J. Physiol. (1953) 119, 224–232

GALACTOSE ABSORPTION FROM THE SURVIVING SMALL INTESTINE OF THE RAT

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(*Received* 1 *July* 1952)

In the surviving intestine of the rat, the rate of absorption of glucose depends upon its initial concentration in the lumen and also upon the position in the small intestine of the absorbing segment (Fisher & Parsons, 1950, 1953). Many authors, for example Verzár & McDougall (1936) and Westenbrink (1936), have given estimates of the comparative rates of absorption of glucose and galactose; but the interpretation of such results is very difficult unless the effects on absorption of both concentration and segment position are known for the two sugars. A study of the absorption of galactose in the surviving intestine has therefore been undertaken, and the results obtained are reported below.

METHODS

The rat-intestine preparation of Fisher & Parsons (1949) was used. Galactose was determined as non-fermentable reducing substance by the method of Hulme & Narain (1931). The reduction of ferricyanide by galactose was linear under the conditions used, 1 ml. of N/40 ferricyanide being reduced by 1·06 mg galactose, and the total reduction by mixtures of glucose and galactose was additive. During the fermentation of solutions containing galactose a small quantity was removed by the yeast. The magnitude of this effect was always determined by control fermentations; it never amounted to more than 3–4 % of the galactose present, but varied with different batches of yeast. The collection of the circulating fluids, the method of fermentation, the measurement of water content of intestinal segments, and the determination of reducing substances in the intestinal wall have been described in the previous paper (Fisher & Parsons, 1952).

RESULTS

The active absorption of galactose

Galactose movements were measured in intestinal segments which were exposed, on both sides, to Ringer solutions containing initially 0·5 % galactose; the outer fluids also contained 0·5 % glucose and the experiments were of 60 min duration. Typical results are shown in Table 1 and demonstrate loss of galactose from the intestinal lumen and the appearance of extra galactose in

the fluid bathing the segments. Such an effect can only be accounted for by an 'active' transfer of galactose. The rates of absorption are less than those already reported for glucose under corresponding conditions, and the effects of segmental position do not appear to be so marked (Fisher & Parsons, 1949).

TABLE 1. Galactose movements across surviving segments of rat intestine. Initially 0·5 % galactose was present in both inner and outer fluids; the outer fluids also contained 0·5 % glucose. The mean values given are weighted to allow for the varying lengths of the segments

	Galactose movements (mg/cm/hr)			
	Disappearance from inner fluid		Appearance in outer fluid	
Animal	Jejunum	Ileum	Jejunum	Ileum
1	1·83	1·05	0·57	0·65
2	0·91	1·02	0·50	0·41
3	0·87	0·82	0·13	0·32
4	1·10	0·95	0·60	0·16
5	1·24	0·99	0·65	0·25
6	1·15	1·14	0·54	0·27
Weighted means	1·20	0·99	0·53	0·39

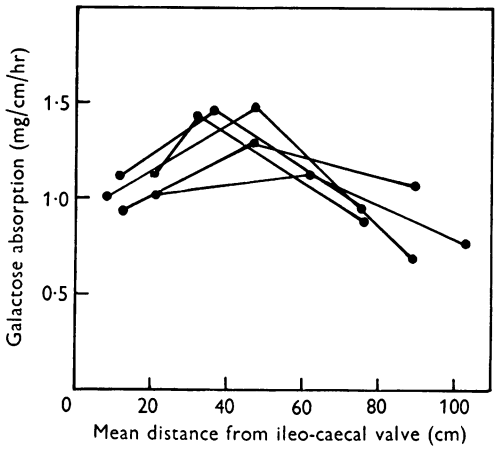

Fig. 1. The relation between galactose absorption and mean distance of surviving segments from ileo-caecal valve. The whole small intestine was used from each of five animals; each intestine was set up as three separate segments. All results for one animal are connected by straight lines. Experiments of 60 min duration. Initial inner fluid galactose concentration 0·5 % (w/v). Outer fluids contained initially 0·5 % glucose and 0·5 % galactose.

The gradient of galactose absorption in the surviving intestine

In a further series of similar experiments, the whole intestine was set up on circulation units as three separate segments. Fig. 1 shows the results obtained. Here the mean absorption for each segment is plotted against the 'mean distance' of the segment from the ileo-caecal valve. Each set of lines in the figure links the results obtained for one animal. It will be seen that in every case the absorption from the middle segment is greater than for either of the extreme segments.

