XXXIV. THE METABOLISM OF GALACTOSE.

II. THE BEHAVIOUR OF THE RAT TOWARDS MODERATE AMOUNTS OF GALACTOSE.

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THE immediate purpose of the work reported in this and the previous paper [Harding and Grant, 1932-33] has been to trace as far as possible, by methods of direct analysis, the fate of orally administered galactose. The use of galactose-acclimatised yeast [Harding and Grant, 1931-32] or *S. marxianus* [Harding, Nicholson and Grant, 1932-33] as an analytical reagent enables free galactose in extracts or preparations of blood, urine and tissues to be estimated with precision. The previous paper showed that the oral administration of moderate amounts of galactose (40 g.) to adult man resulted in a rapid entrance of galactose into the blood stream, with a peak amount at 30-60 mins. followed by a fall to zero by the end of 2 hours. A small increase in arterial blood-glucose was also noted. The urine contained amounts not greater than 2 g. galactose. With this amount of galactose, all the sugar was disposed of in 2 hours, but with a larger dose (80 g.) the excretion into the urine sometimes continued until the fourth hour. No evidence was found in the blood or urine of any new reducing compound formed from galactose, nor did the urine show any trace of non-reducing substance which yielded galactose on hydrolysis, beyond those small, and as yet doubtful, amounts demonstrated in the fasting condition by Harding and Grant [1931-32]. The present communication traces the absorption of galactose from the intestine of the rat and its disappearance from the tissues.

EXPERIMENTAL AND ANALYTICAL METHODS.

Young adult white rats (both sexes) were starved for 24 hours. To Series A was given by stomach tube [Cori, 1925] 1 ml. of 10 % galactose solution. The rats were placed in a cage with provision for urine collection. Five minutes previous to the desired time 15-20 mg. amytal were injected. At the end of 5, 20, 60 or 180 mins. from the administration of the galactose, the alimentary tract was ligatured at the oesophagus and anus and removed. Any urine was removed from the bladder by a small syringe, the bladder being washed out 3 or 4 times with distilled water. The muscle specimen, if desired, was taken from the hind-leg by dissecting away the skin, freezing by solid CO₂ *in situ* and then removing. The liver, if desired, was removed and placed at once in solid CO₂. If the whole animal was desired, it was frozen by solid CO₂ immediately after the removal of the urine. In a few instances a blood specimen was taken from the tail previous

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to the freezing. To Series B was given 1 ml. of 20 % galactose solution, and the animals were examined at the end of 20, 60, 120 and 180 mins. A few control animals were examined at the end of 20 mins.

Preparation previous to analysis. The excised alimentary tract was at once opened throughout its entire length and washed out with distilled water three or four times, gently macerating the interior lining. The extract was treated with 10 % sodium tungstate and $2/3 N H_2SO_4$. After standing for a few minutes this was filtered. The filtrate was shaken with purified Lloyd's reagent [Hamilton, 1928] and refiltered. The frozen muscles were weighed, dropped into 15 ml. of boiling water and extracted for 15 mins. The solution was decanted and the residue ground in a mortar and re-extracted with 10 ml. of boiling water for a second 15 mins. interval. The solution was again decanted and the residue placed in a test-tube and extracted on a boiling water-bath with three successive portions of 10 ml. of hot water, taking 30 mins. for the final extraction. The residue was strained with pressure through a piece of cheese cloth. The total cooled extract was made up to 60 ml. The frozen liver was extracted in exactly the same manner as the muscle tissue, except that the residue was separated by centrifuge.

When an examination of the whole animal (*minus* intestinal contents and urine) was required, the frozen carcass was minced, placed in 100 ml. of boiling water and extracted for 15 mins. The solution was centrifuged and the residue re-extracted with three successive portions of boiling water (100 ml.), the last extraction interval being 30 mins. The cooled extract was made up to 500 ml.

