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ON THE CONVERSION OF FRUCTOSE TO GLUCOSE BY GUINEA PIG INTESTINE

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SUMMARY

Inverted intestinal sacs of the guinea pig, but not of the rat, convert fructose to glucose during its passage through the intestinal wall. An exchange of ^{14}C-activity between C-1 and C-6 of the glucose formed from [1-^{14}C]fructose under these conditions has been observed. Fructokinase and glucose-6-phosphatase have been found to be present in the intestinal mucosa of the guinea pig and it has been confirmed that the latter enzyme is absent in the same tissue of the rat. These observations lead to the conclusion that the conversion of fructose to glucose by the guinea pig intestinal mucosa occurs by the same mechanism as that operating in the liver, involving fructose-1-phosphate and triose phosphates as intermediates. It is suggested that the inability of rat intestine to convert fructose to glucose is due to the absence of glucose-6-phosphatase.

INTRODUCTION

It has been recognized for some time that a conversion of fructose to glucose occurs during intestinal absorption of fructose[1–4]. The mechanism involved in this conversion

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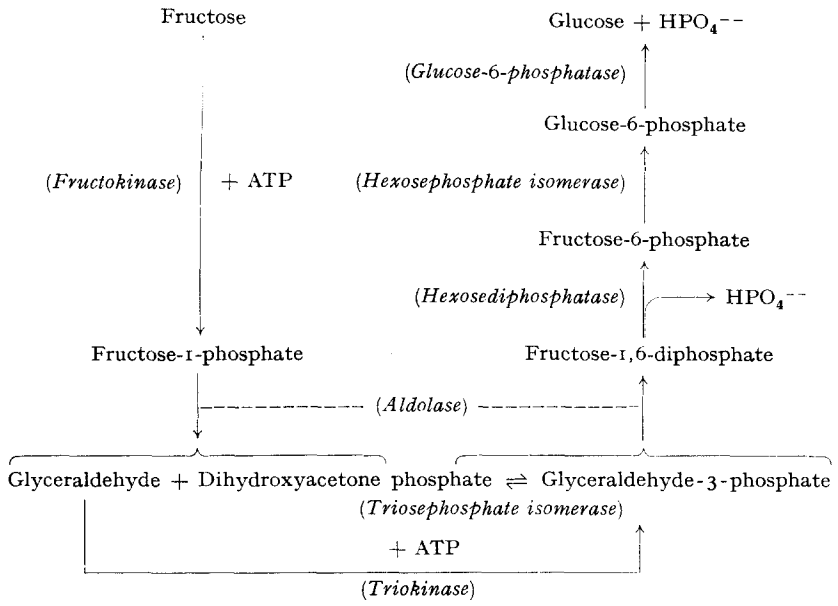
has not, however, been clarified and several pathways may be considered. The first one, which occurs in liver[5], involves fructose-1-phosphate, triose phosphates, fructose-diphosphate and hexose-6-phosphates as shown below:



A second possibility, which has some similarity with the first one, is a direct phosphorylation of fructose to fructose-6-phosphate by hexokinase, thus by-passing the triose phosphates. A third possibility is that an oxidation–reduction is involved in the conversion, with an intermediate such as glucosone or sorbitol. The latter compound is believed to be an intermediate in the reverse transformation in seminal vesicles[6]. Finally, the fourth pathway which may be considered, is a direct isomerization of fructose to glucose similar to the reaction catalysed by the xylose isomerase of *Pseudomonas hydrophila*[7].

The first pathway can be characterized by a randomization of ^{14}C-activity in glucose formed from specifically labeled fructose, due to the intermediate formation of triose phosphates[8]. It requires the presence of fructokinase and of glucose-6-phosphatase. The latter enzyme, however, could not be detected in the intestine of the rat[9]. The other pathways do not involve splitting of the hexose molecule, but the second one also requires glucose-6-phosphatase to allow the selective liberation of glucose from a mixture of hexose phosphates. If route 3 were operative, one might expect sorbitol or glucosone to be converted to glucose by the mucosa. Route 4 is a one-step reaction that could possibly be detected in a tissue extract.

