GALACTOSE TOXICITY IN THE CHICK: TISSUE ACCUMULATION OF GALACTOSE AND GALACTITOL

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Received 12 September 1969

1. Introduction

The ingestion of the sugar D-galactose by the chick results in a toxicity syndrome characterized by shivering and shaking with tetanic and clinic spasms of leg and wing muscles, eventually resulting in death [1–3]. Susceptibility to galactose toxicity varied with the breed, Rhode Island Reds having a higher mortality than Leghorns, and with sex, males being more resistant than females of a given breed [4].

Very little is known regarding the mechanism by which galactose produces its toxic effect on chicks and biochemical observations are meagre. High levels of plasma galactose without change in plasma glucose have been reported [1, 2]. Liver uridine diphosphohexose levels appear to be increased [4] while glycogen stores are decreased [1]. Amino acid excretion in cloacal contents is increased during galactose feeding.

Because the neurotoxicity of the galactose-fed chick may be an exaggeration of more subtle events occurring in mammals subjected to galactose toxicity, we have undertaken an investigation of galactose metabolism in the chick. This report describes the accumulation of galactose and galactitol in various tissues of chicks ingesting galactose.

2. Materials and methods

In each of 10 experiments newly hatched Rhode Island Red chicks (16 males and 16 females) were fed a non-medicated mash diet and were given galactose (5% w/v) in the drinking water. Controls were given either 5% glucose or plain water. Deterioration in the physical consition of the chicks began after 3–4 days on galactose. About 23% of females and 16% of males died by the 4th day of feeding. At day 7, only 10% of the females and 20% of the males survived.

Animals were sacrified at specified intervals by ether anaesthesia and exanguination by cardiac puncture. Tissues were removed and frozen at –45° until analysis. Galactose and galactitol were isolated by extraction of whole tissues in boiling water and deproteinized [5]. After deionization of the extract, analysis of the neutral sugars as their trimethylsilyl ether derivatives by gas-liquid chromatography was accomplished [6]. Gas chromatographic analysis was carried out using a 3% OV-1 on Gas Chrom Q packing (100–120 mesh) in both a Hewlett-Packard Model 402 and a Packard Instrument Model 3372 equipped with hydrogen flame detectors. The 6 ft glass columns were maintained at 190°.

3. Results and discussion

Tissue galactose and galactitol levels of both male and female chicks are shown in fig. 1. After 3 days of galactose feeding, there is an accumulation of galactose in all tissues, with brain and heart having the lowest concentrations. Glactose levels increased as long as feeding continued, with little or no differences between male and female chicks. In-

