both unsaponifiable lipid and phospholipid between the cells and plasma α- and β-lipoproteins were observed. There was no exchange of saponifiable lipid between the cells and β-lipoproteins. Exchanges were observed between the cells and the other plasma lipids.

Fatty acids of human blood

Peters and Man (78) studied the fatty acid composition of human blood. Brun (29) and Kirk (79) observed that
esterified and free cholesterol in blood cells was different from that of plasma in healthy individuals. Studies by Chevallier et al. (80) and Evans et al. (81) have indicated that the partition of the unsaturated fatty acids in blood cells was quite different from that in plasma.

Patil et al. (27) reported the level of oleic acid in blood cells was somewhat lower than that of plasma. In the polyunsaturated fatty acid group, dienoic acid was the major component in plasma, whereas tetraenoic acid was the major component in blood cells. The level of dienoic acid in blood cells was quite low as compared with that of plasma. The levels of pentaenoic acid and hexaenoic acid in blood cells and plasma were low, although there was more of both acids in the blood cells. Trienoic acid was present in small quantities in plasma, whereas it was absent from blood cells. Evans et al. (81) were also unable to show the presence of trienoic acid in blood cells.

The cellular components of human blood are shown to be a convenient system for studies of fatty acids and lipids synthesis and exchange (23). After incubation of whole blood with acetate-2-14C all the common saturated and unsaturated acids are found to be labeled, including the mono- and poly-unsaturated acids of a variety of chain length.

The long chain fatty acids are incorporated into triglyceride and some neutral lipids of undefined structure,
as well as into phospholipids, but not into cholesterol esters (55). The triglycerides and other neutral lipids are rapidly secreted into plasma, where they are incorporated into the plasma α-lipoproteins but not into β-lipoproteins. The phospholipids are more readily incorporated into the plasma β-lipoproteins.

Information on the unsaturated fatty acid contents of human blood has been fragmentary and conflicting (24, 78). Wilson and Hansen (82) have reported unsaturated fatty acids in human plasma and suggested an average iodine number of 108. In 1937, Brown and Hansen (83) showed differences in the amounts of linoleic and arachidonic acid in sera of normal and eczematous children; and later Brown and other (84) demonstrated a decrease of plasma unsaturated acids in the adult man during a prolonged low fat diet. The lipid of human blood cells has been less extensively studied. Analysis by Erickson et al. (28) have shown that practically all blood cell lipids is in stroma, mainly as phospholipid. Wiese and Hansen (85) reported a semi-micromethod for the estimation of Blood serum unsaturated fatty acid, but at that time did not possess constants for the pure natural acids and did not clearly characterize human blood lipid.

Nucleotide

The discovery of the first purine nucleotide, inosinic acid, a deamination product of adenylic acid, goes back to
1847 (86). Seventy years elapsed before adenylic acid was identified in red blood cells (87) and in muscle (88). In 1928, Lohmann (89) and Fiske and SubbaRow (90) independently discovered ATP. Subsequently, and slowly, other nucleotides such as NAD (91, 92), NADP (93, 94) and UDP-glucose (95-98) were identified in different cells and plasma.

Nucleotides are known to be present in blood. Some investigators (99, 100) have determined the amounts of certain nucleotides only, while others (101, 102) have been more interested in the relative amounts of the various nucleotides present in the tissue.

Maizuno et al. (103) isolated some compounds with properties similar to the following nucleotides from normal calf blood thrombocyte: CDPE, CMP, ADP, GDP, ATP, UTP, GTP, and DPN. Of these compounds, adenosine triphosphate is present in comparatively larger amount.

Bishop et al. (104) reported that the use of Dowex-1-formate column and a system of nongradient elution the following nucleotides have been found in normal human blood: DPN, TPN, AMP, ADP, ATP and GTP. Inosinic acid was not found in freshly drawn blood. With the exception of uric acid, all of the purines and pyrimidines in human whole blood seem to be present as nucleotides, with none appearing as free bases or ribonucleotides.
From the work of Nakai et al. (105) it shows that human leukocytes contain at least 16 nucleotides, with UDPG and UDPAG being present in somewhat greater quantities in leukocytes from patients with AML and CML. There appears to be no good correlation between UDPG and UDPAG content with cellular maturity. The minor nucleotides such as CDP, CTP, UMP, GMP and GDP were identified only by their characteristic $R_f$ values. No deoxyribonucleotides were identified.

