Enzymes of Glycogen Metabolism in White Blood Cells

I. Glycogen Phosphorylase in Normal and Leukemic Human Leukocytes*

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SUMMARY

Glycogen phosphorylase has been assayed in normal leukocytes and in those obtained from patients with chronic granulocytic and chronic lymphocytic leukemia. Assay was performed in the direction of both glycogen degradation and glycogen synthesis with and without adenylic acid. The results indicate that (a) a major portion of phosphorylase activity exists in the active or a form as evidenced by 30-70 per cent activity in the absence of adenosine-5'-phosphate, (b) wide variations in activity were noted in leukocytes from different donors, (c) leukocyte phosphorylase activity in leukemic cells did not differ significantly from the normal. Some of the properties of leukocytic glycogen phosphorylase and its relation to phosphorylase from liver and muscle are discussed.

Leukocytes contain significant amounts of glycogen (19, 20). Little has been done, however, to elucidate the various factors concerned in controlling the glycogen level in these cells. As in the case of muscle cells where mechanical work is made possible through energy-yielding metabolic reactions, one of the primary functions of leukocytes, phagocytosis, has been shown to be an energy-dependent process (14). Whether glycogen plays an essential role as an energy store in leukocytes as it does in muscle cells is not clear. Leukocyte glycogen is diminished in certain hematologic diseases such as chronic granulocytic leukemia and elevated in polycythemia vera and in leukocytes of infection (17). Similarly, leukocyte glycogen is elevated in certain types of glycogen storage disease (21). A change in leukocyte glycogen level may reflect a change in glycogen synthesis or in glycogen degradation. It seems important then to investigate the control mechanisms involved in glycogenesis and glycogenolysis in leukocytes. We have accordingly initiated studies concerned with enzymes of glycogen metabolism in these cells. The recent studies demonstrating a UDPG-linked pathway for glycogen synthesis have provided strong evidence for the existence in tissues of a metabolic cycle in which the UDPG-pathway is linked with the synthesis and phosphorylase catalyzes the degradation of glycogen (8, 9, 13, 15, 18). This paper describes the glycogen phosphorylase levels in normal leukocytes and those of chronic granulocytic and chronic lymphocytic leukemia and some preliminary observations on the properties of this enzyme. It will be seen that (a) a major portion of leukocyte glycogen phosphorylase exists in the active or a form (active without adenylic acid); (b) phosphorylase levels in leukemic leukocytes did not differ significantly from the normal.

MATERIALS AND METHODS

Preparation of leukocyte extracts.—Blood was collected in siliconized 50-ml. graduated test tubes, and neutral Na-EDTA1 was added to a final concentration of 5 × 10⁻⁵ M. Leukocytes were isolated by Dextran sedimentation, and contaminating red cells were lysed by transient exposure to a hypotonic medium as described by Walford (22). The leukocyte button was homogenized in 40 volumes of distilled water at 0° C. for 1½ minutes in a Virtis 45 homogenizer set at 45,000 r.p.m. The homogenate was centrifuged at 15,000 × g for 15 minutes, and the clear colorless supernatant was used for phosphorylase assay.

Phosphorylase assay.—Phosphorylase assay was performed by the spectrophotometric method of Wu and Racker as described by Hülsmann et al. (4). In this method the rate of reduction of triphosphopyridine nucleotide (TPN+) is measured in the presence of excess inorganic phosphate, glycogen, and phosphoglucomutase2. The following abbreviations are used in this paper: EDTA = ethylenediaminetetraacetic acid; AMP = adenosine-5'-phosphate; TPN+ = triphosphopyridine nucleotide.

1 The authors are indebted to Dr. V. Najjar for his generous supply of phosphorylase and its relation to phosphorylase from liver and muscle are discussed.

† Leukemia Society, Inc., Scholar.

Received for publication October 7, 1963.

* Supported by grants from U. S. Public Health Service # CA 06213-02, HE 00022-17; by American Cancer Society Institutional Research Grant to Washington University # IN36D; and by gifts in memory of Philipp Hunkel, Mark Edison, Bill Burns, and Joe Blanchard.

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(prepared by the method of Najjar [11]), glucose-6-phosphate dehydrogenase (Zwischenferment), and TPN+ at pH 7.5, phosphorylase (leukocyte extract) being the limiting enzyme. Excess phosphoglucomutase and glucose-6-phosphate dehydrogenase were always checked by replacing glycogen and the leukocyte extract with glucose-1-phosphate. The assay was performed with AMP (10⁻³ M) and without. The reaction was started by the addition of TPN+ and in some instances by the addition of leukocyte extract. Protein concentration in the extract was measured by the method of Lowry (10). Phosphorylase activity was expressed as (µmole glucose-1-phosphate/hour/mg protein), with a molecular extinction coefficient for TPNH of 6.22 × 10⁻³ sq. cm. × mole⁻¹ at 340 nm (3).

In some instances phosphorylase was assayed in total leukocyte homogenates and extracts in the direction of glycogen synthesis by a modification of the method of Sutherland as described by Williams and Field (23). The activity was expressed as µg inorganic phosphate (Pi) liberated/30 min/10⁷ cells as determined on total homogenates or µg Pi/30 min/mg protein as determined on cell extracts.

