THE RELATIONSHIP OF GALACTOSE-1-PHOSPHATE ACCUMULATION AND URIDYL TRANSFERASE ACTIVITY TO THE DIFFERENTIAL GALACTOSE TOXICITY IN MALE AND FEMALE CHICKS

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Summary:

An 18% galactose diet resulted in a significantly greater mortality in female chicks than in males. Galactose-1-phosphate was found to accumulate in the brains of both sexes. However, a much higher concentration was present in brains from female chicks. Galactokinase activity was similar in male and female chicks as was the UDP Gal-4-epimerase value. Galactose-1-phosphate uridyl transferase activity was significantly lower in tissues from female chicks as compared to males. This could account for the increased concentration of galactose-1-phosphate in the brain and the higher mortality in the female chick.

INTRODUCTION:

Galactosemia is an inherited disorder in which the affected individuals have no detectable Gal-1-P uridyl transferase activity(1). Therefore, galactose(2), galactitol(3,4,5) and Gal-1-P(6,7) accumulate before the metabolic block. The disorder is clinically characterized by cataracts, failure to thrive, enlarged liver, mental retardation, and often death within the first few months of life. Accumulation of galactitol appears to result in cataract formation(8) while Gal-1-P accumulation may account for the other clinical manifestations(9). This has been supported by studies in patients with galactokinase deficiency (10,11,12). Galactitol and galactose are found in the urine of the affected individuals and the only physical finding is cataracts.

A disease state also results when a high galactose diet is fed to chicks (13,14). A syndrome consisting of shivering and shaking, seizures and eventually death is seen in chicks on a diet containing greater than 10% galactose(15). It has also been demonstrated that female chicks are more sensitive to galactose than males(16). The tissue accumulation of galactose(17), galactitol(17) and UDPGal(18) has been reported. We have been studying this animal model to further elucidate the mechanisms of galactose toxicity.

METHODS:

Newly hatched Leghorn chicks were placed either on a control synthetic diet or galactose diet. A modification of the basal diet described by Kokatnur

et al.(19) was used. Vitamin E, non-nutritive cellulose, and corn oil were added and p-amino benzoic acid was omitted. In the galactose diet, galactose was added at the expense of glucose so that it accounted for 18% of the total diet. After specified intervals, the chicks were sacrificed by decapitation and the appropriate tissues removed.

For the enzymatic assays, the tissues were homogenized in glass-teflon homogenizers with two volumes of 0.01 M tris-HCl buffer, pH 7.8, containing 0.14 M KCl, 0.01 M mercaptoethanol, and 0.001 M EDTA. The homogenate was centrifuged at 27,000 x g for 30 min. The resulting supernatant was centrifuged at 100,000 x g for 1 hr. Galactokinase was assayed in the 100,000 x g supernatant according to the method of Sherman(20); Gal-1-P uridyl transferase as previously described(21) with excess 6-phosphogluconate dehydrogenase (Type VI, Sigma); and UDPGal-4-epimerase as described by Maxwell et al.(22). Protein was determined by the method of Lowry et al.(23).

Gal-1-P was extracted from brain by homogenizing with two volumes of 0.01 M tris-HCl buffer, pH 7.8, containing 0.14 M KCl. Debris was removed by centrifuging at 27,000 x g for 30 min. The supernatant was placed in a boiling water bath for 3 min and centrifuged at 17,500 x g for 15 min to remove the protein. Gal-1-P concentration was determined by the method of Kurahashi and Anderson(24). For the determination of Gal-1-P in crude extracts, a highly purified transferase was required. Purified transferase was prepared as previously described(21). Galactose and glucose were determined in serum using galactose oxidase and glucose oxidase (Worthington Biochemical Corp.).

RESULTS AND DISCUSSION:

After two days on the galactose diet, the female chicks started to develop toxic symptoms. By 4 days, the mortality was approximately 85% for the female chicks and 20% for the males. To exclude hypoglycemia, a total of 10 control and 17 galactose-fed female chicks were assayed for blood glucose over a 4 day period. The average glucose concentration was 266 mg% (range 244 mg% to 301 mg%) in the controls and 271 mg% (range 216 mg% to 315 mg%) in the galactose fed chicks. No significant difference in blood glucose was observed between the control group and the galactose toxic group. A total of 8 controls and 12 galactose-fed female chicks were assayed for blood galactose. The concentration of galactose ranged from 130 mg% to 500 mg% in the galactose-fed chicks and was minimal in the controls. The blood levels of glucose and galactose were similar to those reported by other investigators (13,14,15,16).