To 60 ml. of the tissue extract were added 15 ml. of 10 % $ZnSO_4 \cdot 7H_2O$ solution and 15 ml. of 0.5 *N* NaOH solution, gradually with shaking of the flask. The precipitates from the muscle and liver extracts were filtered, using an ordinary funnel. The whole animal filtrate was obtained by suction through a Büchner funnel of wide area. It was later found more convenient to precipitate 120 ml. of the whole animal extract, especially in the case of larger animals, as the filtration was slow owing to the presence of fat. In such cases there was occasionally some difficulty in obtaining a clear filtrate. 4.5 g. of purified Lloyd's reagent were added to 50 ml. of the zinc filtrate and the mixture shaken for 2 mins. and filtered. It was found necessary to make this filtrate alkaline with two drops of 40 % NaOH solution to precipitate the excess zinc present. Any excess zinc was found to interfere with the sugar oxidation and also with the removal of the sugars by yeasts. The precipitate of $Zn(OH)_2$ was filtered off, and the filtrate was adjusted to p_H 6–6.5, with 10 *N* H_2SO_4 .

The above details form a slight modification of Somogyi's [1930] directions for the preparation of blood-filtrates suitable for sugar analysis. We were unable to prepare suitable filtrates from tissue by the use of tungstic acid [Power and Clawson, 1928].

Analysis for glucose and galactose. The final clear filtrates from the alimentary tract, muscle, liver or whole animal were analysed for glucose (fermentable sugar) and galactose by the method of Harding and Grant [1931–32]. A double action of ordinary baker's yeast and galactose-acclimatised yeast¹, followed by a double centrifugation previous to the determination of the reducing power, ensured the complete removal of the desired sugar at each stage of analysis. The reducing power was determined by a modified Shaffer-Somogyi reagent [Harding and Downs, 1933]. Galactose in the blood specimens was determined by the method of Grant and Glaister [1933] which is suitable for the estimation of non-fermentable sugar in blood. This method of determining galactose in presence

¹ The authors wish to thank the Standard Brands Inc. New York for the continued preparation of "galac" yeast.

of glucose and the reducing substances in blood corpuscles has been later employed by Harding *et al.* [1933] in the determination of blood-xylose. Urine was analysed according to the previous directions of Harding and Grant.

Recovery of added galactose. Galactose added to muscle, liver or whole animal hash was recovered in 98–105 % yield; added to intestinal contents the recovery yield was 80–90 %.

RESULTS AND DISCUSSION.

The concentrations of glucose, galactose and residual reducing substances together with the total amounts of galactose found in the alimentary tract, urine and whole animal are shown in Tables I (Series A) and II (Series B).

Table I. *Determinations in whole animals of concentrations of tissue "sugars" in individual control rats, and average galactose values at stated intervals after the ingestion of approximately 0.6 g. galactose per kg. body weight (Series A).*

Rat		Dose mg.	Time mins.	Blood- galac- tose mg./ 100 ml.	Tissue "sugars" (mg./100 g.)			Galactose distribution. Total amount in mg. (as galactose)			
No.	Wt. (g.)				Glucose	Galac- tose	Residual reduc- tion (as glucose)	Ali- mentary tract	Urine	Tissues (as free sugar)	Utilised
51	—	—	—	—	60	4	30	—	—	—	—
52	—	0	0	0	38	2	18	0	—	—	—
53	—	—	—	—	41	(1)	18	—	—	—	—
54	—	—	—	—	55	(1)	24	—	—	—	—
Average					48	2	22				
Average of 4	143	92	5	0	39	3	19	76	—	6	10
"	7	162	90	20	41	19	20	40	2	29	17
"	8	153	89	60	44	25	23	4	15	34	36
"	5	147	94	180	49	3	24	0	25	4	64

Table II. *Average concentrations of tissue "sugars" and total amounts of galactose in whole animals after ingestion of approximately 1.2 g. galactose per kg. body weight at end of stated intervals (Series B).*

