INTESTINAL ABSORPTION OF GALACTOSE

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SUMMARY

Galactose absorption from the rat intestine was studied in the intact animal, in the perfused intestine and in the isolated intestinal loop. In the intact animal an average porto-arterial difference of 16.1 milligram per cent galactose was found. At the same time there was no porto-arterial difference in the total reducing sugar.

In the perfused rat intestine, the entire amount of galactose removed from the intestinal lumen could be recovered in the mucosa and the perfusion fluid. In this case too, no additional reducing sugar appeared in the portal blood. The galactose replaced an equivalent amount of glucose. The presence of glucose in the perfusion fluid was an important factor in galactose absorption; its omission decreased galactose transport into the blood by 40–50 %.

Glucose also accelerated galactose transport in the isolated intestine when added to the outer solution, but caused inhibition when introduced into the lumen. Acetate, succinate and pyruvate, up to the concentration of 50 μmoles/ml, were without effect. Higher concentrations of pyruvate accelerated galactose transport to the same extent, irrespective of the site of application.

A working hypothesis is suggested for the resorption mechanism of galactose and other non-metabolized sugars. The relation of this mechanism to that of glucose absorption is discussed.

INTRODUCTION

In the active absorption of glucose from the intestine, the transport of glucose proper across the intestinal cells into the portal blood was supposed by HESTRIN-LERNER AND SHAPIRO[1,2] to be of only secondary importance. According to their assumption, the bulk of the sugar, taken up from the intestinal lumen, is metabolized in the intestinal cells and metabolic intermediary products are transported into the portal blood. This assumption was based on the following findings: (a) Neither in experiments *in vivo* nor in the perfused intestine did the glucose concentration in the portal blood exceed that in the arterial blood, when low concentrations of glucose (0.2–0.4 %) were being absorbed. (b) When [^{14}C]glucose was introduced into the lumen, non-glucose radioactive compounds appeared in the portal blood in concentrations markedly exceeding those in the arterial blood. (c) The rate of glucose metabolism of the intestine was found to be of very high order, and complete combustion of glucose to CO_2 was only a minor factor in the disposition of metabolized glucose.

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This assumption was recently challenged by several investigators[3,4], who showed that during the resorption of [^{14}C]glucose from the intestine the bulk of the radioactivity (80–90 %) in the blood, perfusing the intestinal loop, was associated with glucose and only 10–20 % was found in the form of metabolites like lactic acid, alanine and others. This was considered as proof for the thesis that glucose *per se* is transferred into the portal blood. Moreover, it was shown by TAYLOR AND LANGDON[5], that, following feeding of [1-^{14}C]glucose, liver glycogen had 76–91 % of its radiocarbon in the C_1 position. This was taken to mean that the major part of the glucose was not split into 3-carbon fragments during absorption, since this would have caused a redistribution of the label.

In all these cases, the net transport of glucose was not measured or considered unmeasurable. When repeating the experiments of HESTRIN-LERNER AND SHAPIRO[1] and those of the authors cited above[3,4] it was found by us that all these observations could be confirmed and verified. Thus we were left with the result that, while even with 1 % glucose in the lumen no or negligible portal-arterial differences in glucose could be found, a considerable amount of radioglucose entered the blood from the intestinal lumen.

This discrepancy between the isotopic and chemical results could be explained if one assumed the existence of an exchange of glucose between the mucosal cells and the blood. To test this assumption it was considered of interest to ascertain whether such an exchange exists between mucosal galactose and portal blood glucose. Such an exchange would serve as a possible mechanism for the resorption of galactose and other sugars which are actively absorbed though not metabolized, and at the same time would furnish an explanation of the above stated discrepancy between isotopic and chemical measurements of glucose.

MATERIALS AND METHODS

The experiments were carried out by techniques similar to those in previous reports[1,2] with some modifications as indicated in the following: Rats of 150–200 g were used. All operations were carried out under anaesthesia with pento-barbiturate (5 mg/100 g).

