the uptake and transport of sugar by the intestine (*5*), completely inhibited the effect of galactose on the accumulation of alanine. Preincubation of mucosal strips in galactose for 30 minutes prior to an additional 30-minute incubation with alanine in the absence of galactose caused a slight reduction (16 percent) in the accumulation of alanine.

Influx of alanine across the mucosal border in the presence or absence of sugars is given in Table 3. Neither sugar had any detectable effect. In these experiments, the tissue was exposed to the sugar for only the 60-second interval used to measure the flux of the amino acid across the brush border. However, similar results were obtained in other experiments in which the tissue was preincubated for 40 minutes in 20 m*M* galactose prior to determination of alanine influx.

Table 1. Effect of sugars on flux of alanine from mucosa to serosa. Both bathing solutions contained 5 m*M* alanine. Each pair of fluxes is from a single experiment on tissue from the same animal. Mannitol (20 m*M*) was present in the control solutions.

Sugar (20 m*M*)	Alanine flux (μmole per hour per cm^2)	
	Control tissue	Tissue + sugar
Glucose	1.63	0.86
	1.76	.84
	1.17	.47
Galactose	0.85	.69
	.74	.38
	2.02	1.31

Table 2. Effect of galactose on cellular accumulation of alanine. Data based on five sets of duplicate tissues from five animals. Errors are standard errors of the mean. Alanine 3.3 m*M*, galactose 20 m*M*, and phlorizin 10^{-4}*M* were used.

Additions to bathing solutions	Ratio of cell to medium concentration of alanine
Alanine	9.58 ± 0.86
Alanine + galactose	6.49 ± .45
Alanine + galactose + phlorizin	9.21 ± .82
Alanine, preincubation with galactose	8.01 ± .79

Table 3. Effect of sugars on influx of alanine (5 m*M*) across the mucosal border. All control solutions contained 20 m*M* mannitol. Errors are standard errors of the mean.

Solutions bathing mucosa	Alanine influx (μmole per hour per cm^2)
Control*	2.68 ± 0.17
Galactose (20 m*M*)	2.81 ± .20
Control†	1.55 ± .19
Glucose (20 m*M*)	1.54 ± .17

* Data based on 23 paired samples from seven animals. † Data based on nine paired samples from three animals.

Inhibition by galactose of transmural transport and cellular accumulation of alanine in rabbit small intestine is similar to findings reported for other species (*1–4*). The observation that glucose also inhibits transmural transport of alanine is contrary to the results of Newey and Smyth (*2*) and suggests that this inhibition is unrelated to either the accumulation of galactose-1-PO_4 or the extent to which the sugar is metabolized. Further, the finding that the influx of alanine, which we have shown to be dependent on sodium in the mucosal solution (*7*) and subject to competitive inhibition by other neutral amino acids (*8*), is not inhibited by sugars appears to rule out the hypothesis suggested by Alvarado (*3*).

Since a total of at least four unidirectional fluxes are involved in the overall processes of cellular accumulation and transmural transport, changes in these phenomena cannot be unequivocally ascribed to effects on any single unidirectional flux. Our experiments do, however, suggest that the efflux of alanine from the cell to the mucosal solution is altered by the sugars. If we assume that the transport system can be described by two barriers in series (the mucosal and serosal boundaries of the cell), the transmural unidirectional flux from mucosa to serosa should be given by

$$J_{ms} = J_{mc}\,J_{cs}/(J_{cm} + J_{cs})$$

in which J_{ij} is the unidirectional flux from compartment i to j (*9*). The subscripts m, c, and s denote mucosal solution, cell interior, and serosal solution, respectively. The data in Table 1 show that sugars cause a decrease in J_{ms}. This effect could be due to a decrease in J_{mc} or J_{cs} or to an increase in J_{cm}, but the data in Table 3 show that J_{mc} is unaffected by galactose or glucose. Further, a decrease in J_{cs} should lead to an increase in cellular accumulation of alanine, but accumulation decreases in the presence of galactose (Table 2). Thus, an increase in J_{cm} seems to provide an adequate explanation for the three sets of observations. It is premature to speculate on possible mechanisms of such an effect by sugar since, even if the effect is on this particular flux, there is no information as to the type of transport processes involved. However, the observation that phlorizin prevents galactose inhibition of the accumulation of alanine suggests that the sugar must enter the cell in order to exert its effect. Further studies, including direct measurement of amino acid efflux across the mucosal border, are necessary to clarify the mechanisms involved.