Acid soluble nucleotides are present in human normal and leukemic leukocytes. Bishop et al. (104), Willoughby and Waisman (102) and Ondarza (106) analysed normal and leukemic whole blood. The following compounds were found: DPN, uric acid, AMP, TPN, ADP, ATP, UDPAG, UDPG, GTP, and UTP. Karon (107) analysed acid soluble components in ribonucleic acid (RNA) from human leukemic cells and found no unusual components. Human erythrocytes were analysed by Bartlett and Shafer (108, 109), Corsini et al. (110), Morell et al. (111) and Ockerman (112). The following compounds were found, DPN, uric acid, AMP, ADP, ATP, TPN, IMP, UTP, GTP, CMP, glycogen, glucose, hexose monophosphate and-diphosphate, Pi, 2-3-DPGA and unidentified nucleotides.

Sventsikskaya (113) studied the content of total nucleic acid and adenine and guanine nucleotides in human red blood cells of donors and in cells of patients suffering from chronic leukemia. An increased content of nucleic acids, ATP and guanine nucleotides in the blood of patients was observed.
It has been reported (114-117) that in the course of aging there occurs a marked decrease in nucleoside triphosphates in erythrocytes.

The plasma proteins in leukemia

Extensive study of total protein levels in the plasma or serum, and search for anomalies in component fractions separated by electrophoresis, have revealed no consistent or characteristic changes in any form of leukemia.

In acute leukemia the total amount of serum protein is normal or slightly diminished, while electrophoretic patterns show much variability, though of minor degree. The commonest abnormalities are reduction in the albumin fraction and increases in different globulin constituents, chiefly $\alpha_1$, $\alpha_2$-globulins and $\gamma$-globulins. Fibrinogen levels in plasma remain usually within the normal range. These changes have generally been most marked in state of severe relapse and are probably non-specific responses to infection and hepatic involvement, although Franzini et al. (118) found protein anomalies more common in monocytic, than other forms of acute leukemia and believed that a specific predisposition towards dysproteinaemia might exist in this variety of the disease.

Essentially similar findings have been reported by Brown and his associates (119), Petermann et al. (120), Rundles et al. (121), Creyssel and his colleagues (122).
The abnormally sharp electrophoretic Y-boundaries that are occasionally observed (123, 124), are difficult to interpret unless the presence of macroglobulins has been ruled out. In Hodgkin's disease and malignant lymphoma, the Y-globulin is frequently increased (125), this finding does not appear to be closely related to hepatic involvement, reaction to infection, or the presence of increased number of plasma cells in the bone marrow.

Total protein and protein fractions were detected 3-5 times during the course of disease (126). Hypoproteinemia was found in leukemia experiment during the terminal stage of disease. Decrease of A/G coefficient due to lowering of A fraction was observed. In leukemia α& Y-globulins were markedly increased.

Patients with chronic myelosis showed a globulin increase as a result of increased Y-globulins (127). Residual N remained normal, patients with acute leukemias showed sharp changes in the blood protein compound; the albumin fraction declined and the α1, α2- and Y-globulins increased. None of the therapeutic methods applied altered the blood serum protein picture.

Ionescu (128) reported in electrophoretic investigations that in CLL the Y-globulin fractions were low, parallel with deterioration of the clinical picture. In CML the modifications were significant. In acute leukemia the
total serum protein was decreased as a result of a decrease in albumin accompanied by an increase in \( \alpha_1 \)- and \( \gamma \)-globulin. The modification became more significant with a deterioration of the clinical picture.

Mokhun (129) studied blood plasma proteins, 25 with leukemia, 31 with chronic lymphadenosis and 17 with chronic myelosis. Most patients manifested a hypoproteinemia due to a fall in the albumin fractions and in individual cases, due to a drop in the albumin and globulins. A fall in the A/G ratio in individual cases was the result of an absolute and in some cases of a relative increase in the globulin content. A connection was observed between the frequency of positive Takata-Ara reaction and the formol-gel test, and hypoproteinemia changes in the blood proteins in leukemia, which amounted to more than 60% were accompanied by a disturbance in the liver proteinogenic function. This was shown by positive reactions for amino acids in the urine and by a drop in the Quick-Pytel test.

An analysis of the proteinograms of 40 patients with chronic myeloid leukemia shows a dysproteinemia. There is an increase in \( \alpha_1 \)-globulins, of \( \beta \)-globulins and of \( \gamma \)-globulins (130).

Total blood serum protein of most patients with chronic myelosis increased owing to increased globulins, in particular \( \gamma \)-globulin; the albumins were reduced. Residual
In patients with lymphadenoma total protein was 6.5 - 7.5%, Y-globulins 0.45 - 1.24%, \(\alpha_2\)-globulins 0.37 - 0.8%, \(\alpha_1\)-globulins normal, and residual N increased. Therapy was not generally accompanied by the normalization of the blood protein concentration (131).

The total serum protein in leukemia was decreased with albumin fraction invariably low while the \(\alpha_1\)- and Y-globulin were proportionately increased (132). The serum lipoproteins in leukemia show a decrease in the \(\alpha\)-fraction and a rise in the Y-fraction of the serum glycoproteins the \(\alpha_2\)-fraction, generally associated with Cu showed a notable increase in leukemia.