Total reducing sugar was determined according to the method of SOMOGYI AND NELSON[6].

Galactose was determined as the reducing sugar which remained after glucose was removed by glucose-oxidase. 5 ml of the deproteinized solution were incubated together with 0.3 ml of a 5 % solution of the crude enzyme (Notatin) and 0.05 ml 0.2 *M* phosphate buffer pH 6 for 3 h at 37°. The solution was deproteinized with $ZnSO_4$ (5 %) and $Ba(OH)_2$ (*N*/3) and the reducing sugar in the filtrate was estimated.

Paper chromatography was developed with butanol–pyridin–water (45:25:40). The chromatogram was run for 36 h, dried and scanned for radioactive spots and then sprayed with aniline–oxalate.

[1-^{14}C]galactose was obtained from the National Bureau of Standards, U.S.A.

RESULTS

Porto-arterial differences in sugar concentrations during galactose absorption from the intestine in vivo

When galactose was introduced into the intestine and portal and arterial blood samples were analyzed, it was found that galactose appeared in the portal blood in

concentrations which exceeded those in the arterial vessels markedly and consistently.

The mean porto-arterial difference was 16.1 mg% galactose, a value which may account for the bulk of the galactose disappearing from the intestinal lumen.

However, at the same time, no porto-arterial difference was found in the total reducing sugar concentration, indicating that the galactose which entered the blood replaced an equivalent amount of blood glucose (Table I).

TABLE I

PORTAL ARTERIAL DIFFERENCE IN SUGAR CONCENTRATIONS DURING GALACTOSE RESORPTION

6 ml of a solution containing 50–60 mg galactose in $M/15$ Na_2HPO_4 was injected into the lumen of the small intestine and tied off at both ends. The body cavity was closed and kept warm for 10 min. Blood samples were then extracted simultaneously from the portal vein and from the aorta. Galactose and total reducing sugar were determined in the contents of the lumen, the intestinal tissue and in the blood samples.

mg galactose absorbed	*mg % of total reducing sugar*		*mg % of galactose*		*mg % portal arterial difference**	
	In aorta	*In porta*	*In aorta*	*In porta*	*In total reducing sugar*	*In galactose*
19	126	126	28	44	— 11	+ 12.5
18.7	102	106	26	54	+ 8	+ 30.1
20.3	104	104	24	32	— 5	+ 6.4
17	110	110	24	42	0	+ 18
14.2	120	120	28	44	— 6	+ 13.8

* The portal arterial differences were corrected for shift of water as determined by the haematocrit of portal and arterial blood.

Galactose absorption from the perfused intestine

When the small intestine was perfused *in situ* with artificial blood and a 1% galactose solution was injected into the intestinal lumen, it was again found that galactose could be detected in the perfusion fluid in amounts approaching those disappearing from the intestinal lumen. Here too galactose replaced an equivalent amount of glucose, as no increase in total reducing sugar was found (Table II).

It could furthermore be shown that galactose is transported unchanged and is not metabolized by the intestinal cells. [1-^{14}C]galactose was introduced into the lumen. The intestine was then perfused with artificial blood, and the entire blood leaving the intestine during the absorption period of 30 min was collected.

All of the [1-^{14}C]galactose which disappeared from the lumen could be recovered in the intestinal wall and in the blood without any change in specific activity. Chromatographic analysis confirmed the above assumption: All of the radioactivity was confined to a single spot identified as galactose (Table III).

If the exchange between blood glucose and intestinal galactose was a factor in galactose absorption, the presence or absence of glucose from the perfusion fluid would be expected to influence the rate of galactose absorption. This was actually found to be the case. When the absorbing intestine was perfused with a fluid to which no glucose was added, the rate of absorption was decreased to 40–50% of that when glucose was added. It should be stated that in no case did we obtain a perfusion with a fluid completely free of glucose. Some admixture of glucose from the test animal could not be prevented. In spite of that a considerable increase in absorption was obtained when glucose was added to the perfusion fluid (Table II).

