

demonstrated a variety of patterns. Some samples showed no activity in this region, while others had from one to four bands; moreover, the positions of the bands varied considerably. In three instances there was a three-band pattern resembling that seen in the heterozygous *Peromyscus*. However, with the wide variety of forms, and in the absence of genetic data, these human variations cannot be placed in any simple genetic scheme; nor can one even say whether they are genetically determined.

The anodal G6PD in the human liver extracts were nonvariant except in three samples in which the zone was shifted anodally about 6 mm. These three samples were all from male Negroes, and the variation presumably represents the common sex-linked "A" form described in Negro erythrocytes (3). Unfortunately, blood specimens were not available from these three subjects, but kidney extract from two of them also showed the fast form of the anodal G6PD.

Ohno *et al.* (4) have recently confirmed occurrence of the additional G6PD enzyme in human liver. They have further differentiated the two human enzymes by using the substrate galactose-6-phosphate. The anodal G6PD shows very low activity toward this substrate, while the cathodal enzyme is about equally active with both substrates.

When notified by Ohno of this finding, I compared the activities of human, *Peromyscus*, and horse livers on the two substrates glucose-6-phosphate and galactose-6-phosphate (Fig. 1). In all three species the anodal enzyme was active only on the former substrate, while the cathode enzyme showed approximately equal activity on both.

These findings suggest the homology of the A enzyme of *Peromyscus* with the erythrocyte (or sex-linked) enzyme of man, and further indicate that the B enzyme of deer mouse may be homologous with the more cathodal enzyme of human tissues. However, as noted earlier, the variant patterns in the two organisms are different. The possibility was considered that some of the human variations may represent postmortem change, but incubation of whole tissues at room temperature for up to 24 hours produced no shift in position—only gradual loss of activity. In all 14 horses studied, the cathodal enzyme occurred as a single band, with no variation in position.

Marked increase in intensity of the

B zone was seen in 22 percent of the female deer mice, but not in any males. In most instances the phenomenon was restricted to certain tissues: for example, when it occurred it was usually present in kidney but not in liver of the same animal, which fact indicated a selective increase in production of enzyme by specific organs. The fact that it was seen only in females suggests its involvement with galactose metabolism, although it did not correlate with lactation. Quantitative differences were also seen in the human enzyme patterns, but these could not be evaluated satisfactorily because some of them probably represented postmortem deterioration; evidence of this was the fact that intensities of anodal and cathodal bands usually varied together.

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Hexose-6-Phosphate Dehydrogenase Found in Human Liver

Abstract. *Starch-gel electrophoresis of extracts of human liver revealed the presence of a new hexose-6-phosphate dehydrogenase that was slower-moving at pH 8.6 than the sex-linked glucose-6-phosphate dehydrogenase. When the gel plate was stained, galactose-6-phosphate being used as a substrate, this enzyme band stained intensely, but the sex-linked glucose-6-phosphate dehydrogenase failed to stain. This new human enzyme may well be homologous with the autosomally inherited glucose-6-phosphate dehydrogenase of the deer mouse (*Peromyscus maniculatus*), reported by Shaw and Barto.*

It has been shown that the enzyme glucose-6-phosphate dehydrogenase (G6PD) is produced by a sex-linked gene in other placental mammals (1) as well as in man (2). However, Shaw and Barto (3) have recently shown in

the deer mouse (*Peromyscus maniculatus*) another G6PD which is autosomally inherited.

We now report a similar hexose-6-phosphate dehydrogenase in human liver. This enzyme of man and deer mouse differs from the sex-linked G6PD in that it shows considerable activity as galactose-6-phosphate dehydrogenase (Gal6PD).

Liver samples were obtained on autopsy from eight diseased bodies of various ages. A male newborn and an 11-day-old female were sampled in Mexico City; the cause of death was not apparent. A 6-year-old female with the trisomy-E syndrome and two males with cerebral palsy in their teens were sampled at Costa Mesa, California; pneumonia was the cause of death. Two male and one female middle-aged persons who had died of neoplastic diseases were sampled at Duarte, California.

