Enzymes of carbohydrate metabolism in developing galactose cataracts of rats

Theodore O. Sippel

Information on the nature of alterations in the protein composition of the lens preceding the formation of nuclear cataracts in galactose-fed rats was sought in an investigation of a group of enzymatic proteins. Changes in the levels of five enzymes of carbohydrate metabolism were followed through the progressive phases of cataract development. A marked decrease in the activity (per lens) of aldolase occurs early in the first week of galactose feeding and appears to correlate with the vacuolization of the lens cortex. The other enzymes studied remain at control levels until a few days after the appearance of nuclear cataracts, at the end of the second week. They then decrease up to 50 per cent in activity when half of the total protein is lost from the lens. The sensitivity of an enzymatic protein to lens changes induced by galactose is probably influenced by its intrinsic metabolic stability, regional distribution, and subcellular localization, but is not related to a sulfhydryl requirement for catalytic activity.

Densely white nuclear cataracts develop in the course of a day in eyes of young rats fed a galactose-rich diet for approximately two weeks. The disruption of lenticular protein metabolism which becomes evident within the first week of galactose feeding may promote this relatively sudden alteration of lens structure. One of the presumably critical early changes is the accumulation (despite the arrest of net protein synthesis and the increased proportion of insoluble protein) of a soluble protein which precipitates upon oxidation in vitro.[1, 2] It appears to result from an increase in the availability of sulfhydryl groups in the β-crystallin fraction of the structural lens proteins[2] and may be connected with the striking decrease in the glutathione content of the lens shortly after the animals are placed on the galactose diet.[3] Surprisingly, no other precataractous changes in the physicochemical properties of the soluble structural lens proteins have been demonstrated by fractionation procedures.[4-6]

Further information on the nature of the disturbances in the protein composition of the lens may come from investigation of the changing levels of lenticular enzymes, of which at least three have been reported to decrease in activity prior to the formation of a distinct galactose cataract.[7-9] The enzymatic proteins, which are readily measured on the basis of their catalytic activities, are structurally widely diversified. Several depend in one way or another on sulfhydryl groups and would be expected to be among the first affected during cataract development.[10]

The present report, as part of a survey of

From the Department of Anatomy, The University of Michigan Medical School, Ann Arbor, Mich.

This work was supported by Research Grant NB-03711 from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, and by Grant 165 from the University of Michigan Faculty Research Equipment Fund.

enzymatic changes in the lenses of rats during galactose feeding, concerns five enzymes which participate in lenticular carbohydrate metabolism. Selection of this group stems from reports that certain pathways of glucose breakdown are reduced in activity during the early phases of galactose cataract development.[11, 12]

Materials and methods

A mixture of 50 per cent galactose in ground Purina laboratory chow was fed to female Sprague-Dawley rats initially weighing 50 to 60 grams, while control animals were given ground chow alone. The rats were painlessly put to death by decapitation after periods of up to 40 days, and the lenses were rapidly removed by an anterior approach from the eyes in situ. Nuclear cataract (Stage 4 as previously reported[3]) was noted in 4 of 6 lenses removed on the fifteenth day of galactose feeding and on all subsequent intervals until obscured by total opacification (Stage 5) after approximately 30 days. Each lens was blotted to remove extraneous material, weighed, and homogenized in 1.0 ml. of ice-cold 0.01M sodium phosphate buffer of pH 7.5. The homogenates were frozen for periods ranging from 30 minutes to overnight and then were thawed and centrifuged at 1,000 ×G for 10 minutes in the cold, providing clear extracts.

The enzymes aldolase, lactic dehydrogenase (LDH), malic dehydrogenase (MDH), α-glycerophosphate dehydrogenase (GPDH), and glucose-6-phosphate dehydrogenase (G-6-PDH) were measured as shown in Table I, but not all five were analyzed in every extract. Each requires, or may be coupled with (aldolase), a nucleotide of which the rate of oxidation or reduction was followed at 340 mμ in a Beckman Model B spectrophotometer for a total of 2 to 10 minutes at room temperature. The enzyme activities are expressed below as micromoles of substrate consumed per lens per hour.

Results

The changes in the enzyme levels in the lenses of both galactose-fed and control rats are shown for aldolase and G-6-PDH in Fig. 1 and for LDH (to which MDH was quantitatively similar in all respects) and GPDH in Fig. 2. Curves were fitted to the data points by inspection. All five enzymes appeared to increase in activity in the control lenses, but only LDH (and MDH) and GPDH were significantly more active at the end of 40 days. The average fresh weights of these lenses increased from 17.8 to 32.2 mg. during this time.

The aldolase level in the lens (Fig. 1) was affected by galactose feeding by the end of the first day when it was 10 per cent lower than the starting value ($p = 0.05$, t test). Only 30 per cent of the initial aldolase activity remained after 4 weeks. The presence of nuclear opacities in 3 of the 5 lenses from galactose-fed rats used on the fifteenth day did not appear to change the aldolase level, since the highest and two lowest values were obtained from the cataractous lenses.

