DETAILED STUDY of leukocytes and their metabolism is still a comparatively unexplored field of investigation. In general, three basic technics have been employed. The earliest of these involved the morphologic classification of leukocytes in terms of the conventional methods employed in staining dried films of blood or in terms of wet, supravital preparations, and the characterization of the differential changes in various forms of leukemia, in infection and in other disease entities. The most recent advance brought about by conventional morphology has been the recognition of the lupus erythematosis (L.E.) cells by workers at the Mayo Clinic. Schwind has reviewed current concepts of leukocyte morphology as derived from supravital staining technics.

The second technic, that of selective histochemical localization of biochemical constituents and of enzyme activity, represents a further extension of the conventional morphologic horizons and lends qualitative physiologic significance to morphologic data. Beginning with the work of Gomori on the intracellular localization of alkaline phosphatase, enzymatic activity of leukocytes as well as their nonenzymatic biochemical constituents has been the subject of many investigations. The lipids, nucleoproteins, glycogen, acid and alkaline phosphatase content of leukocytes in various stages of development have all been studied.

It is not the purpose of this review to discuss in detail the large number of important contributions made to the understanding of leukocyte biochemistry by histochemical technics, and only a few of the many papers on the subject are documented. The reader is referred to the reviews of Gersh, Dempsey and Wislocki, and Rheingold and Wislocki. Neither is it intended to discuss here the contributions of the technics of phase microscopy and of physical chemistry to our knowledge of leukocyte physiology.

The third technic employed in the investigation of leukocytes and their metabolism is concerned with the quantitative estimation of biochemical constituents and enzymatic activity by in vitro methods, either on whole blood or on separated leukocytes. It is this phase of study of leukocyte physiology with which this report is largely concerned. Investigations of this type have been greatly facilitated by the recent expansion in the number of readily performed spectrophotometric, cytochemical, and enzymatic methods and, more particularly, by the development of more satisfactory leukocyte separation technics. In our hands, the method described by Buckley et al. has been most useful. It must be recognized, of course, that in vitro studies are open to the criticism that they involve artificial systems outside the body, that where substrates are employed

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in enzyme studies they may not represent the native substrates of the enzyme within the organism, that the complexities of body metabolic processes cannot be reproduced in the test tube, and that the active functions of biochemical substances in the living subject can often not be inferred from data obtained in comparatively simple chemical systems in vitro. None the less, it is possible to establish descriptively a large number of metabolic parameters for leukocytes in both the normal subject and in the subject with a variety of physiologic derangements. Many investigations have dealt with the metabolism of the leukocyte per se without correlation of the biochemical status of the subject with clinical and functional events of the moment. It is becoming increasingly apparent that morphologically identical leukocytes may possess widely varying metabolic patterns, and that at times these patterns seem to possess strikingly uniform correlation with the type of physiologic derangement involved. The emergence of metabolic patterns of leukocytes in different types of disease processes points up the necessity of defining as many parameters as possible in each set of circumstances.

Respiratory and Energy Metabolism

Grafe in 1911 demonstrated that leukocytes consume O2. They split sugar and produce lactic acid from glucose. Bakkar showed that leukocyte respiratory metabolism is less than aerobic glycolysis in exudate white cells, an indication that aerobic glycolysis may be a metabolic phase of cell injury or alternatively that exudate leukocytes resemble tumor cells in which aerobic glycolysis is also high. Schlossman showed that in lymphatic leukemia the leukocytes appear to have a purely oxidative metabolism. He believed that aerobic glycolysis of mature lymphocytes is only a manifestation of dying. Soffer and Wintrobe studied aerobic and anaerobic glycolysis in a series of cases of leukocytoses and acute and chronic myeloid and lymphoid leukemia. Oxygen consumption was found to decrease when the concentration of leukocytes in the blood was high, and the amount of oxygen consumed rapidly decreased as the experiment progressed. No differences in metabolism were noted between mature and immature leukocytes nor between leukocytes of normal and leukemic blood. The oxygen consumption of granulocytes was found to be slightly higher than that of lymphocytes and the glycolytic power of granulocytes was two times greater than that of lymphocytes. It was concluded that the metabolism of granulocytes resembles that of malignant tissues while that of lymphocytes is similar to normal adult tissue. Kempner has extensively reviewed the subject of leukocyte respiratory and glycolytic metabolism. In his studies of leukemic cells myeloblasts and lymphoblasts, in contrast to mature forms, exhibited a purely oxidative metabolism similar to that of uninjured normal young cells rather than that of cancerous tissue. While thus far little additional information as to leukocyte function has been derived from manometric determinations of overall respiratory and fermentative metabolism, the more modern technics involving homogenizations, fortified systems, and manipulation of cofactors, substrates, activators and inhibitors in a controlled manner, offer considerable promise.

