The Carbohydrate Metabolism of Leukocytes: A Review*

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INTRODUCTION

Because leukocytes arise from tissues of unusual growth potential and because of their predilection for leukemic involvement, it has been assumed that they might have metabolic traits in common with embryonic or tumor tissue. This question has attracted students for half a century. The wide diversity in motivation for these studies, ranging from the purely biochemical to the purely clinical, is reflected in a highly contradictory and dissentient literature. In the hope of delineating the present status of this work, it is proposed to review here the various past contributions on leukocyte carbohydrate metabolism with emphasis on studies of circulating blood leukocytes in health and leukemia, together with a critique of the methods used and the materials studied by past workers.

Investigations into the nature and etiology of leukemia have taken many directions, as pointed out recently by Furth (91). He states that most early theories were based upon clinical and morphological observations. The finding of a filtrable agent causing fowl "leukemia" and the subsequent studies on viruses, biological properties of leukemic cells, chemical carcinogenesis, endocrine and genetic influences, effects of radiation, and leukocyte elimination mechanisms have produced a welter of complex data and intriguing hypotheses. Furth states that present knowledge seems to indicate "the essential change in leukemia resides in the leukemic cell and consists of an acquired inability of immature leukocytes to respond to forces normally regulating their proliferation and maturation."

Leukemia is an abnormality that eventually and perhaps inevitably involves more than a single blood constituent, the white cells (13). Although leukocyte proliferation is the most obvious clinical finding, the entity of leukemia apparently includes disturbances in the erythrocytes, thrombocytes, and total body metabolism. It is not improbable that etiological information may be found in systems other than the leukocytes or their precursors. Conceivably, the disorder is one of growth control, a disturbance describable only in quantitative and kinetic terms and one which leaves little or no imprint on the cells themselves.

Even though it has not been established whether the metabolic changes discernible in leukemic cells themselves are a cause, accompaniment, or consequence of the disease process, much work has been done on the biochemistry of leukocytes. The advent of satisfactory methods for separating leukocytes from whole blood (11, 38, 50, 55) has made possible quantitative biochemical measurements with the methods of the tissue physiologist. The contributions of this type have been reviewed (52). In general, it appears that striking differences are to be found in the leukocyte content of various substances in health and leukemia. For example, the leukocytes of chronic myelocytic leukemia are high in histamine (54) and low in alkaline phosphatase (53). Those of neutrophilic leukocytosis show the opposite picture—low histamine and high alkaline phosphatase. The leukocytes of polycythemia vera which has passed into the so-called "leukemic" stage, with elevated leukocyte counts and cell immaturity, show high alkaline phosphatase values, the converse of the picture in typical chronic myelocytic leukemia (6). In addition, the leukocytes of polycythemia have high glycogen

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levels (60). Here, biochemistry provides a basis for classification where morphology has ceased to be adequate.

Circulating leukocytes are uniquely well suited for metabolic study under simulated in vivo conditions. With the exception of spermatozoa and certain unusual tumors such as the Ehrlich ascites tumor, leukocytes and their precursors are probably the only accessible free-floating, nucleated, mammalian cells. Certainly they are the most easily obtainable human cells. They can be accurately enumerated in the hemocytometer, a notable technical advantage, and can be precisely examined by a variety of well known cytomorphological technics.

Because they can be studied in the human patient, the leukocytes provide one of the few meeting grounds for cellular biochemistry and clinical investigation. These cells have the advantage of day-to-day availability in individuals undergoing changes in clinical and therapeutic status, thus permitting controlled biochemical studies with a dimension in time. In addition to leukocytosis and leukemia, leukocytes apparently participate in certain systemic metabolic disorders such as glycogen storage disease (59). Increased deposition of glycogen in leukocytes is reported to follow ACTH injection in rats (45). One wonders to what further extent leukocytes may participate in systemic metabolic or endocrine processes.

