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BLOOD ENZYMES IN CANCER AND OTHER DISEASES*

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I. Introduction

The recognition and description of the enzymes that mediate the various steps in the intermediary metabolic sequences in tissues have constituted one of the great advances in biochemistry during the past 30 years. Many of the studies involved in this development were performed on unicellular organisms and animal tissues, but by the doctrine of the unity of biochemistry were assumed to apply to man. If metabolic processes and the associated enzyme activities in tissues could be reflected in the blood of man, the ready and repeated availability of this fluid would add much to the dynamic study of disease. Analytical studies of the blood for metabolites, frequently evanescent in character and variable in concentration, have played a definite but limited share in this respect.

The investigation of blood enzymes has followed a variable course. A few enzymes, such as the alkaline and acid phosphatases, received much attention because of their diagnostic applicability. The study of the glycolytic enzymes in cancer was given impetus by the suggestion of Warburg and Christian (1943b) that the excessive glycolytic activity of neoplasms might be manifested by the passage of various enzymes of this metabolic sequence into the blood. But, as will be pointed out more fully later, these serum enzyme activities were also found to be altered in other diseases, failed to achieve the specificity of the alkaline and acid phosphatases, and hence have had only limited applicability. On the other hand, it has been shown that in some hereditary diseases the genetic enzymic defects may be reflected in the formed elements or indeed in the serum of blood. The study of the activities of these enzymes has contributed to an understanding of the mechanisms that are involved in this group of disorders.

The present paper will review our available information concerning the enzymes present in the formed elements of blood, in the plasma and serum and, where pertinent, will relate these to the corresponding enzymes in the tissues and other body fluids. It will attempt to consider the origin of these enzymes and the mechanisms regulating their concentration in the blood. It will be especially concerned with the alteration of these enzyme activities in cancer and, to some extent, in other diseases, and with the application of these alterations in diagnosis and

management. Finally, it is hoped to evaluate briefly the channels for future investigations of blood enzymes.

II. Enzymes in the Erythrocyte

A. Localization of Enzymes in the Erythrocyte

The structure of the mature erythrocyte has been under consideration for many years. The fixed framework in these cells has been considered to be the stroma, or the material remaining after treating washed erythrocytes with hypotonic solutions to leach out the hemoglobin and after washing and centrifuging such ghost material. According to Ponder (1948) examination of the erythrocyte in various ways by the ordinary or electron microscope has failed to determine whether there is a surrounding membrane or an internal matrix which maintains the contents of the erythrocyte in its usual shape. Conductivity measurements indicate that much of the stroma is arranged in a surface ultrastructure. The erythrocyte does not possess mitochondria or microsomes (Altman, 1959).

Since some of the enzymes in the erythrocyte may be firmly bound to the stroma and particularly to that portion making up the surface, a few details about the chemical composition are relevant. In man, the stroma constitutes about 3.4% by dry weight of the total material in the red cell. The average total lipid content is about 4.7 mg. per milliliter, and the protein is about 8.6 mg. per milliliter of stroma. According to Ponder's calculations (1948), these components would provide a lipoprotein layer about 70 to 80 Å thick at the surface of the cell.

The following enzymes and enzyme systems have been found to be bound to the stromal portion of the erythrocyte: peptidases, acetylcholinesterase, adenosinetriphosphatase, diphosphopyridine nucleotidase. In contrast, the enzymes of the glycolytic sequence, the pentose phosphate oxidative pathway and the uridyl transferase pathway are, in general, found in the interior of the erythrocyte. There is some evidence that some enzymes of the glycolytic pathway, such as glyceraldehyde-3-phosphate dehydrogenase may on occasion be located on the cell surface and may play a role in the transport of metabolites into and out of the erythrocyte. Such a function may, of course, be postulated more definitely for the stromal enzymes.

B. The Glycolytic Sequence

The formation of lactic acid from glucose in shed whole blood appears to have been known for many years. In recent studies Bird (1947) reported that heparinized whole human blood produced an average of

49 cu. mm. of CO_2 per hour per milliliter of blood anaerobically, and 43 cu. mm. aerobically. The glycolytic capacity, Q_G, expressed as the number of cubic millimeters of CO_2 produced in one hour per milligram of red cell dry weight, is 0.28 under anaerobic conditions, and 0.25 aerobically. The contribution of white cells to these values was calculated as being no greater than about 0.04 cu. mm. As is well known, the glycolytic capacity of most normal adult tissues is zero in the presence of oxygen and ranges between about 3 and 8 cu. mm. in anaerobiosis (Burk, 1939). The glycolytic enzymes that have been found to be present in human red cells and their activities are listed in Table I.