After rinsing in physiological saline, small cubes of liver were homogenized in an equal volume of 0.01M potassium phosphate buffer containing 5 mg of ethylenediamine tetraacetate and one drop of β -mercaptoethanol per 50 milliliters; after 2-hour centrifugation at 15,000g and at 4°C, the clear supernatant was used for electrophoresis. A continuous system of vertical starch-gel electrophoresis (2) at pH 8.6 employed borate buffer; each starch-gel plate contained triphosphopyridine nucleotide (TPN) at 5 mg/50 ml. Electrophoresis was continued for 16 hours at 4°C, with a gradient of 4 volt/cm. The 10 ml of staining solution for G6PD contained 10 mg of glucose-6-phosphate dipotassium salt, 2 mg of 3-(4,5-dimethylthiazolyl-1,2,5)-diphenyl-tetrazolium bromide, 2 mg of phenazine methosulfate, and 2 mg of TPN; 0.1M tris HCl buffer, pH 8.0. For detection of Gal6PD activity, galactose-6-phosphate (ICN or Sigma) was substituted for glucose-6-phosphate; disodium salt was used.

Figure 1, *a* and *b*, illustrates the situation found in seven of the eight individuals. When one slice of the gel plate was stained for G6PD, a single narrow band of the slower-moving component was recognized in addition to a broad, intensely stained band of the sex-linked component. Under the experimental conditions, this slower moving component migrated 13 to 15 mm toward the anode (Fig. 1*a*). On the other slice of the plate, which was stained for Gal6PD, the broad, sex-linked band was not recognizable, while the slower-

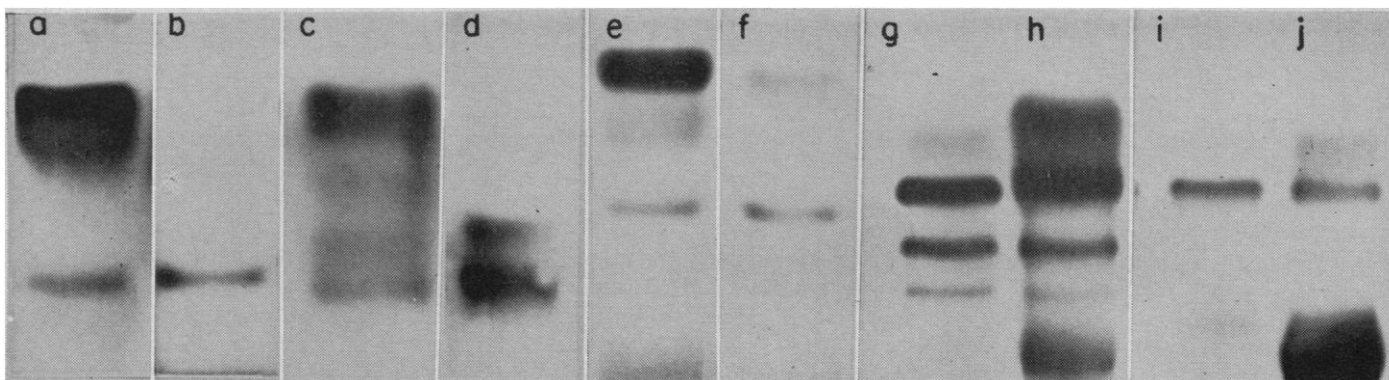


Fig. 1. Starch-gel plates at the same magnification; the starting point is at the bottom and the anodal direction is upward, *a* and *b* are from a 6-year-old female with the trisomy-E syndrome: *a* is stained for G6PD; *b*, for Gal6PD. *c* and *d* are from a male with cerebral palsy in his teens: *c* is stained for G6PD; *d*, for Gal6PD. *e* and *f* are from a liver extract from a male deer mouse (*Peromyscus maniculatus hollisteri*): *e* is stained for G6PD; *f*, for Gal6PD. *g*–*j* are from an erythrocyte extract (*g* and *i*) and a liver extract (*h* and *j*) from a 1-year-old male rainbow trout: *g* and *h* are stained for G6PD; *i* and *j*, for Gal6PD.

