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METABOLISM OF FRUCTOSE IN THE SMALL INTESTINE

I. THE EFFECT OF FRUCTOSE FEEDING ON FRUCTOSE TRANSPORT AND METABOLISM IN RAT SMALL INTESTINE

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SUMMARY

1. The metabolism and transport of fructose in rat small intestine following fructose feeding for up to 20 days has been measured. A preparation of villus epithelial cells was used for the measurements.

2. In confirmation of the results of other workers fructose was poorly metabolised in rat small intestine. Small conversion to lactate and glucose was observed.

3. Fructose feeding had virtually no effect on the conversion of fructose to lactate or glucose.

4. Fructose feeding for 3 days resulted in a significant increase (approx. 2-fold) in the activities of fructokinase (EC 2.7.1.1.) and fructose-1-phosphate aldolase (EC 4.1.2.7) in rat small intestine whereas after fructose feeding for 15 days these enzyme activities were not significantly different from enzyme activities in the small intestine of animals fed on normal laboratory chow.

5. Measurements of the rate of fructose transport in segments of intestine taken from rats fed on normal laboratory chow or the fructose diet were made. The rate of fructose uptake in segments of intestine taken from animals fed for 3 days on the fructose diet was about twice that of animals fed on normal laboratory chow. The rate of fructose uptake was not further altered if segments were obtained from intestine of animals which had received the fructose diet for 15 days.

INTRODUCTION

There is a marked difference in the metabolism of fructose in the small intestine of rats and guinea pigs[1–3]. In the rat small intestine fructose is poorly metabolized and appears in the portal blood primarily as fructose together with a small amount of lactate[1]. In the small intestine of the guinea pig, fructose is mainly converted to glucose[2,3]. The reasons for this species difference are not clear. Ginsburg and Hers[2] attempted to explain the lack of conversion of fructose to glucose in rat small intestine on the basis of the absence of glucose-6-phosphatase (EC 3.1.3.9). Subsequently, glucose-6-phosphatase was found in the rat small intestine[4], but its measurement was complicated by the presence of a naturally occurring inhibitor[5].

Heinz and Lamprecht[6] observed very low activities of fructokinase (EC 2.7.1.1) and fructose-1-phosphate aldolase (EC 4.1.2.7) in the rat small intestine, compared with the enzyme activities in the guinea pig small intestine. These authors inferred that the low activities of these rate limiting enzymes in rat small intestine precluded fructose metabolism.

Stifel *et al.*[7] have found that fructose feeding for a period of three days results in a marked increase in the activities of fructokinase and fructose-1-phosphate aldolase in rat jejunum.

From these results it was proposed that fructose metabolism in rat jejunum may be accelerated after fructose feeding and that this increased metabolism may increase the rate of absorption of fructose into the jejunal epithelium.

The purpose of the experiments described in this paper was to investigate the proposition that fructose metabolism in rat small intestine is restricted by low activities of rate limiting enzymes and that the rate of fructose metabolism can be accelerated by increasing the levels of these enzymes by fructose feeding. Furthermore the rate of fructose transport following fructose feeding has been studied.

METHODS AND MATERIALS

Animals

Male Wistar rats weighing between 250–350 g were housed in pairs in individual cages with water *ad libitum*. They were fasted for one day prior to feeding. The fructose diet consisted of fructose (60%, w/w), casein (17%), cellulose powder (10%), American Lard (8%), and a vitamin and mineral mix (5%). No significant difference was noted in the weight gains and food consumption of the animals when compared to control animals fed on normal laboratory chow.

Enzymes and chemicals

Enzymes and coenzymes were purchased from Boehringer Corp. (London) Ltd, London W.5, Great Britain. D-Fructose 1-phosphate (barium salt) was obtained from Sigma (London) Chemical Co., London S.W.6. D-[U-^{14}C]Fructose was purchased from the Radiochemical Centre, Amersham, Bucks., Great Britain. D-[1-^{3}H]-Mannitol was bought from NEN Chemicals GmbH, 6072 Dreiechenheim bei Frankfurt/Main, Germany. All other chemicals were of A.R. grade.

Collection and preparation of tissues

The rats were killed by cervical fracture and the whole of the small intestine was removed. The intestinal lumen was rinsed with ice-cold isotonic saline. The intestine was slit longitudinally and the mucosa was scraped lightly with a scalpel. This intestinal preparation was shown histologically to contain mostly epithelial cells which were derived from the tips of the villi and will be referred to as the epithelial cell preparation. For fructose transport determinations the intestine was everted on a glass rod and cut into segments approx. 1.5 cm in length. The segments were pooled and utilized at random.