The activities of G-6-PDH (Fig. 1) and of LDH and MDH (Fig. 2) did not differ

Table I. Composition of enzyme assay reaction mixtures (final concentrations are shown)

Enzyme and reference	*Extract (μL/ml.)*	*Buffer* (50 mM.)*	*Coenzyme† (0.1 mM.)*	*Substrate‡ (1.0 mM.)*	*Additions*
Aldolase[13]	100	Phosphate	NADH	Sodium fructose-1,6-diphosphate	TPI/GPDH§ (1.7 μg/ml.)
LDH[14]	10	Phosphate	NADH	Sodium pyruvate	—
MDH[15]	10	Phosphate	NADH	Oxaloacetic acid‖	—
GPDH[16]	100	Phosphate	NADH	Dihydroxyacetone phosphate¶	—
G-6-PDH[17]	100	Tris-HCl	NADP	Sodium glucose-6-phosphate	$MgCl_2$ (10 mM.)

*pH 7.5, except for Tris-HCl which was at pH 8.0.

†NADH, reduced nicotinamide-adenine dinucleotide (formerly DPNH); NADP, oxidized nicotinamide-adenine dinucleotide phosphate (formerly TPN).

‡Except sodium fructose-1,6-diphosphate, 5 mM.

§Mixture of triosephosphate isomerase and GPDH (Calbiochem).

‖Freshly dissolved and neutralized just before use.

¶Prepared from cyclohexylamine salt (Sigma), stored frozen, and neutralized just before use.

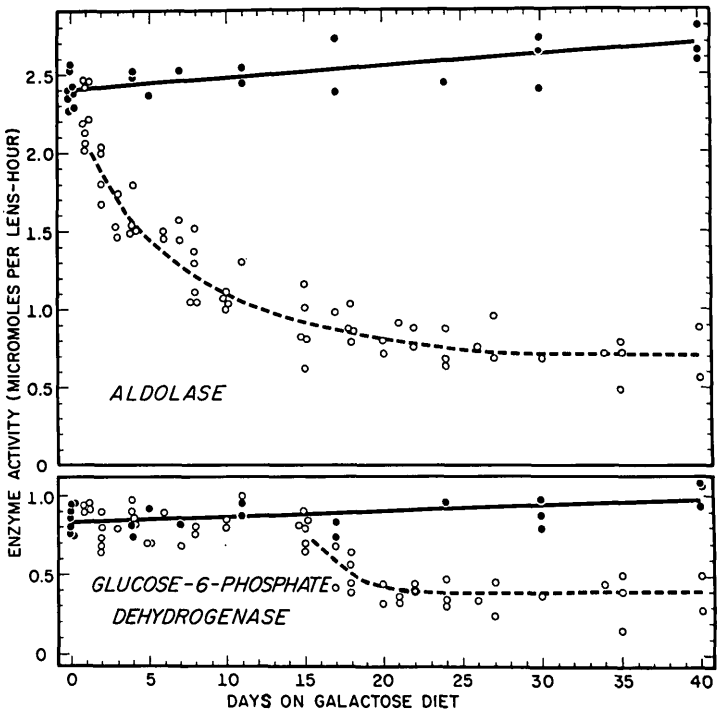

Fig. 1. Changes in the levels of aldolase and G-6-PDH in lenses of rats fed 50 per cent galactose (open circles) and of control rats on a normal chow diet (filled circles). G-6-PDH activity is not corrected for NADP reduction by 6-phosphogluconic dehydrogenase.

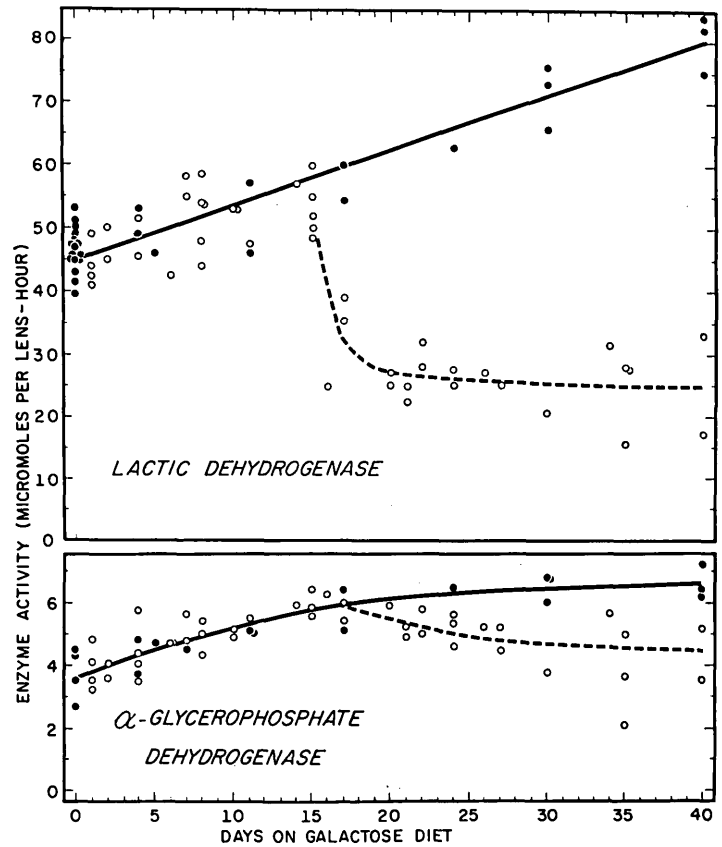

Fig. 2. Changes in the levels of LDH (and MDH; see text) and GPDH in lenses of rats fed 50 per cent galactose (open circles) and of control rats on a normal chow diet (filled circles).