TABLE II

GALACTOSE ABSORPTION IN THE PERFUSED RAT INTESTINE IN THE PRESENCE AND ABSENCE OF GLUCOSE FROM THE PERFUSION FLUID

Perfusion was carried out as described in a previous publication[1]. The perfusion fluid in these experiments consisted of two parts of bovine erythrocytes previously washed 5–6 times with Ringer solution dissolved in three parts of 5% bovine albumin (Pentex) in Ringer solution. The pH was adjusted to 7.4 with 6.5% $NaHCO_3$. Glucose was added to a final concentration of approx. 100 mg %. The fluid was saturated with O_2-CO_2 (95:5) and was warmed to 40° by passing through a coil which was placed in a thermostatic water bath. 50–60 mg galactose in 6 ml of $M/15$ Na_2HPO_4 was injected into the lumen of the intestine. The portal blood was collected in ice cooled tubes during the resorption period which was 10 min.

Blood flow (ml/min)	*Reducing sugar concentration in mg %*		*Galactose concentration in portal blood in mg %*	*mg galactose absorbed**
	In perfusion blood	*In portal blood*		
		With glucose in perfusion fluid		
4.5	86	88	20	15
6	86	88	23	12
5.4	86	88	25	10
3.4	106	96	16	12
6	94	96	12	10
6	90	98	28	19
9	88	90	16	19
4.7	94	90	22	9
4	94	88	16	6.3
1.5	98	76	22	3.2
2.5	74	68	15	4.5
2.3	96	86	27	4
		Mean	20.16 ± 2.19	10.33 ± 1.39
		Without glucose in perfusion fluid		
2	0	4	4	3
5	0	30	12	13
3.5	0	22	12	4
1.5	0	32	18	5
3	2	26	13	4
4.5	2	24	11	5
3	1.5	14	14	5
2.8	2	12	12	3
1.7	0	20	13	4
		Mean	12.11 ± 1.45	5.11 ± 0.25

* Calculated from the amount of galactose which disappeared from the lumen minus the amount retained by the intestinal tissue.

Absorption of galactose from an intestinal loop in vitro

Further evidence for an exchange between glucose and galactose was obtained in experiments with the isolated intestine. When the galactose was introduced into isolated small intestine and this was placed into a tyrode bath, it was found that the penetration of galactose from the lumen into the outer fluid was increased markedly by the presence of glucose in the outer solution. This effect of glucose increased with increasing glucose concentrations up to 55 μmoles/ml. Beyond this concentration the rate of galactose absorption decreased (Table IV).

TABLE III

ISOTOPIC AND CHEMICAL DISTRIBUTION OF [1-^{14}C]GALACTOSE IN THE LUMEN, INTESTINAL TISSUE AND PORTAL BLOOD DURING GALACTOSE RESORPTION

Perfusion was carried out as described in Table II but with [1-^{14}C]galactose in the lumen and 0.1 % glucose in the perfusion fluid.

Isotopic distribution				
Counts/min in lumen before resorption	*Counts/min/after 30 min resorption*			
	In lumen	*In intestinal tissue*	*In portal blood*	*Total*
825,000	583,500	114,000	159,760	857,260
546,000	300,000	50,000	152,000	502,000
592,000	821,000	52,000	208,000	541,000
Chemical distribution				
mg galactose in lumen before resorption	*mg galactose after 30 min resorption*			
	In lumen	*In intestinal tissue*	*In portal blood*	*Total*
50.7	33.1	5.8	9.9	50.8
51	32	4.4	13.6	50
49.8	28.2	5.2	16	49.4

TABLE IV

ABSORPTION OF GALACTOSE FROM AN INTESTINAL LOOP *in vitro* IN THE PRESENCE OF GLUCOSE IN THE OUTER FLUID

Resorption from intestinal loop was studied as reported before[2]. The loop was filled with a solution of 1 % galactose in tyrode, tied at both ends and placed in a beaker containing 50 ml of tyrode solution at 37°. The outer solution contained initially glucose at various concentrations or no glucose at all. CO_2–O_2 (5:95) was bubbled through the outer fluid. Numbers in brackets present the number of the experiments. P was determined according to "Student's Method" in relation to the means of the experiments without glucose.

