ABSORPTION OF GLUCOSE FROM THE INTESTINE

II. *IN VIVO* AND PERFUSION STUDIES*

by

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In our previous communication[1], dealing with the active uptake of glucose by the isolated small intestine of the rat, it was shown that most of the changes, occurring in the lumen of the intestine during glucose resorption *in vivo*, can be demonstrated *in vitro* as well. Glucose disappeared rapidly from the lumen of the isolated intestine, even when no concentration gradient existed between the inner and outer solution. The rate of uptake was of the same order of magnitude as that found during resorption *in vivo* from similar solutions and the specificity towards various sugars was also similar in both types of experiments. Phlorrhizin inhibited the uptake in both cases.

In the *in vitro* studies, glucose was taken up from the lumen but the outer solution showed no increased glucose content, nor could any significant portion of the glucose be recovered from extracts of the intestine even after prolonged acid hydrolysis. Experiments with radioactive ^{14}C-glucose showed that only a negligible part of the sugar was combusted and that most of it was transferred as an intermediary metabolite, which diffused gradually into the outer solution. So far, the metabolite has not been identified with any known intermediate of glucose metabolism. These experiments showed that, in the isolated intestine, glucose is transported across the intestinal barrier in the form of a metabolite and not in its original form.

It was of interest to ascertain, whether this form of transport plays a role in glucose resorption *in vivo* as well, or whether it is an artefact peculiar to the conditions prevailing in the *in vitro* studies.

It is generally believed that glucose, after penetrating the intestinal cells, is changed into a derivative which is reconverted to glucose at another site within the cell. The glucose is then transported as such to the portal system. However, very little evidence, that glucose actively absorbed from the lumen can be recovered in the portal vein, has been provided by the literature. Most papers, concerned with active absorption, dealt only with the fate of glucose in the intestinal lumen but had very little to say about its appearance on the other side of the intestinal barrier. There is no doubt that, when glucose is given in large amounts and enters the lumen of the intestine as a highly concentrated solution, part of it will appear in the portal system by pure diffusion. Thus, in several recent studies on the composition of the blood in collateral veins (appearing in patients suffering from liver cirrhosis and supposed to present portal blood)

* Based partly on a Ph.D. thesis by S. H–L.

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a higher glucose concentration was often found in the collateral veins than in the systemic blood[2,3,4]. In these experiments, large doses of glucose were given (50–100 grams). Even under these conditions the portal-systemic difference was low and in some cases was not present at all. This type of experiment is inconclusive as to the fate of actively resorbed glucose, since diffusion might play a marked role under these conditions. Furthermore, no data are available on the amounts of glucose taken up from the intestine and so it is not possible to calculate the part of the resorbed glucose recovered in its original form in the portal system.

Most of the older data on this point, summarized by VERZAR[5], may be questioned on the same grounds. It should, however, be stressed that most investigators failed to find any increase in portal glucose concentration above that in the systemic blood, during resorption from low glucose concentrations. In all the cases cited no concomitant determinations were made on the quantities of glucose taken up. In view of our results with the *in vitro* system, it was felt to be worthwhile to reexamine this point and to establish whether any considerable portion of the glucose resorbed from comparatively dilute glucose solutions (active resorption) could be recovered in the portal blood.

For this purpose two types of experiments were carried out. In one series, the portal-arterial difference in glucose concentration during the resorption of known amounts of glucose was examined. In a second series, the intestine was perfused in situ with blood of known composition and the entire blood perfusing the intestine was collected from the portal vein and examined.

A. PORTAL-ARTERIAL DIFFERENCE DURING GLUCOSE RESORPTION

Experimental

The body cavity of the rat, under nembutal anaesthesia, was opened. The small intestine was tied off at both ends and 8–10 ml of a solution containing 20–55 mg glucose were injected into the duodenum. The concentration of glucose (approximately 0.4%) was low in order to minimize glucose resorption by diffusion. The medium used was tyrode or phosphate buffer $M/15$ at pH 7.4. The body cavity was closed and the animal kept warm for 4–15 minutes. A tuberculin syringe was then used for the extraction of one ml of blood from the portal vessel and one ml from the aorta. After this, the small intestine was excised and reducing material remaining in the lumen and in the intestinal wall was determined. Analytical methods were the same as in our previous communication[1].

