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METABOLISM OF FRUCTOSE IN THE SMALL INTESTINE

II. THE EFFECT OF FRUCTOSE FEEDING ON FRUCTOSE TRANSPORT AND METABOLISM IN GUINEA PIG SMALL INTESTINE

DENIS A. MAVRIAS AND R. JOHN MAYER

Department Biochemistry, Medical School, University Nottingham, Nottingham NG7 2RD (Great Britain)

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SUMMARY

1. The metabolism and transport of fructose in guinea pig 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, most of the fructose metabolized by guinea pig small intestine appears as glucose, the rest appearing as lactate.

3. Fructose feeding had no effect on the protein content of the epithelial cell preparation, however, the DNA content decreased markedly after 10 days on the fructose diet.

4. Fructose metabolism decreased initially on fructose feeding. Subsequently a gradual increase occurred which became very pronounced after 10 days on the fructose diet.

5. Fructose feeding for 3 days resulted in no change in the activities of fructokinase and fructose-1-phosphate aldolase in the guinea pig small intestine.

6. Measurements of the rate of fructose uptake in segments of intestine taken from guinea pigs 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 was the same as that of animals fed on normal laboratory chow. There was a slight increase in the rate of fructose uptake in intestinal segments taken from animals fed the fructose diet for 20 days.

7. Histological section of intestine taken from animals fed the fructose diet for 20 days showed an accumulation of a yellowish material in the sub-epithelial region at the tips of the villi. This material appeared to be present within macrophages. Preliminary evidence indicates that the material is a protein containing a carbohydrate component.

INTRODUCTION

Guinea pig small intestine metabolizes fructose predominantly to glucose[1–3] unlike the rat small intestine which poorly metabolizes fructose[1]. In the previous paper

the effects of fructose feeding on the metabolism and transport of fructose in rat small intestine was described.

A similar diet was fed to guinea pigs in order to establish how this diet affected fructose metabolism and transport. It was hoped that a comparison of the effects of fructose feeding on fructose metabolism and transport in the guinea pig and the rat would allow resolution of some of the reasons for the fundamental differences in fructose metabolism in the small intestine of the two species.

METHODS AND MATERIALS

Animals

Guinea pigs weighing between 350–500 g were housed in pairs in individual cages with water *ad libitum*. They were fasted for 1 day prior to feeding. They were fed on a 60 % fructose diet, as previously described[4]. In additon, they received ascorbic acid (2.5 %, w/v) in their drinking water. Control animals were fed on powdered laboratory chow supplemented with ascorbic acid (2.5 %, w/v) in the drinking water. There was considerable difference in the weight gain of the animals fed on these two diets. The control animals gained an average of 6.0 g per day whereas the guinea pigs fed on the fructose diet lost an average of 4.0 g per day, the largest weight loss being in the first 3 days. A number of guinea pigs which had been on the diet for longer than 2 weeks died of unkown causes.

Collection and preparation of tissues and assays

The collection and preparation of tissue, assays of fructose, glucose and lactate, enzyme assays, and measurement of fructose transport were the same as described in the preceding paper[4].

Chemicals

All chemicals used were those indicated in the previous paper[4]. The protease preparation used was obtained from Sigma (London) Chemical Co.

RESULTS

The effects of fructose feeding on fructose metabolism in intestinal epithelial cells from guinea pig intestine are shown in Fig. 1. The protein content of the epithelial cell preparations remained relatively constant for 20 days on the fructose diet. The DNA content of the epithelial cell preparations, however, showed a marked decrease after approx. 3 days on the diet, reaching 25 % of the initial level after 15 days on the diet.