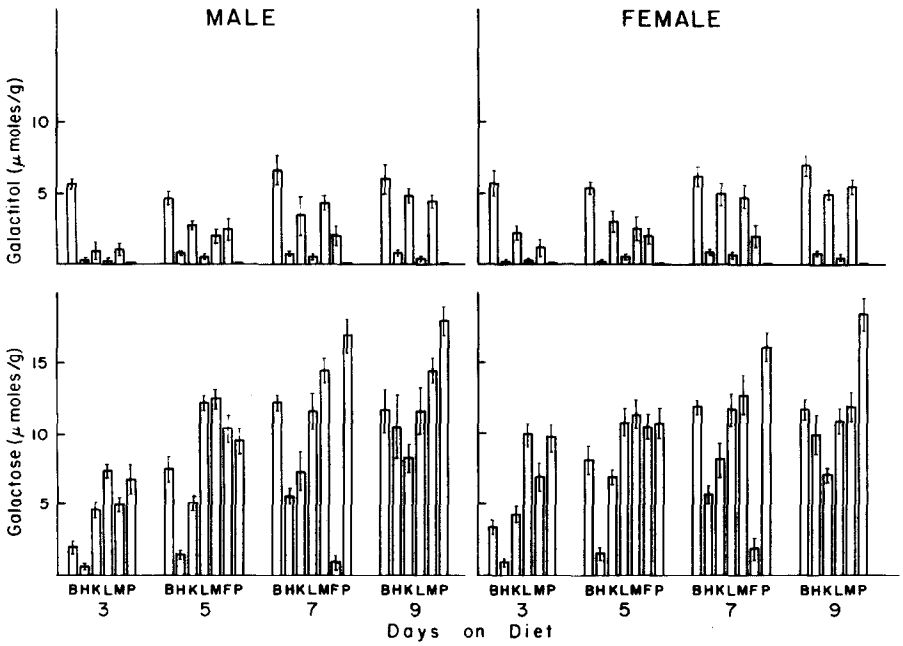

Fig. 1. Galactose and galactitol accumulation by male and female chicks. The chicks were maintained on the diet for the specified times. The results of tissues from 6 to 12 chicks are expressed as μmoles/gram of wet weight and are mean values ± standard errors.

terestingly, the amount of galactose in heart muscle was found to be considerably less than that found in skeletal muscle (1.53 vs 12.5 μmoles/g).

Galactitol accumulates in brain more rapidly than in any other tissue. After 3 days of galactose feeding chick brain has reached galactitol levels equal to or greater than that found in other tissues after 9 days. No difference in brain galactitol concentration between male and female was observed. The lack of differences in tissue galactitol levels between male and female suggests that the increased mortality observed in the female cannot be attributed to the amount of galactitol present. Galactitol levels were comparable to that found in brains of rats fed galactose [7] but less than that found in brains of galactosemic humans [8].

Galactitol was never detected in plasma and only very small quantities were observed in liver even after 9 days of galactose-feeding. Both galactose and galactitol have been detected in the cloacal contents after 5 and 7 days feedings, but analysis of the 3 and 9 days specimens was not done.

Increased levels of galactose and galactitol were maintained as long as galactose feeding was continued. Within 24 hr after removal of the diet from chicks fed the diet for 7 days, galactose concentration dropped to zero in all tissues except muscle, where it remained at a level of 1.58–2.00 μmoles/g for 3 days. It had completely disappeared by day 5. Although the chick normally never ingests galactose, chick tissues possess the enzymes of uridine nucleotide pathway of galactose metabolism [8]. This would account for the galactose elimination.

A concomitant disappearance of galactitol from various tissues was also noted. Galactitol content of brains dropped from 6 μmoles/g to less than 0.6 μmoles/g, while muscle galactitol dropped from 4.28 μmoles/g to 0.11 μmoles/g in 24 hr. Galactitol was completely absent from both tissues 3 days after removal from diet. The absence of galactitol in the cloacal contents and plasma suggests that the depletion of galactitol from the tissues is due to further metabolism of the sugar alcohol. Extensive metabolism of galactitol, however, has been thought to be

non-existent [10]. The galactose toxicity syndrome abated with the cessation of galactose feeding. By the time galactose and galactitol had disappeared from the tissues the chicks appeared normal.

Galactitol accumulation due to activity of aldose reductase has been shown to be an important etiologic factor in lens cataract formation in rats fed escessive amounts of galactose [11]. Presence of this polyol in other tissues, with especially high levels observed in the brain of galactose toxic rats and humans, has suggested the possibility that galactitol may play a role in causing the mental retardation seen in human galactosemia. The etiology of the severe galactose toxicity seen in the chick remains to be determined. The fact that tissue galactitol levels are highest in the brain after 3 days of galactose feeding, at a time when the neurologic syndrome is manifest, may indicate some relationship of the polyol to the observed disorder.

There appears to be no inherited animal counterpart to human galactosemia, so that feeding escessive galactose to the young rat has been used as an animal model. Recently, the combination of galactose plus ethanol feeding with resulting severe mortality in rats has been advocated as a better model [12]. Although the syndrome of galactose toxicity in the chick has been known for many years, extensive studies of this model system have not been made. Because the chick does not normally ingest galactose, exposure to the sugar produces more devastating effects than those seen when mammals whose diet includes galactose are given excessive amounts. We feel that the chick represents an excellent model for the study of biochemical galactose toxicity and are pursuing further studies to elucidate the underlying cause.

Acknowledgement

Supported by a grant from the National Institutes of Health (AM 10894).

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