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Hexokinase and TPN-Dependent Dehydrogenases of Leucocytes in Leukaemia and Other Haematological Disorders

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It has been known for a long time that leukaemic leucocytes undergo marked changes involving their glycolytic and oxidative metabolism; for example, their aerobic glycolysis and respiration, when studied in phosphate medium, decrease to about one-third (Beck and Valentine, 1953), mainly as a result of a lower hexokinase activity (Beck, 1958a)). This enzyme seems to be the rate-limiting factor of the glycolytic pathway, both in normal and in leukaemic leucocytes.

Beck (1958b) also reported decreased leucocyte enzyme activity in chronic myelocytic and lymphocytic leukaemia with respect to several other glycolytic enzymes and also glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, both of which require co-enzyme II (triphosphopyridine nucleotide, TPN, Beck, 1958b).

These results have been partially confirmed by our previous studies (Ghiotto, De Sandre and Perona, 1960). In this paper we present some data concerning the enzymic pattern of the leucocytes of 68 patients affected by different haematological disorders. Hexokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase and isocitric dehydrogenase activities have been determined in chronic myelocytic leukaemia, chronic lymphocytic leukaemia, acute leukaemia, Hodgkin's disease and osteosclerotic anaemia.

MATERIAL AND METHODS

All the patients were hospital in-patients. The clinical diagnosis was supported by histological study of bone marrow or lymph node, or both. Some patients were under treatment (1-4,dimethanesulphonyloxybutane, prednisone, nitrogen mustard).

Control subjects were selected from a group of individuals in good health and without a history of known haematological disease.

The enzymic activities were measured on samples of leucocytes from the peripheral blood separated from red cells by sedimentation in dextran, and disrupted by freezing and thawing four times. Our procedures, given in detail elsewhere (Ghiotto, De Sandre and Cortesi, 1959; Cortesi, Perona, Ghiotto and De Sandre, 1962) were based on the photometric determination of the rate of reduction of TPN measured at $\lambda 366$ mμ, in an assay system containing the leucolysate of $2.5–3.0 \times 10^6$ cells (in 0.5 ml. isotonic KCl) in a total volume of 2.5 ml. (Table I).

The assays were not significantly affected by the presence in the samples of two to three red cells for each leucocyte before lysis, erythrocyte enzymic activities being greatly inferior to those of leucocytes (about one-fiftieth).

The enzyme activities are expressed as the change in extinction at 366 mμ (Δ_E) per minute per 10^9 cells.

RESULTS

Enzyme Activities of White Cells in Different Haematological Disorders

Hexokinase (Table II). The average activity was decreased in chronic lymphocytic and in chronic myelocytic leukaemia, normal in Hodgkin's disease and osteosclerotic anaemia, and variable in acute leukaemia. In a single case of basophilic leukaemia the activity was markedly increased.

TABLE I

COMPOSITION OF REACTION MIXTURES IN ENZYME ASSAYS

All concentrations are expressed as Moles per ml.

Enzyme substrate used	*Enzyme to be assayed*			
	Dehydrogenases			*Hexokinase*
	Glucose-6-phosphate (sodium salt)	*6 Phosphogluconate (sodium salt)*	*Isocitrate (sodium salt)*	*Glucose*
Substrate conc.	8×10^{-6}	8×10^{-6}	4.8×10^{-7}	8.3×10^{-6}
TPN	2.4×10^{-7}	2.4×10^{-7}	2.4×10^{-7}	3.75×10^{-7}
$MgCl_2$	1.6×10^{-5}	1.6×10^{-5}	—	1.3×10^{-5}
$MnCl_2$	—	—	5.2×10^{-7}	—
NaF	—	—	—	1.7×10^{-6}
ATP	—	—	—	1.6×10^{-6}
Glucose 6-phosphate dehydrogenase (Boehringer)	—	—	—	Dilution 1/100
pH of TRIS buffer	7.5	9.0	7.5	7.5