Ten healthy children and 20 children with acute leukosis (age 2 1/2 - 15 years) were examined (133). Total protein in healthy children averaged 7.5%. In 19 ill children total protein fell from 6.76 to 3.5%, during the peak of the disease. Two groups of patients were distinguished in respect to the ratio of serum protein fractions. In one group Y-globulin increased to 20.5 - 30 and A dropped to 25 - 46%. In the second group Y-globulin dropped to 13.17 and A often dropped to 48-55%. In both groups \(\alpha_1\)-globulin increased from 3.5 - 9.5%, \(\alpha_2\)-globulin from 11.9 to 22.9% and \(\beta\)-globulin from 10.5 to 16.5%. These changes grew more pronounced as the children's condition worsened. During remission the blood protein picture tended to normalize.
Matsneva (134) found in 47 of 69 patients with CML the total protein content in the blood serum was normal, in 13 it was above normal (8.28 - 9.35 gm %) and in 9 it was subnormal (5.25 - 6.4 gm %). In most of the patients A had decreased to 44.3% (av) (Normal 58.1 gm %) while globulins increased to 51.6 - 59.8% and in some cases to 63.8 - 68.8% vs. a normal of 41.9%; Y-globulin especially increased to 24.9 - 36.1 %; normal 18.8%). In 51 patients β-globulin was normal (11.8%) and in 18 reduced to 6.3 - 9.0%. An elevated α1-globulin content was found in 18 and elevated α2-globulin content in 24 patients. In 26 of 40 patients fibrinogen was increased to 5.9 - 10.7% (normal 3.0 - 4.9%). Changes in the protein picture of the blood usually were correlated with the gravity of the illness.

Before treatment, the total protein concentration in blood serum was normal (6.5 - 8.15 g %) in 25 patients with AL (135), elevated (8.3 - 9.1 g %) in 5, and low (4.92 - 6.41 g %) in 13. Albumin concentration was low (39%) in the majority of patients, the concentration of globulins elevated. The average A/G ratio was 0.63. A significant rise in the fibrinogen concentration (15.7%) compared with normal (3.0 - 4.9%) was seen with deterioration in the clinical condition of the patients. Successful treatment with cortison improved the protein picture of the blood. During progress of the disease, A concentration in the serum continued to decrease and G concentration to rise. The observed shifts in the protein
composition of the blood in patients with acute leukemia were not correlated either with the number of leukocytes of the morphological composition of the blood.

Metabolism

(a) Carbohydrate

The carbohydrate metabolism of leukemic leukocyte is a debatable subject. The chief aim of most investigators has been to establish whether metabolism of these cells resembles those regarded by many workers, in consonance with Warburg's views, as characteristic of malignancy. No account was taken by older workers of the fact that this malignant metabolism, with its preponderance of aerobic glycolysis over an aerobic respiration, is itself a very insecure method of classifying tissues, since a number of undoubtedly normal ones like retina, kidney medulla and jejunal mucosa, also have a "tumor-like" metabolism. The metabolism of leukemic leukocytes being characterised as "malignant" by some (136-138), as normal by others (139) and as typical of injured cells by yet another group (140). The careful analysis of Beck and Valentine (141, 142) have thrown much light on the reasons for these contradictions by showing that the carbohydrate metabolism of all leukocytes is extremely sensitive to the nature of the substrate in which they exert their functions. They have shown that, though both normal and leukemic leukocytes have a high aerobic glycolysis, that of the former is
much greater than that of leukocytes from chronic granulocytic as well as chronic lymphocytic leukemia. Similarly, leukemic leukocytes consume less oxygen and use less glycogen than normal leukocytes.

More recently, Beck (143, 144) has sought to explain the reasons for these differences in glycolysis. He found that leukemic leukocytes had a deficiency of several of the enzymes active in the glycolytic cycle, the most important deficiency being that of hexokinase which is a rate limiting factor in glycolysis. Furthermore, leukemic granulocytes were found to be deficient in ADP-generating system, including not only those dependent on hexokinase but also others, like ATPase. Again, phosphogluconate pathway of glucose metabolism is more predominant in leukemic leukocytes than the normal (143). Beck (144) reported the existence of parallelism between the concentration of ATPase and alkaline phosphatase activity in the several types of leukemic leukocytes. Stave and Oehme (145) also found the low level of hexokinase activity and a depression of glucose-6-phosphate dehydrogenase and of glycer-aldehydephosphate dehydrogenase.