	Glucose concentration in the outer fluid in μmoles/ml				
	0	*11*	*27*	*55*	*110*
Mg galactose in the outer fluid after 10 min	1.3 ± 0.2 (9)	2.6 ± 0.1 (9) P < 0.01	3.4 ± 0.3 (3) P < 0.01	6.8 ± 0.5 (5) P < 0.01	14 ± 1 (3) P < 0.01
Mg galactose in the outer fluid after 20 min	3.8 ± 0.2 (9)	4.4 ± 0.3 (9) P = 0.1	6 ± 0.9 (3) P < 0.05	8 ± 0.3 (9) P < 0.01	15 ± 1 (3) P < 0.01

It was further tested whether the activating effect of glucose was due to the fact that it serves as a metabolite of the intestine. Glucose was therefore replaced by other metabolites, like pyruvate, succinate and acetate. The two latter compounds had no effect whatsoever (Table V). Pyruvate was also without effect when tested at concentrations up to 50 μmoles/ml, although these concentrations sustain maximum respiratory rate (Table VI). Only when the pyruvate concentration was raised above 50 μmoles/ml did a significant effect on galactose transport appear.

TABLE V

ABSORPTION OF GALACTOSE FROM AN INTESTINAL LOOP *in vitro* IN THE PRESENCE OF PYRUVATE, SUCCINATE AND ACETATE IN THE OUTER FLUID

The experiments were carried out as described in the heading to Table IV. Figures in the table present μmoles galactose transported into the outer fluid after 10 min.

Metabolite	*Concentration of metabolite in outer fluid in μmoles/ml*				
	0	*10*	*20*	*50*	*100*
Sodium pyruvate	1.3 ± 0.2 (9)	1.6 ± 0.1 (5) P = 0.5	2 ± 0.5 (5) P = 0.2	2.6 ± 0.1 (7) P < 0.01	3.8 ± 0.4 (5) P < 0.01
Sodium succinate	1.3 ± 0.2 (4)	2.1 ± 0.2 (4) P = 0.05	1.9 ± 0.2 (5) P > 0.05	2 ± 0.2 (4) P = 0.05	1.6 ± 0.7 (3)
Sodium acetate	1.3 ± 0.2 (9)	0.9 ± 0.2 (2)	1.2 ± 0.3 (3)	1.5 ± 0.2 (4)	

TABLE VI

RESPIRATION OF INTESTINAL TISSUE IN THE PRESENCE OF PYRUVATE

About 100 mg of fresh tissue was introduced into small Warburg vessels containing Krebs–Ringer solution with pyruvate at the indicated concentrations, with 10% KOH and filter paper in the centre well.

Concentration of pyruvate μmoles/ml	*μl O_2 consumed/h/100 mg fresh tissue*
0	27
10	67
20	57

TABLE VII

ABSORPTION OF GALACTOSE FROM AN INTESTINAL LOOP *in vitro* IN THE PRESENCE OF GLUCOSE, PYRUVATE AND SUCCINATE IN THE LUMEN

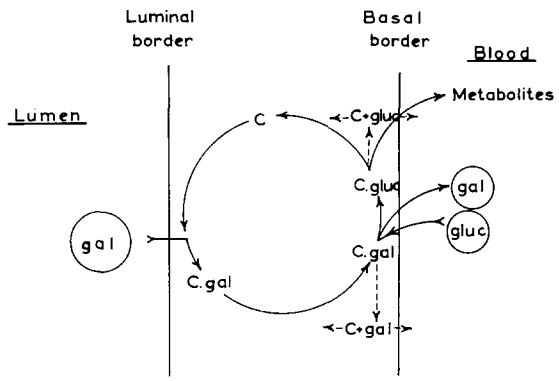
The experiment was carried out as described before[2]. Glucose, pyruvate and succinate at various concentrations were introduced together with galactose (55 μmoles/ml) into the inner solution. Galactose in the outer solution was determined after 20 min.