Galactose absorption and galactose accumulation in the intestinal wall

We have shown that glucose absorption from the surviving intestine is hardly affected by large alterations in the glucose concentration in the fluid bathing the segments (Fisher & Parsons, 1953). During glucose absorption, however, there is a marked accumulation of it and of water in the intestinal wall, and these accumulations are associated with a depression of glucose absorption. The absorption of galactose has now been examined to see if there are similar effects. Galactose absorption from 0·5 % solution has been measured in experiments of 30 and 60 min duration. The outer fluid galactose concentration was either 0, 0·5 or 1 %, and 0·5 % glucose was always present. The results of these experiments are given in Table 2. They show that the absorption of galactose from the lumen is depressed by increasing the initial concentration of galactose in the outer fluid.

TABLE 2. The effects of duration of experiment and of galactose concentration in outer fluid on the rate of galactose absorption. Initial galactose concentration in the lumen was 0·5 %

Initial outer fluid concn. (%)		Duration of expt. (min)	No. of segments	Mean galactose absorption for whole intestine (mg/cm/hr)
Galactose	Glucose			
0·0	0·5	30	6	1·23±0·18
0·0	0·5	60	12	1·17±0·12
0·5	0·5	60	16	1·01±0·07
1·0	0·5	60	12	0·75±0·10

In a further series of experiments of 30 min duration we measured the accumulation of non-fermentable reducing substances in the intestinal wall during galactose absorption from 0·5 % solution. The outer fluids contained 0·5 % glucose and 0, 0·5 or 1 % galactose. Control values, and values for segments absorbing 0·5 % glucose, were also determined. The results obtained did not depend upon the position of the segment in the intestine, and the mean values, calculated as galactose, are given in Table 3. The results show that there is an accumulation of non-fermentable reducing substance in the wall of the

TABLE 3. Accumulation of non-fermentable reducing substances, calculated as galactose, in the intestinal wall during the absorption of galactose from 0·5 % solution. The outer fluids contained 0·5 % glucose. Duration of all experiments, 30 min. The control segments were taken from anaesthetized rats

Sugar in circulating fluids		Non-fermentable reducing substance in the intestinal wall (mg/cm) (weighted mean)
Outer	Inner	
1·0 % galactose	0·5 % galactose	0·406±0·027 (6)
0·5 % galactose	0·5 % galactose	0·324±0·020 (6)
None	0·5 % galactose	0·266±0·021 (6)
0·5 % glucose (glucose control)	0·5 % glucose	0·064±0·006 (8)
Control		0·106±0·003 (7)

surviving intestine during galactose absorption, the extent of which appears to depend upon the outer fluid galactose concentration. There is no accumulation of non-fermentable reducing substances when glucose is absorbed.

The water content of the intestinal wall of surviving segments absorbing 0·5 % glucose or galactose has been determined in experiments of 30 min duration, and the results are shown in Table 4. The water content of the glucose-absorbing segments (Fisher & Parsons, 1953) is included here for comparison. It will be seen that the presence of galactose in the lumen is not associated with the accumulation of water, although marked accumulation does occur during the absorption of glucose; very little water accumulation is evident when glucose and galactose are absorbed together. We have frequently observed that water absorption proceeds much more rapidly from glucose-containing solutions in the lumen than it does from solutions containing galactose, so that it appears that absorbed water accumulates in the intestinal wall and that in surviving intestine water absorption is inhibited during galactose absorption.

TABLE 4. Dry weight and water content of intestinal segments absorbing glucose or galactose from the lumen. Each set of results is the weighted mean for six segments. Initial concentration of each sugar, 0·5 %; outer fluids contained 0·5 % glucose only; survival time 30 min. The control segments were taken directly from anaesthetized rats

Sugar absorbed	Dry weight (mg/cm)	Water content (mg/cm)
Controls	10·23±0·31	23·44±0·44
Galactose	10·71±0·57	24·23±0·52
Glucose	12·03±0·44	51·25±2·14
Glucose and galactose together	10·02±0·44	30·80±1·50

The data presented in Tables 3 and 4 show that at least 160 μg/cm of galactose accumulated in the intestinal wall after 30 min absorption from 0·5 % solution in the lumen, none being initially present in the outer fluid. Even if it penetrates the whole of the intestinal tissue water, the galactose concentration must be nearly 0·7 %; if galactose is confined to only one-third of the total intestinal water, its concentration would be about 2 % after 30 min absorption. When 1 % galactose is initially present in the outer fluid, the corresponding figures are 1·2 % and nearly 4 %.