Results

Experiments presented in Table I show that in no case was the expected minimal portal-arterial difference in glucose concentration found. In most cases the difference in concentration between the blood leaving the intestine (as represented by the portal blood) and the concentration of the blood supplying the intestine (represented by blood from the aorta) was negligible or even negative. The only instances in which a significantly higher glucose concentration was obtained in the portal system were those with the lowest glucose resorption or with no resorption at all (Exp. 5 and 7). It may be assumed that factors other than glucose resorption are responsible for a certain rise in portal glucose concentration in these cases.

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TABLE I

PORTAL-ARTERIAL DIFFERENCE IN GLUCOSE CONCENTRATION DURING GLUCOSE RESORPTION

Exp. No.	*Duration of resorption min*	*mg glucose* expected to reach the blood*	*mg% of glucose*		*mg% portal-arterial difference*	
			in aorta	*in porta*	*expected***	*found*
1	6	23.0	115	116	77–144	— 1
2	$5^1/_2$	10.4	145	158	38–76	—13
3	5	8.8	98	109	35–70	—11
4	5	9.5	91	93	36–72	+ 2
5	$5^1/_2$	5	125	110	20–40	+15
6	6	7	93	90	23–46	+ 3
7	7	0	84	67	0	+17
8	12	20.0	109	106	29–58	+ 3

* Calculated from the amount of glucose which disappeared from the lumen minus the amount retained by the intestine. A separate series of experiments showed that non-absorbing intestinal tissue contained about 3 mg glucose equivalents. This figure was substracted from the glucose values found in the intestinal tissue after the absorption experiment.

** Calculated from the amount of glucose expected to reach the portal vein per minute divided by the blood flow through the intestine. The latter figure was assumed to be 2.5–5.0 ml/min for a 200 g rat. These figures were obtained from the hepatic blood flow of 80 ml/min/100 g liver, as measured by BIRNIE AND GRAYSON[6], and assuming that the portal flow comprises 80% of the total liver flow. From these data a portal flow of 5 ml/min is obtained. If the 50% decrease in blood flow due to nembutal narcosis, found by BIRNIE AND GRAYSON, is taken into account, a flow of 2.5 ml/min is obtained.

These results suggest that most of the glucose transported reaches the portal system in the form of a non-reducing metabolite, rather than as glucose itself. This is in agreement with the previously reported *in vitro* result. Further evidence for this assumption is supplied by an experiment in which radioactive ^{14}C-glucose was introduced into the intestinal lumen. It can be seen in Table II that the concentration of glucose as deter-

TABLE II

RESORPTION EXPERIMENT WITH ^{14}C GLUCOSE

	Uptake from the lumen total	*Found in*		*Intestinal extract*
		Aorta	*Porta*	
Glucose by reduction	13.5 mg	139 mg%	132 mg%	0 mg
Counts/min	29.400	350/cc	520/cc	10.500
Counts/min after fermentation		75/cc	185/cc	8.200

mined by reduction was 7 mg% lower in the portal blood than in the arterial blood. Nevertheless the concentration of radioactive material was higher in the portal blood. Though no glucose was retained in the intestinal tissue, a considerable part of the radioactivity, which had disappeared from the lumen, could be recovered in it. Thus, it seems that the glucose taken up by the intestinal cells was transformed into a metabolite and that this metabolite, rather than glucose, diffused into the portal blood.

Further analysis of the radioactive material in the intestinal extract and in the blood filtrates showed that most of the radioactivity in the extract was associated with a non-fermenting substance, whereas the substance present in the arterial and portal blood was partly fermentable. The non-fermentable fraction in the portal system was

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larger than that in the arterial system. These results are in agreement with the assumption that the non-fermenting metabolite formed in the intestine, diffuses into the portal blood and is rapidly transformed into a fermenting substance by some other organ in the body.

B. PERFUSION EXPERIMENTS

Experimental

The solution injected into the intestine consisted of tyrode or phosphate buffer $M/15$ at pH 7.4. The concentration of glucose in the solution was usually 0.4%, in one case 0.8% was used, and in the experiments with radioactive glucose 0.2% ^{14}C glucose was employed.

The perfusion fluid consisted of equal parts of bovine serum, previously dialyzed against tyrode for 48 hrs, and bovine erythrocytes washed in Ringer solution. Glucose was added to a concentration of approximately 100 mg %.