Circulating leukocytes lack many of the disadvantages inherent in solid tumor material, among which are the difficulties in obtaining homogeneous, stroma-free tumor cell preparations, the impossibility of accurately counting the cells of a solid tissue and, hence, the difficulty in expressing data, the uncertainty as to vascular and nutritional uniformity throughout the tumor mass, the possibility of immunological reactions between transplanted tumor and implanted host and the question of appropriateness of choice of normal or control homologous tissue. Morphological identification of cell type of origin may be extremely difficult in anaplastic tumors. This last difficulty is, of course, shared by leukocytes, concerning questions which are constantly arising as to the role of cell type and cell immaturity in determining the biochemical picture. This problem can sometimes be circumvented—e.g., by the study of patients in remission or under varying states of the differential formula, by inference from histochromic data, and by manipulations of the cell suspension which take advantage of the differences in specific gravity and sedimentation behavior of various cell types. The problem which plagues the biochemist studying all tissues, including leukocytes, still remains. It is the problem of somehow connecting, if not causally then by some other consistent correlation, the events of metabolism with the patterns of biological behavior. Here, leukocytes present no advantages, and one always risks the interpretive perils of transitional states which are neither black nor white, hazards which are deeply rooted in the issues of semantics.

**REVIEW OF THE LITERATURE**

The difficulties which arise in attempting to compare the results of different workers are chiefly three: (a) The materials studied differ widely in species of origin, anatomical source, cell type, stage of maturity, and mode of preparation. The literature reviewed will largely include studies on human and animal peripheral blood leukocytes from normal and leukemic individuals and exude leukocytes obtained variously from human and animal empyema cavities, peritoneal exudates, sterile blisters, etc., although mention is made of pertinent collateral studies on solid lymphoid tissue, bone marrow, and "leukemic tumors." Where possible, technical details are given. (b) Differing analytical methods and incubation media have been used. It is concluded here that results are profoundly affected by the composition of the medium. (c) Metabolic data have been referred variously to dry weight, cell numbers, and to cell nitrogen. The authors consider cell numbers to be the most plausible reference basis in leukocyte studies and in reviewing past work we will, where possible, convert data into millimoles per $10^8$ cells per hour. Where the "Q" notation is used (indicating activity/mg dry weight/hour), an empirical factor of 1,960 is used to convert dry weight to $10^{10}$ cells.1

**Glycolysis and respiration.**—It was early known that sugar disappeared from blood on standing, and, in the more primitive days of enzymology and analytical chemistry, this was variously attributed to blood "decomposition," and protein, sugar, or amino acid breakdown. In 1911, Slosse (48) noted that blood contained lactic acid which increased on standing. The source of the lactic acid was shown to be glucose, not protein or amino acids. The following year, Levene and Meyer (81), using quantitative methods, studied the conversion of glucose to lactic acid in leukocytes obtained from the pleural cavity of dogs after prior injection of tur-

1 Obtained from the studies of Bird, Clements, and Becker (9) on nonleukemic cells and data of our own which are substantially in agreement.
pentine, and concluded that all glucose utilized could be accounted for by the lactic acid produced. Glucose was measured reductimetrically and lactic acid gravimetrically. Phosphate was found essential for activity. Because of the contemporary argument whether lactic acid was derived from carbohydrate or protein, this work had general significance. Hematologic data on the leukocyte material are not given, but it is known (14) that such exudate procedures yield a population of cells consisting almost exclusively of polymorphonuclear neutrophils. Levene and Meyer (32), extending their observations, found that fructose, mannose, and galactose, but not pentoses, were transformed into lactic acid by leukocytes.

Rona and Arnheim (46) compared the glycolytic activity of white and red cells and found the latter greater, no doubt because of the larger number of red cells present in blood since MacLean and Weir (36) in 1915 showed, by comparing bloods with varying leukocyte counts, that the ratio of glycolytic activity (per cell) of white to red cells varied from 200:1 to 1000:1. In five experiments on a plasma suspension rich in normal leukocytes and a glucose concentration of 0.0015 M, glucose utilization averaged 3.50 mM/10^{10} cells/hr, as measured by a reducing method.

