Rates of Oxidation of Galactose and Glucose in Erythrocytes and Liver after Lactose Feeding

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Fiegelson & Conte (1954) could find no adaptive increase in galactose oxidation in adult rats fed on a galactose diet. Young rats grew better when fed on 30% of dry matter of the diet as lactose (Whittier, Cary & Ellis, 1935; Chowdhury & Sadhu, 1958), while high lactose or galactose feeding is toxic to rats (Ershoff & Deuel, 1944; Guha, 1931). These studies and those in galactosaemia (Handler, 1947; Schwarz, Goldberg, Konrower & Holzal, 1956) led us to study the rates of oxidation of galactose and glucose in erythrocytes and liver tissue in young rats fed on a lactose diet, since it was thought that the young animals were likely to show better adaptive formation of enzymes for galactose oxidation.

EXPERIMENTAL

Series 1. Albino rats of the Bengal Veterinary College stock weighing about 30 g. were divided into two groups and fed *ad lib.* Group 1 was kept on a diet containing 70% of sucrose, 15% of casein, 5% of cod-liver oil, 5% of coconut oil, 4% of salt mixture (Hubbell, Mendel & Wakeman, 1937) and 1% of yeast. In group 2, instead of 70% of sucrose, 50% of sucrose + 20% of lactose were given, the other constituents of the diet being the same as in group 1. Direct measurement of glucose and galactose oxidation in erythrocytes was carried out by the procedure of Fiegelson & Conte (1954) with a few modifications. The animal was anaesthetized with ether-chloroform, the chest was opened and blood was collected into a heparinized syringe directly from the heart. The blood was centrifuged at 2500 rev./min. at 5–10° for 10 min. The erythrocytes were suspended in 3 vol. of ice-cold Krebs–Ringer phosphate buffer (pH 7.4) (Umbreit, Burris & Stauffer, 1957) and centrifuged at 2500 rev./min. at 5–10° for 10 min. This washing was repeated twice and finally the packed erythrocytes (centrifuged at 2500 rev./min. for 10 min.) were suspended in 2 vol. of ice-cold Krebs–Ringer phosphate buffer (pH 7.4).

A similar method was applied to study liver galactose-oxidizing activity; 10% liver homogenate in Krebs–Ringer phosphate buffer (pH 7.4) was used. A portion of the right lobe of the liver was placed on a watch glass put on cracked ice; it was dried by filter paper, weighed in a torsion balance and quickly transferred to Krebs–Ringer phosphate buffer (pH 7.4) in the homogenizer tube, which was placed in a beaker containing cracked ice and the tissue homogenized while the tube was in ice.

In the main vessel of the Warburg flask were placed 1 ml. of erythrocyte suspension, 0.9 ml. of Krebs–Ringer phosphate (pH 7.4), 0.3 ml. of mm-adenosine triphosphate (ATP) solution, 0.2 ml. of 0.05% methylene blue or (for liver) 1 ml. of homogenate, 1 ml. of Krebs–Ringer phosphate (pH 7.4) and 0.4 ml. of mm-ATP solution. In the side arm was placed 0.5 ml. of 33 mm-glucose or galactose or 0.5 ml. of Krebs–Ringer phosphate (pH 7.4) for the blank; the central cup contained 0.1 ml. of 30% potassium hydroxide solution with 1 cm.² filter paper. Oxygen consumption was measured for 1 hr. at 38° by tipping over the substrate from the side arm after 10 min. temperature equilibration.

Series 2. Rats weighing 30–35 g. were divided into three groups. Groups 1 and 2 were fed *ad lib.* in the same way as groups 1 and 2 of series 1. Group 3 was fed on a diet similar to that of group 1 except that it contained 40% of lactose and 30% of sucrose. The apparent galactose oxidation by liver in rats of series 1 might have been affected by the erythrocytes contained in the same tissue; therefore in series 2 the liver was first perfused with cold Ringer solution through the hepatic artery till the liver was quite blanched. The nitrogen content of the tissues was determined by the standard micro-Kjeldahl method. Other details were as in series 1.

