α-D-Glucosidase in Normal and Leukemic Lymphocytes

The purpose of this study was to determine whether a deficiency of the enzyme α-D-glucosidase exists in the peripheral blood lymphocytes of patients with chronic lymphocytic leukemia in order to provide a potential biochemical basis for the accumulation of excess glycogen in the cytoplasm of this neoplastic cell[1]. This enzyme was chosen for investigation because its deficiency leads to a clinical form of glycogenosis, Pompe's or type III glycogen storage disease[2], because normal activity of other enzymes involved in glycogen metabolism, such as phosphorylase[3] and UDPG-glucosyltransferase[4], could not explain the increased amounts of PAS-positive material in leukemic lymphocytes, and because its assay, particularly in the lymphocyte as opposed to other white blood cells[5], is without known recorded precedent.

The method described depends upon the enzymatic hydrolysis of the chromogenic substrate 6-bromo-2-naphthyl α-D-glucopyranoside. The liberated 6-bromo-2-naphthol is able, under appropriate circumstances, to combine with tetrazotized *o*-dianisidine to form an azo dye whose color density may be measured spectrophotometrically[6].

Materials and methods. (A) Lymphocyte donors. Lymphocytes for enzyme assay were obtained from 11 patients with chronic lymphocytic leukemia, 54–85 years old, who had their disease for 1–17 years. Chlorambucil was being administered to 4 of these individuals at the time of the study; the remainder had never required any form of specific antileukemic treatment. When stained with the PAS reagent[7], all their lymphocytes contained excess glycogen as determined semiquantitatively[8]. The PAS-reactivity disappeared completely after the peripheral blood smears were incubated with saliva in a manner described previously[8]. Eight volunteers from the hospital staff, 23–39 years of age, were a source of normal control lymphocytes.

(B) Lymphocyte preparation. Depending on the total white blood cell count, 20–40 ml venous blood were defibrinated by rotation in an Erlenmeyer flask containing a glass bead for each ml blood[9]. The blood, without its coagulum, was transferred to a 40 ml, round-bottom test-tube containing an equal volume of 3% dextran solution (mol. wt. 188,000), mixed, and incubated in the vertical position in a 37 °C water bath for 30–45 min. The supernatant, with a residual trail of sedimenting red cells, was transferred to a 15 ml centrifuge tube and spun at 90 *g* (No. 269 head, PR-2 centrifuge) for 30 min. The supernatant was removed, transferred to another tube, and centrifuged at 1200 *g* for 5 min to deposit the lymphocytes. Examination under phase microscopy indicated the cell suspension consisted of 95% lymphocytes, 5% red cells, and no polymorphonuclear leucocytes, inferring that enzyme activity would be lymphocyte-derived. The cell button was washed 3 times with TC 199 (Difco, Detroit, Michigan) and resuspended in a final volume of 1 ml phosphate buffer, pH 8.0 (0.1 *M*). A white cell count was performed by hemocytometer, and the lymphocytes disrupted by ultrasound using a sonifier (Branson Instrument Co., Danbury, Conn.), at peak output. The fragmented cells were centrifuged in the cold at 9000 *g* for 10 min and the clear supernatant removed to be used for enzyme quantitation as described below.

(C) Enzyme determinations. To delineate the suitability of the proposed assay, saliva was used initially as enzyme source in a system somewhat modified from that originally outlined[6]. 1 ml freshly collected saliva, from 7 healthy subjects, cleared by centrifugation, was added separately to 0.5 ml of substrate solution made by dissolving 6-bromo-2-naphthyl α-D-glucopyranoside (Dajac Laboratories, Philadelphia, Pa.) in 2-methoxyethanol (0.5 mg/ml). These reactants and 2 ml of phosphate buffer, pH 6.3 (0.1 *M*), were incubated in a 37 °C water bath for 3 h. After this period, 0.5 ml phosphate buffer, pH 8.0 (0.1 *M*), and 1 ml of a 0.1% solution of tetrazotized *o*-dianisidine (Dajac Laboratories) made in cold water were added to the incubated tubes. After 1 min, but not later than 3 min, the O.D. of the solution was measured spectrophotometrically at a wave-length of 560 nm. Enzyme activity, as μg 6-bromo-2-naphthol liberated/h, was calculated from a standard curve made by dissolving known concentrations (50–200 μg/ml) of 6-bromo-2-naphthol in 2-methoxyethanol and repeating the dye coupling reaction. Lymphocyte α-D-glucosidase was assayed in an identical fashion except that lymphocyte extract replaced saliva in the procedure detailed above.

Results. The suitability of the present method to detect glucosidase activity was demonstrated by the production of the requisite[6,10] pink color for spectrophotometric assay by all the salivas tested. This indicates destruction of substrate glycosidic linkages in a fashion similar to the enzymatic hydrolysis of α1–4 and α1–6 bonds by serum amylase and duodenal and intestinal juice[11]. The destruction by saliva of histochemical, PAS-reactive lymphocyte glycogen supports this assumption. Amounts of 6-bromo-2-naphthol liberated by the different salivas varied rather widely from 10.5–27.5 μg/h.