The perfusion apparatus: The pressure necessary for the perfusion was supplied by a water filled separating funnel at a height of 80–100 cm, and connected by rubber tubing to a washbottle. The pressure was relayed from this to another washbottle containing the perfusion fluid, warmed to 40° C by a water bath. The perfusion fluid leaving the wash bottle passed through a rubber tube ending in a metal cannula. The cannula was inserted into the aorta descendens below the kidney. When the clamp on the rubber tubing was released, the perfusion fluid flowed at approximately arterial pressure into the aorta.

Procedure. The perfusion fluid was saturated with alveolar air before being placed in the perfusion apparatus. Anaesthesia was induced by the intraperitoneal injection of 5 mg/100 g of pentobarbital. The rat was kept warm during the perfusion. The abdominal cavity was opened and a metal cannula was introduced into the aorta descendens in the region below the kidneys. 0.4 ml of a 4% sodium citrate solution was injected into the aorta through the cannula in order to prevent blood clotting. The cannula was then connected to the rubber tubing leading from the reservoir containing the perfusion fluid. The rat's blood supply was then shut off by clamping the aorta just below the diaphragm and the perfusion fluid was run into the aorta. A glass cannula was inserted into the portal vessel close to the liver. The portal blood was first collected for some time. The glucose solution was then injected into the duodenum and the small intestine was tied off at both ends. The portal blood was then collected throughout the resorption period.

Glucose concentration was determined in (1) the perfusion fluid administered; (2) the portal blood collected prior to the glucose injection; (3) the portal blood collected during the resorption period; (4) the solution injected into the intestine; (5) the solution remaining in the intestinal lumen at the end of the experiment; (6) the extract ot the intestinal wall. Lactic acid was determined according to BARKER AND SUMMERSON[7]. Oxygen consumption was measured by the determination of the oxygen content of the perfusion blood and the portal blood[8].

Results

By perfusing the intestine with blood of known composition and by collecting all the blood that leaves the intestinal circulation, the rise in glucose concentration expected, on the assumption that it is glucose which is transferred to the portal vein,

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TABLE III

GLUCOSE ABSORPTION IN THE PERFUSED RAT INTESTINE

Exp. No.	Duration of resorption min	Blood flow in porta ml min		Glucose uptake from lumen* in mg	Glucose concentration in mg%			Expected increase in glucose concentration in porta**
		Before resorption	During resorption		Perfusion blood	Portal blood		
						Before resorption	During resorption	
1	10	1.4	1.6	7.0	95	56	60	44
2	27	1.0	0.8	6.2	100	62	71	28
3	20	1.0	1.4	10.7	90	69	68	38
4	15	1.0	1.2	6.3	154	128	120	35
5	16	1.6	1.2	17.4	82	68	70	91
6	12	1.6	1.8	17.2	105	81	94	80
7	20	0.65	0.55	8.3	143	86	83	78

* Corrected for the amount retained in the intestinal tissue as in Table I.
** Corrected for the amount of fluid shifted between lumen and blood.

can be easily calculated. As can be seen from Table III, this expected rise was never found. In fact, glucose concentration in the portal blood was always lower than that in the perfusion fluid. Furthermore, no marked differences were found in glucose concentration in the portal blood, collected before and during resorption. Only in one case did the difference exceed 10 mg%, while the expected increase in concentration was 80 mg%.

As in our own *in vitro* experiments[1], only small proportions of the resorbed glucose were combusted to CO_2. Assuming that all the oxygen consumed was used for the complete combustion of glucose, 0.8 mg of glucose in experiment 3, 1.6 mg in experiment 4, 0.5 mg in experiment 5, and 2.8 mg in experiment 6 should have disappeared for this reason. Furthermore, only small differences were found in oxygen consumption before and during resorption. Complete oxidation of glucose by the intestinal tissue at most accounted for the glucose consumption of the non-resorbing intestine.

Lactic acid production. Lactic acid was 5 mg% higher in the resorptive period than in the pre-resorptive period in perfusion 5 (*i.e.* an additional 0.96 mg of lactic acid was produced during the 16 min of the resorptive period). In perfusion 6, 1.76 mg lactic acid were added to the portal blood during the resorptive period and 2.6 mg were found to have accumulated in the intestinal lumen. In this experiment about ¼ of the glucose resorbed might have been converted to lactic acid.