The kinetics of galactose absorption

The rate of absorption of glucose is related to the concentration in the lumen by an equation of the Michaelis-Menten type (Fisher & Parsons, 1953). If the average absorption rate is R and the initial concentration in the lumen is x_0

$$R = \frac{ax_0}{K_m + x_0}, \qquad (1)$$

where a and K_m are constants, this may be written

$$\frac{1}{R}=\frac{K_m}{a}\frac{1}{x_0}+\frac{1}{a}, \tag{2}$$

so that if the kinetics of absorption are of the Michaelis-Menten (1913) type, the reciprocals of the average absorption rate and initial concentration should be linearly related. This relation has been tested for galactose absorption. The initial concentrations used were 0·2, 0·3, 0·5 and 1·0 %. The experiments

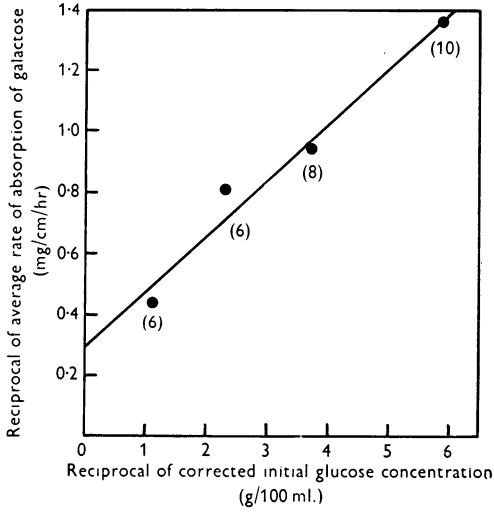

Fig. 2. The relation between the reciprocal of the initial concentration of galactose in the lumen of the surviving rat intestine and the reciprocal of the mean rate of galactose absorption. The initial concentrations have been corrected for the fall in concentration that occurs during absorption. The figures in brackets below each point represent the number of observations from which each weighted mean value was determined. Experiments of 30 min duration. Outer fluids contained initially 0·5 % glucose.

lasted for 30 min, the outer fluids initially containing only glucose. The results are shown in Fig. 2. Although there is more scatter than was observed for glucose (Fisher & Parsons, 1953), the points are related in a linear fashion. It appears that galactose absorption depends upon concentration, the relation being of the Michaelis-Menten type. Analysis of the data of Fig. 2 in the manner already described for glucose gives 0·63 % (35 mM) as an estimate of the apparent half-saturation concentration (K_m). The apparent limiting velocity of absorption is 3·4 mg/cm/hr.

The interactions of glucose and galactose during absorption

Table 5 gives the results of experiments of 60 min duration when glucose and galactose were present together in the lumen. The initial concentrations of each sugar were 0·5 % in both inner and outer fluids. For comparison, the

galactose-absorption rates found with this outer fluid composition and reported in Table 2 are also given. It will be seen that when the two sugars are present together in the lumen, the rate of glucose absorption is about three times that of galactose, the absorption of which is depressed to about half the usual rate. Since glucose will accumulate in the intestinal wall in these conditions, the effect of glucose on galactose absorption might be due to this accumulation. We tested this by measuring the absorption of galactose when the outer fluid contained glucose, but no galactose, at concentrations of 0·1 or 1·0 %. Earlier work had shown that glucose would enter the intestinal wall from the outer fluid at rates dependent upon the outer fluid concentration when no glucose was present in the lumen. The results are also given in Table 5 and show that galactose absorption from the lumen is not affected by the concentration of glucose in the outer fluid.

TABLE 5. The mutual effects of glucose and galactose during absorption. Initial concentration of each sugar in lumen, 0·5 %; experiments of 60 min duration

Sugar absorbed	Absorption rate (mg/cm/hr)	No. of segments	Initial composition of outer fluid (%)	
			Glucose	Galactose
Galactose in presence of Glucose	0·53±0·02	12	0·5	0·5
	1·59±0·11			
Galactose alone	1·01±0·07	16	0·5	0·5
	1·04±0·17	6	1·0	0·0
	0·99±0·10	6	0·1	0·0