Measurement of fructose metabolism

Approx. 100-mg samples of epithelial cells were weighed on a torsion balance

and placed in 25-ml conical flasks containing 4 ml of ice-cold Krebs–Henseleit[8] medium containing 5 mM fructose previously gassed with O_2–CO_2 (95:5, v/v) for 1 h. The flasks were placed in a shaking water bath at 37 °C. The contents of each flask were gassed with O_2–CO_2 (95:5, v/v) for 5 min to allow temperature equilibration after which the flasks were sealed and incubated for a further 30 min. After the incubation 0.1 ml of 72% $HClO_4$ was added to each flask, the contents mixed and the material from each flask was sonicated (1 min at 21 kHz in an MSE sonicator) in order to disrupt the cells. Each sonicated preparation was neutralised with solid $NaHCO_3$ and centrifuged (75000 × g·min). Samples of the resulting supernatants were used for analyses of fructose[9], glucose[10] and lactate[11] using a Technicon autoanalyser. These analyses were corrected using values obtained with identically treated unincubated controls. Each analysis was carried out in triplicate.

Enzyme assays

These were carried out either on particle-free supernatants of epithelial cell homogenates prepared by the method of Weiser and Quill[12] or on particle-free supernatants from liver prepared in a similar way. All enzyme assays were performed in duplicate at 30 °C. Fructose-1-phosphate aldolase was assayed by a modification of the method of Rajkumar *et al.*[13]. The buffer used was 0.5 M Tris–HCl (pH 7.5) and the incubation cocktail did not contain triosephosphate isomerase since only fructose-1-phosphate aldolase was being determined. D-Fructose 1-phosphate (sodium salt) was prepared from D-fructose 1-phosphate (barium salt) as described by Spolter *et al.*[14]. The final concentration of D-fructose 1-phosphate was determined using the phosphate method of King[15]. Prior to the assay of fructokinase the particulate-free supernatant was subjected to a further acid purification step as described by Weiser and Quill[12] in order to remove most of the hexokinase activity. Fructokinase was assayed by a modification of the method of Adelman *et al.*[16]. Each incubation mixture contained in a final volume of 3 ml the following (final concentrations): 50 mM Tris–HCl buffer (pH 7.4), 100 mM KCl, 0.45 mM NADH, 15 mM ATP (previously adjusted to pH 7.4 with $NaHCO_3$), 10 mM phosphoenolpyruvate (previously adjusted to pH 7.4 with $NaHCO_3$), 15 mM NaF, 20 mM $MgCl_2$, 5 mM fructose, 10 units each of pyruvate kinase (EC 2.7.1.40) and lactate dehydrogenase (EC 1.1.1.27), and 0.2–0.7 mg of protein of the preparation to be assayed. The reaction was started by the addition of fructose. There was a considerable reaction in the absence of fructose which was subtracted from the fructokinase reaction

Measurement of fructose transport

Intestinal segments were housed in chambers as described by Semenza[17]. Fructose uptake was measured using a modification of the method of Gracey *et al.*[18]. Each incubation (final vol. 25 ml) contained 5 mM fructose, D-[U-^{14}C]fructose (2.5 μCi) and D-[1-^{3}H]mannitol (3.75 μCi). Incubations were at 37 °C for various time periods up to 3 min. Subsequent treatments of the samples were carried out as described by Gracey *et al.*[18] except that a triton scintillator was used[19]. All estimations of D-[U-^{14}C]fructose uptake were corrected for passive diffusion by subtraction of dpm due to D-[1-^{3}H]mannitol.

Protein and DNA determinations

These were carried out as described by Hübscher *et al.*[20].

RESULTS

The effects of fructose feeding on fructose metabolism in epithelial cell preparations are shown in Fig. 1. The protein and DNA content of the epithelial cell preparations remained relatively constant for the 20 days of fructose feeding. Fructose disappearance from the incubation was very small and not significantly different when preparations of epithelial cells from the intestine of animals fed on control or fructose diet were used. In cell preparations from animals fed on laboratory chow lactate production was slightly greater than glucose production although only very small amounts of both metabolites were produced. The production of lactate and glucose by cell preparations from animals fed on the fructose diet was not significantly different from that in preparations from animals fed the control diet.