The results obtained in experiments with the resorption of ^{14}C glucose by the perfused intestine (Table IV) are in agreement with the assumption that the substance transferred to the portal system during glucose absorption is mainly a metabolite of

TABLE IV

RESORPTION OF ^{14}C GLUCOSE IN THE PERFUSED INTESTINE

	Amount introduced into intestine	Recovered after 12 min		Increase in portal blood above perfusion blood
		in lumen	in intestine tissue	
mg glucose (by reduction)	26.3	18.0	0	—40 mg%
cts/min	83,000	64,000	12,000	6,000

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glucose and not the sugar itself. In spite of the fact that no additional glucose could be detected in the portal blood, about $^1/_3$ of the radioactivity taken up from the lumen was recovered in the portal blood. The rest was found in the extract of the intestinal tissue and was not associated with any reducing substances. It could, furthermore, be shown that the radioactive substance appearing in the portal blood was mainly non-fermentable. Of the 6000 counts found, 4400 remained in the solution after fermentation and drying.

DISCUSSION

The results of this investigation show that in all three types of experiments (with the isolated intestine, the perfused intestine, and during glucose resorption *in vivo*) actively resorbed glucose is transported into the blood in the form of a metabolite. If regeneration of glucose in the intestinal cells occurs at all, it takes place at most to a very limited extent. This finding markedly simplifies the theory for the active absorption of glucose from the intestine. Glucose penetrating into the intestinal cells is quickly changed into a metabolite and a high concentration gradient is thus maintained between the lumen and the cells. Glucose is not regenerated at a second site within the cell and no spatial separation of various enzyme systems must be assumed. The metabolite accumulating in the intestinal cells diffuses out into the blood stream and is carried to the liver and into the general circulation. The regeneration of glucose presumably takes place in some other organ. In the meantime, some evidence has been obtained[9] that the liver is capable of transforming the non-fermenting metabolite into a fermenting substance. The results obtained with the resorption of ^{14}C glucose *in vivo* are in accord with this assumption. The metabolite that accumulated in the intestinal tissue was almost completely non-fermentable, however a large part of the radioactive substance in the blood stream of the *in vivo* experiments was fermentable. The blood collected before the liver (portal blood) had a higher content of non-fermentable metabolite than that collected from blood which had already passed through the liver (arterial blood). When the blood was collected without passing the liver, as in our perfusion experiments, the metabolite found was non-fermentable.

The function of the intestine as a resorbing organ would thus be made possible by a peculiar type of metabolism, in which the amount of glucose metabolized exceeds the amount which is completely combusted to such an extent, that most of the glucose attacked accumulates in the tissue as an intermediate. The identity of this intermediate is not yet known. In our previous communication[1] the radioactive intermediate was isolated from *in vitro* incubation media and was shown to contain only extremely low concentrations of phosphorus. Under certain conditions, especially with deficient oxygen supply, part of the metabolite may accumulate as lactic acid.

SUMMARY

1. When glucose is resorbed *in vivo* from low concentrations (0.4 %), no significant increase in the concentration of glucose in the portal vein, above that in the aorta, can be found.

2. Estimation of the amount of glucose taken up from the lumen showed that if this amount were transferred to the portal vein as glucose and assuming a portal blood flow of 2.5–5.0 ml/min, marked concentration differences should have existed between the portal vein and the aorta.

3. When radioactive ^{14}C glucose was introduced into the lumen, the portal blood was found to contain more radioactive substances than the aorta. The main difference was found in the amount of non-fermentable radioactive substances.

4. When the intestine was perfused with blood of known glucose concentration and all the blood

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perfusing the intestine was collected from the portal vein, no additional glucose could be found in the perfusion fluid, whilst glucose was taken up from the lumen.

5. When ^{14}C glucose was resorbed by the perfused intestine during 12 minutes, $^2/_3$ of the radioactivity taken up was recovered in the intestinal tissue and the rest was found in the perfusion fluid as a non-fermenting substance.

6. Complete combustion of glucose accounted for only a negligible part of the glucose which disappeared from the lumen. Lactic acid was formed to some extent in the perfused intestine, accounting at most for $^1/_4$ of the glucose which disappeared.