The first study of the respiratory activity of leukocytes was that of Grafe (24) in 1911. He attempted to explain the marked increase in the total O_{2} consumption of the body in leukemia with data on the gas exchange of the blood leukocytes. The cells were obtained by centrifuging whole blood, removing the plasma, and resuspending red and white cells in a salt solution containing saponin. Using a Haldane-Barcroft apparatus, he found O_{2} consumption rates of 0.087–0.089 ml/10^{6} cells/hr (0.017–0.037 mM/10^{10} cells/hr) in a group of myelocytic and lymphocytic leukemias. Glycolysis was not measured. The gas exchange of the polymorphonuclear leukocyte was considerably higher than that of the mononuclear.

The semi-quantitative studies of Fukushima (20) in 1922 and Löhner (34) in 1926 on glucose utilization by leukocytes obtained from rabbit peritoneal exudate, horse blood, and human empyema pus showed that sugar breakdown continues in vitro for about 72 hours and that the washed leukocytes themselves contain little sugar or reducing substance.

Are Leukemic leukocytes “embryonic” or “malignant” cells?—During this period, the classic studies of Warburg (83) made their appearance. It was observed that slices of cancer tissue possessed a greater capacity for aerobic and anaerobic transformation of glucose into lactic acid than did normal tissue, tumor tissue exhibiting a deficient Pasteur effect. High anaerobic glycolysis was also found in embryonal tissue, suggesting a connection between anaerobic glycolysis and rapid growth. In the embryo, however, glycolysis was largely abolished in oxygen. It was concluded that neoplastic transformation involved fundamental changes in energy-supplying metabolic reactions. A vast number of confirmatory reports strongly implied that here one had criteria for the state of malignancy: high aerobic and anaerobic glycolysis, a low relative Pasteur effect, and a moderately high respiratory rate. The eventual discovery of exceptions, of normal tissues possessing these characteristics such as retina, kidney medulla, and jejunal mucosa, made it clear that the specificity of the tumor metabolism pattern was illusory. An attempt was made to attribute the so-called “cancer pattern” when found in normal tissues to injury or damage in handling. Thus began a long cycle of studies which sought to label the various tissues as normal, injured, cancer-like, or embryo-like.

Bakker (2) believed that leukocytes obtained from rabbit peritoneal exudate (presumably polymorphonuclear neutrophils) behaved like cancer cells, because he observed a moderate oxidative level, a high anaerobic and a fairly high aerobic glycolysis. Using a saline suspension of intact leukocytes, he found in typical experiments that the addition of glucose (0.1 per cent) increased the 1-hour O_{2} consumption by 72 per cent and that the ratio of aerobic glycolysis to respiration was approximately 25:1.

Daland and Isaacs (18) measured the O_{2} consumption of human whole blood in six normal individuals and ten patients with leukemia. Activity in the normal cells was too low to measure and that in the cells of myelocytic leukemia averaged 765 μ1/100 ml of blood/hr. It seemed to the authors that the results could be best correlated with the number of mature polymorphonuclear leukocytes present, that the Q_{o_{2}} of the immature cells was probably low, and, therefore, they resembled tumor cells more than embryonic or young tissue.

This conclusion was disputed by Fujita (19), who observed that leukocyte metabolism resembled that of normal embryonic, not malignant, tissue. He incubated a centrifuged fraction of rat blood containing 60 vol. per cent of intact leukocytes in a citrate-plasma medium and found a Q_{o_{2}} of 9.2 (0.80 mM/10^{10} cells/hr) a Q_{l} of 2.6 (0.56 mM/10^{10} cells/hr), and a Q_{l} of 20.6 (1.80 mM/10^{10} cells/hr), indicating a “replacement” of anaerobic glycolysis by respiration. The ratio of 1Q_{o_{2}} and Q_{l} signify mg of lactic acid produced/mg dry weight/hour in N_{2} and O_{2}, respectively.
Q, to Q, in terms of glucose equivalents was 0.85 to 1. Only two experiments were quoted by the author, and technical details were not given. Presumably, the leukocyte population had the type distribution of average normal rat blood, although this is not made clear. The author stated without qualification that prolonged centrifugation and cooling to 0° C. significantly lowered the leukocyte respiratory rate.