RESULTS

Phosphorylase activity was assayed in leukocytes obtained from nine normal donors, ten patients with chronic lymphocytic leukemia, thirteen patients with chronic granulocytic leukemia, and two patients with polycythemia vera. The results of the assays are shown in Table 1. A wide variation is noted in the levels of phosphorylase activity in leukocytes obtained from different donors. The ratio of phosphorylase activity with AMP to that without AMP ranged between 0.26 and 0.75, indicating that a significant portion of enzyme exists in the active or a form (active without AMP). The level of enzyme activity in leukemia cells did not differ significantly from the normal. Although the mean activity level in lymphocytes from donors with chronic lymphocytic leukemia was slightly higher than that of leukocytes from chronic myelocytic leukemia and normal donors, there was a significant overlap in the individual cases. Leukocytes from two patients with polycythemia vera showed normal phosphorylase activity.

In Table 2 are listed the phosphorylase activities as measured in the direction of polysaccharide synthesis.

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<th>Donor</th>
<th>µg Pi/30 min/10⁷ cells</th>
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- Values in parentheses represent µg Pi/30 min/mg protein in the extract.
lymphocyte appeared to possess an activity about one-fourth to one-fifth that of the granulocyte. However, when expressed as μg Pi/30 min/mg protein in the extract both cell types appeared to have comparable activities.

Leukocyte phosphorylase activities as measured by both methods was not significantly affected by the addition of cysteine.

**DISCUSSION**

Cori, Cori, and Green (1) found that glycogen phosphorylase from rabbit skeletal muscle exists in two forms: One designated as phosphorylase a exhibits 65 per cent of its maximal activity in the absence of AMP; the other, phosphorylase b, is inactive in the absence of the nucleotide. Extracts of resting muscle were found to contain the enzyme virtually completely in the b form (5). Similar results were obtained with rabbit heart muscle and human autopy skeletal muscle (24, 25). Phosphorylase b can be readily converted to phosphorylase a; the mechanism of conversion has recently been elucidated through the work of Krebs et al. (7). It involves phosphorylation and dimerization to form phosphorylase a according to the following reaction: 2 phosphorylase b + 4 ATP $\rightarrow$ phosphorylase a + 4 ADP. The enzyme catalyzing this reaction has been termed phosphorylase b kinase. When assayed in resting muscle, phosphorylase kinase is found to be inactive at pH 7.0 but shows its maximal activity at pH 8.6. This inactive kinase can be activated in several ways: by Ca$^{+2}$ ions, Mg-ATP, cyclic AMP, or Mg-ATP-cyclic AMP (6); the enzyme now exhibits activity at pH 7.0 and is called active kinase. The rate of glycogenolysis in a given tissue presumably depends upon the ratio of phosphorylase a to b which in turn depends upon the state of kinase activity. The physiologic mechanism which triggers phosphorylase kinase activation leading to glycogenolysis is the subject of extensive study and is not completely understood at present. The existence of phosphorylase in the b form in resting muscle is compatible with the presence of inactive kinase.

If one assumes that leukocyte phosphorylase a also exhibits 65 per cent of its maximal activity without AMP, then the present study shows that leukocyte phosphorylase exists almost entirely in the a form. It is of interest that preliminary observations in this laboratory indicate that leukocytes also contain phosphorylase kinase which readily converts crystalline rabbit muscle phosphorylase b to the a form at pH 7.0. The implications of these observations have to await further studies. Extensive studies on liver phosphorylase by Sutherland and co-workers have demonstrated wide differences in the properties of this enzyme and that of muscle (16). Thus, liver phosphorylase exhibits over 50 per cent of its activity without AMP, is activated by glucagon, its activity is not stimulated by cysteine or glutathione, and it can be phosphorylated by a kinase enzyme but without dimerization. In addition, liver and muscle phosphorylase have been shown to be immunologically different (2).

Evidence from other studies and the present one indicates that leukocyte phosphorylase is closely related to liver phosphorylase rather than to that of muscle. Thus, leukocyte phosphorylase shows considerable activity in the absence of AMP and is activated by glucagon (23), and cysteine does not stimulate its activity. In addition, low leukocyte phosphorylase has been demonstrated in cases of glycogen storage disease caused by deficiency of liver phosphorylase (4, 23). This latter finding indicates that both enzymes may share a common genetic control. Further characterization of this relation should await purification of leukocyte phosphorylase.

The results of phosphorylase assays in normal leukocytes described here are in close agreement with those of Hülsmann (4) and Williams and Field, who reported on leukocyte phosphorylase activity in glycogen storage disease and normal controls (23). Wide variations in activity were also noted by these authors. Hülsmann attributes these variations to possible differences in the secretion of epinephrine and glucagon. Williams and Field were able to demonstrate considerable activation of leukocyte phosphorylase by glucagon in vitro (23).

The normal phosphorylase activity in leukemia cells is of interest in view of the recent report by Nirenberg (12) describing the absence of glycogen phosphorylase activity in some ascites tumors. More interesting is the fact that cells from these tumors were devoid of glycolgen but contained normal amounts of phosphorylase kinase. Although the present study reveals no difference in phosphorylase activity between normal and leukemic leukocytes, it is possible that future studies may reveal differences in the mechanisms controlling glycogenolysis in these cells. Furthermore, other types of human leukemias may be of interest to study from this aspect.

**REFERENCES**


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_Cancer Res_ 1964;24:489-492.

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