RESULTS AND DISCUSSION

The results of series 1 are given in Tables 1 and 2. It will be observed from Table 1 that after 20 days there was no difference between the two groups in the rates of oxidation by the erythrocytes. Oxygen consumption with galactose was small. When the feeding period was increased to 45 days, galactose-oxidizing capacity was considerably increased in group 2, i.e. in the lactose-fed group. The rate of oxygen consumption with galactose substrate was more than 50% of that obtained with glucose substrate. The rate of glucose oxidation was about the same in the two groups.

Galactose-oxidizing activity could not be detected in liver homogenate by this method after 1½ months (the reading of the blank was the same as the reading with galactose as substrate). When feeding was continued for 3 months there was a small amount of galactose-oxidizing activity in the lactose-fed group but not in the sucrose-fed control group (Table 2). These results show an increased

Table 1. Galactose- and glucose-oxidizing activity of erythrocytes after lactose feeding

Rats were fed on a diet containing *ad lib.* either 70 % of sucrose (group 1) or 50 % of sucrose + 20 % of lactose (group 2). Each figure represents the average of estimation in two rats.

No. of days of feeding (approx.)	Group	Average gain in body wt. (g./week)	O ₂ consumed (μl./ml. of packed erythrocytes for 30 min.)	
			With glucose as substrate	With galactose as substrate
20	1	6.2	126	3.7
	1		120	4.2
	1		110	3.6
	2	6.3	129	5.3
	2		98.5	3.3
	2		127.3	3.2
45	1	5.5	122	7.0
	1		115	6.3
	1		120.5	5.6
	1		125	7.5
	2	6.8	109.3	68.3
	2		124	72.8
	2		123.2	71.7
	2		129.3	73.1
	2			

Table 2. Galactose-oxidizing activity of liver after lactose feeding for 3 months

Each figure represents the average of estimations in two rats. Groups are as in Table 1.

Group	Average gain in body wt. (g./week)	O ₂ consumed (μl./ml. of liver homogenate in 30 min.)	
		No substrate	With galactose as substrate
1	4.5	22.6	21.9
1		23.4	23
1		20.1	21
2	5.5	20.9	35.75
2		18.7	26.65
2		20.7	33.6

Table 3. Galactose- and glucose-oxidizing activity of erythrocytes and of liver after 6 months' lactose feeding

Groups 1-3, received 0, 20 and 40 % respectively of lactose substituted for sucrose and fed *ad lib.* Q_{O_2} is determined as μl. of O₂/mg. of N/hr. in erythrocytes and as μl. of O₂ consumed/mg. of dry tissue/hr. in liver at 38°.

No. of group	No. of experiments	Average gain in body wt. (g./week)	Tissue	Q_{O_2} with glucose substrate (mean ± s.e.)	<i>t</i> (D.F. 10) compared with group 1	Q_{O_2} with galactose substrate (mean ± s.e.)	<i>t</i> (D.F. 10) compared with group 1
1	6	4.5	Erythrocytes	3.62 ± 0.47	—	0.84 ± 0.04	—
2	6	5.5	Erythrocytes	2.78 ± 0.24	3.8	2.88 ± 0.26	19
3	6	3.2	Erythrocytes	2.40 ± 0.27	5.1	1.35 ± 0.15	8
1	6	4.5	Liver	0.62 ± 0.15	—	0.28 ± 0.11	—
2	6	5.5	Liver	0.42 ± 0.07	4	0.62 ± 0.11	5.3
3	6	3.2	Liver	0.103 ± 0.029	10.5	0.33 ± 0.056	1*

* Difference is not significant.

galactose oxidation in erythrocytes and liver after a prolonged feeding of lactose, such as 1½ months after feeding of lactose in erythrocytes and after 3 months in liver. Table 3 shows the results of a study of glucose and galactose oxidation after feeding rats for six months (series 2). It will be observed from Table 3 that Q_{O_2} value of erythrocytes with galactose substrate was much higher in group 2 than in group 1. In these rats of group 2 the average value of Q_{O_2} with galactose substrate was very near to the Q_{O_2} with glucose substrate. In

Group 3, where the diet included 40 % of lactose, the Q_{O_2} value with galactose substrate was less than in group 2, but it was still significantly higher than in group 1.