Glycogen

Wagner has presented a method of determining blood glycogen and reviewed the literature relative to leukocyte glycogen prior to 1946. The method utilizes
1 ml. of whole blood and involves heating with KOH, alcohol precipitation of glycogen and alcohol washing of the precipitate, hydrolysis of the washed precipitate with HCl, neutralization, and determination of the sugar content by the method of Somogyi. In a companion sample the same procedure is followed except the hydrolysate is fermented with yeast at pH 4–6 prior to the sugar determination. The difference between the two samples represents true glycogen. A similar procedure may be employed with isolated leukocytes. The average glycogen content per 100 ml. of normal human blood was found to be 5.5 mg. No detectable glycogen was found in plasma, erythrocytes, or blood platelets and experiments with leukemic leukocytes of uniform cell type indicated no measurable amounts in blast cells or lymphocytes. The granulated cells from the myelocyte stage on appeared to be the only significant carriers of blood glycogen. In studies on separated leukocytes an average of 2.54 μg. of glycogen per million total leukocytes or 4.23 μg. per million granulated cells was found. Isolated leukocytes contained somewhat less than the expected amount of glycogen and this was shown to result from an increased enzymatic cleavage of glycogen in the separated preparations in contrast to those of whole blood. Some enzymatic loss of glycogen was inevitable in the time required for isolation of the cells.

The same author showed that in all cases of chronic lymphatic leukemia and all cases of blastic leukemia studied the average glycogen content per million WBC was less than 1 μg. (average 0.32 and 0.29 respectively). The glycogen content per ml. of blood was markedly increased in cases of chronic myeloid leukemia, the increase being in almost linear proportionality with that of the number of cells. In polycythemia vera (4 cases), however, the glycogen content per ml. of blood appreciably exceeded normal values, but, in addition, the content per million WBC was very high in three of the four subjects studied. It is of considerable interest that in Von Gierke's disease glycogen up to 25 μg. per million WBC (3.05 per cent) was observed. This is in confirmation of the high values obtained by Bridge and Holt and again indicates that the leukocytes share the general metabolic abnormality in this disease. Both quantitative and histologic evidence suggest that the ability to store energy in the form of glycogen appears in the myelocytic stage and may increase with increasing maturity. The increased glycogen content of the leukocytes in polycythemia vera is unexplained but constitutes a metabolic parameter differing from that in normal subjects or subjects with chronic myeloid leukemia. A qualitative increase in granulocyte glycogen in polycythemia has also been demonstrated by histochemical staining technics. Other histochemical studies dealing with glycogen have been mentioned. There is some disagreement as to whether reducing substances observed in platelets and lymphocytes histochemically are actually glycogen or, as Wagner contends, nonglycogen material. Dempsey and Wislocki have commented upon the possible relationship of alkaline phosphatase to glycogenesis.

Histamine

Histamine or some pharmacologically indistinguishable substance has for a number of years been known to exist in bound form in the cellular components of blood. It has been clearly shown that in man this constituent is present almost entirely within the myeloid leukocytes. Histamine can be released from its
pharmacologically inactive form within the leukocytes by trichloroacetic acid and, after suitable preparation, assayed utilizing the guinea pig ileum as the assay object. The method employed in this laboratory has been Code's modification of the technic of Barsoum and Gaddum. Normal values vary slightly from laboratory to laboratory but in general are below 0.1 \( \mu g \) per ml. of whole blood. The histamine content per \( 10^6 \) myeloid leukocytes averages approximately .02 \( \mu g \) per \( 10^6 \) myeloid cells. Low to normal values are found in a wide variety of clinical conditions with the exception of allergy, chronic myeloid leukemia, polycythemia vera and terminal liver disease. Values are variable or only moderately elevated in allergy and in terminal liver disease. However, in chronic myeloid leukemia tremendous elevations up to 250 times the upper limits of normal have been observed. In polycythemia vera a pattern similar to, but less dramatic, than that seen in chronic myeloid leukemia, is often observed in those cases presenting with leukocytosis. Blast cells and lymphocytes appear to possess little or no histamine. The eosinophil seems to have no special significance as a carrier of blood histamine. Subjects with chronic myeloid leukemia possess the metabolic capacity of incorporating histamine in normal or increased amounts into myeloid leukocytes almost ad infinitum. With therapy, a falling count is accompanied by a fairly proportional fall in blood histamine, but the metabolic abnormality does not depend solely upon the increased numbers of leukocytes nor upon the presence of cell immaturity. Individuals with normal counts and a differential picture temporarily indistinguishable from normal continue to exhibit substantially elevated total and unit cell histamine values. In marked contrast elevations in leukocyte count associated with infection and malignancy, even when the blood picture is leukemoid, show low to normal total blood histamine values and, consequently, unit cell histamine values are often far below the normal range. In polycythemia vera the total blood histamine is elevated in the presence of leukocytosis and the unit cell histamine low normal to normal. The data emphasize the markedly different biochemical constitutions of the leukocytes in superficially similar circumstances. The physiologic role of granulocyte histamine is not known. It is of interest that where granulocytes are the major components of exudates vasodilatation, redness and heat are often prominent, whereas exudates composed largely of mononuclear cells are referred to as "cold abscesses" and are usually devoid of these features.