The loss of fructose from the incubations was very small using preparations of epithelial cells from animals fed laboratory chow. An initial decrase in fructose disappearance from the incubations occurred with preparations from animals fed the fructose diet, followed by a gradual increase which was more pronounced after 10 days of fructose feeding. Virtually all of the fructose disappearance from the incubation could be accounted for as glucose and lactate. Glucose production in cell preparations from animals fed on laboratory chow was greater (3-fold) than lactate production. There was an initial decrease in both glucose and lactate production following fructose

feeding. Subsequently, an increase in glucose and lactate production occurred which was most marked after 10 days on the fructose diet.

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. There was no significant change in the enzyme activities of either the intestine or the liver from animals fed on the high fructose diet for a period of 3 days.

The results presented in Fig. 2 show that the rate of fructose uptake is linear for 2 min in segments of intestine taken from animals fed laboratory chow or fructose diet. The K_m for fructose transport in segments of intestine obtained from animals fed the control diet was 4 mM (Fig. 3). Fructose uptake in the 2-min period was reduced considerably (Table II) when segments were incubated in the presence of dinitrophenol ($5 \cdot 10^{-4}$ M). The rate of fructose uptake in segments of intestine taken from animals which had received the fructose diet for 3 days was the same as that in segments of intestine prepared from animals which received the control diet. The rate of fructose