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Glucose-6-Phosphate Dehydrogenase: Homologous Molecules in Deer Mouse and Man

Abstract. *Two forms of glucose-6-phosphate dehydrogenase, A and B, have been reported in deer mouse tissues. The B enzyme, which showed autosomally controlled polymorphism, is now found to be equally active toward glucose-6-phosphate and galactose-6-phosphate; the A enzyme is specific for the former. Human and horse livers also have two forms of glucose-6-phosphate dehydrogenase which exhibit the same substrate specifities as those in the deer mouse. A wide variety of electrophoretic patterns was seen in the human galactose-active enzyme.*

I have previously reported (*1*) the occurrence of two different forms of glucose-6-phosphate dehydrogenase (G6PD) in tissues of the deer mouse *Peromyscus maniculatus*; called A and B, they were demonstrated by starch

gel electrophoresis. The A enzyme was present in all tissues studied: erythrocytes, liver, kidney, lung, brain, spleen, and testis; the B form, in all such except erythrocytes.

A genetic variant of the B enzyme was described in which homozygous animals presented a single zone of activity at one of two different positions, while the heterozygote showed bands at both positions plus a third midway between; this three-band pattern indicated that the enzyme is a dimer composed of randomly associated subunits. Genetic analysis showed that the enzyme is controlled by a single autosomal locus.

Since only the A form of G6PD occurs in deer-mouse erythrocytes, it was suggested that this molecule may be phylogenetically homologous with G6PD of human erythrocytes, which is controlled in man by an X-linked gene (*2*). Examination of this enzyme in approximately 400 deer mice has disclosed no genetic variant, so that one cannot determine whether it is X-controlled in this species. However, my studies of liver tissues of man and deer mouse provide evidence of homology between the A enzyme of deer mouse and the erythrocyte enzyme of man; they further show that there is another form of G6PD in human tissues which is probably homologous with the B enzyme of *Peromyscus*. Both types of G6PD were also found in horse liver.

Fresh liver tissue was obtained at autopsy from 50 human adults of both sexes who had died from various causes, and from 14 freshly slaughtered horses. The tissues were homogenized in distilled water in a proportion of 1:3 (weight:volume), frozen and thawed twice, and centrifuged at 25,000*g* in the cold until the supernatant was clear. The supernatant extract was pipetted directly into the sample slots of the gel; after electrophoresis, the gel was sliced in half and stained for G6PD.

Both human and horse livers showed gel patterns similar to that of *Peromyscus*, with a rather broad, diffuse zone at the anodal end of the gel, and lighter, well-defined bands about midway between the former and the origin (Fig. 1, left). These cathodal bands were relatively weak, especially in some human extracts, so that in certain preparations prolonged incubation (up to 8 hours) was required for their demonstration. Red cell hemolyzates from three persons showed only the anodal G6PD.