Addition		*mg galactose transported*
To outer fluid	*To inner fluid*	
—	—	3.8 ± 0.2
Glucose 27 μmoles/ml	—	6.0 ± 0.9
—	Glucose 27 μmoles/ml	3.8 ± 0.4
Glucose 55 μmoles/ml	—	8.0 ± 0.3
—	Glucose 55 μmoles/ml	2.1 ± 0.28
Pyruvate 20 μmoles/ml	—	4.4 ± 0.4
—	Pyruvate 20 μmoles/ml	4.7 ± 0.6
Pyruvate 50 μmoles/ml	—	5.4 ± 0.2
—	Pyruvate 50 μmoles/ml	4.8 ± 0.3
Succinate 20 μmoles	—	4.0 ± 0.5
—	Succinate 20 μmoles/ml	4.3 ± 0.2
Succinate 50 μmoles	—	5.8 ± 0.3
—	Succinate 50 μmoles/ml	4.3 ± 0.3

Glucose and pyruvate differ in their behaviour towards galactose absorption in another sense, in addition to the quantitative differences. While pyruvate exerted the same effect whether this substance was applied at the serosal or mucosal side (Table VII), the glucose effect showed a marked polarity, *i.e.* addition to the mucosal side together with the galactose caused inhibition of galactose uptake, as has been repeatedly shown[7–10].

DISCUSSION

The results presented in the present paper lead us to the postulation of the following scheme for galactose absorption:



The galactose which penetrates the mucosal cells from the lumen is reversibly bound to a carrier (c). This carrier–galactose complex migrates to the serosal side and there, glucose derived from the blood competes with galactose for the carrier and galactose is replaced by glucose and migrates into the extracellular space and portal blood. The carrier–glucose complex is decomposed by the metabolism of glucose and removal of the metabolic products to the blood stream. The carrier is thereby liberated and can combine at the mucosal side with an additional molecule of galactose.

This scheme is based on the following considerations:

The assumed exchange between galactose and glucose is corroborated by the finding that, in all levels of experiments, the total reducing sugar in the blood or bathing solution remained unchanged, while galactose replaced part of the glucose. Furthermore, blood glucose was shown to accelerate galactose transport. The assumption that both sugars combine with the same carrier is based on the finding presented here and made previously by others[7–10] that glucose competes with galactose when both sugars are introduced together into the lumen. In these experiments, FISHER AND PARSONS could determine a Michaelis constant for the resorption process, which was $8.3 \cdot 10^{-3}$ for glucose[11] and $3.5 \cdot 10^{-2}$ for galactose[9]. These constants may be actually the dissociation constant of the sugar–carrier complexes as postulated above. The lower dissociation constant of the glucose than that of the galactose-carrier complex makes glucose an efficient exchanging agent. Just as glucose competes with galactose for the carrier when introduced into the lumen, it replaces galactose from the carrier at the blood border and thereby increases the concentration of free galactose at this site and causes an acceleration in the transfer of galactose into the blood. The scheme

would thus explain the polarity of the glucose effect, demonstrated here, competing at the luminal border and accelerating at the blood border.