(b) Glycogen:

Chemical methods (146, 147) have shown that the glycogen in whole blood is contained exclusively in the leukocytes fraction. With cytochemical estimation, it was found that platelets also contain small amounts of
glycogen (148, 149). Some chemists have measured glycogen per wet weight of leukocytes (146) and others per unit number of cells (147). Granulocyte shows a maximum concentration of glycogen in the cell. Valentine et al. (147) claimed that glycogen content per unit number of leukemic granulocytes is only one-half that of normal granulocytes. It is possible that this finding is explained by the rapid carbohydrate metabolism which is found in leukemic granulocytes. Lymphocytes and myelocytes have a similar content of glycogen as determined by chemical methods (150). Successful treatment of CLL leads to a return to normal of the lymphocytic glycogen (151).

The mature polymorphonuclear leukocytes from normal subjects possess a high aerobic lactic acid formation (152). These cells do not exhibit the "Pasteur effect" in that the lactate formation is not increased by exclusion of oxygen from the environment (140). Myeloid leukemic cells have a low lactic acid formation in the presence of oxygen and exhibit the "Pasteur effect". Lactic acid production by leukocytes is also supported by Slosse (153). Levene and Meyer (154) studied the conversion of glucose to lactic acid in leukocytic exudates obtained in dogs. Grafe (155) in 1911 first studied the respiratory activity of leukocytes in an attempt to explain the marked increase in oxygen consumption in leukemic subjects.

The most recent views are in agreement that leukocytes from normal subjects and subjects with chronic granulocytic
leukemia possess a substantial aerobic glycolysis, while those from subjects with chronic lymphocytic leukemia have a comparatively low glycolytic rate. Striking differences between normal and leukemic leukocytes are noted in oxygen consumption, glucose utilisation and lactic acid production (141) in a fortified homogenate system.

The mechanism behind the significantly lower lactic acid production in leukemic leukocytes has been extensively studied by Beck (142, 143, 156, 157) who has attempted to define the rate limiting reactions in normal and leukemic material by analysis of individual glycolytic enzymes. Several enzymes of the Embden-Meyerhof cycle were found to possess substantially low activity in leukemic leukocytes. Hexokinase, phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase pyruvate kinase and lactic dehydrogenase activities were well below normal levels in chronic granulocytic leukemic cells when compared on the basis of activity per $10^{10}$ leukocytes. On the other hand, phosphohexose isomerase, aldolase and triosephosphate isomerase were entirely normal or slightly elevated in the chronic granulocytic leukemic leukocytes.

In tumor tissue, aldolase was found to be present in limited quantity (158) and in the leukocytes of horse, Wagner has shown enzymatic degradation of glycogen, however, there were no reducing intermediates between glycogen and lactic acid in the intact cell (159). In dialyzed cells, reducing
intermediates were found to be present, and the lactic acid formation was proportional to the phosphate concentration (160, 161). The intermediates isolated were shown to be G-l-P, G-6-P, Fr-6-P, Fr-l-6-diP and PGA. He also showed the presence of hexokinase (162).

The chronic granulocytic leukemic leukocytes, however, comparatively hexokinase deficient, and additions of purified hexokinase increased overall glycolysis 50 per cent in normal material and 300 per cent in leukemic material. The leukemic cells were also shown to be deficient in ADP generating mechanism. Glucose metabolism in leukocytes chiefly resulted in the formation of lactic acid via pyruvate, and lactic acid was demonstrated by radioisotopic techniques to be reutilized (163).

The investigations on the phosphogluconate pathway of glucose metabolism, Beck (144) has presented evidence indicating that glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activity are reduced in cells of chronic leukemia. Phosphopentose isomerase activity was comparable in chronic granulocytic leukemia and normal cell. The dehydrogenases, however, were present in ten times greater concentration than necessary for their observed metabolic load. It has been found that, less than 10% of glucose was metabolized via the phosphogluconate pathway, but the per cent was higher in leukemic cells. The levels of phosphogluconate pathway
metabolism were nearly the same in intact cells as in homogenate systems. It is thus demonstrated that the metabolic pattern in leukemic cells is definitely abnormal and these aberrations may play a role in the cell's growth and differentiation abnormalities characteristic of the leukemic state.

Wagner (59, 164) pointed out that low α-glycerophosphate dehydrogenase activity in leukemic leukocytes could result from defects in any of a series of enzymes necessary to obtain oxygen uptake.

From the foregoing résumé it will appear that the leukemia is a very complex problem and its etiology is greatly obscure, though approach to this problem has been made from various disciplines. Biochemical studies also received early recognition and the present study has been made from that angle which mainly consists of the study of the nature, composition and synthesis of lipids in leukemic cells as compared to normal. In addition to the study of lipids the following investigation has been made with respect to nucleotides, phosphate esters and proteins of both normal and leukemic cells in order to get some exploitable differences which might exist between the two conditions.