TABLE I

ENZYMES OF THE GLYCOLYTIC SEQUENCE IN THE HUMAN ERYTHROCYTE[a]

Enzyme	Activity as μmoles substrate utilized per hour per 10^{11} RBC	Reference
Glucokinase	20	Bartlett and Marlow (1951)
Phosphoglucose isomerase	9,000	Bruns and Vahlhaus (1956)
Phosphofructokinase	384	Blanchaer *et al.* (1955) Altman (1959)
Aldolase	549	Löhr *et al.* (1958)
Triosephosphate isomerase	46,130	Löhr *et al.* (1958)
D-Glyceraldehyde-3-phosphate dehydrogenase	2,610	Löhr *et al.* (1958)
3-Phosphoglycerate kinase	1,630	Löhr *et al.* (1958)
3-Phosphoglyceromutase	?	Not recorded
Enolase	1,180	Löhr *et al.* (1958)
Phosphopyruvate kinase	2,400	Löhr *et al.* (1958)
Lactate dehydrogenase	10,200	Löhr *et al.* (1958)

[a] Determinations by Löhr were at 25°C. and pH 7.4. The activity for phosphofructokinase was determined at 36°C. and the value is that of Altman.

Although the reactions that have been listed above are on the direct pathway toward the formation of lactic acid, there are a number of side reactions which are of interest. These will now be discussed briefly.

C. PHOSPHOGLUCOMUTASE

This enzyme mediates the conversion of glucose-1-phosphate, the initial product of the phosphorolytic change of starch or glycogen, to glucose-6-phosphate. At equilibrium the ratio of glucose-1-phosphate to glucose-6-phosphate at 37°C. is 94:6 (Colowick and Sutherland, 1942). The action of phosphoglucomutase thus serves to bring glucose-1-phosphate or, through it, any of its precursors into the glycolytic sequence.

Although glucose-1-phosphate has not been found to be present in the human erythrocyte, the concentration of glucose-6-phosphate is 0.08–0.10 μmoles per liter of erythrocytes (Bartlett, 1959). The activity of phosphoglucomutase is 31 μmoles of substrate converted per hour per milliliter erythrocytes at 37°C. (Noltmann and Bruns, 1958). This is equivalent to approximately 280 μmoles substrate changed in one hour by 10^{11} erythrocytes. The phosphoglucomutase activity per milliliter serum is much less—about 0.5 μmoles substrate converted.

D. The Rapoport-Luebering Cycle

The erythrocytes of man and many other mammalian species contain a high concentration of 2,3-diphosphoglyceric acid. In man half of the total organic acid-soluble phosphorus, approximately 55 mg. phosphorus per 100 ml. erythrocytes, is in the form of this compound (Rapoport and Guest, 1941). Rapoport and Luebering (1950) found that rabbit erythrocytes, which also have a high concentration of 2,3-diphosphoglyceric acid, contain a specific diphosphoglyceromutase which mediates the following interaction:

$$\text{1,3-diphosphoglycerate} \rightleftharpoons \text{2,3-diphosphoglycerate}$$

A diphosphoglycerate phosphatase, also present in the erythrocytes, splits the product of the reaction into 3-phosphoglycerate and inorganic phosphate. According to Altman (1959), the endergonic reactions utilizing ATP and the ATPase system regulating the concentration of ADP and ATP have atrophied in the mature human erythrocyte. The Rapoport-Luebering cycle thus affords a channel for "wasting" energy and a bypass from 1,3-diphosphoglycerate, the product of the action of D-glyceraldehyde-3-phosphate dehydrogenase, to 3-phosphoglycerate, the substrate for phosphoglyceromutase action. ATP and 1,3-diphosphoglycerate do not accumulate, and the concentrations of ADP and inorganic phosphate do not fall—events which would retard or even halt glycolysis.

E. Changes in Activities of Enzymes of Glycolytic and Associated Sequences in Disease

The over-all glycolysis in erythrocytes is usually increased in anemias due to a hemolytic process. Hollingsworth (1955) obtained an average value in normal subjects of 43 (± 8.8) mg. glucose utilized per 100 ml. of packed cells per hour at 37°C., and noticed the following typical values in various patients: hereditary nonspherocytic hemolytic anemia, 77; hereditary spherocytosis before splenectomy, 96; sickle-cell anemia, 64, 151; homozygous C-hemoglobin disease, 156; carcinoma of cervix with acquired hemolytic anemia, 162. The increase in rate of

glycolysis was generally, but not always, proportional to the number of reticulocytes present. However, a decreased rate of glycolysis has been observed in hemolytic anemia without spherocytosis (Selwyn and Dacie, 1954), in idiopathic thrombocytopenia (Vaccari *et al.*, 1959) and in metabolic acidosis (Mackler and Guest, 1953).