TABLE II

THE ENZYMIC ACTIVITIES OF LEUCOCYTES IN NORMAL SUBJECTS AND IN PATIENT WITH LEUKAEMIA AND OTHER DISEASES

	Hexokinase		*Glucose-6-phosphate dehydrogenase*		*6-Phosphogluconate dehydrogenase*		*Isocitric dehydrogenase*	
	No.	Δ_E/min./10^9 cells (mean ± S.D.)	*No.*	Δ_E/min./10^9 cells (mean ± S.D.)	*No.*	Δ_E/min./10^9 cells (mean ± S.D.)	*No.*	Δ_E/min./10^9 cells (mean ± S.D.)
Normal subjects	15	2.10 ± 0.52	20	16.89 ± 4.55	20	7.78 ± 2.16	20	2.53 ± 0.88
Hodgkin's disease	9	1.88 ± 0.80	16	19.27 ± 4.15	8	10.91 ± 0.84*	12	4.01 ± 1.62*
Osteosclerotic anaemia	1	1.80	3	17.17 ± 2.09	2	3.20 ± 0.57*	3	6.06 ± 2.69*
Acute leukaemia	9	1.47 ± 1.10*	18	10.55 ± 7.72*	10	5.20 ± 2.38*	15	3.53 ± 1.77*
Chronic lymphocytic leukaemia	10	1.10 ± 0.94*	13	6.63 ± 4.68*	6	2.67 ± 1.64*	11	1.72 ± 1.30*
Chronic myelocytic leukaemia	11	1.27 ± 1.02*	17	16.22 ± 4.90	6	8.44 ± 1.44	11	3.74 ± 1.46*
Basophilic leukaemia	1	4.77	1	25.20	1	9.40	1	3.80

* Difference statistically significant ($P < 0.05$).

TPN-Dependent Dehydrogenases (Table II)

Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. The activities of both these enzymes were decreased to about one-third of normal in chronic lymphocytic leukaemia and to about two-thirds of normal in acute leukaemia. In chronic myelocytic leukaemia activities were indistinguishable from normal. In the single case of basophilic

leukaemia the activity of both enzymes was markedly increased. 6-Phosphogluconate dehydrogenase was decreased in osteosclerotic anaemia and increased in Hodgkin's disease.

Isocitric dehydrogenase. The activity was increased in chronic myelocytic leukaemia, acute leukaemia, osteosclerotic anaemia and Hodgkin's disease, and decreased in chronic lymphocytic leukaemia.

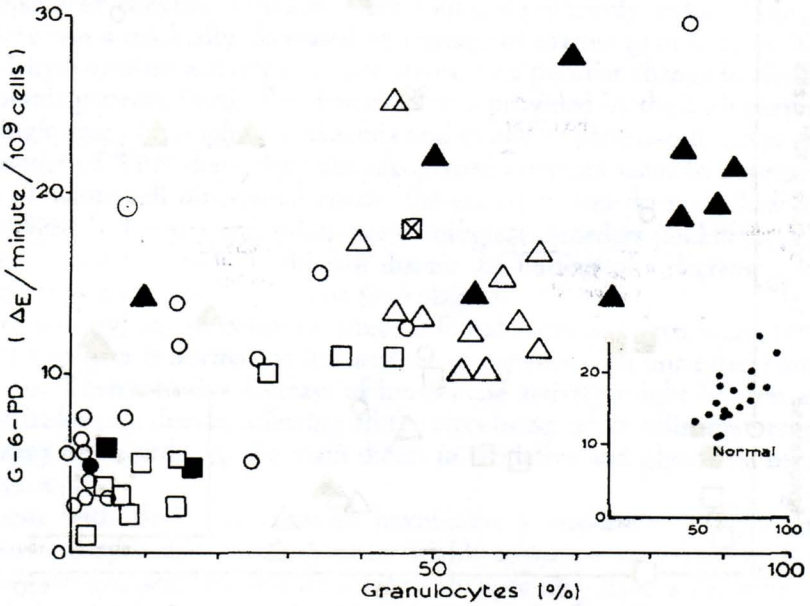

FIG. 1 Estimates of glucose-6-phosphate dehydrogenase activity plotted against percentage of granulocytes in blood samples from patients with various types of leukaemia. ○ = Acute leukaemia; △ = Chronic myeloid leukaemia; □ = Chronic lymphatic leukaemia; △with bar = Basophilic leukaemia; ⊙ = Plasma cell leukaemia; ⊠ with cross = Chronic lymphatic leukaemia with splenectomy. Solid symbols represent treated cases. Inset: observations in normal subjects.

The present results for the TPN-dependent dehydrogenases in chronic myelocytic leukaemia do not agree with the observation of Beck (1958b) who obtained low results for these enzymes. It was thought that this difference might be accounted for by different cell populations in the leucocyte samples examined and that variations in cell population might also account for the wide range of values and high standard deviation in the leukaemia cases. Fig. 1 shows that there is a definite correlation between the activity of TPN-dependent dehydrogenases and percentage of granulocytes, although such correlation is not good in the case of hexokinase (Fig. 2). By performing differential white-cell counts and estimating white-cell enzyme activities in a group of normal persons we were able to calculate approximately the enzyme activity of each type of normal cell. The enzymic activity of the immature cells in leukaemia were then calculated in a similar way by assigning the values obtained for the normal cells to the corresponding mature cells in the leukaemia population (Fig. 3).