RÉSUMÉ

1. La concentration du glucose dans la veine porte ne dépasse pas de façon nette sa concentration dans l'aorte au cours de la résorption *in vivo* d'une solution de glucose faiblement concentrée (0.4 %).

2. La détermination de la quantité de glucose résorbée montre que si cette quantité était transmise à la veine porte sous forme de glucose, le débit sanguin dans la veine porte étant de 2.5 à 5.0 ml/min, la concentration dans la veine porte serait nettement différente de la concentration dans l'aorte.

3. Quand du glucose marqué par ^{14}C est introduit dans le lumen, le sang de la veine porte renferme plus de substances radioactives que le sang aortique.

4. Quand l'intestin est perfusé avec du sang, le sang recueilli dans la veine porte après perfusion renferme la même quantité de glucose que le sang avant perfusion, quoique du glucose ait été résorbé.

5. Après résorption de glucose ^{14}C pendant 12 minutes par l'intestin perfusé, les $^2/_3$ de la radioactivité résorbée se retrouvent dans le tissu intestinal, et le reste dans le liquide de perfusion sous forme d'une substance non fermentescible.

6. Le glucose qui subit une combustion totale ne représente qu'une partie négligeable du glucose qui a disparu du lumen. Il se forme une certaine quantité d'acide lactique dans l'intestin perfusé, quantité qui correspond, au plus, au $^1/_4$ du glucose disparu.

ZUSAMMENFASSUNG

1. Bei der Resorption von Glucose *in vivo* bei niedrigen Konzentrationen (0.4 %) kann kein bedeutsames Anwachsen der Glucosekonzentration in der Pfortader, das grösser als das in der Aorta wäre, gefunden werden.

2. Die Bestimmung der aus dem Lumen aufgenommenen Menge Glucose zeigte, dass wenn diese Menge zur Pfortader als Glucose weitergeleitet worden wäre und bei Annahme einer Strömungsgeschwindigkeit des Blutes in der Pfortader von 2.5–5.0 ml/min, beträchtliche Konzentrationsunterschiede zwischen der Pfortader und der Aorta bestehen müssten.

3. Bei der Einführung von radioaktiver ^{14}C Glucose in das Lumen, wurde gefunden, dass im Blut der Pfortader mehr Radioaktive Stoffe enthalten waren als in der Aorta. Der Hauptunterschied trat bei der Menge der nicht vergärbaren radioaktiven Substanzen auf.

4. Wenn die Gedärme mit Blut einer bekannten Glucosekonzentration durchströmt wurden und all das die Gedärme durchströmende Blut aus der Pfortader gesammelt wurde, konnte keine zusätzliche Glucosemenge im Perfusat gefunden werden, während aus dem Lumen Glucose aufgenommen wurde.

5. Bei der Resorption von ^{14}C Glucose im durchströmten Gedärme innerhalb von 12 Minuten wurde $^2/_3$ der aufgenommenen Radioaktivität im Darmgewebe und der Rest im Perfusat als nichtvergärbare Substanz zurückgefunden.

6. Die vollständige Verbrennung der Glucose erklärte nur einen zu vernachlässigenden Teil der aus dem Lumen verschwundenen Glucose. In gewissem Ausmass wurde in den übergossenen Gedärmen Milchsäure gebildet, für die im besten Fall $^1/_4$ der verschwundenen Glucose veranschlagt werden kann.

REFERENCES

1 S. HESTRIN-LERNER AND B. SHAPIRO, *Biochim. Biophys. Acta*, 12 (1953) 533.
2 S. SHERLOCK AND W. WALSHE, *Clin. Sci.*, 6 (1946) 113.
3 J. D. MYERS, *J. Clin. Invest.*, 29 (1950) 836.
4 W. B. BEAN, M. FRANKLIN, J. F. EMBICK AND K. DAUM, *J. Clin. Invest.*, 30 (1951) 213.
5 F. VERZAR, *Absorption from the Intestine*, p. 128. London 1936.
6 J. H. BIRNIE AND J. GRAYSON, *J. Physiol.*, 116 (1952) 189.
7 J. B. BARKER AND W. H. SUMMERSON, *J. Biol. Chem.*, 138 (1941) 535.
8 P. B. HAWK, B. L. OSER AND W. H. SUMMERSON, *Practical Physiological Chemistry*, p. 649. Philadelphia 1949.
9 B. SHAPIRO, unpublished results.

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