muscorum, and *S. lividus,* cystine is the least abundant amino acid; however, in the *Cyanidium caldarium* phycocyanin, histidine is the least abundant. The amino acid residues calculated on the cystine basis for *C. caldarium* show some real deviations; however, if histidine is used as the arbitrary basis, agreement with the other phycocyanins is quite good. The interesting deviation in the phycocyanins in both *Porphyra tenera* and *C. caldarium* is in the ratio of histidine residues to whole-cystine residues. Most striking is the fact that the molecular weight calculated from these analyses for a minimum molecular unit for five of the phycocyanins is between 28,000 and 31,000. The sixth (*P. tenera*) is in the 39,000 region. The molecular weight of the minimum unit observed in sedimentation studies is calculated to be in this same 30,000 molecular weight region.

The evidence presented seems to point to a minimum molecular weight unit of 30,000 for C-phycocyanin. There is good agreement between physical studies and analyses of amino acid content of the phycocyanins. It should be emphasized that in nearly all physical studies the smallest molecular weight component is not present in the largest amount. The conditions of preparation may be altered to cause a reversible formation of larger or smaller amounts of the smallest component.

DONALD S. BERNS
EDITH SCOTT
KATHLEEN T. O'REILLY
Division of Laboratories and Research, New York State Department of Health, and *Department of Biochemistry, Albany Medical College, Albany*

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Erythrocyte Glucose-6-Phosphate Dehydrogenase in Caucasians: New Inherited Variant

Abstract. *A new inherited variant of glucose-6-phosphate dehydrogenase having both a lowered enzyme activity and an altered electrophoretic mobility was discovered in two unrelated American families, one of Irish and the other of German ancestry. Family studies of the trait indicate that it is due to a sex-linked gene.*

Deficiency in erythrocytic glucose-6-phosphate dehydrogenase (G-6-PD) activity is recognized as a heterogeneously expressed, sex-linked, enzyme defect which has been found in all major races of man in varying frequencies. The enzyme defect is manifest clinically by hemolytic anemia in apparently healthy individuals upon ingestion of the antimalarial drug primaquine, fava beans, or a large number of other hemolytic compounds. The general subject has been recently reviewed (*1*). In enzyme-deficient Negro males (13 percent of the American Negro male population), the erythrocytic G-6-PD activity is 8 to 15 percent of normal (*2*). Porter *et al.* (*3*) reported two electrophoretic forms (fast and slow) of erythrocytic G-6-PD in American Negroes, and they were designated as types A+ and B+ respectively (*4*); a plus (+) indicates normal enzyme activity. Studies by Kirkman and Hendrickson (*5*) demonstrated that these two phenotypes distinguishable by electrophoresis follow a sex-linked mode of inheritance. The G-6-PD in deficient Negro males usually migrates to the type A position and is designated A—. The B+ form of the enzyme is the common type found in Americans of European origin and in Mediterranean Caucasians (*3, 4*). Enzyme-deficient Mediterranean Caucasian males have 3 to 6 percent of normal enzyme activity (*2*), and their G-6-PD migrating to the B position is designated type B— (*4*). In addition to the above G-6-PD variants, other variants, all rare, have been reported in which electrophoretic and/or kinetic alterations are associated with the enzyme deficiency (*2, 6, 7*).

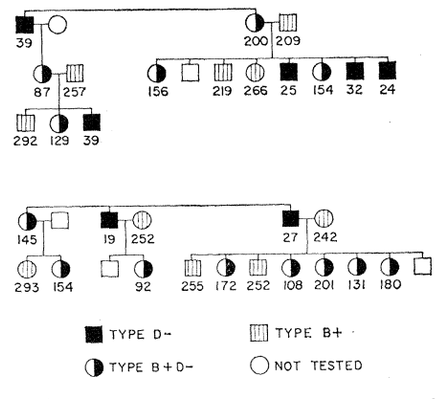

Fig. 1. Pedigrees showing segregation of the glucose-6-phosphate dehydrogenase variant, D— (*11*). The numbers beneath the tested individuals give G-6-PD activity in μmoles of reduced triphosphopyridine nucleotide (TPNH) formed per gram of hemoglobin per hour assayed at 22.5°C. The G-6-PD activity of the erythrocytes of normal males is 237 ± 36.