Enzyme Activities of Types of White Cells (Fig. 3)

TPN-dependent dehydrogenases. All three enzymes gave similar results for each type of white cell. The highest values were obtained for eosinophils, followed respectively by neutrophils, myelocytes, myeloblasts, leukaemic lymphocytes and normal lymphocytes. There was no difference in enzyme activity between normal and leukaemic mature cells.

Hexokinase. The results for the activity of this enzyme in different types of cells were similar to results for the activity of TPN-dependent dehydrogenases with the following exceptions. Eosinophils were shown to have an extremely low hexokinase activity and the lymphocytes of chronic lymphocytic leukaemia gave lower values than normal lymphocytes.

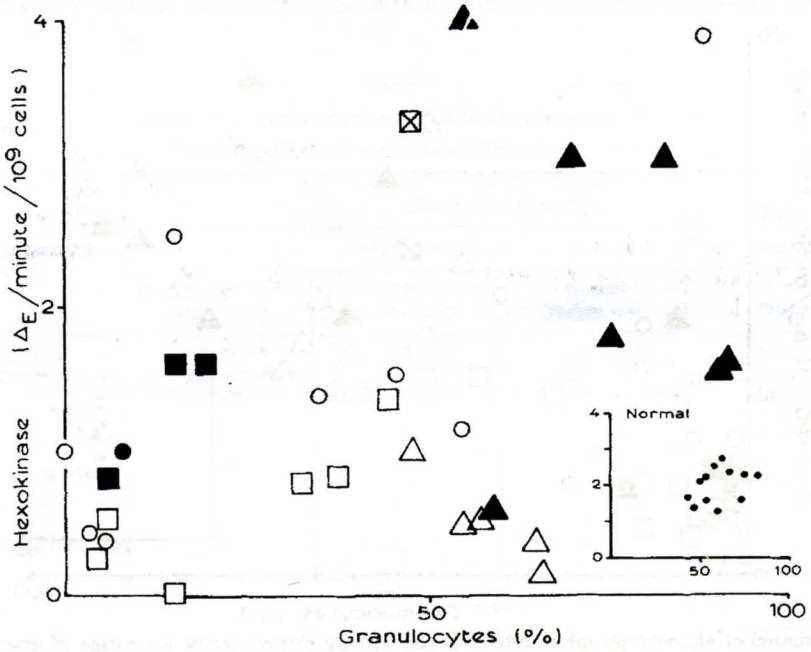

FIG. 2. Estimates of hexokinase activity plotted against the percentage of granulocytes in blood samples from patients with various types of leukaemia. ○ = Acute leukaemia; △ = Chronic myeloid leukaemia; □ = Chronic lymphatic leukaemia; ▲ = Basophilic leukaemia; ⊠ with cross = Chronic lymphatic leukaemia after splenectomy. Solid symbols represent treated cases. Inset: observations in normal subjects.

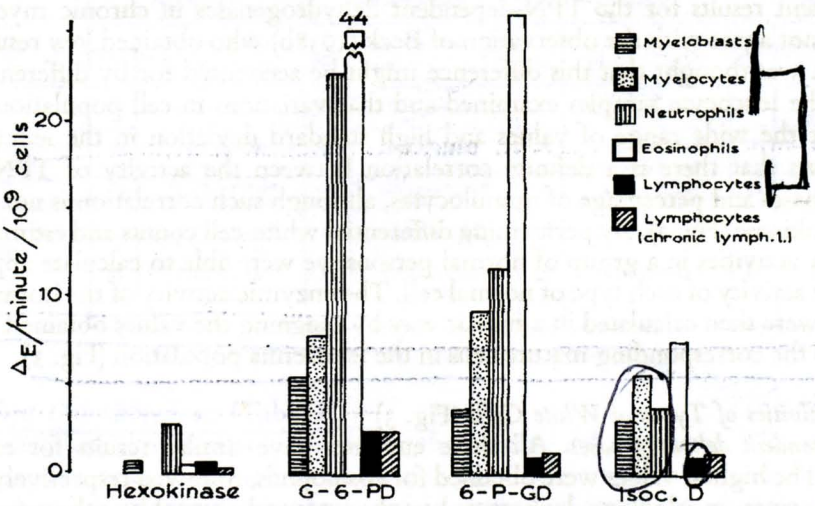

FIG. 3. Estimates of enzyme activity in different types of white cell.

normal or leukemic sample?