No. of exps.	Average		Time mins.	Blood- galac- tose mg./ 100 ml.	Tissue "sugars" mg./100 g.			Galactose distribution. Total amount in mg. (as galactose)			
	Rat wt. (g.)	Dose mg.			Glucose	Galac- tose	Residual reduc- tion (as glucose)	Ali- mentary tract	Urine	Tissues (as free sugar)	Utilised
6	156	184	20	86	39	21	16	119	3	32	30
6	134	180	60	126	49	63	19	12	42	82	45
5	148	185	120	57	47	23	21	3	75	34	73
6	150	191	180	20	58	6	20	(1)	76	9	105

At the outset we wish to emphasise the relatively small dose of galactose we have given. Series A received an average of 0.6 g. per kg. and Series B, 1.2 g. per kg. These amounts are comparable with the feeding of 40 and 80 g. galactose to the adult man. True, even these amounts are much in excess of what either the rat or man would receive under natural conditions, but they are much less than those administered by Cori [1925], who gave 8–9 g. per kg. by mouth to the rat, or the 2 g. per kg. per hour given intravenously by Wierzuchowski *et al.* [1931] to the dog. The dose given by Cori to the rat is equivalent to 500–600 g. galactose given to man.

Absorption from the intestine. Both in Series A and B, the absorption of galactose is practically complete at the end of an hour. Galactose is known to be rapidly absorbed in the dog [Nagano, 1902], cat [Hewitt, 1924], rabbit [Hédon,

1900], and rat [Cori, 1925]. Mainly from a study of glucose absorption, but stating that the figures on the absorption of other sugars support the same conclusion, Cori has formulated laws of absorption of sugars from the intestine. "The rate of absorption of a sugar is independent of the amount or concentration of the sugar fed." These conclusions for the absorption of glucose have been questioned by other observers [MacKay and Bergman, 1933]. Cori's absorption rate for galactose is 1.96 [1925] and 1.82 [1926] g. per kg. per hour. From his average figures it can be seen that at the end of 3 hours there were left unabsorbed 1.31, 3.47 and 3.94 g. galactose per kg. body weight. Only the first is comparable with our highest amounts fed in Series B. In Table III we show the

Table III. *Rate of absorption of galactose over short periods of time when given in moderate amounts.*

	After 20 mins. Amount absorbed		After 60 mins.
	mg./100 g.	mg./100 g. per hr.	Amount absorbed mg./100 g. per hr.
Series A	31	93	55
Series B	42	125	125

absorption rates (calculated from the data of Tables I and II) of galactose when administered in amounts below those investigated by Cori. In no case does the absorption rate approach 1.82 g. per kg. per hour. Our figures are few but the most significant give a value of 1.25 g. per kg. per hour. The very short time of our absorption periods, however, may introduce an error tending to low results, but in Series B the rate in the first 20 minutes is the same as in the next 40 minutes. Other differences in the experimental conditions are to be noted. Our animals were starved for 24 hours previous to the feeding, Cori's for 48 hours. By analogy with the absorption of glucose and fructose this should raise our rates of absorption rather than decrease them [Cori and Cori, 1927]. Amytal anaesthesia also lowers the rate of absorption about 30 % [Cori, 1931]. Only a few minutes elapsed, however, between the administration of the amytal and the excision of the alimentary tract. Nor did we divide the sugar remaining in the alimentary tract between the stomach and intestine. Any galactose left in the former would of course raise the calculated rate of absorption from the intestine. McCance and Madders [1930] and Trimble *et al.* [1933] have pointed out the part taken by the pyloric discharge in determining rates of intestinal absorption. The subject of absorption of sugars at low initial concentrations invites further investigation.

Fermentable sugar was also found in irregular amounts in the intestinal contents. It is to be noted that both in Series A and B, at the end of 3 hours, when galactose was still present in the blood stream, none was present in the alimentary tract.