Since Gal-1-P has been postulated to be the toxic agent in galactosemia and the symptoms in the galactose toxic chicks are mainly neurological, its concentration was determined in brain(Fig.1). Gal-1-P was found to accumulate

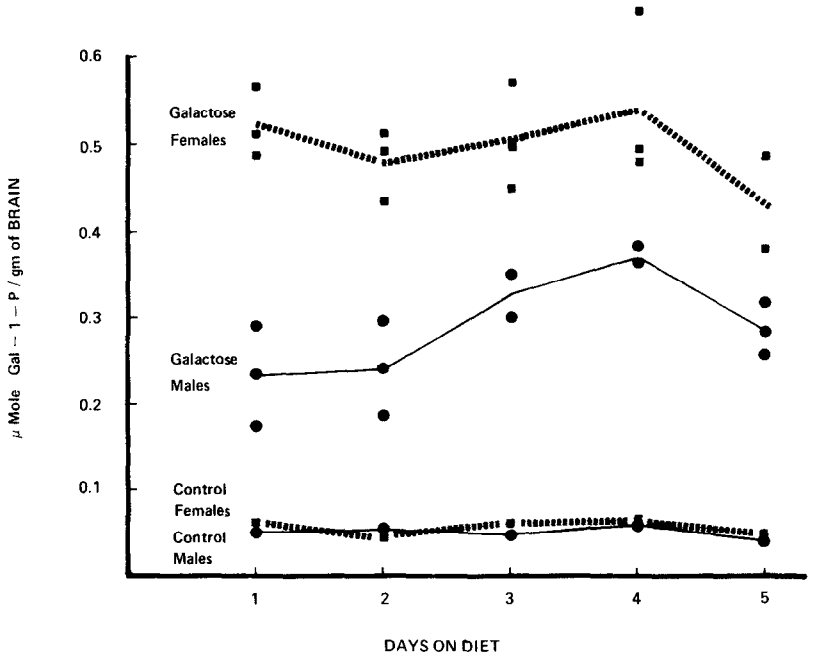

Fig. 1. Galactose-1-phosphate concentration in brains from galactose-fed and control chicks

in brains from chicks on a galactose diet. Gal-1-P concentration was higher in brains from female chicks than in males. This appeared to correlate with the higher mortality in female chicks.

While this paper was in preparation, Kozak and Wells (25) reported the analysis of galactose-P as well as other compounds in the brain from galactose-fed male chicks. For galactose-P analysis, they measured the increase in galactose after incubation with alkaline phosphatase. Their value for galactose-P was slightly higher than ours. This could be explained by the fact that they fed a higher percentage of galactose in the diet or other galactose-P besides Gal-1-P could have contributed to the total galactose-P.

In order to determine the possible cause of Gal-1-P accumulation, the galactose enzymes were assayed in brain and liver from day old male and female chicks(Table 1). Activity was similar in male and female chicks for both galactokinase and epimerase, but transferase activity in the tissues from male chicks was approximately twice the female chick value. This would account for the higher accumulation of Gal-1-P in the brain of female chicks.

Table 1

ACTIVITIES OF GALACTOSE ENZYMES IN TISSUES FROM MALE AND FEMALE CHICKS

Sex	Tissue	No.	Galactokinase*	No.	Transferase*	No.	Epimerase*
Male	Brain	12	1.08 ± 0.31	12	0.96 ± 0.37	11	3.93 ± 0.64
Female	Brain	10	1.22 ± 0.27	10	0.52 ± 0.13	9	4.12 ± 0.88
Male	Liver	8	3.71 ± 0.71	8	3.48 ± 0.43	8	2.68 ± 0.40
Female	Liver	7	3.40 ± 0.73	8	2.20 ± 0.31	8	2.96 ± 0.39

*Activity is given as μMoles x 10^3 of product formed per min per mg of protein and as average $\pm$ standard deviation.

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