moving component was clearly stained (Fig. 1*b*); the latter was clearly a relatively specific dehydrogenase, because glucose, galactose, mannose, mannose-6-phosphate, fructose-6-phosphate, and 6-phosphogluconate, used as substrates, failed to stain the band. A nonspecific dehydrogenase of human liver moved rapidly toward the cathode and was not studied further. The slower-moving component of G6PD, with Gal6PD activity, appeared to exist in appreciable quantity only in liver; concentrated extracts of kidney, leukocytes, and erythrocytes revealed the presence of only the sex-linked G6PD.

The homology between the slower-moving human component and the autosomally inherited G6PD of the deer mouse (Fig. 1, *e* and *f*) is suggested. The autosomal G6PD of the latter also occurs in a small quantity that moves more slowly than the major component, and it, too, demonstrates Gal6PD activity; furthermore, the autosomal G6PD is not found in erythrocytes (3).

The liver homogenate from one male with cerebral palsy demonstrated three evenly spaced bands of this component when stained for G6PD. The slowest-moving of these three bands corresponded to the single band of the component demonstrated by the other seven (Fig. 1*c*); it may be that this male was heterozygous for a faster-moving variant of the hexose-6-phosphate dehydrogenase. The middle band may represent a hybrid dimer enzyme; if this interpretation is correct, autosomal inheritance of this component is indicated in man. The autosomal G6PD of the deer mouse forms a hybrid dimer in heterozygotes (3), but Gal6PD activity was noted on only the slowest-moving and middle bands of the component of this male (Fig. 1*d*). Thus the signifi-

cance of this observation on one male remains unclarified.

For further biochemical study, the sex-linked G6PD and the hexose-6-phosphate dehydrogenase with both G6PD and Gal6PD activities were separated with ion-exchange resin. The centrifuged and dialyzed homogenate was passed through IRC-50 and eluted with 0.05*M* phosphate buffer, pH 6.8, containing TPN at 20 mg/100 ml (fraction I). The adsorbed enzyme was then eluted with 0.5*M* sodium acetate solution (fraction II).

Electrophoresis of the two fractions showed that fraction I consisted almost entirely of the sex-linked major component, while fraction II consisted almost entirely of the slower-moving component. By use of either glucose-6-phosphate or galactose-6-phosphate as a substrate, the rate of reduction of TPN catalyzed by these separate fractions was studied (4). Fraction I had approximately 12 times as much G6PD activity (corrected for 6-phosphogluconate dehydrogenase activity) as Gal6PD activity. Fraction II, in contrast, had approximately the same activity with galactose-6-phosphate as with glucose-6-phosphate.

The hexose-6-phosphate dehydrogenase with both G6PD and Gal6PD activities was also identified in the liver of cattle; occurrence in three widely separated species suggests that it may be common to all placental mammals. Furthermore, our study of the rainbow trout indicated that this component arose early in the phylogeny of vertebrates; two major and two minor bands of G6PD can be distinguished in extracts from erythrocytes of the rainbow trout (Fig. 1*g*). In liver, an additional minor band is found, which is slower moving than any of the four

(Fig. 1*h*). While only slight Gal6PD activity by one major component is detectable on the erythrocyte extract (Fig. 1*i*), the slowest-moving component from liver showed very intense Gal6PD activity (Fig. 1*j*).

The function of Gal6PD in the liver remains obscure. It may serve to remove galactose-6-phosphate, which can be formed in the liver by the action of phosphoglucomutase on galactose-1-phosphate, which in turn is formed from galactose. Indeed, in galactosemic individuals in whom a large amount of galactose-1-phosphate accumulates, galactose-6-phosphate has been identified in erythrocytes (5).

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