**Sulfhydryl Content**

Contopoulas and Anderson have reviewed the literature relative to sulfhydryl content of leukocytes including the possible role of \(-SH\) groups in the control of cell division. To this they have added data on 6 normal subjects, 3 patients with chronic and 1 with acute lymphatic leukemia, 4 with chronic myeloid leukemia, and 1 with agranulocytosis. Normal reduced GSH values averaged 3.91 ± 0.14 mg. per 100 Gm. wet weight of leukocytes. The corresponding values of oxidized GSH ranged from 0.13 to 0.28 mg. per 100 Gm. In acute lymphatic leukemia the markedly elevated value of 15.00 mg. per 100 Gm. of tissue was obtained. The patients with chronic lymphatic leukemia likewise exhibited increases in leukocyte \(-SH\) content ranging from 7.15 to 8.12 mg. per 100 Gm. The sub-
jects with chronic myeloid leukemia showed variations between the chronic and acute lymphatic leukemias, while the subject with granulocytic aplastic anemia showed values below the normal range. Further, after epinephrine injection the —SH components increased more promptly and to a greater degree in leukemia than in normal individuals. The authors point out the possible diagnostic and prognostic implications of these observations and raise the question as to whether high cellular sulfhydryl components suggest malignancy or immaturity of leukocytes. In any event, definite alterations in these constituents occur in leukemia and in granulocytopenia, and they constitute another parameter for evaluating leukocyte metabolism.

**Enzymes**

Many enzymes have been found in circulating leukocytes including amylase, lysozyme, lipase, proteolytic ferments, catalase, nucleotidase, phoshpatase and β-glucuronidase. Barnes and Rebuck have reviewed many of the contributions to the subject. However, much of the data is qualitative and much is uncorrelated with the physiologic status of the subjects whose leukocytes were examined. This review will consider chiefly recent quantitative observations in normal human subjects and subjects with a variety of hematopoietic abnormalities.

**The Phosphatases**

Kay in 1929 first suggested that phosphatase may be contained in leukocytes. In the next few years several investigators were able to demonstrate phosphatase activity in both granulocytes and lymphocytes obtained from animals. Iwatsuru and co-workers found strong phosphatase activity in the blood of a patient with chronic myeloid leukemia and normal values in acute lymphatic leukemia. They also concluded that eosinophils as well as neutrophils contain large amounts of phosphatase. A large number of investigators have noted the presence of acid and alkaline phosphatase in fixed, specially stained preparations of leukocytes. These histochemical observations will not be reviewed here except to mention Wachstein’s observation that the intensity of reaction disclosed by alkaline phosphatase staining technics appeared qualitatively increased in subjects with infection and decreased in chronic myeloid leukemia. Rossiter has shown that such surface active substances as saponin increase the amount of demonstrable phosphatase activity of leukocyte preparations in vitro. Using isolated leukocytes, saponin and disodium phenylphosphate as substrate, Haight and Rossiter found alkaline phosphatase to reside chiefly in the polymorphonuclear leukocytes and acid phosphatase chiefly in the lymphocytes of normal subjects. Acid phosphatase was also thought to be present, though in lesser concentration, in the granulocytic cells. No data are recorded on the leukocytes of subjects with dyscrasias. Quantitative analysis of alkaline and acid phosphatase activity in leukocytes from normal subjects and patients with acute and chronic leukemias, with leukocytoses of various etiologies, and with a variety of miscellaneous hematologic abnormalities have recently been completed in this laboratory. Employing Rossiter’s saponin technic but utilizing sodium β-glycerophosphate as substrate, phosphatase determinations...
were made on separated leukocytes at pH 9.9 and pH 5.0. In 23 normal subjects the alkaline phosphatase activity expressed as mg. of P liberated per hour by $10^{10}$ leukocytes ranged from 13.4 to 58.0 with a mean of 25.8. In striking contrast the similarly expressed range in subjects with leukocytosis was 35.4 to 276.5 with a mean of 119.1, nearly five times that of normal subjects. This was true, further, for a time after antibiotic therapy had returned the leukocyte count to normal in subjects with acute infections. On the other hand, the values in 22 determinations on 14 subjects with chronic myeloid leukemia, with one exception, were very low ranging from 0.0 to 14.4, the mean being 4.0. The single exception exhibited higher than normal values and also was atypical as regards the histamine content of his blood.

