Galactose-Metabolizing Enzymes in the Rat (35936)

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The induction of galactose-type cataracts in rats with a yogurt diet (1) resembles the natural cataractogenic action of the milk sugars in patients with galactosemia, a hereditary disease characterized by galactose-1-phosphate uridyl transferase deficiency. The role of the transferase and other galactose-metabolizing enzymes in the pathogenesis of cataracts in rats has not been assessed, however, although enzymes in the glycolytic (2–4) and oxidative shunt pathways (5, 6) are inhibited when rats are fed strict galactose diets. Indeed, the decreasing activities of the liver enzymes, galactokinase (7), transferase (8, 9), and uridine diphosphogalactose-4-epimerase (10) in the aging rat suggest that one or all of the enzymes of the sugar nucleotide pathway limit carbohydrate metabolism in rats fed on galactose. There appears to be a similar age-dependence in the respective red cell enzymes.

Materials and Methods. Male Wistar rats were bled from the heart at 1, 2, 3, 8, and 16 weeks of age in groups of three or more. Galactose-1-phosphate uridyl transferase was assayed in the red cells by a fluorometric method (11, 12) and galactokinase, by an isotopic method (13). Red cells from five healthy men and women, ranging in ages from 21 to 50 years, served as controls for the conditions of the assays, which had been selected for enzymes from human tissues. The production of reduced triphosphopyridine nucleotide (TPNH) at constant rates (Fig. 1) by the transferases from the two species assured us, for example, that the substrate conditions during the 30 min incubation period were adequate for the rat transferase. Nor was differential absorbance of rat hemoglobin a source of error in calculating transferase activities, as the hemoglobins of both species approached their maxima at 410 nm. Storage of whole blood samples for 18 hr at 4° before assay caused a 1% loss of initial activity or less.

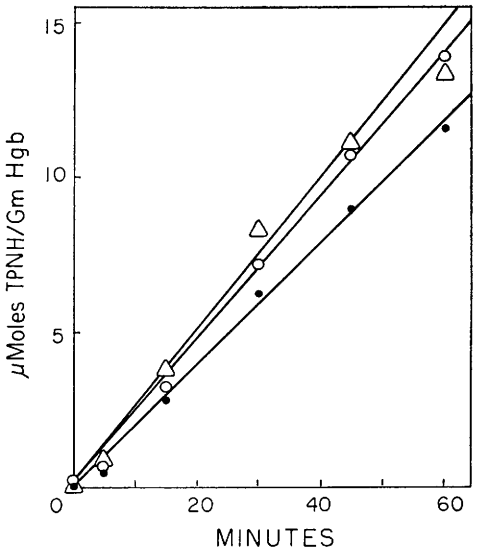

FIG. 1. Production of reduced triphosphopyridine nucleotide by red cell transferases from 8-week-old rats (●), a 45-year-old man (Δ); and a 23-year-old woman (○). Ten microliter samples of whole blood were incubated with 100 μl of reaction mixture, made up in 6 ml amounts, as follows: 4.0 mg of uridine-5′-diphosphoglucose; 6.0 mg of galactose-1-phosphate; 4.0 mg of triphosphopyridine-nucleotide; 2.0 ml of 0.75 *M* Tris–acetate buffer, pH 8.0; 0.8 ml of saturated digitonin; 0.9 mg of ethylenediamine tetraacetic acid; 2.4 mg of magnesium chloride; 3.2 ml of water.

Electrophoretic mobilities of the transferases from the two species were compared on horizontal starch gel (14, 15).

Results. Mean activities of erythrocyte transferase were greatest in the week-old rats and declined to minimum stable levels when they were 2 months old (Table I). Erythrocyte galactokinase activities were also greatest in younger rats; they declined gradually and reached minimum stable levels in the 2-month-old rats.

TABLE I. Activities of Galactose-Metabolized Enzymes in the Red Cells of Rats of Different Ages.

Age of Rats (weeks)	Activities	
	Transferase (units/gm of Hgb)	Kinase (μmoles of galactose phosphorylated/ml of RBC/hr)
1	24 ± 0.9 (2)[a]	3.3 (5)[b]
2	21 ± 0.8 (4)	2.6 ± 0.02 (3)
3	18 ± 0.4 (4)	3.0 ± 0.32 (4)
8	11 ± 0.4 (10)	2.2 ± 0.30 (4)
16	10 ± 0.7 (2)	2.2 ± 0.20 (3)

[a] Results are expressed as the mean ± SE. Number of animals is given in parentheses.

[b] Assay of a pooled sample from five rats.

The transferase in two month-old rats consisted of two isozymes with electrophoretic mobilities greater than those of either the normal human or Duarte variant transferases.

Mean activities of erythrocyte transferase and galactokinase in the healthy adults were 12 to 16 units/g of Hgb and 0.3 to 0.5 units (μmoles of galactose phosphorylated/ml of RBC/hr), respectively, and resembled known values obtained by the same methods (11, 16).

Discussion. The decreasing activities of red cell transferase and galactokinase with increasing age of the rat parallel the observations of their respective activities in liver (7–9). Since weaned rats are relatively deficient in these enzymes, especially in the transferase, they apparently can not use the excessive sugar of a galactose-rich diet.

The more rapid appearance of the cataract in younger rats, given the same diet as older rats (1), may also be iatrogenic, as rats growing at a logarithmic rate (from 35 to 100 days old) consume more food than older rats (17). Their galactose load, therefore, is greater than in older rats on the same diet, without compensatory increases in activity of galactose-metabolizing enzymes at the very time when the levels are declining.

The age-dependent levels of transferase in maturing rats, as opposed to the age-independence of the human transferase (11), may have phylogenetic significance, when the great change in the rodent feeding habits at weaning is considered. They suggest, further, that the rat biochemical counterpart in man, the carrier of galactosemia with a lifelong suboptimal level of transferase, may be equally liable to the cataractogenic effect of galactose-rich diets.

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