DISCUSSION

The results suggest that galactose must be absorbed from the surviving intestine entirely by an 'active process'. After survival for 30 min the galactose concentration in the intestinal wall must be greater than that in the lumen, but absorption still continues. This 'active process', like that responsible for the absorption of glucose, has kinetics which, over the range studied, are related to the concentration in the lumen by an equation of the Michaelis-Menten type. The question arises whether this 'active process' is common to the two sugars. If it were, then one could expect that when glucose and galactose are present together in the lumen of the intestine there would be competition, and the absorption rates of both sugars would be depressed. Cori (1926), measuring absorption on intact rats, has shown that the absorptions of glucose and galactose are mutually depressed when a mixture of the sugars is fed. The data of Westenbrink & Middlebeek (1936) suggest that a similar depression occurred in their experiments. The data presented here (in Table 5) indicate that the presence of 0·5 % glucose depresses the absorption of 0·5 % galactose from 1·01 to 0·53 mg/cm/hr, i.e. to 52 % of its original rate. The absorption of glucose, on the other hand, is 1·59 mg/cm/hr when galactose is present, and

this figure can be compared with our estimate of $1{\cdot}86 \pm 0{\cdot}06$ mg/cm/hr for glucose absorption (Fisher & Parsons, 1953). Thus the presence of galactose inhibits the absorption of glucose to only 85 % of its original value. The figures given by Cori (1926) show that, in his conditions, the galactose absorption was inhibited to 38 % and the glucose absorption to 61 % of the original values.

The amount of interaction to be expected between the two sugars during absorption by a common rate-limiting process with Michaelis kinetics may be calculated using the relations known to exist for competitive inhibition (see, for example, Massart, 1950). If K_m constants for glucose of 0·15 % (Fisher & Parsons, 1953) and 0·63 % for galactose (this paper) are used, such calculations show that when the two sugars, each at an initial concentration of 0·5 %, are absorbed simultaneously from the lumen, the galactose absorption would be depressed to 34 % of the standard rate and the glucose absorption to only 86 %. Quantitative agreement between the expected and observed depression of glucose absorption in our experiments is thus good, but is poor for galactose. Such a discrepancy would occur if the common process were normally rate-limiting for only one of the sugars.

If galactose and glucose are absorbed by mechanisms having a common rate-limiting process, the gradients of absorption should be the same along the intestine; yet in the surviving intestine they are very different, for galactose absorption appears to occur most rapidly from segments in the middle of the small intestine. Also, in the surviving intestine, the average absorption of galactose from 0·5 % solution is only 54 % of the rate we have previously found for glucose; most reports in the literature suggest that, in the living rat, galactose is absorbed at least as fast as glucose, although the concentrations used were much higher than in our experiments. The answer to some of these difficulties may lie in the fact that when galactose is absorbed from the surviving intestine it accumulates rapidly in the intestinal wall to even higher concentrations than does glucose, and, as can be seen from Tables 2 and 3, this accumulation is associated with the depression of galactose gradient found in the surviving intestine. As we have already mentioned, the extent of the galactose accumulation apparently does not depend upon the position in the small intestine of the absorbing segment, but there is no reason why the local concentration of galactose in the tissue water should not vary with position in the intestine. We may thus conclude that, while there is nothing inherently improbable in the suggestion that glucose and galactose are absorbed by mechanisms having common processes, the properties of the surviving intestine are such that it is difficult to test this hypothesis conclusively.

In the surviving intestine, water absorption from the Ringer in the lumen is always greater when glucose rather than galactose is being absorbed. This fact is reflected in the figures for the water content of the intestinal wall given in Table 4. The intestinal wall does not accumulate water when galactose is being

absorbed, but it does so in the case of glucose. It is difficult to see how the absorption and accumulation of water can be due to the osmotic effects of sugar accumulated in the intestine. This would require glucose and galactose to be distributed very differently from one another between the water compartments of the intestinal wall. On the other hand, if the glucose-absorbing process is closely linked with the water-absorbing process, it seems unlikely that galactose and glucose could be absorbed by a common mechanism. We do not know the answer to these problems, but it is of interest that observations on the reabsorption of glucose and galactose in the kidney by Gammeltoft & Kjerulf-Jensen (1943) have shown that saturation of the glucose-absorbing mechanism reduces the simultaneous reabsorption of galactose, both in the cat and in man.