Summary of lymphocyte α-D-glucosidase assays

μg 6-bromo-2-naphthol liberated/lymphocyte/h		
Leukemic lymphocytes		Normal lymphocytes
8.3×10^{-8}		7.0×10^{-8}
8.0×10^{-8}		1.2×10^{-7}
1.7×10^{-7}		2.5×10^{-7}
6.0×10^{-8}		4.3×10^{-7}
6.3×10^{-8}		7.3×10^{-7}
1.0×10^{-7}		2.1×10^{-7}
1.1×10^{-7}		3.7×10^{-7}
3.3×10^{-8}		7.7×10^{-7}
1.4×10^{-7}		
2.8×10^{-7}		
3.1×10^{-7}		
1.2×10^{-7}	means	3.7×10^{-7}

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The results of the lymphocyte α-D-glucosidase determinations are shown in the Table. Extracts from both normal and leukemic lymphocytes were able to liberate 6-bromo-2-naphthol, indicating the presence of α-D-glucosidase in these cells. As observed with the salivary enzyme, the hydrolytic potencies of leukemic lymphocyte extracts showed considerable variability, unrelated to disease duration, treatment administered, or amount of glycogen detected with the PAS reagent. Normal lymphocyte extracts demonstrated similar differences but their mean value was higher than that quantitated for leukemic enzyme activity.

Discussion. The overlapping of individual glucosidase activity values obtained with normal and leukemic lymphocyte extracts questions the significance of the mean differences detected by these assays. It appears that leukemic lymphocytes contain appreciable amounts of α-D-glucosidase making it unlikely that a deficiency of this enzyme is responsible for the excess histochemically demonstrable glycogen seen in these cells.

The consistent failure, up to the moment, to show that lymphocyte glycogen storage is related to abnormalities per se of 3 different enzymes involved in the synthesis or catabolism of glycogen suggests an alternative metabolic defect. It is conceivable, for example, that a block in either the EMBDEN-MEYERHOF or hexose monophosphate (HMP) shunt pathways might lead to an accumulation of glycolytic intermediates which could ultimately enter the glycogen synthesis cycle and become manifest as increased PAS-positive intracytoplasmic material. Experiments in this laboratory, measuring quantitative evolution of $C^{14}O_2$ from glucose-1-C^{14} as an endpoint, imply that leukemic lymphocytes metabolize proportionately less carbohydrate via the HMP pathway[12] than do their normal counterparts. These preliminary studies, which support, but do not prove, the stated hypothesis, are being extended to more precisely localize this metabolic abnormality[13,14].

Zusammenfassung. Der einfache Nachweis von α-D-Glukosidase in leukämischen Lymphozyten gelang spektrophotometrisch. Dies lässt darauf schliessen, dass ein Enzymmangel nicht Ursache für die Glykogenspeicherung dieser Zellen sein kann. Es scheint sich um eine Ansammlung von glykolytischen Zwischenprodukten und nicht um einen Defekt der Glykogenolyse zu handeln.

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The Activities of Phenylalanine Hydroxylase and Hypoxanthine Dehydrogenase in Chick Embryos and an Attempt to Induce these Enzymes

Genic defect of metabolic enzymes in man results in congenital metabolic disturbances. Phenylalanine hydroxylase and hypoxanthine dehydrogenase are 2 such enzymes, the lack of which causes phenylpyruvic oligophrenia and xanthinuria, respectively. The fetal mammalian liver contains very little of the former enzyme, which starts to develop several days after birth[1,2]. The latter enzyme, which is present in embryonic chick liver, is also negligible, although it increases abruptly immediately after hatching[3,4]. The activity of hydroxylase in young rats[5] and of dehydrogenase in rat and chick embryos[4,6] varies with the administration of substrate or non-substrate substances. Among many inducers of enzymes in rat liver[7], X-irradiation has been reported as an effective stimulator of tryptophan pyrrolase in which the hypophysis-adrenal function appears to be a controlling factor[8]. In view of this, observations have been made on the developmental patterns of these enzymes in chick embryos and on the inducibility of these by X-irradiation.

Developmentally staged chicks of ages between 14 days of incubation and 12 h after hatching were used. For the 2 dose groups of irradiated embryos, a single dose of either 600 or 800 r was administered into the embryo side of the eggs by an X-ray apparatus (250 kv, 30 mA, HVL 1.4 mm Cu, 295 r/min, distance 60 cm), and they were immediately returned into the incubator. These and sham-irradiated control embryos were sacrificed simultaneously after 2 h of treatment. The procedures for preparation and incubation for measuring the activities of hydroxylase and dehydrogenase, from the supernatant after 16,000 and 34,000 *g* centrifugation, respectively, of the pooled liver homogenate, were the modified methods[2,3] of corresponding original workers. Crude preparations were used to secure all the enzyme content of the cells which might be present in multiple forms. At least 5 determinations were made from each batch of eggs at

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