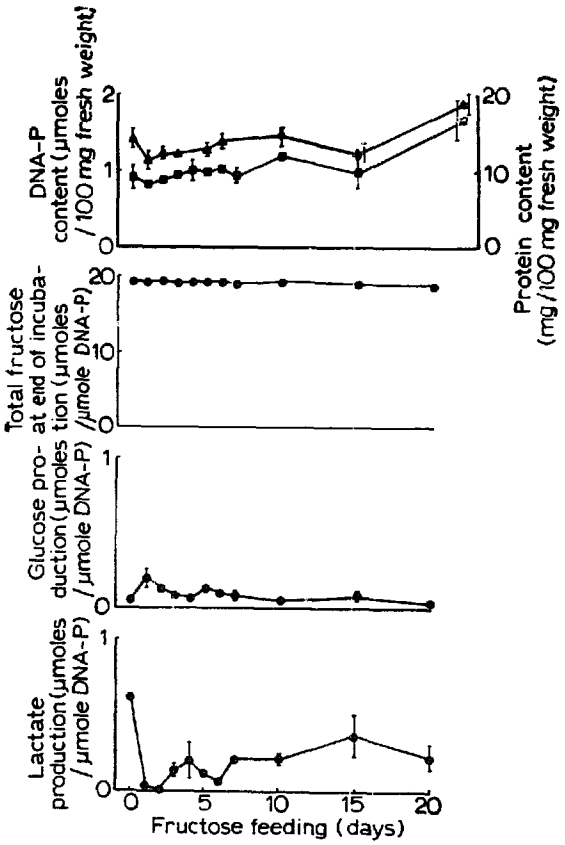

Fig. 1. The effect of fructose feeding on fructose metabolism in rat intestine. Rat intestinal epithelial cells were incubated in 4 ml of Krebs–Henseleit[8] medium containing 5 mM fructose for 30 min at 37 °C. The values represent the mean ± half the range of measurements on two animals. ■—■, DNA-P (DNA-phosphorus); ▲—▲, protein.

Fructokinase and fructose-1-phosphate aldolase activities in intestine and liver from animals fed the control or high fructose diet are shown in Table I. A significant increase in fructokinase and fructose-1-phosphate aldolase activities in the intestine was observed after the animals had received the fructose diet for three days. The activities of these two enzymes were not significantly different from control values after the animals had received the fructose diet for twenty days. In rat liver the changes in enzyme activities of fructose feeding were similar to those in the in-

TABLE I

EFFECT OF FRUCTOSE FEEDING ON FRUCTOKINASE AND FRUCTOSE-1-PHOSPHATE ALDOLASE ACTIVITIES IN RAT INTESTINAL EPITHELIAL CELLS AND IN RAT LIVER

Enzyme activities are expressed as nmoles substrate reacted/min per mg protein. The results are given as mean ± S.D. The numbers in parentheses indicate the number of animals used. N.S., no significance.

Tissue	*Days on diet*	*Fructokinase*	*Significance (P)*	*Fructose-1-phosphate aldolase*	*Significance (P)*
Intestine	0	39.8 ± 3.6 (3)	Null	69.6 ± 9.0 (3)	Null
	3	80.3 ± 9.8 (3)	<0.01	186.3 ± 26.0 (3)	<0.01
	15	38.6 ± 10.0 (3)	N.S.	115.0 ± 35.7 (3)	N.S.
Liver	0	76.1 (1)	—	114.3 (1)	—
	3	136.6 (1)	—	123.6 (1)	—
	15	106.0 (1)	—	105.6 (1)	—

