LEUCOCYTE METABOLISM IN HEALTH AND DISEASE

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INTRODUCTION

A direct enzymic study of cellular metabolism in man is greatly hindered by the danger and difficulties encountered in collecting tissue samples by biopsy. However, peripheral leucocytes can be obtained repeatedly without much trouble, and, in this way, a close study of cellular metabolism during various diseases or during their treatment is now possible.

Blood leucocytes originate from many different sources and have specialized functions. Several methods have been proposed for the separation of different cell types. We found, from our personal experience, that this could be achieved also by density-gradient centrifugation of blood leucocytes in sucrose. This, in conjunction with microscopical–cytochemical methods, permits a further refinement in metabolic studies.

Metabolic changes in leucocytes may be due to three causes:

(1) Blood metabolite or hormone concentrations are altered in various pathological or physiological conditions. The leucocyte response is that of altered enzyme activity.

(2) Genetic metabolic defects, often not restricted to leucocytes.

(3) Intrinsic disturbances, specifically arising in leucocytes themselves, during division and maturation, as, *e.g.*, leukaemia.

The last group of causes will not be considered here except for the remark that leukaemic cells cannot be used any longer in studies of metabolism during normal cell-maturation.

ALKALINE PHOSPHATASE IN LEUCOCYTE METABOLISM

Hormonal influences on enzyme activity

An example illustrating the actual technical possibilities is given by the study of Borel *et al.*[1], from which we borrow the data on enzyme activities in different cell types shown in *Table 1.*

Table 1. Enzyme activity expressed as 10^{-9} μmol of substrate transformed per leucocyte and per minute

	Granulocytes	*Lymphocytes + Monocytes*
Aldolase	5·0	4·0
Glucose-6-phosphate dehydrogenase	1·0	2·2
Acid phosphatase	6·8	3·2
Alkaline phosphatase	2·9	0·02

A typical granulocyte enzyme is shown to be strongly influenced by blood hormone levels.

In women, enzyme activity is doubled during the second week of the menstrual cycle, and is quadrupled in pregnancy. Oral doses of 5 mg of oestradiol propionate gave similar results in healthy man. These data conflict somewhat with the results of the microscopical–cytochemical studies of Tsutsumi[2], who noted an increase of alkaline phosphatase activity during the menstrual period and a decrease immediately afterwards. He noted an increase of both alkaline and acid phosphatase during the summer, and a diurnal rise with a maximum at noon. These effects could be due to adrenal gland activity.

Alkaline phosphatase is markedly increased in such diverse pathological states as infection, trauma, diabetic acidosis, acute gout, intestinal haemorrhage, cerebral vascular accidents, acute urinary retention and myocardial infarction[3]. In all cases an increase in pituitary–adrenal response can be expected. For reasons which are not clear, uncomplicated but active rheumatoid arthritis, cases of uncomplicated rheumatoid fever, and cases of lupus erythematosus, do not show high alkaline phosphatase values. Substantial doses of ACTH (40 units in 3 days) or 17-hydroxy-corticosteroids give rise to a three-fold increase in unit leucocyte alkaline phosphatase activity; functioning adrenal tissue is necessary for this to occur. Berga[4] has noted an increasing percentage of reacting neutrophils in pyogenic infections, metastatic cancer, polycythaemia vera, and Hodgkin's disease. Several substances such as histamine and procaine raise leucocyte alkaline phosphatase activity, possibly through their stressor activity[5]; they stimulate phagocytosis also. Total body X-radiation (400 r) of rats, even of those which have been adrenalectomized, is followed by high enzyme levels in peripheral leucocytes[6].

The possible role of alkaline phosphatase in cellular physiology

To solve this question, one must know what is the function of alkanine phosphatase in leucocyte metabolism. In this connection we suggest an hypothesis based mainly on the results of Wagner *et al.*[7] Glycogen metabolism (in neutrophils) is illustrated in *Figure 1*. From this it is apparent that alkaline phosphatase, acting on ATP, could have a regulatory function on glycogenolysis. The inhibitory action of glucose on the former could thus be explained; the fact that respiration is increased when exogenous glucose consumption decreases[7] also becomes understandable.