With liver homogenate similar results were obtained, i.e. galactose oxidation was significantly higher in group 2 (20 % of lactose) than in group 1 (control group), but there was no significant difference between groups 3 and 1. The oxidation rate of liver homogenates was rather low; the use of a homogenate with Ringer-phosphate would not be

expected to give maximum rates of carbohydrate oxidation (Wenner, Dunn & Weinhouse, 1954).

All these results show that an adaptive increase in the oxidation of galactose in erythrocytes and liver takes place in rats when the diet contains 20 % of lactose; this increase is lowered when lactose content of the diet is increased to 40 %. Galactose oxidation by liver and erythrocytes in group 1 of series 2 was higher than that of group 1 of series 1. This indicated that the galactose-oxidizing capacity increased with age. Glucose oxidation was decreased in erythrocytes and liver of rats receiving 20 % of lactose in the diet and such decrease was still greater in erythrocytes of rats receiving 40 % of lactose (Table 3).

The increased oxidation of galactose might be due to an increase in enzyme concentrations, permeability effects or alterations in cofactor concentrations. It has not been possible to differentiate between these causes in the present work. The control rats used by Fiegelson & Conte (1954) showed high galactose oxidation and the oxidation rate in these adult rats could not be increased by galactose administration, whereas in our experiments young rats were used where galactose oxidation in the control rats was low and lactose feeding increased the galactose oxidation. The galactose-oxidation capacity of our control rats increased with age, which is in conformity with the initial high galactose-oxidation capacity of the adult rats used by Fiegelson & Conte (1954).

SUMMARY

1. Three groups (1-3) of albino rats (30-35 g.) were placed on diets in which respectively 0, 20 and 40 % of lactose was substituted for sucrose.
2. After 45 days' feeding, galactose-oxidizing

activity in the erythrocytes had increased considerably in group 2, but not in group 1.

3. After 3 months' feeding, galactose-oxidizing activity of suspensions of liver in Ringer-phosphate had increased in group 2 but not in group 1.

4. After 6 months' feeding the rates of consumption of oxygen (/mg. of N of erythrocytes or /mg. dry wt. of liver) with galactose as substrate had increased in group 2 compared with group 1.

5. In group 3 this increase was less in the erythrocytes and insignificant in liver. The rates of consumption of oxygen by erythrocytes and liver with glucose substrate were lower in group 2 than in group 1, and still lower in group 3.

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Studies on Gastric Proteolysis

4. PROTEINASE ACTIVITY OF GASTRIC JUICE AND GASTRIC-MUCOSAL EXTRACTS AT pH 6 TO 8*

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There have been several reports of the existence in gastric juice and gastric-mucosal extracts of a weak proteinase with maximal activity at neutral pH (Willstätter & Bamann, 1929; Klein & Wilkinson, 1933; Griffiths, 1934; Emerson & Helmer, 1936; Taylor, Castle, Heinle & Adams, 1938; Fox, 1949;

Glass & Boyd, 1954; Janowitz & Hollander, 1954). Except for that of Willstätter & Bamann, these reports have been concerned with gastric mucolysine (Glass & Boyd, 1954; Janowitz & Hollander, 1954), an enzyme which liberates tyrosine-containing products from human gastric mucus, or with the intrinsic anti-pernicious anaemia factor of Castle (1929). This factor occurs in gastric juice and

* Part 3: Taylor (1959c).