The glycolytic activity of erythrocytes was reported by Ultmann *et al.* (1957) to be, in milligrams of glucose consumed per 5 million erythrocytes per hour, 11.8 ± 1.6 mg. in 19 control patients and 14.2 ± 2.9 mg. in 27 patients with biopsy-proved malignancy. The elevation was considered to be statistically significant. The mean reticulocyte counts of the two groups were 0.9% and 0.8%, respectively. These investigators pointed out, as had Hollingsworth, that the red cell population might be younger in patients with cancer, yet be unassociated with reticulocytosis. Increased erythrocytic glycolysis also occurs in patients with chronic disease.

The individual erythrocytic enzymes of the glycolytic and associated sequences have also been assayed in various disorders. Merten and Ess (1958) reported that the activities of phosphoglucomutase, phosphoglucose isomerase, and aldolase were elevated in anemias, but did not note an increase for lactate dehydrogenase. Bock *et al.* (1958) found that the activities of triosephosphate isomerase, D-glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, enolase, phosphopyruvate kinase, and lactate dehydrogenase were increased in cirrhosis and in hepatic coma, but only lactate dehydrogenase was increased in hepatitis and obstructive jaundice. In view of the increased glycolytic rate of erythrocytes in cancer, it would not be surprising to find increased activities of the individual glycolytic enzymes.

F. Respiration of the Erythrocyte

The respiration of the adult mammalian and, more specifically, of the human erythrocyte, is extremely low, if not actually absent. Whereas most tissues take up 5 to 20 ml. of oxygen per milligram of dry weight in one hour (Q_{O_2} value), the washed human erythrocyte has been reported to have a Q_{O_2} of only 1% or less of this value or about 0.05 (Ramsey and Warren, 1930; Damble, 1933). The question may well be raised whether even the small oxygen uptake exhibited by the washed erythrocytes may not be entirely due to entrapped reticulocytes that are normally present to the extent of about ½ to 1% of the total erythrocytes. However, as Harrop and Barron (1928) demonstrated, the addition of methylene blue in even as low a concentration as 0.005% to 0.0005% in a suspension of erythrocytes caused an enormous increase in oxygen consumption—to values about fortyfold the normal value. This

observation indicates, as will presently be shown in greater detail, that a sequence of enzymes required for oxidation is present in the erythrocyte but is not normally operative. These are the enzymes of the glucose-6-phosphate oxidation sequence.

G. The Glucose-6-Phosphate Oxidation System

This sequence is also known as the monophosphate shunt, the phosphogluconate oxidation pathway or the pentose phosphate cycle. The series of reactions may be briefly summarized as follows (Racker, 1957; Dickens, 1958): glucose-6-phosphate → 6-phosphogluconolactone → 6-phosphogluconate → ribulose-5-phosphate and CO_2. The D-ribulose-5-phosphate may then go either to D-ribose-5-phosphate as the result of phosphoribose isomerase action, or to D-xylulose-5-phosphate through the action of phosphoribulose epimerase. These reactions constitute a cycle, for the two pentoses interact reversibly through the mediation of transketolase and thiamine pyrophosphate as cofactor to form D-glyceraldehyde-3-phosphate and D-sedulohептulose-7-phosphate; the formation of D-glyceraldehyde-3-phosphate constitutes a re-entry to the glycolytic sequence. Moreover, the D-glyceraldehyde-3-phosphate and D-sedoheptulose-1-phosphate interact reversibly under the influence of transaldolase to form D-erythrose-4-phosphate and D-fructose-6-phosphate. The formation of the latter constitutes another link with the glycolytic sequence.

The activities of these enzymes, expressed as μmoles of substrate utilized per hour per 10^{11} erythrocytes, have been determined as follows: glucose-6-phosphate dehydrogenase, 848 (Löhr *et al.*, 1958); phosphoribose isomerase, 27,300 to 50,000 (Bruns *et al.*, 1958a); phosphoribomutase, 3140 (Guarino and Sable, 1955) transketolase, 24 to 67 (Bruns *et al.*, 1958b). Marks (1958) has submitted a value of 0.66 for the activity of phosphoketopentose isomerase, in terms of the change in optical density at 340 mμ per minute per 10^6 erythrocytes.

In spite of the presence of these enzymes in the erythrocyte, glucose is not oxidized through this pathway. The failure of this pathway to operate is chiefly due to the following factors. First, methemoglobin reductase and glutathione reductase are not sufficiently active to regenerate enough TPN from TPNH for the continued action of glucose-6-phosphate dehydrogenase and phosphogluconate dehydrogenase. Secondly, no electron transport system is available in the erythrocyte through which molecular oxygen can be utilized for the reoxidation of TPNH. The addition of methylene blue to erythrocytes supplies a means for this reoxidation. Glucose may then be utilized, and the oxygen uptake increases greatly.