This theory, however, does not apply to eosinophils which have no visible glycogen reserve although showing a strong microscopical reaction for alkaline phosphatase. They have phagocytic power and they can spread on surfaces as well as the other granulocytes containing alkaline phosphatase. Phagocytosis and spreading require a great cellular plasticity. Here the ATPase activity of the enzyme can be invoked for the folding and unfolding of protein molecules during the processes just mentioned.

Glycogenolysis and phagocytosis are connected in the following way: glycogenolysis is favoured by increased alkaline phosphatase activity, thus leading to increased lactic acid production, as a result of which the phagocytic power is enhanced.

The enzyme is localized in the cytoplasm[8]. According to Cohn and Hirsch[9], alkaline and acid phosphatases, as well as a number of other hydrolases, are localized in the specific leucocyte granules resembling the well-known lysosomes. Those granules are present in rabbit exudate cells and decrease during phagocytosis. In this connection we should mention the experiments of Hiraki *et al.*[10] with cover-slip cultures of human peripheral

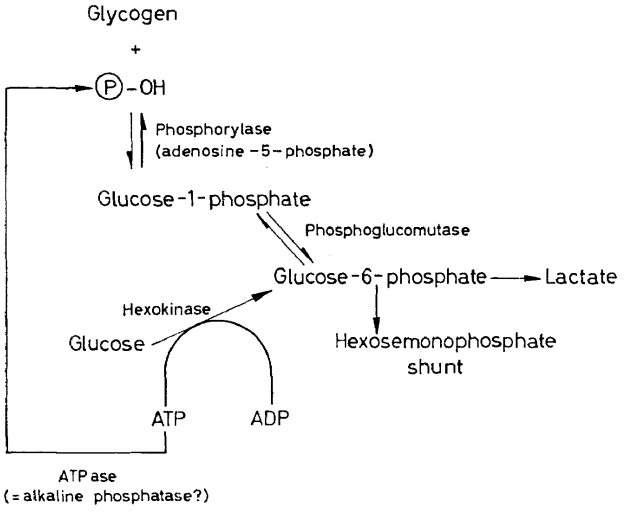

Figure 1. Scheme illustrating glycogen metabolism in neutrophils

leucocytes: the different migratory patterns observed are so characteristic that they can be used for diagnostic purposes.

LEUCOCYTE METABOLISM IN MAPLE-SYRUP URINE DISEASE

Dancis *et al.*[11] have shown that an early diagnosis of maple-syrup disease is possible by the study of the metabolism of leucine, isoleucine and valine by peripheral white blood cells. Tracer studies revealed that the leucocytes lack enzyme systems for the decarboxylation of branched β-keto-acids arising from the metabolism of these amino-acids.

LUPUS ERYTHEMATOSUS

Another example of the use of leucocytes in diagnosis is the study of the alteration of nuclear structure in systemic lupus erythematosus. Carrera *et al.*[12] have demonstrated that chicken and horse granulocytes are most susceptible to this lupusery thematosus phenomenon. Kurnick *et al.*[13, 14] suggested that the intracellular DNAase and the DNAase-inhibitor participate in the depolymerization of DNA which characterizes the lupus erythematosus phenomenon. The derangement of the intracellular DNAase–DNAase-inhibitor system is not primary in the cell. It can be induced in

normal leucocytes, the use of which highly increases the sensitivity of diagnosis.

The determination of the redox activity of leucocytes has also been proposed as an early diagnostic aid in systemic lupus erythematosus[15]. Depressed redox activity was found in all 14 cases of known systemic lupus erythematosus, and in 8 additional patients with systemic lupus erythematosus prior to definite diagnosis.

CONCLUDING REMARKS

We hope that these few examples have proved the usefulness of metabolic studies on peripheral leucocytes as a diagnostic aid. However, a few points must be borne in mind. Many metabolic studies have been performed on exudate leucocytes which may differ appreciably from normal circulating cells[16]. In addition, poor handling and the use of unsuitable media may decrease oxygen consumption.

It is quite clear that leucocyte physiology is still a broad field for both fundamental and applied research.

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