This paper describes experiments designed to distinguish between the four pathways given above. The conversion of fructose to glucose by the intestine was conveniently studied by using the *in vitro* technique of WILSON AND WISEMAN[10]. As published in a preliminary communication[11], the results obtained by this technique favour the first pathway. This conclusion was supported by enzymic studies on cell-free extracts of the mucosa. In a recent paper, SALOMON AND JOHNSON[12] arrived independently at a similar conclusion.

MATERIALS AND METHODS

Sugars

Glucosone was kindly supplied by Dr. S. BAYNE of the University of St. Andrews. Uniformly labeled fructose, [1-^{14}C]glucose, [6-^{14}C]glucose and [1-^{14}C]sorbitol were purchased from The Radiochemical Centre, Amersham. [1-^{14}C]Fructose was prepared from [1-^{14}C]sorbitol by incubation with sheep seminal vesicle slices under O_2[13]. The fructose formed was purified from the incubation mixture by paper chromatography with water-saturated phenol. To prepare [6-^{14}C]fructose, [6-^{14}C]glucose was converted to a mixture of glucose-6-phosphate and fructose-6-phosphate by commercial hexokinase and phosphohexoisomerase (Sigma) and the hexoses were liberated by the action of alkaline phosphatase. Microsomes from sheep kidney were used as a source of this enzyme. The free sugars were then separated by paper chromatography with water-saturated phenol and butanol–acetic acid–water as solvents[14].

Analytical methods

Inorganic phosphate was determined by the method of FISKE AND SUBBAROW[15], lactic acid by the method of BARKER AND SUMMERSON[16], glucose by the use of glucose oxidase[17] or by its reducing power[18] and fructose by the resorcinol method[19].

The distribution of ^{14}C in the glucose samples was determined by *Leuconostoc mesenteroides* fermentation followed by chemical degradation of the products[8].

Preparation of biological material

Inverted intestinal sacs were prepared from the small intestines of guinea pigs and rats by the method described by WILSON AND WISEMAN[10]. The sacs, approximately 10 cm long, were filled with 1.5 ml of the bicarbonate buffer of KREBS AND HENSELEIT[20] and placed in the same buffer containing the appropriate sugar. After incubation in a Dubnoff shaker at 37° in an atmosphere of 95 % O_2 and 5 % CO_2, the sacs were removed and washed with water. Chemical analyses were carried out on the sac contents after deproteinization by the method of SOMOGYI[21]. When the isolation of glucose was desired, the sac contents were passed directly through a column of Amberlite MB-3 and reduced to a small volume in a vacuum desiccator. The glucose was then isolated by paper chromatography with butanol–acetic acid–water[14].

The particulate fraction from intestinal mucosa was prepared as follows: The mucosa was scraped off with a blunt spatula and 1.5 g of the scraped tissue was suspended in 9.0 ml of 0.25 *M* sucrose. After homogenizing in a Potter–Elvehjem apparatus[22], the preparation was centrifuged at 25,000 rev./min for 6 min in a Spinco Model L centrifuge, using a No. 40 head. The cloudy supernatant solution was decanted and recentrifuged for 30 min at 38,000 rev./min. The supernatant solution was discarded and the pellet suspended in 3 ml of H_2O.

RESULTS

I. Fructose to glucose conversion by the isolated intestinal sac

Species difference. Although *in vitro* experiments by DARLINGTON AND QUASTEL[2] and by WILSON AND VINCENT[3] have shown that isolated guinea pig or hamster intestines readily convert fructose to glucose during its absorption, the results

presented by KIYASU AND CHAIKOFF[4] indicate that such a conversion is of much less importance in the rat. The data presented in Table I show a qualitative difference in the fate of fructose absorbed by intestinal sacs of guinea pig and rat. While glucose is the major product appearing inside the guinea pig sac, little, if any, is formed by the rat.

Experiments with radioactive sugars. Guinea pig sacs were incubated with [1-^{14}C]fructose and [1-^{14}C]glucose and the radioactive glucose samples appearing inside the sacs were isolated. The samples were then degraded in order to determine their distribution of ^{14}C-activity. The results are given in Table II.