Several of the enzymes in the glucose-6-phosphate oxidation sequence are altered in pathological processes. Hockwald and his associates (1952) found that the administration of the antimalarial compound primaquine produced intravascular hemolysis in about 10% of Negroes, but rarely in Caucasians. The erythrocytes of persons sensitive to primaquine were found to possess the following characteristics: low reduced glutathione, glutathione instability, decreased or absent glucose-6-phosphate dehydrogenase activity, and increased activities of glutathione reductase and aldolase (Alving *et al.*, 1959). Tarlov and Kellermeyer (1959) have more recently found that catalase is also reduced.

The genetic character of primaquine-sensitive hemolytic anemia was established by further studies of the erythrocyte enzyme and enzyme-linked reactions. When erythrocytes are incubated with 5 mg. acetylphenylhydrazine per milliliter, the content of reduced glutathione (GSH) of the erythrocytes in normal persons changes negligibly in 2 hours. In drug-sensitive individuals GSH decreases from a normal level of 50 to 80 mg. per 100 ml. of packed erythrocytes to 20 mg. or less per 100 ml. of packed erythrocytes (Beutler, 1957). Beutler (1959) noted that about 9% of Negro males, but none of a large group of Caucasians, exhibited this decrease in GSH. The remaining Negroes and all of the white males had GSH values of over 40 mg. per 100 ml. packed erythrocytes after the 2-hour incubation period. In another survey by Childs *et al.* (1958), 14% of Negro males and only 2% of females had values below 22 mg. per 100 ml., and 2% of males and 5% of females had values in the zone between 23 and 40 mg. per 100 ml. More detailed considerations have shown that GSH instability, and hence primaquine sensitivity, were transmitted as a sex-linked recessive.

The deficiency of erythrocyte glucose-6-phosphate dehydrogenase and its hereditary nature were demonstrated by Carson *et al.* (1956) in persons sensitive to primaquine-induced hemolytic anemias and by R. T. Gross *et al.* (1958) in subjects sensitive to fava beans and to naphthalene, and in occasional individuals with anemia of unknown cause. The enzyme deficiency was encountered in 4.6% of an unselected population of 305 subjects, and the defect was more common among Negroes (7.2%) than among healthy Caucasians (1.3%). The deficiency was consistently associated in adults with GSH instability.

The activities of enzymes in the aging cell have been the subject of several studies (Löhr *et al.*, 1958; Marks *et al.*, 1958). Of the important enzymes involved in the erythrocyte glucose metabolism, glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, and phosphohexose isomerase are relatively high in the young erythrocyte and diminish markedly with aging *in vivo*.

There appear to be no specific studies on the changes of the activities of the enzymes of this sequence in patients with cancer. As has been noted, erythrocyte enzyme activities tend, in general, to rise in reticulocytosis. The activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase are elevated in patients with reticulocytosis (Marks *et al.*, 1958) and insofar as patients with neoplasm would fall into such a group, they too might be expected to show elevated levels of these two enzymes.

H. Enzymes of the Citric Acid Cycle

The erythrocyte does not contain the complete functioning citric acid cycle. The presence of malic dehydrogenase has been noted, and its electrophoretic properties studied (Vesell and Bearn, 1958), but evidence for the other enzymes of the cycle is either vague or absent.

I. The Uridyl Transferase System

Lactose, the disaccharide found in the milk of mammals, is hydrolyzed to glucose and galactose, and the latter monosaccharide passes into the stream of carbohydrate through the following series of reactions (Leloir, 1951):

$$\text{galactose} + \text{ATP} \xrightleftharpoons{\text{galactokinase}} \text{galactose-1-phosphate} + \text{ADP}$$

$$\text{galactose-1-phosphate} + \text{uridine diphosphate glucose} \;\underset{\text{uridyl transferase}}{\rightleftarrows}\; \text{glucose-1-phosphate} + \text{uridine diphosphate galactose}$$

$$\text{uridine diphosphate galactose} \xrightleftharpoons{\text{epimerase}} \text{uridine diphosphate glucose}$$

$$\text{pyrophosphate} + \text{uridine diphosphate glucose} \xrightleftharpoons{\text{pyrophosphorylase}} \text{uridine triphosphate} + \text{glucose-1-phosphate}$$

V. Schwarz *et al.* (1956) showed that the feeding of milk to a galactosemic child was associated with an abnormal accumulation of galactose-1-phosphate in the erythrocyte; this compound was not found in normal children under comparable circumstances. It was soon demonstrated that the accumulation of galactose-1-phosphate was due to a virtual absence of the galactose-1-phosphate uridyl transferase from the erythrocyte. The activities of the remaining enzymes in the sequences were present at normal levels (Kalckar *et al.*, 1956; Isselbacher *et al.*, 1956). The transferase is also absent or present in very small amounts in the liver of the patient with galactosemia. It is of compelling interest that the erythrocyte which, of course, does not bear the general burden of over-all metabolism as does the liver, should reflect a metabolic deficiency. The opportunity for the presence or absence of the transferase