DISCUSSION

Our results show that TPN-dependent dehydrogenase activities are significantly reduced in chronic lymphocytic leukaemia cells, in agreement with Beck's data. In chronic myelocytic leukaemia we did not observe the decreased values reported by Beck. In acute leukaemia the TPN-dependent enzymic activities were found significantly reduced only in subjects in whom there was a markedly decreased percentage of mature granulocytes. Therefore the decrease of dehydrogenase activity does not appear as a peculiar change in circulating white cells of leukaemic patients. Further evidence of this is provided by the high enzymic activities found in a single case of basophilic leukaemia and in one of plasma-cell leukaemia.

As the changes of TPN-dependent dehydrogenase activities seem to depend directly on the changes of white-cell differential count, the enzymic data do not allow a differential diagnosis between leukaemia and other haematological disorders (leukocytosis, Hodgkin's disease, osteosclerotic anaemia). In this last disease the finding of a decreased activity of 6-phosphogluconic dehydrogenase remains unexplained.

Hexokinase activity, on the contrary, may be found decreased even when the proportion of mature granulocytes is normal, as for instance in patients with untreated chronic myelocytic leukaemia. Therefore the decrease of hexokinase activity might be seen as a primary peculiarity of leukaemic disease, affecting all the circulating white cells, mature granulocytes included. It may be regarded as the main defect in oxidative and glycolytic metabolisms of leukaemic leucocyte.

In agreement with Beck, we consider hexokinase a rate-limiting factor in glycolytic metabolism of leucocytes since this enzyme invariably appears to be less active than the TPN-dependent dehydrogenases. Hexokinase activity changes may have a negative influence on energy metabolism of leukaemic cells, affecting indirectly the efficiency of hexose–monophosphate shunt. On the other hand, the dehydrogenase activity changes probably have less effect, both because their activity in the intact cell is controlled by the hexokinase reaction rate, and because their activity varies widely if observed in different kinds of normal cells. It can be presumed that only a very marked decrease of glucose-6-phosphate dehydrogenase or 6-phosphogluconate dehydrogenase is able to affect the function of the hexose–monophosphate shunt. A possible primary disorder of the hexose–monophosphate shunt may be found in leucocytes of patients with congenital nonspherocytic haemolytic anaemia with deficiency of erythrocyte and leucocyte glucose-6-phosphate dehydrogenase. In two patients we have recently observed a leucocyte glucose-6-phosphate dehydrogenase activity of 1.43 (Δ_E per minute per 10^9 cells) corresponding to about 6 per cent of the normal average (Ghiotto, Perona and De Sandre, 1962).

It is known, on the other hand, that the hexose–monophosphate shunt enzymes furnish the cells with pentose phosphates and TPNH, which is an essential co-enzyme in several reductive synthetic processes (Horecker and Hiatt, 1958). In this connection the low glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase activity of leukaemic cells is in striking contrast with the high degree of TPN-dependent dehydrogenase activities of the cells in proliferative states, as, for instance, neoplastic (Weber and Cantero, 1959; Vergano, Colajacomo, Luzzatto and Missale, 1959) and regenerating tissues (Rossi and Zatti, 1962).

It probably means that some vital processes, such as the efficiency of organic synthesis and perhaps the proliferative power, are hindered in circulating leukaemic cells. This conclusion agrees with the data obtained from the study of the incorporation by circulating cells of

thymidine labelled with tritium, the rate of which probably reflects the rate of synthesis of DNA; this is far lower with myeloblasts of patients with acute leukaemia than with normal myeloblasts (Gavosto, Maraini, and Pileri, 1960).

SUMMARY

The activities of hexokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase and isocitric dehydrogenase have been determined in circulating white cells of 68 patients with haematological disorders (leukaemia, Hodgkin's disease, osteosclerotic anaemia).

The results showed that the TPN-dependent dehydrogenase changes were correlated with the differential count of the leucocyte sample examined. The activity of dehydrogenases in granulocytes was greater than in lymphocytes; the activity was less in immature than in mature cells. The enzymic activity of the leukaemic leucocytes was markedly decreased only in the cases presenting a noticeable change of leucocyte composition.

In comparison, hexokinase was sometimes depressed in leukaemia even when the mature granulocyte percentage was normal.

The behaviour of leukaemic cells differed from that of neoplastic tissue, in which the TPN-dependent dehydrogenases were very active. The deficiency of these enzymic activities may signify that some vital processes are reduced in the circulating immature leukaemic white cells.

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