TABLE I

FRUCTOSE, GLUCOSE AND LACTIC ACID RECOVERED FROM INTESTINAL SACS AFTER THE ABSORPTION OF FRUCTOSE

The intestinal sacs were incubated for 2 h in 3 ml of buffer containing 9 mg fructose.

Intestinal sac	*Fructose absorbed (μg)*	*Product appearing in sacs (μg)*		
		Fructose	*Glucose*	*Lactic acid*
Guinea pig	4,860	92	830	251
Rat	3,260	354	< 6	492

TABLE II

PERCENTAGE DISTRIBUTION OF ^{14}C IN GLUCOSE RECOVERED FROM GUINEA PIG INTESTINAL SACS

Intestinal sacs were incubated for 2 h in 2 ml of buffer containing 2 mg [1-^{14}C]fructose or [1-^{14}C]glucose. The radioactive glucose appearing inside the sacs was isolated and degraded as described under METHODS. The values in the table are expressed as percentages of the total activity in the glucose samples as determined by persulfate oxidation[31].

Sugar supplied	*Carbon atom*					*Recovery of activity*
	1	*2 + 3*	*4*	*5*	*6*	
[1-^{14}C]Fructose	55	2	0	0	51	108
[1-^{14}C]Glucose	97	2	0	0	1	100

It can be seen that the ^{14}C-activity in the glucose formed from [1-^{14}C]fructose is almost equally distributed between C-1 and C-6. Since no randomization occurred in the glucose during its transport across the intestinal wall, it is reasonable to assume that the observed randomization when [1-^{14}C]fructose was used occurred prior to the formation of glucose. TAYLOR AND LANGDON[23] have shown that no redistribution of ^{14}C-activity occurred in liver glycogen formed by the rat from [1-^{14}C]glucose administered by stomach tube.

These data support the first pathway, which involves the formation of glyceraldehyde and triose phosphate. The nearly equal degree of labeling found in positions 1 and 6 might be taken as an indication that the newly formed glucose arises only from the top half of fructose and that the glyceraldehyde formed is not utilized for glucose formation. In order to check this possibility, intestinal sacs were incubated with uniformly labeled fructose or fructose specifically labeled in carbon 1 or 6. Glucose appearing inside the sac was isolated and its specific activity was determined

TABLE III

SPECIFIC ACTIVITY OF GLUCOSE FORMED FROM [1-^{14}C]FRUCTOSE, [6-^{14}C]FRUCTOSE, OR UNIFORMLY LABELED FRUCTOSE BY GUINEA PIG INTESTINAL SACS

Intestinal sacs were incubated for 2 h in 2 ml of buffer containing 2 mg of the radioactive fructose samples. The glucose appearing inside the sacs was isolated as described under METHODS. The values in the table are expressed as percentage of the specific activity of the fructose supplied and represent the mean of different experiments. The standard error of mean is also given, and the figures in brackets represent the number of experiments.

Sugar supplied	*Specific activity of glucose formed*
[1-^{14}C]Fructose	122 ± 16.6 [5]
[6-^{14}C]Fructose	81.5 ± 10 [2]
[U-^{14}C]Fructose	73 ± 3.5 [5]

and compared with that of the fructose substrates. The results are shown in Table III. Although the large experimental errors preclude a quantitative evaluation of the data, it appears that C-6 of the fructose molecule is readily utilized for glucose formation. The significantly higher activities in the glucose obtained from [1-^{14}C]fructose (in some cases higher than the original fructose) would seem to indicate that not all of the glyceraldehyde arising from carbon atoms 4, 5 and 6 of fructose is metabolized by phosphorylation to triose phosphate. The recent observation by LAMPRECHT AND HEINZ[24] that D-glyceraldehyde can be metabolized in the liver via glyceric acid is of interest in this regard.

Experiments with glucosone and sorbitol. No glucose was formed inside or outside the sacs when sorbitol or glucosone were used as substrates in the incubation mixture. However, when glucosone was added simultaneously with glucose, a larger amount of glucose was recovered inside and outside the sac. This phenomenon was interpreted as being due to the inhibition of hexokinase by glucosone[25], with a resulting decrease in glucose utilization.