In human livers, the cathodal enzyme

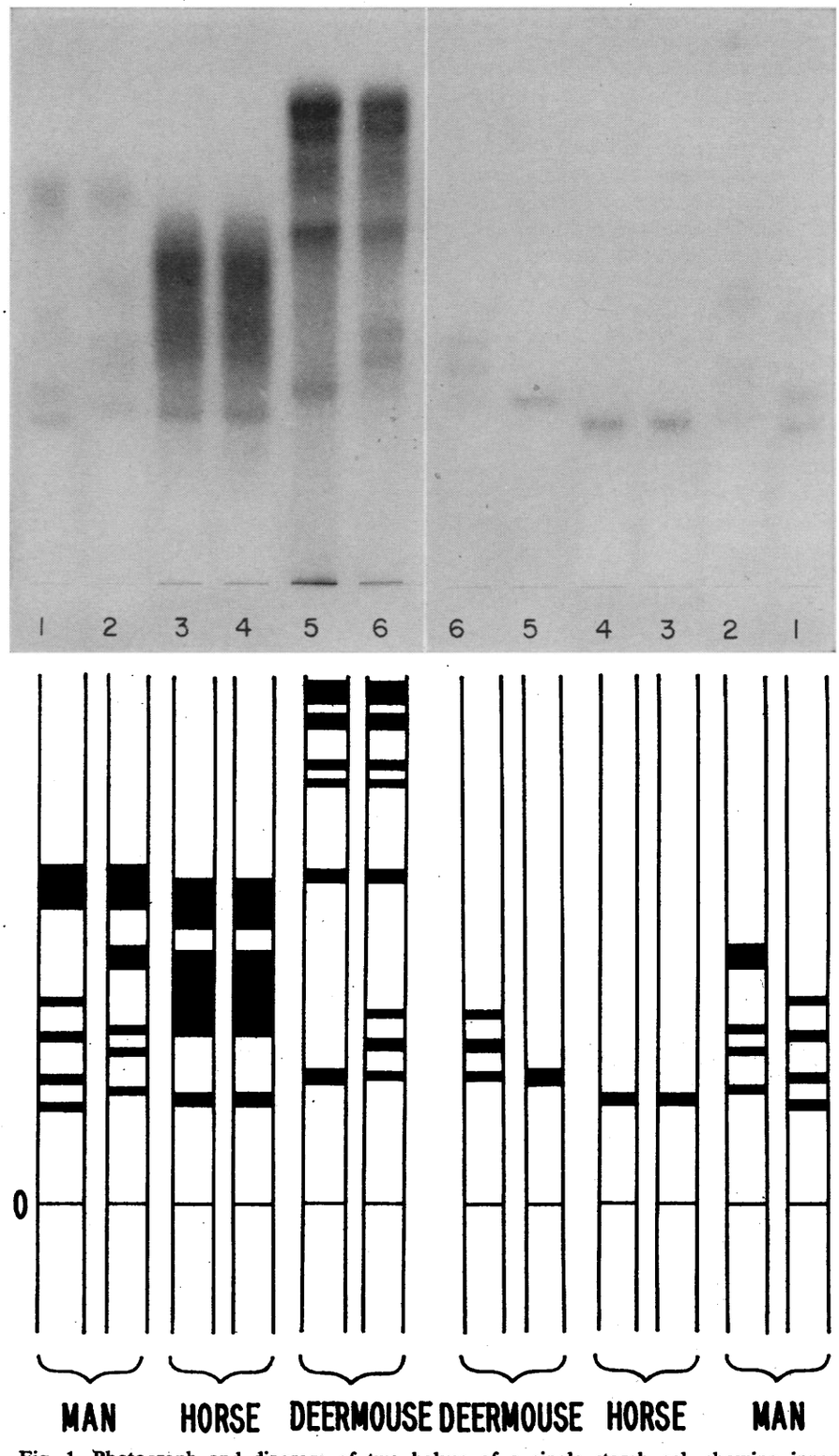

Fig. 1. Photograph and diagram of two halves of a single starch gel, showing inner surface of each slice so that the patterns are mirror images. The left half was developed in glucose-6-phosphate; right half, in galactose-6-phosphate. Anode is at the top. Six different liver extracts were used as the enzyme source: two human, two horse, and two *Peromyscus*. Note the three-band heterozygous pattern of the cathodal enzyme in one *Peromyscus* and the single, homozygous band in the other. Gel buffer was tris EDTA–sodium borate, *p*H 8.0; total molarity, 0.05. Bridge buffer was the same mixture but 0.5*M*. Vertical electrophoresis was performed in the cold for 18 hours at a gradient of 7.5 volt/cm. Gels were incubated for 4 hours (longer in some instances) at 35°C in a 100-ml mixture containing 50 mg of nitro-blue tetrazolium, 50 mg of nicotinamide adenine dinucleotide phosphate, 2 mg of phenazine methosulfate; 10 ml tris buffer, *p*H 6.8; and 5 ml glucose-6-phosphate or galactose-6-phosphate, 1.0*M*.

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 0.01*M* potassium phosphate buffer containing 5 mg of ethylenediamine tetraacetate and one drop of β-mercaptoethanol per 50 milliliters; after 2-hour centrifugation at 15,000*g* and at 4°C, the clear supernatant was used for electrophoresis. A continuous system of vertical starch-gel electrophoresis (*2*) at *p*H 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-dimethylthiazoyl-1,2)2,5 - diphenyltetrazolium bromide, 2 mg of phenazine methosulfate, and 2 mg of TPN; 0.1*M* tris HCl buffer, *p*H 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-