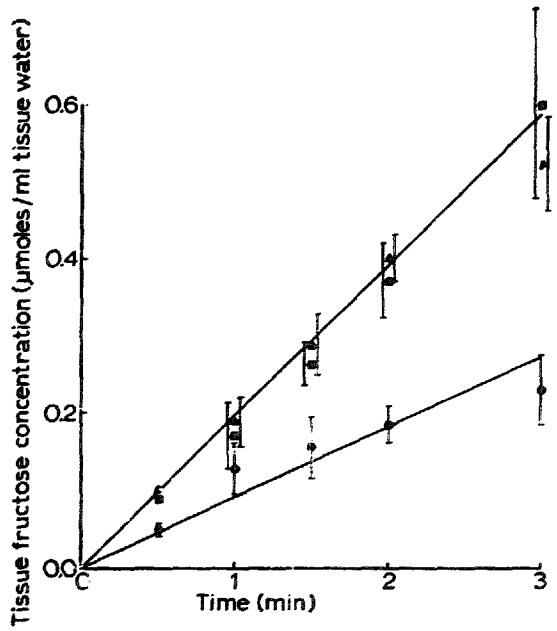

Fig. 2. The effect of fructose feeding on the rate of fructose uptake in rat intestine. Everted segments of rat intestine were housed in plexiglass chambers and incubated at 37 °C in Krebs–Henseleit[8] medium containing 5 mM [U-^{14}C]fructose and [1-^{3}H]mannitol. The [U-^{14}C]fructose uptake at each time point was corrected for the presence of [1-^{3}H]mannitol which is an index of passive diffusion and extracellular space. The values represent the mean ± S.D. of 3 animals. ●—●, control animals; ▲—▲, animals fed on fructose diet for 3 days; ■—■, animals fed on fructose diet for 15 days. The rates of fructose uptake following both 3 and 15 days of fructose feeding were significantly greater ($P < 0.001$) than the rate observed with control animals.

testine although the effect on fructose-1-phosphate aldolase was not as pronounced as that seen in the intestine.

The results presented in Fig. 2 show that the rate of fructose uptake is linear for 3 min in segments of intestine taken from animals fed the control (correlation coefficient 0.97) or fructose diet. The rate of fructose uptake in segments of intestine taken from animals which had received the fructose diet for 3 days was about twice that in segments of intestine prepared from animals which received the control diet.

The rate of fructose uptake was not further altered in segments of intestine prepared from animals which received the fructose diet for 15 days. Although the rate of fructose uptake in segments of intestine increased on fructose feeding the steady-state amounts of fructose in preparations from rats fed the control or fructose diet were too low to be measured.

DISCUSSION

The results presented in Fig. 1 and Table I clearly show that no correlation exists between the activities of fructokinase and fructose-1-phosphate aldolase in the intestinal epithelial cell preparation and the metabolism of fructose to glucose and lactate. The enzyme levels were significantly elevated after 3 days of fructose feeding, with no concomitant increase in fructose metabolism, while after 15 days of fructose feeding the enzyme levels were not significantly different from values obtained with preparations from control animals fed laboratory chow.

The results shown in Fig. 1 show no increase in fructose disappearance from the incubations with preparations obtained from animals fed for up to 20 days on the fructose diet. These results preclude any significant metabolism of fructose to any metabolites other than glucose or lactate.

The levels of fructokinase and fructose-1-phosphate aldolase measured in preparations of epithelial cells from animals fed on laboratory chow were considerably higher than the values reported for these enzymes in rat small-intestinal mucosa by Heinz and Lamprecht[6]. These differences may be explained in part by use of different preparations, control diets, and in the case of fructokinase a different enzyme assay. However, the present results do not substantiate the proposal of Heinz and Lamprecht[6] that low levels of fructokinase and fructose-1-phosphate aldolase limit the metabolism of fructose in rat intestine. Furthermore elevation of the levels of fructokinase and fructose-1-phosphate aldolase after 3 days of fructose feeding was not accompanied by an increase in fructose metabolism. This elevation of enzyme levels confirms the results of Stifel *et al.*[7] although the activities of the enzymes measured in the present study were higher than those reported by these authors. This may be due to the fact that measurements were made at 30 °C in the present study, whereas Stifel *et al.*[7] made measurements at 25 °C and that different intestinal preparations were used.

The levels of fructokinase and fructose-1-phosphate aldolase in preparations from animals fed for 15 days on the fructose diet were not significantly different from those of animals fed on the control diet unlike the levels in preparations from animals fed for 3 days on the fructose diet. The reasons for the decline in the elevated enzyme levels on prolonged fructose feeding are not apparent.

Fructose transport in rat small intestine was reported to occur by a process of "facilitated diffusion". The process was considered not to require energy or to show Michaelis–Menten kinetics[21]. Recently, however, Gracey *et al.*[18] showed that fructose was actively transported in rat small intestine by a process demonstrating Michaelis–Menten kinetics.

Crouzoulon-Bourcart *et al.*[22] have recently shown a significant increase (2-fold) in the absorption of fructose *in vivo* in rat intestine following feeding of a 10% solution of fructose for a period of 3 days. The 2-fold increase in fructose uptake in

intestinal segments prepared from rats fed for 3 days on the fructose diet (Fig. 2) is in complete agreement with the results obtained *in vivo* by these authors. Fructose feeding results in an increase in fructose transport in rat small intestine whereas fructose metabolism is unaffected. If fructose transport is an energy-requiring process then it would be interesting to determine the source of energy for the process since fructose metabolism is not increased.

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