II. Experiments with cell-free extracts

The conversion of glucose to fructose by the first pathway requires the action of fructokinase and glucose-6-phosphatase. These two enzymes, in contrast to the other enzymes participating in this pathway, have a limited distribution in tissues. Experiments were carried out in order to determine whether they are present in the intestinal mucosa of guinea pigs.

Fructokinase. Fructose-1-phosphate, which is the product of fructokinase, has been isolated by KJERULF-JENSEN[26] from the intestinal mucosa of rats and rabbits, where it accumulates during the course of fructose absorption. A fructokinase is known to be present in the intestinal mucosa of the rat but could not be demonstrated in the cow[27]. No published data were found for the guinea pig.

The phosphorylation of fructose by ATP, catalysed by an extract of guinea pig intestinal mucosa, was tested in the presence and absence of glucose. As shown in Table IV the phosphorylation of fructose was not inhibited by glucose. This is presumptive evidence for the presence of a fructokinase, since the phosphorylation of fructose by hexokinase is almost completely inhibited by an equimolar concentration of glucose[28].

The fructose phosphate formed by the mucosal preparation was isolated as a barium precipitate in ethanol and tested for its lability in acid. The results of this experiment are given in Table V. It was found that 96 % of the esterified fructose was liberated as free sugar in 20 min at 100° in *N* HCl. This lability is characteristic of fructose-1-phosphate as contrasted with fructose-6-phosphate, which is only hydrolysed approximately 18 % under these conditions[19].

TABLE IV

EFFECT OF GLUCOSE ON THE PHOSPHORYLATION OF FRUCTOSE BY A CELL-FREE EXTRACT OF GUINEA PIG INTESTINAL MUCOSA

The reaction mixture contained 2.2 μmoles fructose, 8 μmoles ATP, 8 μmoles $MgCl_2$, 900 μmoles KCl, 20 μmoles cacodylate buffer, pH 7, 100 μmoles KF and 1 ml of a 20 % aqueous homogenate of intestinal mucosa in a total volume of 2 ml. After 40 min incubation at 37°, the mixtures were deproteinized by the method of SOMOGYI[21] and fructose was measured in the protein-free filtrate. As fructose was not metabolized in the absence of ATP, fructose disappearance was taken as a measure of fructose phosphorylation.

Glucose added (μmoles)	*Fructose phosphorylated (μmoles)*
—	1.80
0.44	1.76
2.2	1.70
4.4	1.63
11	1.82
22	1.81

TABLE V

RATE OF HYDROLYSIS OF THE FRUCTOSE PHOSPHATE FORMED BY A CELL-FREE EXTRACT OF GUINEA PIG INTESTINAL MUCOSA

Fructose phosphate was enzymically prepared by the experimental conditions given in Table IV. Deproteinization was carried out by the addition of perchloric acid. After filtration, the acid extract was neutralized with KOH and refiltered. The phosphate esters were then precipitated by the addition of $BaCl_2$ and 2 volumes ethanol. The barium precipitate was washed 3 times with 60 % ethanol and then dissolved in 1 *N* HCl. This solution was heated at 100° and aliquots were withdrawn at appropriate times for free fructose analyses after adsorption of the remaining phosphate esters by $Ba(OH)_2$–$ZnSO_4$[32]. Total fructose was determined in an aliquot not treated with the barium–zinc reagent.

Time of hydrolysis (min)	*Total fructose liberated (%)*
5	59
10	81
20	96
40	95

Glucose-6-phosphatase. Glucose-6-phosphatase, a microsome-bound enzyme[29], is known to be present in liver and kidney. It could not be found, however, in the intestinal mucosa of the rat[9]. In confirmation of this observation, the results presented in Fig. 1 show that microsomes from rat intestinal mucosa do not hydrolyse glucose-6-phosphate more rapidly than glucose-1-phosphate. In contrast, guinea pig microsomes hydrolyse glucose-6-phosphate faster than glucose-1-phosphate. The greatest

difference in rate of hydrolysis was at pH 6.0, which is the pH optimum of liver glucose-6-phosphatase[30].