This report describes a new inherited variant of G-6-PD which is distinguished phenotypically by both a diminished enzyme activity and an altered electrophoretic mobility. In the course of a study of erythrocytic G-6-PD activity of 215 Caucasians (*8*), in which the enzyme was assayed by both a spectrophotometric method (*9*) and the methemoglobin reduction test (*10*), two individuals (a male and a female) with significantly lowered enzyme activity were found in two unrelated families, one of German and the other of Irish ancestry. In both families, there was a total of eight male members with the lowered enzyme activity. Their erythrocytic G-6-PD activity was 8 to 16 percent of normal (Fig. 1), an amount equivalent to that usually found in enzyme-deficient Negro males (8 to 15 percent of normal), and higher than that usually observed in enzyme-deficient Caucasian males (3 to 6 percent of normal) (*2*).

Electrophoretic studies were carried out on the erythrocytic G-6-PD of members of these two families. Under the electrophoretic conditions used (Fig. 2), all enzyme-deficient males had a single band of G-6-PD activity which migrated about 10 percent more slowly than the B form and 15 percent more slowly than the A form (Fig. 2). This slowly migrating band will be referred to here as D— (*13*). In eight of 25 male relatives tested, D— was the only band observed; all eight were enzyme deficient. In 15 of 25 female relatives tested, B+ and D— bands were present; the enzyme activity in these 15 females ranged from 35 to 80 percent of normal.

The data from the two pedigrees (Fig. 1) are entirely compatible with sex-linked inheritance. Mothers of all affected males are heterozygous, and the D− band appears in all the daughters but not in the sons of affected males.

Thus far most surveys for G-6-PD variants have been directed toward Negro and other populations indigenous to tropical regions while similar studies of unselected Caucasian subjects have been limited almost entirely to several Mediterranean populations (*1*). The discovery of two individuals with the D− variant of G-6-PD, in a rather small sample of Caucasians of non-Mediterranean origin, suggests that it may be a characteristic G-6-PD variant in populations of northern European origin.