A comparison of the metabolism of blood leukocytes (of geese) with exudate leukocytes (of the rabbit) was made by Fleischmann and Kubowitz (18), using Ringers-phosphate as the incubation medium. It was found that the aerobic glycolysis of exudate leukocytes (Q,g = 14.0) was higher than that of blood leukocytes (Q,g = 1.8). The high aerobic glycolysis rate in exudate was taken to mean that aerobic glycolysis in leukocytes is not proof of the malignant state but due instead to injury, since "leukocytes are dying more rapidly in exudate than in blood." No comment was made on the possible significance of species difference. The reported Q, rates were around 4.5, 10 times higher than those reported by Bakker (3).

A succession of authors in the 1930's continued debating over the possibility of differences between normal and leukemic cell metabolism, on the relationship between cell maturity and cell metabolism and on the issue of whether leukemic leukocytes were indeed malignant, normal, embryonic, or injured. The controversy failed to be resolved. In 1930, Peschel (41) examined the lymphocytes of four cases of human lymphatic leukemia. The cells were isolated by centrifugation and were suspended in 0.016 M bicarbonate containing 160 mg per cent glucose. The mean Q, was 5.8, Q, zero, and Q,s 11.0—seemingly a pure oxidative metabolism with no aerobic glycolysis. This was confirmed by Schlossmann (47), both authors concluding that the lymphocytes of lymphatic leukemia were young normal cells. The appearance of aerobic glycolysis in these cells was an "accompaniment of cell aging and death."

The studies of Bossa (10) on human leukocytes obtained by brief centrifugation showed a high aerobic glycolysis (Q,g = 4.2–37.3) in myelocytic leukemia but a very low aerobic glycolysis in leukemic lymphocytes. Bossa considered that these findings signified, not cancer metabolism, but the results of cell injury. Rin (44) was unable to find any evidence of aerobic glycolysis in normal and leukemic leukocytes and concluded that these cells were of the embryonic type.

The field was reviewed by Kempner (30) in 1939. He studied patients with myelocytic and lymphocytic leukemia and concluded that aerobic glycolysis occurring in mature leukocytes was a symptom of aging and dying. As Fleischmann (17) said, "The aging of a cell in the blood stream or its wandering out of the blood into an exudate results in an aerobic glycolysis."

In attempting to characterize leukemic cell metabolism, Victor and Potter (50) found in the lymph nodes of mice, with spontaneous and transmitted lymphocytic leukemia, consistent increases in both aerobic and anaerobic glycolysis over the values obtained with normal nodes. Hall and Furth (25) presented similar results. Their data showed little difference between the oxygen consumption of lymph nodes of leukemic mice and that of normal lymph nodes.

Effect of cell immaturity.—In 1929, Barron and Harrop (4) undertook to compare the metabolic activities of human granulocytes and nongranulocytes and to determine the possible significance of cell maturity and certain experimental conditions. The authors first found that the metabolism was greatly influenced by the in vitro conditions, respiration being adversely affected by overcrowding of cells in the experimental flask, the use of citrate as an anticoagulant, prolonged experiments, cooling, and centrifugation. These restrictions necessitated quick, mild centrifugation of fresh blood and immediate transfer of an aliquot of cells (red and white) into the respirometer, avoiding cooling. Glucose disappearance was determined by the Benedict method, and glycolysis was assumed equivalent to glucose disappearance. Data were expressed per 10^6 cells. It was concluded that there were no differences in the metabolism of mature and immature granulocytes or of granulocytes from cases of leukocytosis and myelocytic leukemia. The conclusions are perhaps open to question in that myelocytic leukemia and leukocytosis were lumped together as the "polynuclear group," and chronic lymphocytic leukemia and dog lymph lymphocytes as the "mononuclear group." The latter cells were stated to have a higher O_2 consumption than lymphocytes from lymphocytic leukemia, but this was speculatively attributed to crowding in the leukemic material. In five cases of myelocytic leukemia, the average O_2 consumption was 0.80 μl/10^6 cells/hr (0.35 mm/10^8 cells/hr), and the glucose utilization 0.0058 mg/10^6 cells/hr (0.20 mm/10^6 cells/hr). The data suggested that the aerobic glycolytic activity in "polynuclear" cells was 5 times greater than that in the "mononuclear" cell.