We have previously shown (Fisher & Parsons, 1950, 1953) that there is a net loss of glucose at a rate of about 1 mg/cm/hr from the whole of the surviving intestine preparation when it is being absorbed from 0·5 % solution in the lumen. Yet the data reported here show that the intestine absorbs galactose as rapidly as ever when glucose is present in the outer fluid at a concentration of only 0·1 %. At this concentration the rate of entry of glucose into the intestinal wall from the outer fluid must be very small. Inspection of Table 1 shows the discrepancy between the galactose disappearing from the inner fluid and that appearing in the outer fluid is about 0·6 mg/cm/hr; of this, as may be seen in Table 3, more than half can be recovered from the intestinal wall. We may say, then, that galactose 'utilization' (see Fisher & Parsons 1953) by the galactose-absorbing surviving intestine is much less than the glucose utilization during glucose absorption. Also the surviving intestine will still exhibit 'active' absorption when its glucose supply is cut to very low levels.

It is clear that statements such as 'the comparative rate of absorption of glucose and galactose are as 100 to 110' do not, by themselves, have very much meaning. Since both the sugars appear to be absorbed by processes which have Michaelis kinetics, comparisons of the two absorptions should be made on the basis of either the Michaelis constants (K_m) or the limiting velocities (a), and the site of absorption should be specified. While it is quite likely that the apparent limiting velocity of absorption of galactose is greater than that of glucose at all points along the intestine of the living animal, it appears that the K_m for glucose is lower than that for galactose. However, more detailed investigation of these points requires another type of intestinal preparation.

SUMMARY

1. In the surviving intestine of the rat, galactose is absorbed from the lumen and moved across the intestinal wall against a concentration gradient. At 0·5 % initial concentration the average absorption rate is only half that

observed for glucose in similar conditions. The middle third of the surviving small intestine appears to absorb galactose most rapidly.

2. The absorption rate of galactose depends upon the concentration in the lumen, the relation being of the Michaelis-Menten type. The apparent half-saturation concentration of the process is about 35 mM.

3. During absorption, galactose accumulates in the intestinal wall in high concentration. The accumulation of galactose is associated with a depression of its absorption.

4. When glucose and galactose are present together at the same concentration in the lumen, the absorption of galactose is depressed to a much greater extent than is that of glucose.

5. When glucose is absorbed from the surviving intestine there is a marked accumulation of water in the intestinal wall; this does not occur during the absorption of galactose.

6. These findings are shown to be consistent only to a limited extent with the hypothesis that galactose and glucose are absorbed by a common mechanism.

We wish to thank Sylvia Smith and H. Smith for skilled assistance.

REFERENCES

CORI, C. F. (1926). The rate of absorption of a mixture of glucose and galactose. *Proc. Soc. exp. Biol., N.Y.*, **23**, 290–291.

FISHER, R. B. & PARSONS, D. S. (1949). A preparation of surviving rat small intestine for the study of absorption. *J. Physiol.* **110**, 36–46.

FISHER, R. B. & PARSONS, D. S. (1950). Glucose absorption from the surviving rat small intestine. *J. Physiol.* **110**, 281–293.

FISHER, R. B. & PARSONS, D. S. (1953). Glucose movements across the wall of the rat small intestine. *J. Physiol.* **119**, 210–223.

GAMMELTOFT, A. & KJERULF-JENSEN, K. (1943). The mechanism of renal excretion of fructose and galactose in rabbit, cat, dog and man (with special reference to the phosphorylation theory). *Acta physiol. scand.* **6**, 368–384.

HULME, A. C. & NARAIN, R. (1931). The ferricyanide method for the determination of reducing sugars. A modification of the Hagedorn-Jensen-Hanes technique. *Biochem. J.* **25**, 1051–1061.

MASSART, L. (1950). Enzyme inhibition. In *The Enzymes*, **1**, pt. 1, pp. 307–342, ed. SUMNER, J. B. New York: Academic Press Inc.

MICHAELIS, L. & MENTEN, M. L. (1913). Die Kinetik der Invertinwirkung. *Biochem. Z.* **49**, 333–369.

VERZÁR, F. & MCDOUGALL, E. J. (1936). *Absorption from the Intestine.* London: Longmans, Green and Co.

WESTENBRINK, H. G. K. (1936). Über die Spezifität der Resorption einiger Monosen aus den Darme der Ratte und der Taube. *Arch. néerl. Physiol.* **21**, 433–454.

WESTENBRINK, H. G. K. & MIDDLEBEEK, A. (1936). Über die Korrelationen zwischen den Geschwindigkeiten der Darmresorption einiger einfacher Zucker. *Arch. néerl. Physiol.* **21**, 283–293.