This may represent a variant in the usual metabolic pattern of the disease. Further, the characteristic pattern persisted in treated subjects with essentially normal leukocyte pictures, and in the few cases where it was possible to observe the effects of superimposed infections, only slight elevations were observed (highest 14.4). In acute leukemias and in chronic lymphatic leukemia the alkaline phosphatase values were low, while in cases of polycytemia vera with leukocytosis the pattern resembled that of leukocytosis of infection. The acid phosphatase content of leukocytes showed less striking variation, tending to be normal in leukocytosis and normal to high in chronic myeloid leukemia. Low values were found in chronic lymphatic and blastic leukemias. In a single case of agnogenic myeloid metaplasia of the spleen, high alkaline phosphatase values very different from the low values of chronic myeloid leukemia were found. Again, the quantitative data on phosphatases points up the widely different biochemical constitution of leukocytes under different circumstances.

**Beta-glucuronidase**

Fishman and co-workers have reported marked glucuronidase activity in the buffy coat layer of human blood. Most of the activity was considered to be in the leukocytes rather than in the platelets. Rossiter and Wong, in addition to presenting data on the content of this enzyme in rabbit leukocytes, have reviewed the literature on $\beta$-glucuronidase and speculated upon its possible role in the leukocyte in the tissue reactions of inflammation. In a subsequent study, data were presented indicating that approximately 15,000 units of glucuronidase activity per $10^{10}$ leukocytes could be demonstrated on the average under the conditions of their experiments. A unit is defined as the amount of enzyme capable of liberating 1 $\mu$g of phenolphthalein from the substrate (phenolphthalein glucuronide) in one hour. Statistical studies indicated that the activity may be fairly evenly divided between polymorphonuclear leukocytes and lymphocytes. Since the enzyme appears to be largely concerned with the hydrolysis of glucuronides, Rossiter and Wong postulate it may be concerned with hydrolysing some glucuronic acid derivative normally present in tissues. Anlian and associates found 1000 to 3000 units $\beta$-glucuronidase per Gm. of buffy coat in normals and nonleukemic patients. In chronic lymphatic and myelogenous leukemia, values were well below 1000 units, rising to normal with successful treatment. However, in acute and terminal chronic myelogenous leukemia,
levels rose as high as 9000 units. Values were normal in acute lymphatic leukemia.

Peptidase

Stern and co-workers have reported on the hydrolysis of glycylglycyglycine by components of human blood. The leukocytes volume for volume were 500 times as active as plasma, and cell for cell, the leukocytes of young people were 40 times more active than erythrocytes. In senile subjects, the rate of hydrolysis of substrate by leukocytes was about 70 per cent greater on the average than that of young subjects.

Trace Metals

Gibson et al. (quoted by Vallee and Altschule) found the zinc content per unit of leukocytes to be sharply reduced in leukemic subjects with elevated counts. This rose as the count was reduced by therapeutic measures. The role of zinc and other trace metals in leukocyte metabolism is not understood.

Discussion

It has been impossible to document this review with all of the contributions made to our knowledge of leukocyte metabolism. It is apparent from the material recorded that morphologically identical leukocytes present substantially different metabolic patterns under varying physiologic and pathologic circumstances. These patterns in some instances appear highly characteristic of the disease process. For example, unit cell histamine is markedly increased in chronic myeloid leukemia and greatly reduced in neutrophilic leukocytosis and leukemoid reactions, while the converse is true of alkaline phosphatase activity. The histamine metabolism of leukocytes in polycythemia vera suggests a kinship with leukemia, while the alkaline phosphatase activity in this condition resembles that of the leukocytes of infection in cases presenting with elevated leukocyte counts.

Similar differences are described for other metabolic parameters. These have obvious implications in regard to the diagnosis and prognosis of certain dyscrasias, yet the usual morphologic criteria will certainly be adequate in most instances from the clinical standpoint. More important are the possible relationships of these variations to physiologic derangements. For example, if the usual marked elevation of leukocyte alkaline phosphatase activity in infection is a purposeful reaction enhancing resistance, does the converse situation of low alkaline phosphatase in chronic myeloid leukemia relate to the vague lowered resistance of subjects with this condition?

Also, since the leukocytes possess many enzyme systems in common with other body tissues and since, as in glycogen storage disease, they may share a general metabolic abnormality, the possibility of studying certain derangements in terms of multiple biopsies of this readily available tissue is an intriguing possibility. The investigation of leukocyte metabolism is at present largely in the descriptive phase. For the most part, the significance of the findings is poorly understood or not understood at all. The findings present, however, a challenge for the future. Emerging metabolic patterns may ultimately clarify some of our
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concepts of leukocyte function and eventuate in a better understanding of the derangements inherent in the leukemias. It is to be hoped that future work may establish clues for rational therapy.

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