Galactose in the tissues. In our control animals the examination of individual muscle or whole animal always showed the presence of small amounts of substance removable by "galac" yeast. This was not removable by the two previous treatments with ordinary baker's yeast, and while the first treatment with "galac" yeast always effected a small removal the second treatment failed to show any further removal. The amounts varied from 1 to 6 mg./100 g. tissue or muscle (Tables I and IV). Such amounts are just at, or very slightly above, our experimental error. Consequently we cannot place too much reliance on them. Liver extracts showed no evidence of such a removal, but these extracts were much more dilute. No free galactose was found in control rat blood or urine,

Table IV. Concentrations of muscle and liver "glucose," galactose and residual reducing substances one hour after ingestion of 190 mg. galactose by the rat.

mg./100 g.						
Muscle			Liver			
	Glucose	Galactose	Residual reduction (as glucose)	Glucose	Galactose	Residual reduction (as glucose)
Controls	25	0	4	—	—	—
	34	0	5	—	—	—
	26	2	4	101	0	21
	32	6	8	114	0	19
	38	3	7	162	0	20
	33	2	5	97	0	21
	22	2	9	80	0	17
Average	30 ± 6	2.5 ± 2	6 ± 2	111 ± 20	0	18 ± 3
One hour after galactose	34	33	7	98	104	17
	33	27	14	129	129	25
	35	36	10	144	111	20
	38	33	9	97	130	35
	33	37	5	143	98	21
Average	35 ± 2	33 ± 4	9 ± 3	122 ± 21	114 ± 14	23 ± 7

nor did Harding and Grant find any in normal human blood and urine. However, the dilutions of 1 : 10 used by them would fail to show amounts less than 2-3 mg./100 ml. In view of the fact that free galactose is readily excretable both in man and the rat it seems unlikely that our analytical finding represents galactose as a free constituent of living muscle.

Corresponding to the rapid absorption of galactose from the intestine there is a rapid rise of this sugar in the blood and tissues. Our observations are too infrequent for us to do more than state that the maximum concentration of galactose in the tissues and blood occurs somewhere within or about the first hour, with either the smaller or larger dose. This same maximum in the blood occurs in man. Are we to infer that the absorption of galactose from the intestine of man is as rapid as in the rat?

At the end of 3 hours after the smaller dose, the galactose content of the whole animal has fallen to the low indefinite figure of the control. After the larger dose, it is sufficiently low (4-9 mg./100 g.) to enable us to state that all but traces of the ingested sugar have disappeared.

Fermentable sugar, residual reducing substances and glycogen after galactose. Our method of analysis gives us, in addition to galactose, the concentration of glucose (fermentable sugar) and residual reducing substances. Kosterlitz and Wedler [1933] examined the sugar of the liver after galactose ingestion. They found slight increases in fermentable sugar but not in such amount as to lead to any definite conclusion. Their residual reducing fraction, which included galactose, was increased. Examination of our whole animal experiments shows a slight average increase in "glucose" at the end of the higher dose experiments, but in view of the large variations little importance can be attached to the figures. Harding and Grant found increases in blood-glucose in man after galactose feeding. The normal variations in blood-glucose are small, however, and experimental fluctuations are thus more easily ascertained. Certainly, in the rat, there occurs no transformation of ingested galactose into glucose sufficiently rapid to cause an accumulation of this latter sugar. In an endeavour to check more strictly the possible occurrence of rapid galactose-glucose conversion, we

examined a section of leg muscle and the liver, one hour after the feeding of the higher dose of galactose. The results are shown in Table IV. The increase in the concentration of galactose is plain in both muscle and liver; but again, the big variation in the "glucose" prevents any definite conclusion. Our figures for "fermentable" sugar in rat muscle (30 ± 6 mg./100 g.) are much higher than those recently published by Cori *et al.* [1933] (10–12 mg./100 g.), though these workers obtained much higher figures for the resting muscle of the amygalised animal. They agree more nearly with those of Power and Clawson [1928] and Bischoff and Long [1932]. The occurrence of any extensive glycogenolysis would impair the value of any experiments designed to measure an increase of that sugar after galactose feeding. Our animals were extremely quiet, however, and our muscle freezings and excisions were rapid. It is interesting to note that our figures for the non-fermentable sugar fraction of muscle extract obtained with $\text{Zn}(\text{OH})_2$ as a precipitating agent followed by Lloyd's reagent are lower (4–9 mg./100 g.) than those of Cori *et al.* [1933] (8–22 mg./100 g.), who used $\text{HgSO}_4\text{--BaCO}_3$, though two of our animals after galactose gave 10 and 14 mg./100 g.