significantly from the values in control lenses through the first two weeks of galactose feeding. Variations in the measurements made on the lenses of galactose-fed rats on day 15 were not correlated with the presence or absence of nuclear opacities. In the following 5 days, however, the activities of all three enzymes decreased 50 per cent in the cataractous lenses and remained at a low level thereafter.

GPDH activity in the lens was least affected by galactose feeding (Fig. 2). In the first 20 days, the level increased to the same extent as in control lenses. Beyond this time the difference between the average activities measured on the two groups was less than 30 per cent.

Discussion

The prompt decrease in aldolase activity soon after the start of galactose feeding is correlated with the progressive vacuolization of the lens cortex and begins at about the time that most of the nonprotein sulfhydryl (glutathione) disappears from the lens.[3] The catalytic activity of aldolase, in contrast to that of the more persistent GPDH, is not particularly dependent upon sulfhydryl groups.* The aldolase molecule is, however, unusually rich in sulfhydryl[26] and so may require a high glutathione level for its maintenance. An intrinsic metabolic lability of the enzyme is suggested by the evidence that during normal aging the decrease in unit activity in the lens nucleus is greater for aldolase than for several other enzymes of carbohydrate metabolism;[19] replacement protein synthesis probably does not occur in this region.[20] The combination of a low level of stability of the molecule, a possible requirement for glutathione, and the localization of a large part of the activity in the lens cortex may render aldolase particularly sensitive to galactose-induced cortical vacuolization.

The sodium-potassium activated adenosine triphosphatase of the rat lens also has been found to decrease in activity soon after the beginning of galactose feeding.[9] Because of its distinctive properties, i.e., a close association with cell membranes and the localization of two thirds of its total activity in the lens epithelium (with the decrease occurring entirely in the epithelial component), this enzyme is probably affected by factors which differ from those proposed to control aldolase activity.

Most of the lenticular enzymes which have been followed during long-term galactose feeding maintain at least their initial levels of activity throughout the vacuolar phase of cataract development. This group includes 6-phosphogluconic dehydrogenase[7] and ATP-creatine phosphotransferase[21] in addition to four of the enzymes examined in the present study. Of these, LDH, MDH, and G-6-PDH decrease 50 per cent in activity soon after the appearance of nuclear cataracts. As soluble enzymes of less than 150,000 molecular weight, they presumably escape through the lens capsule following the postcataractous breakdown of lens fibers when half of the total lenticular protein is lost.[3, 22] The smaller and somewhat delayed decrease in GPDH (and its continuing accumulation during the preceding vacuolar phase) probably reflects the localization of a part of the activity of this enzyme in the mitochondrial fraction of the lens.[23] Mitochondria are contained almost exclusively in the epithelium and equatorial cortex, two areas not visibly damaged during the development of galactose cataracts. There is in fact a rapid proliferation of epithelial cells which is thought to account for the eventual recovery of Na-K activated adenosine triphosphatase activity beyond its initial level[9] and which probably modifies the extent of the changes in GPDH activity.

The current results do not confirm the report that the activity of G-6-PDH is decreased before the onset of visible changes in the lens during galactose feeding.[7] They are, however, in accord with

*In developing x-ray cataracts of rabbits, sulfhydryl-dependent enzymes are among the first and last affected (e.g., acetaldehyde oxidase and hexokinase, respectively). Aldolase, although more sensitive than several other enzymes of carbohydrate metabolism, is not decreased in activity until late stages. A relationship specifically to glutathione appears most characteristic of enzymes losing activity earliest (e.g., glyceraldehyde-3-phosphate dehydrogenase, glyoxalase, and glutathione reductase).[10] The eventual decrease in the glutathione reductase activity in lenses of rats fed galactose probably is not significantly greater, however, than the change in G-6-PDH activity.[18]

the finding that aldolase activity may be considerably reduced while G-6-PDH remains unaffected.[8] It has been suggested that the decrease in the aldolase level is responsible for the lowering of the glycolytic rate in the lenses of galactose-fed rats.[8, 24] A comparison of the progressive change in aldolase activity reported here with earlier measurements of glycolysis in the lenses of similarly treated rats[12] shows that both decrease about one third in the first two days of galactose feeding. However, in view of the evidence that aldolase activity is not normally rate-limiting for lenticular glycolysis,[25] and since the glycolytic rate becomes relatively constant after two days of galactose feeding while aldolase activity continues to decrease, it is unlikely that their changes are directly related.

I wish to thank Miss Monica Baumeister for providing excellent technical assistance.

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