Glover, Daland, and Schmitz (22) believed that the metabolism of leukemic whole blood was certainly related to the maturity of the leukocytes. In extremely blastic leukemia, the O_2 consumption
was lower and the aerobic glycolysis greater than in bloods with more mature cells. The authors determined the glycolytic rate by subtracting from the total sugar utilized the amount oxidized, assuming that all oxygen utilized was used for the oxidation of sugar. The $Q_o$ of normal human leukocytes was 7.1 (0.62 mm/10^10 cells/hr) and that of chronic myelocytic leukemic cells 2.5 (0.23 mm/10^10 cells/hr). Lymphocytic leukemic leukocytes varied from 2.4 to 5.8 (0.21 to 0.51 mm/10^10 cells/hr).

The studies of Soffer and Wintrobe (49) failed to confirm Daland and Isaacs, and Glover, Daland, and Schmitz. These workers, studying whole bloods containing varying numbers of cells, concluded that the respiratory and glycolytic rates of leukemic cells were slightly higher than the normal although the differences were small. Furthermore, they could make no correlations with the level of maturity. They found the metabolism of granulocytes to resemble that of malignant tissues and lymphocytes that of normal adult tissue.

Ponder and MacLeod (48) attempted to evaluate the effects on metabolism of cell immaturity by producing rabbit peritoneal exudates in rapid succession, so that the cells became younger and younger due to bone marrow stimulation. The $O_2$ consumption of the first exudate was 0.18 mm/10^10 cells/hr and that of the fourth exudate containing many immature forms was higher by 50 per cent. An increase of 400 per cent could be obtained by repeated exudates in rapid succession.

Keibl and Spitz (27) recently reported studies on a group of human normal and leukemic leukocytes. Cells were isolated by erythrocyte sedimentation, and it is stated that centrifugation was not harmful. The authors compared respiration, and aerobic and anaerobic glycolysis in a group of selected cases showing graded variations in the differential formula. From these studies, differences are reported, for example, between cases of lymphocytic leukemia with 80 per cent lymphocytes and 20 per cent neutrophils and cases with 90 per cent lymphocytes and 10 per cent neutrophils, the latter reportedly showing significantly lower aerobic glycolytic rates. The authors conclude that myeloid cell respiration, both normal and leukemic, increases with increasing maturity. Unfortunately, there is no statement of the standard error encountered in making comparisons such as the above. The authors present only the mean results of their groups, and no mention is made of the large probable error in the differential counts which were determined "by the usual clinical methods."

Effect of added serum and cell crowding.—Workers have varied in their choices of suspending media for leukocyte preparations, some using serum and others different saline solutions. It is not always clear whether the serum medium was normal or leukemic serum, and seldom are the reasons given for the authors' choices. Victor and Potter (58) demonstrated that mouse leukemic serum is typically hypoglycemic. A number of reports have compared the activity of cells with and without added serum. Victor and Potter (37) showed that normal mouse lymphoid and leukemic cells have higher respiratory rates in normal serum than in Ringer's solution, while the anaerobic glycolytic rates are the same in both media. Typical experiments showed $Q_o$'s in leukemic cells of 6.1 (in serum) and 4.9 (in Ringer's); and, in normal cells, 5.5 (in serum) and 4.6 (in Ringer's). The effect of the suspending medium was also studied by MacLeod and Rholes (37). Using suspensions of rabbit peritoneal exudate leukocytes, the authors observed in Ringer's-phosphate an average $Q_o$ of 4.6, $Q_o$ of 17 and $Q_o$ of 25.4 Serum, neutralized with HCl, increased the respiration to a mean $Q_o$ of 7.0, an increase of 50 per cent. Similar findings were reported by Pages and Delaunay (40). The enhancement of respiration reported to occur on addition of serum probably reflects the addition of catalytic ions, coenzymes, and substrates, although Warren (64) failed to identify definitely the stimulatory factor of serum, his evidence indicating the possibility of a dicarboxylic acid.