In view of the large difference between the glucose-6-phosphatase activity of microsomes obtained from rat and guinea pig intestinal mucosa, it was of interest

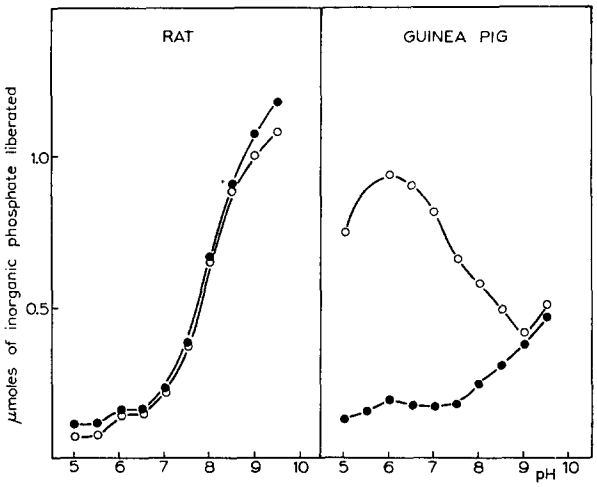


Fig. 1. Phosphatase activity of microsomes obtained from rat and guinea pig intestinal mucosa. 10 μmoles of glucose-6-phosphate or glucose-1-phosphate, 100 μmoles of buffer and a suspension of microsomes were incubated for 1 h at 37° in a total volume of 0.55 ml. Acetate was used as a buffer for pH 5.0, an equimolar mixture of tris(hydroxymethyl)aminomethane and cacodylate for pH 5.5–9.0 and glycine for pH 9.5. The microsomes in each incubation mixture were obtained from 5 mg of mucosa in the case of the rat and 25 mg of mucosa in the case of guinea pig. O, glucose-6-phosphate; ●, glucose-1-phosphate.

to examine microsomes obtained from the mucosa of other animals for this enzymic activity. The rabbit was found to resemble the guinea pig in that a peak of glucose-6-phosphatase activity at pH 6.0 was noted. The hamster was found to have no clear-cut peak at this pH but the hydrolysis of glucose-6-phosphate proceeded 2 to 3 times more rapidly than that of glucose-1-phosphate in the range of pH 5–7, while in the pH range of 8–10, glucose-1-phosphate was hydrolysed more rapidly than glucose-6-phosphate. Microsomes from human intestinal mucosa were found to contain acid and alkaline phosphatase activities but no evidence for a glucose-6-phosphatase peak at pH 6.0 was obtained.

Other experiments. Negative results were obtained in attempts to show either a reduction of glucosone to glucose in the presence of TPNH or an oxidation of sorbitol in the presence of methylene blue by cell-free preparations of guinea pig intestinal mucosa. It was also not possible to demonstrate a direct isomerisation of fructose to glucose.

DISCUSSION

The data presented in this paper allow a choice between several possible routes for the conversion of fructose to glucose by guinea pig intestine. The randomization of ^{14}C-activity between positions 1 and 6 of the glucose formed from [1-^{14}C]fructose as well as the presence of fructokinase and glucose-6-phosphatase in this tissue is strong

evidence that the first pathway is responsible for the conversion. The operation of the other routes to any significant extent was not indicated by our experiments.

The nearly equal degree of labeling of carbon 1 and 6 of the glucose formed from [1-^{14}C]fructose indicates either that triosephosphate isomerase is very active in the intestine and allows a complete equilibration of carbon between the two triose phosphates, or that the glyceraldehyde formed from fructose-1-phosphate is not converted to glucose. That the latter is not the case is shown by the conversion of [6-^{14}C]fructose to labeled glucose.

As previously shown in the case of fructokinase[27], the enzymic equipment of the intestinal mucosa seems to vary from one species to the other. It appears possible that the inability of the rat intestine to convert fructose to glucose is related to the absence of glucose-6-phosphatase in this tissue. If this is true, the fact that glucose-6-phosphatase could not be detected in human intestine might indicate that fructose is not converted to glucose in the course of digestive absorption in man.

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