THE DISTRIBUTION OF GALACTITOL IN TISSUES OF RATS FED GALACTOSE

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Van Heyningen (1959a) demonstrated that galactitol accumulated in the lens of rats fed a 35% galactose-containing diet. The only other tissues found to contain galactitol were cardiac and skeletal muscle though only in trace amounts. In the course of the development of quantitative methods for carbohydrate analysis by gas-liquid chromatography, Wells et al. (1964a) discovered galactitol in the urine and plasma of galactosemic patients and subsequently in the brain of two patients who had died with the disease (Wells, et al., 1965a). Rats fed 35% galactose-containing diets for several weeks excreted significant amounts of galactitol in the urine (Chin, 1964). The quantity of galactitol in the urine was not diminished if the lens of the rats were removed. These observations suggested that tissues other than lens must be capable of converting galactose to galactitol. We wish to report the distribution and content of galactitol in various tissues of rats fed a 35% galactose-containing diet for 16 days.

Sixteen weanling male albino rats of the Holtzman strain were divided into two equal groups and housed in individual cages. The control group was fed a diet of the composition, 72.8% sucrose, 18% casein, 4.0% Wesson salt mix,* 5.0% cotton seed oil (supplemented with cod liver oil and α-tocopherol), 0.1% choline chloride and 0.1% vitamin mix. The experimental group was fed a diet of the same composition except for the substitution of 35% of galactose at the expense of an equal amount of sucrose. At the end of the 16 day feeding period

*Nutritional Biochemicals Corporation, Cleveland 28, Ohio

the rats (ether anesthetized) were killed by exsanguination with a heparinized syringe. The blood was centrifuged at 3,000 rpm for 15 minutes at 4°. The blood cells, plasma, lens, brain, heart, kidney, liver, small intestine and leg muscle were immediately frozen until analyzed.

Plasma sugars were determined by the method of Wells, et al., (1964b). For the analysis of sugars from red blood cells, a modification of the method of Wells, et al., (1965b) was employed. Two ml of a suspension of blood cells diluted with 3 volumes of water were shaken vigorously with 2 ml of 10% Trichloroacetic acid (TCA) and allowed to stand for two hours. After centrifugation the residue was re-extracted with 2 ml of 5% TCA. The combined TCA extracts were washed 7-10 times with diethyl ether until the ether extract was free of TCA. An aliquot was dried before trimethylsilylation and gas chromatographic analysis. The brain, heart, kidney, pooled lenses and representative samples (1g) of liver, small intestine and leg muscle were weighed, and the lipids extracted by the procedure of Folch, et al. (1957). The water washes from the Folch procedure as well as the water extract of the residue were combined. Aliquots were dried and trimethylsilylated prior to gas chromatographic analysis.

The galactitol concentration of several tissues is summarized in Table I. With the exception of the kidney, the tissue from the control animals contained no detectable acylic polyol. In agreement with van Heyningen (1959a), eye lens contained the highest concentration of galactitol. Skeletal and cardiac muscle accommodated significant quantities of the polyol, 22.32 and 18.06 μmoles /g tissue, respectively. Assuming respresentative skeletal muscle [45.4% of body weight (Donaldson, 1924)], the total amount of galactitol deposited in the muscle would amount to 185mg/100g body weight. Lesser but significant quantities of galactitol were observed in the brain, kidney, small intestine and packed blood cells of the galactose-fed rats. The liver and plasma were essentially devoid of the hexitol even at the level of sensitivity provided by the gas-liquid chromatography technique. The gas chromatographic records of the

TABLE I

Free Galactitol Concentration (μmoles /g fresh tissue) in Tissues of the Rat

Tissue	Galactose-fed	Sucrose-fed
Eye lens*	44.38	none
Skeletal Muscle	22.32±2.79**	"
Cardiac Muscle	18.06±3.75	"
Brain	5.17±0.63	"
Kidney	3.18±0.91	0.34±0.10+
Small Intestine		
Proximal 1/3	1.96±0.28	none
Distal 2/3	1.16±0.44	"
Blood Cells++	0.67±0.12	"
Liver	none	"
Plasma	"	"

* Pooled sample of 12 lenses
** Standard deviation, $s = \pm \sqrt{\frac{\Sigma X^2}{N} - \left(\frac{\Sigma X}{N}\right)^2}$, N = 8
\+ Tentitatively identified as sorbitol
++ Concentration is based on μmoles/ml of packed cells

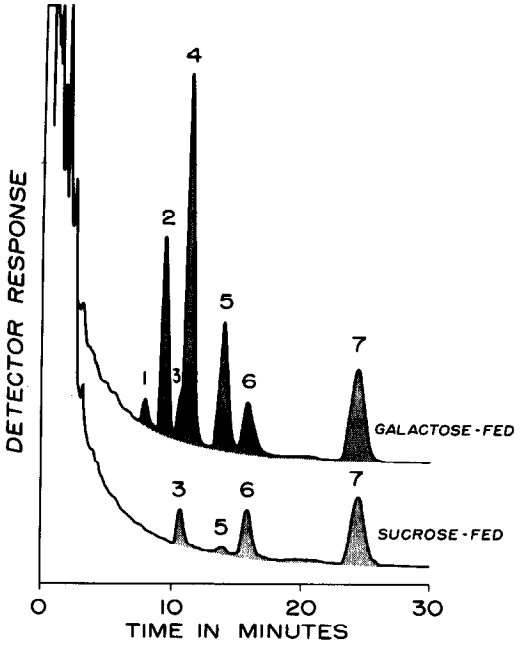

Figure 1

free sugar trimethylsilyl ethers from the kidneys of control and galactose-fed rats are shown in Figure 1. In the upper trace, peaks corresponding to γ, α and β galactose (1, 2 and 4), α and β glucose (3 and 6), galactitol plus a trace of sorbitol (5) and myo-inositol (7) may be identified. In the lower trace only α and β glucose, sorbitol and myo-inositol can be seen.

Although the finding of galactitol accumulation in a given tissue does not necessarily indicate that the tissue is the actual site of galactitol biosynthesis, the low permeability of cellular membranes to the sugar polyols (Le Fevre and Davies, 1951; Wick and Drury, 1951) and the absence of galactitol in the plasma strongly supports this hypothesis. Eye lens contain the enzyme aldose reductase (van Heyningen, 1959b; Hayman and Kinoshita, 1965) a NADPH requiring enzyme, but this enzyme has not yet been successfully demonstrated in the other tissues examined in this report, though the enzyme has been located in the seminal vesicle and placenta of the sheep (Hers, 1960).

The pathogenicity of feeding galactose to galactosemics is believed to be due to the toxicity of a galactose derivative rather than to the accumulation of the free sugar in the tissues. For example, galactose-1-phosphate has been found to accumulate in red blood cells of galactosemic patients (Schwarz, et al. 1956) and inhibit the action of phosphoglucomutase (Ginsberg and Neufeld, 1957; Sidbury, 1957). Galactitol formation may be a new candidate for the toxic metabolite of galactose or it may simply be an important alternate pathway for the disposition of galactose by the galactose-1-phosphate uridyl transferase-less human.

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