It has been reported by some that leukocytes are peculiarly susceptible to crowding, i.e., respiratory rates do not increase linearly with an increase in cell numbers in the incubation flask. This problem was encountered by Soffer and Wintrobe (49) and, as mentioned above, by Barron and Harrop (4). Ponder and MacLeod (48) pointedly denied that centrifugation, cooling, or crowding interfered with leukocyte respiration, although the latter authors were dealing with rabbit peritoneal exudate leukocytes and the former with human material.

More recently, Hartman (26), using guinea pig exudate leukocytes in various concentrations, found that a disproportion between $O_2$ consumption per cell and cell concentration occurred only when the cells were suspended in serum but that proportionality existed when the leukocytes were washed and suspended in a physiological salt solution. In a typical experiment a serum suspension containing 0.315 X 10^6 leukocytes consumed 0.06 $\mu l$ $O_2/10^6$ cells/hr (0.48 mm/10^10 cells/hr) and one

$Q_o$ and $Q_o$ signify $\mu l$ of $O_2$ produced by action of formed acid on added bicarbonate/mg dry weight/hr in $O_2$ and $N_2$, respectively.
Effect of phagocytosis.—Baldrige and Gerard (3), working with dog leukocytes in serum, observed that the addition of *Sarcina lutea* caused an increase in *O₂* consumption. The rise began immediately, lasted 10–15 minutes and ceased in 1½–2½ hours. No respiratory change accompanied ingestion of India ink. Ado (1) determined the effect of phagocytosis on the aerobic glycolysis and *O₂* consumption of dog and guinea pig exudate leukocytes in Ringer’s solution. The *Qₙₐₐₐ₈* averaged 5.2 (0.45 mm/10⁶ cells/hr) regardless of the presence or absence of phagocytosis. The so-called *Qₙₐₐₐ₈* (amount of lactic acid produced/mg dry weight of tissue/hr in a system made “anaerobic” with KCN) averaged 0.05 (1.10 mm/10⁶ cells/hr) in the absence of phagocytosis, 0.032 in the presence of starch granules, and 0.017 in the presence of staphylococci. The results obtained by Baldrige and Gerard were confirmed by Delaunay, Pages, and Maurin (15) and by Marinellarena (38).

Effect of therapy.—Keibl and Spitz (28) studied the effects of radiation, urethan, nitrogen mustards, and colchicine on chronic myelocytic leukemia leukocyte metabolism and concluded that observed changes were attributable to therapy-induced alterations in the differential formula. The main change was increased respiration. Glycolysis was affected variably. Reports of various authors have shown that leukocyte respiration is decreased by potassium arsenite (65) and thiouracil (66) and is stimulated by menadione (51) and ascorbic acid (39).

Recent studies on isolated leukocytes.—Using newer technics of leukocyte isolation, measurements were made of *O₂* consumption, glucose utilization, and lactic acid production in human nonleukemic and leukemic material by Bird, Clements, and Becker (9). Hematologic data are not given. Cells obtained by the bovine fibrinogen method (11) were incubated in a mixture of plasma, Ringer’s solution, and added glucose (final concentration, 500 mg. per cent). Measurements were made of lactic acid formed, and glucose was determined by the reduction method of Benedict. Metabolic rates, first calculated per 10⁶ cells/hr, were converted to a dry weight basis by using a universal factor determined separately on 10 specimens of normal cells. The use of this factor (1 mg dry wt. = 5.1 × 10⁶ cells) for both nonleukemic and leukemic cells was justified by the statement that the nitrogen content of both normal and abnormal cells was similar. The data, here reconverted to a reference basis of 10⁶ cells (and to mm), indicated a mean *O₂* consumption of 0.23 in nonleukemic leukocytes and 0.10 in both chronic myelocytic and lymphocytic leukemia. Lactic acid accounted for only 75 per cent of the total acid formed anaerobically by myelogenous leukocytes. The authors believed their data showed no significant difference between the metabolism of nonleukemic and myelocytic leukemia cells. A low glucose utilization and lactic acid formation was found in lymphocytic leukemia leukocytes and was considered statistically significant.