The proposed mechanism for galactose absorption may be a more general one, facilitating the transfer of all compounds which have affinity for the carrier, *i.e.* show certain structural similarities with glucose and galactose[12–14]. It seems also to be the basis for the penetration of radioactive glucose into the portal blood, without a corresponding net increase in glucose concentration. The results presented demonstrate that it is technically possible to detect net increase in the portal blood of a substance which is absorbed at the rate of galactose and of glucose, provided that it gets into the blood unchanged.

Glucose differs from the other sugars in being not only bound by the carrier but also metabolized. It is not readily released as free glucose from the carrier complex, because of its low dissociation constant. The metabolic breakdown of glucose liberates the carrier and the metabolic products penetrate into the blood.

The findings of TAYLOR AND LANGDON[5] that little randomization occurs in ^{14}C in liver glycogen (9–24 %), following [1-^{14}C]glucose resorption may also be explained by the penetration of intact [1-^{14}C]glucose as a result of this exchange process. This penetration would obscure the part of carbon which had been randomized due to the main process of resorption, *i.e.* the metabolism of glucose. It is interesting to note, in this connection, that SIU AND WOOD[15] re-examined this distribution of ^{14}C in liver glycogen. They introduced [2-^{14}C]glucose and [2-^{14}C]galactose, rather than the C_1 labelled compounds, in order to account also for that part of the sugar which is degraded *via* the pentose cycle, which loses its radioactivity with the decarboxylation of C_1. With these markers they found that with glucose there was randomization of 20–30 % whereas with galactose this was less than 10 %. This difference was partly attributed to the fact that glucose is degraded in the intestine to a considerable extent while galactose is not.

The nature of the metabolic products of glucose has been shown to be lactic acid[3,4,16–18]. Other compounds also seem to appear in smaller amounts. The results of our own experiments varied and smaller or larger amounts of lactic acid were found. The exact nature of the products seems to depend on the conditions, as suggested by ATKINSON *et al.*[4].

It should further be stated that WILSON AND WISEMAN[16] and WILSON AND VINCENT[19] could show with the aid of everted hamster intestine a considerable capacity to concentrate glucose and galactose against a concentration gradient. This would point to a mechanism different from that suggested here. It remains to be settled what the relative importance of these various mechanisms is. It is quite possible that in this respect there exist marked species differences, the mechanism suggested here predominating in the rat and the active concentration of glucose in the hamster.

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STUDIES ON THE BIOLUMINESCENCE OF *RENILLA RENIFORMIS*

I. REQUIREMENTS FOR LUMINESCENCE IN EXTRACTS AND CHARACTERISTICS OF THE SYSTEM*

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SUMMARY

A method is described for the preparation of luminescent extracts from the sea pansy, *Renilla reniformis*. Light production requires the presence of *Renilla* luciferase, an adenine-containing nucleotide (either ATP, ADP, or AMP), *Renilla* luciferin, and oxygen. The reaction is specific for adenine-containing nucleotides and *Renilla* luciferin. The luminescent reaction was shown to involve a nucleotide-dependent oxidation of *Renilla* luciferin.

Various factors that affect the system were studied. These include the effect of pH, enzyme concentration, temperature, inhibitors, nucleotide concentration, and luciferin concentration.

The evidence presented indicates that ATP is involved indirectly in this sytem by functioning as a generating system for ADP and AMP via an ATPase reaction.

Abbreviations used: AMP, ADP, and ATP are the mono-, di- and triphosphates of adenosine respectively; CMP, CDP and CTP are the mono-, di- and triphosphates of cytidine respectively; UMP, UDP, and UTP are the mono-, di- and triphosphates of uridine respectively; GMP, GDP, and GTP are the mono-, di- and triphosphates of guanosine respectively; GSH, reduced glutathione; PCMB, *p*-chloromercuribenzoate; ATPase, adenosine-5′-triphosphatase; FMN, $FMNH_2$, flavin mononucleotide and dihydroflavin mononucleotide respectively; FAD, flavin adenine dinucleotide.

* This is contribution No. 18 from the University of Georgia Marine Institute, Sapelo Island, Georgia.