The constancy of the concentration of residual reducing substances indicates the thoroughness of our methods of extraction and sugar analyses. It indicates also the improbability of any conversion of galactose into another extractable reducing carbohydrate at such a rate that accumulation occurs. It may be noted that Bischoff and Long [1932] claim to have proved the absence of hexose-phosphate from muscle extracts precipitated by $\text{Zn}(\text{OH})_2$. Harding and Grant noted also in man the absence of any new reducing carbohydrate in blood and urine after administration of galactose. Bierry *et al.* [1932] have claimed that they have proved the occurrence in liver of a galactose-containing polysaccharide. We did not examine the hydrolysis products of our animal or muscle hash after extraction to see if any combined or non-extractable galactose occurred. We, however, examined both liver- and muscle-glycogen in 2 rats 4 hours after the ingestion of 400 mg. galactose. The glycogen was extracted by the modification of Pflüger's method detailed by Good *et al.* [1933]. The glycogen obtained was hydrolysed and analysed for glucose and galactose. Only glucose was found.

Excretion into the urine. At the end of 20 minutes small amounts of galactose were found in the urine in both Series A and B. At the end of 3 hours 25 % of the smaller dose of galactose was found in the urine and 40 % of the larger. Cori [1926] found 60 % excretion in 4 hours. In both our series the excretion into the urine is greater than in man; 4 % excretion was the highest noted by Harding and Grant in man after the ingestion of 40 g. galactose, and 8 % after the ingestion of 80 g.

CONCLUSIONS.

The essential difference between the rat and man in their behaviour to moderate doses of galactose appears to be in the slower rate of utilisation by the rat. Parallels with other sugars are not wanting—thus the rat utilises intravenous glucose at a much more rapid rate than the dog or man [Cori and Cori, 1927]. The low rate of metabolism of intravenously administered galactose is also seen in the dog (0.2 g. per kg. per hour) [Wierzechowski, 1931]. From Table II it can be seen that an average of 73 mg. galactose disappears in 2 hours from 150 g. body weight. This means an average utilisation of 0.25 g. per kg. per hour. After the corresponding dose to man Harding and Grant found a disappearance of over 90 % of the galactose in 2 hours—a disappearance rate of approximately 0.5 g. per kg. per hour. Whilst these figures, calculated as they are from experiments varying widely in conditions, must be taken as a very rough

approximation, they nevertheless support the idea that galactose utilisation in man is much better than experimental work on other animals has led us to anticipate. This low rate of change in the rat, coupled with the high rate of absorption and excretion, is responsible, according to Guha [1931], for the failure of this animal to grow or maintain life when galactose is the sole carbohydrate. In man and woman [Duel *et al.*, 1932; 1933] galactose is active as a nitrogen-sparing carbohydrate and an anti-ketogenic body. In this latter capacity it is even claimed that it is superior to glucose, its effect lasting 2 or 3 days. These two extreme statements may well be reconciled by a difference in the rate of transposition in different species.

SUMMARY.

Galactose, fed to rats at levels comparable with those at which it may be administered to man (0.6 g. and 1.2 g. per kg.), is almost completely absorbed from the intestine within an hour.

With both dosages of galactose, all but traces of the free sugar has disappeared from the tissues at the end of 3 hours.

There is proportionately a much larger excretion of the ingested galactose into the urine in the rat than in man. There thus appears to be a lower rate of conversion of galactose into its metabolites in the rat.

No evidence was found indicating the conversion of galactose into glucose or into any other reducing carbohydrate. The glycogen formed after galactose ingestion, either in the muscle or the liver, gives only glucose on hydrolysis.

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