There have been several reports on the effect of varying substrate and coenzyme conditions in leukocyte material. Marinellarena (38) reported the respiratory and glycolytic activities of guinea pig exudate leukocytes with various substrates and inhibitors. Using intact, washed cells suspended in Ringer’s solution, and conventional manometric technic, the author typically found an *O₂* consumption around 0.46 μl/10⁶ cells/hr and a glycolytic rate of 0.89 μl of CO₂/10⁶ cells/hr. Homologous serum benefited respiration. Added glucose, hexose diphosphate, and fructose increased glycolysis, and succinate increased respiration. Glycolysis was decreased 76 per cent by fluoride, 0.1 m, and 88 per cent by iodoacetate, 0.001 m. KCN, 0.001 m, decreased respiration 67 per cent and malonate, 0.04 m, decreased it by 17 per cent.

McKinney, Rundles, and Martin (35) isolated intact normal human leukocytes by the dextran method and found, after incubation in a system containing plasma and Hank’s solution, oxygen consumption of 0.28 mm/10⁶ cells/hr. This value was not increased by addition of citrate, α-ketoglutarate, succinate, fumarate, malate, oxalacetate, or glutamate in final concentrations of 0.01 m. Added cytochrome c (5 × 10⁻⁴ m) did not stimulate the respiration of the intact cells. However, when the cells were disrupted by grinding with alumina gel, stimulation of *O₂* consumption did occur on addition of succinate, α-ketoglutarate, oxalacetate, and cytochrome c. The possible effect of adsorption of enzyme protein by alumina gel is not discussed.

Beck and Valentine (7, 8) studied the respiration and aerobic glycolysis of leukocytes isolated by the fibrinogen method (11) from normal and leukemic blood. Three clinically uniform groups of patients were assembled for study: normals; chronic myelocytic leukemias (average leukocyte count 100,000/cc mm, with 96 per cent myeloid cells, including chiefly myelocytes, metamyelocytes and band forms); chronic lymphocytic leukemias
(average leukocyte count, 124,000/c mm, with 98 per cent lymphocytes). Leukocytes were suspended in alkaline isotonic KCl, homogenized, and incubated in a buffered (pH 7.4), fortified system containing added DPN (0.0007 M), ATP (0.0011 M), cytochrome c (1.4 × 10⁻⁶ M), phosphate (0.01 M), Mg²⁺ (0.005 M), and various substrates. Data were expressed per 10⁹ cells. Lactic acid was measured chemically and glucose utilization by a modification (5) of the glucose oxidase method of Keilin and Hartree (29), which, being more specific than reducing methods, gave demonstrably higher disappearance values. With glucose and hexose diphosphate (0.0053 M) as substrates, the following mean values were found for O₂ consumption, glucose utilization, and lactic acid production in mm/10⁹ cells/hr. (Standard deviations are shown):