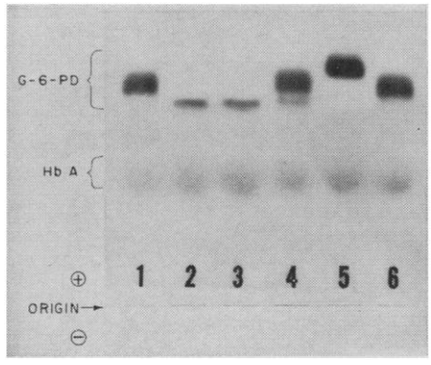

Fig. 2. Phenotypes of erythrocytic glucose-6-phosphate dehydrogenase after starch gel electrophoresis. *1*, Caucasian male, type B+; *2*, deficient male of Irish descent, type D−; *3*, deficient male of German ancestry, type D−; *4*, heterozygous mother of 3, type B+D−; *5*, Negro male, type A+; *6*, Negro male, type B+. Distance from origin to leading edge of A band is 9 cm. Vertical electrophoresis (4 to 5 v/cm) was carried out for 16 hours at 2° to 4°C in gel buffer containing 0.021*M* tris, 0.02*M* boric acid, and $5.4 \times 10^{-4}M$ EDTA. Five milliliters of $2.5 \times 10^{-3}M$ triphosphopyridine nucleotide (TPN) was added to the molten gel before degassing. The gel was prepared as described by Smithies (*12*). The bridge buffer solution contained 0.21*M* tris, 0.15*M* boric acid, and $4.7 \times 10^{-3}M$ EDTA adjusted to *p*H 8.0 with HCl. The cathode tray next to the gel contained $3.4 \times 10^{-5}M$ TPN. Whole blood was collected in ACD (acid-citrate-dextrose) solution, and stromata-free hemolysates were prepared by lysing washed red cells with one volume of distilled water and extracting with four-tenths volume of toluene, the toluene layer being discarded. Hemolysates were then centrifuged at 30,900*g* for 20 minutes, and about 0.025 ml of hemolysate was placed in each gel slot. After electrophoresis, the gels were incubated from 3 to 5 hours at 32°C in a staining solution composed of 10 ml of 0.05*M* tris-HCl, *p*H 8.0, $6 \times 10^{-4}M$ glucose-6-phosphate, 1 to $2 \times 10^{-4}M$ TPN, 10 mg nitro blue tetrazolium, $2 \times 10^{-4}M$ phenazine methosulfate, and $5 \times 10^{-3}M$ $MgCl_2$ made up to 100 ml with distilled water.

Kirkman *et al.* (*2*, *7*) have recently reported a variant (Seattle I) which seems to fit into this same category. They describe a male of Scotch-Welsh ancestry whose red cells have 8 to 15 percent of normal G-6-PD activity, and on electrophoresis this enzyme migrates 10 percent more slowly than normal. Although this variant has not been tested in our laboratory as yet, it is possible that the variant reported here and the Seattle mutant are identical.

THOMAS B. SHOWS, JR.
RICHARD E. TASHIAN
GEORGE J. BREWER
Department of Human Genetics, University of Michigan Medical School, Ann Arbor
RAYMOND J. DERN
Department of Medicine, Stritch School of Medicine, Loyola University, and *Department of Hematology, Hektoen Institute for Medical Research, Cook County Hospital, Chicago, Illinois*

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Thymidine Incorporation by Mitochondria in Physarum polycephalum

Abstract: *Cytoplasmic particles of the slime-mold,* Physarum polycephalum, *incorporate tritiated thymidine in a form which is insoluble in acid and in ribonuclease, and soluble in deoxyribonuclease. Evidence for the mitochondrial nature of these thymidine-incorporating particles is based on their distribution, size, and staining behavior.*

Extranuclear DNA has been reported to occur in mitochondria (*1*), in chloroplasts (*2*), and in other cytoplasmic particles (*3*, *4*) which as yet are unidentified. We report here on the incorporation of tritiated thymidine into the mitochondria of the slime-mold, *Physarum polycephalum*, in a form which is insoluble in acids and can be extracted with deoxyribonuclease but not with ribonuclease.

Mitotically synchronized plasmodia of *P. polycephalum* were prepared, by fusion, from microplasmodia growing submersed in agitated culture (*5*), as described previously (*6*). The nuclei of such plasmodia divide in synchrony until the plasmodia reach a size of approximately 5 cm (*6*). At different times between the second and the third mitosis after fusion, the plasmodia were exposed for 3-hour periods to a growth medium (*5*) containing tritiated thymidine (*7*); smear preparations were then fixed in duplicate on cover slips. Fixation was in 95 percent ethanol, followed by ethanol–acetic acid (3 : 1 by volume).

After all the smears had been incubated with ribonuclease (*8*) according to the method of Plaut and Sagan (*3*), one slide from each set of preparations was treated with deoxyribonuclease (*3*, *9*), while the duplicate slide from each set was incubated with a portion of the buffer used as the solvent for the deoxyribonuclease and thus served as a control. The preparations were then covered with Kodak AR-10 stripping film, and after exposure for 4 weeks the autoradiographs were developed in Kodak D-19 developer (10 minutes at 20°C).

During all phases of the mitotic cycle, thymidine-H^3 was incorporated in considerable quantities by numerous, evenly distributed particles having diameters of approximately 2 μ in the thinnest areas of the preparation. The