<table>
<thead>
<tr>
<th></th>
<th>O₂ consumption</th>
<th>Glucose utilization</th>
<th>Lactic acid production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.40 ± 0.17</td>
<td>1.40 ± 0.18</td>
<td>3.01 ± 1.60</td>
</tr>
<tr>
<td>Chronic myelocytic leukemia</td>
<td>0.18 ± 0.03</td>
<td>0.55 ± 0.28</td>
<td>1.27 ± 0.50</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>0.11 ± 0.01</td>
<td>0.31 ± 0.08</td>
<td>0.48 ± 0.19</td>
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The oxygen consumption, glucose utilization, and lactic acid production are significantly higher in normal than in leukemic homogenates. It is seen that, in this system, leukocytes have a predominantly aerobic glycolytic metabolism. In the presence of glucose and HDP, the ratio of aerobic glycolysis to respiration (in terms of glucose equivalents) is about 30 in normal and myelocytic leukemia and about fifteen in lymphocytic leukemia. In the absence of added substrate, the glycolysis to respiration ratio in normals is 3.5; myelocytic leukemia, 10.0; and lymphocytic leukemia, 1.6. Succinate stimulated oxygen uptake to a greater extent in normal than in leukemic material, and the omission or manipulation of added co-factors profoundly affected metabolic rates: both types of leukemic leukocytes had a greater cytochrome c requirement for maximal oxygen uptake and lymphocytic leukemic leukocytes had a greater DPN requirement for maximal lactic acid production than did normal leukocytes.

Metabolic pathways of leukocytes.—The many reported biochemical differences between normal and leukemic leukocytes suggest possible different pathways of metabolism in the two tissues. Studies, such as the authors', indicating differing responses to added substrates and coenzymes, support this possibility. The intermediary carbohydrate metabolism of leukocytes is being most carefully studied by Wagner and his associates. This group (61) studied the breakdown of glycogen in leukocytes and noted that the reaction was catalyzed by a phosphorylase. In leukocytes isolated by flotation techniques from citrated horse blood, they demonstrated the formation of an intermediary reducing substance in the enzymatic breakdown of glycogen to lactic acid. This could be shown only by use of cell suspensions which had been dialyzed against distilled water. Without dialysis, the reducing intermediate did not accumulate, and the cells utilized inorganic phosphorus.

The presence in the reaction mixture of adenosine-5-phosphate was essential for the formation of the reducing substance. Addition of NaF inhibited lactic acid formation without interfering with glyco-

gen degradation and reducing substance formation. More recent studies (62) demonstrated inorganic phosphorus utilization in dialyzed cells. Organic phosphate intermediates, reducing and non-

reducing, were isolated and identified as glucose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate, phosphoglyceric acid, and fructose 1,6-diphosphate.

DISCUSSION AND SUMMARY

Both the older and the more recent literature would indicate that normal and leukemic myeloid cells are characterized by a substantial aerobic glycolysis. Conversely, lymphatic leukemic lymphocytes and blast forms have low aerobic glycolytic rates. Hence, it is improper to make conclusions regarding the neoplastic nature of leukemia on the basis of their "tumor metabolism."

The concept of "metabolism of injury" is also clearly hazardous, inasmuch as the claim that cells are injured is frequently based on the metabolic results obtained and on no other criteria. The absence of marked metabolic differences between intact cells and homogenates (7) and, in fact, the considerable difficulties in preparing leukocyte homogenates cast further doubt on the role of injury in producing a characteristic metabolic pattern.

It appears that normal myeloid leukocytes possess higher rates of respiration and glycolysis than do leukemic cells. It may be that leukocyte glycolysis suits these cells for existence in the comparatively anoxic environment of exudates. It has been shown (17) that phagocytosis persists in the presence of KCN as long as glucose is present. Fluoride and iodoacetate inhibit phagocytosis. Bone marrow is reported to have a similarly high aerobic glycolytic rate (16) and low oxygen consumption (28).

The problem of precisely what material to use as control in studying leukocyte abnormalities is admitted far from solution. Ideally, leukemic lym-
phocytes should be compared to normal lymphocytes, blasts to blasts, and so on. As yet, such rigorous standards are all but unattainable, and one is forced to make inferences from more indirect approaches.

Leukocytes are extremely sensitive to the composition of the incubation medium. Recent results indicate certain qualitative and quantitative differences in the responses of normal and leukemic material to addition of various substrates and coenzymes. It is hoped that, in the light of more modern biochemical knowledge, further clarification may be brought to this problem.

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