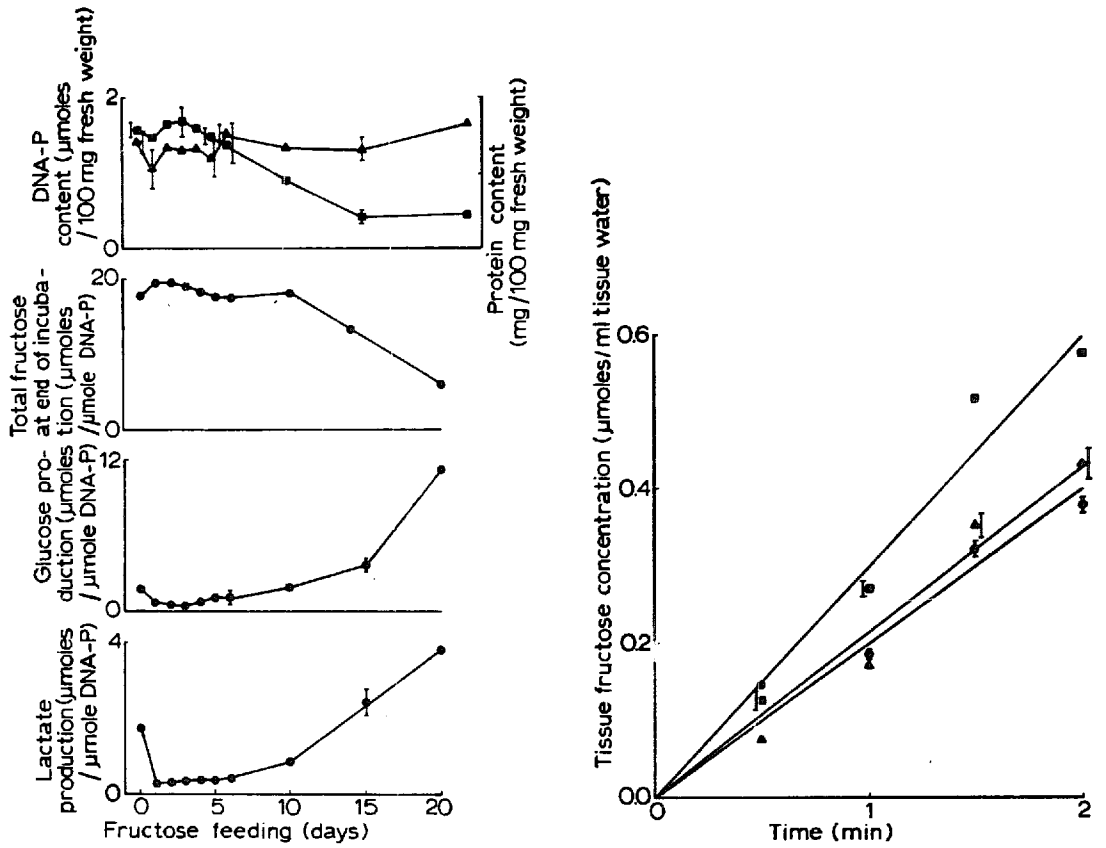

Fig. 1. The effect of fructose feeding on fructose metabolism in guinea pig intestine. Guinea pig intestine epithelial cells were incubated in 4 ml of Krebs–Henseleit[13] medium for 30 min at 37 °C. The values represent the mean ± half the range of measurements on two animals. ■—■, DNA-P (DNA-phosphorus); ▲—▲, protein.

Fig. 2. The effect of fructose feeding on the rate of fructose uptake in guinea pig intestine. Everted segments of guinea pig intestine were housed in plexiglass chambers and incubated at 37 °C in Krebs–Henseleit[13] 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 20 days. There was no significant difference in the rate of fructose uptake following fructose feeding.

TABLE I

EFFECT OF FRUCTOSE FEEDING ON FRUCTOKINASE AND FRUCTOSE-1-PHOSPHATE ALDOLASE ACTIVITIES IN GUINEA PIG INTESTINAL EPITHELIAL CELLS AND 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	77.9 ± 8.2 (4)	Null	128.0 ± 4.5 (4)	Null
	3	83.7 ± 9.3 (4)	N.S.	126.1 ± 25.3 (4)	N.S.
Liver	0	14.8 (1)	—	32.0 (1)	—
	3	14.0 (1)	—	37.0 (1)	—

TABLE II

EFFECT OF 2,4-DINITROPHENOL ON THE RATE OF FRUCTOSE UPTAKE IN SEGMENTS OF GUINEA PIG INTESTINE

Fructose uptake is expressed as μmoles/ml of tissue water. The results were obtained with segments of intestine from one animal fed the control diet.

Fructose in medium (mM)	*Dinitrophenol ($5 \cdot 10^{-4}$ M)*	*Fructose uptake*	*Inhibition (%)*
1	—	0.193	—
1	+	0.051	73.6
5	—	0.517	—
5	+	0.214	58.4

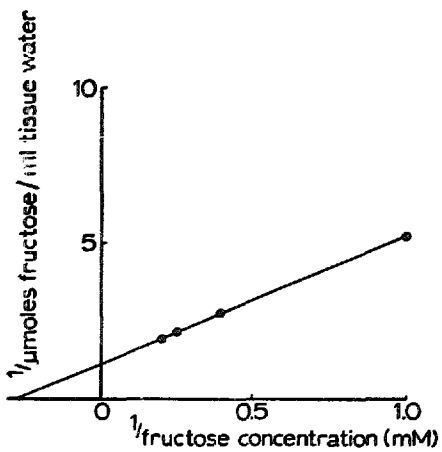

Fig. 3. K_m of fructose uptake in guinea pig intestine. Fructose uptake was measured as described in the legend to Fig. 2. Values represent the means of two determinations on segments of intestine from one animal. Individual values were essentially identical.

uptake in segments of intestine taken from animals which had received the fructose diet for 20 days was increased over the rate seen in the intestine from control animals although this increase was not statistically significant.

The appearance of the intestinal mucosa from guinea pigs fed on the high fructose diet for a period of 10 days or longer was different from that of the control animals.

The intestine from the fructose-fed animals was yellowish-orange in colour. Histological sections of intestine from animals fed the diet for 20 days (Fig. 4B) show an accumulation of material in the sub-epithelial region at the tips of the villi. This material shows up clearly in unstained sections due to its yellow colour. None of the material was found to be present in either the lacteals or the capillaries. High power magnification indicates that the material may be trapped within macrophages. On periodic acid oxidation of the material in the sections followed by Schiff's base formation the material showed some degree of red staining indicating the possibility of a carbohydrate component.

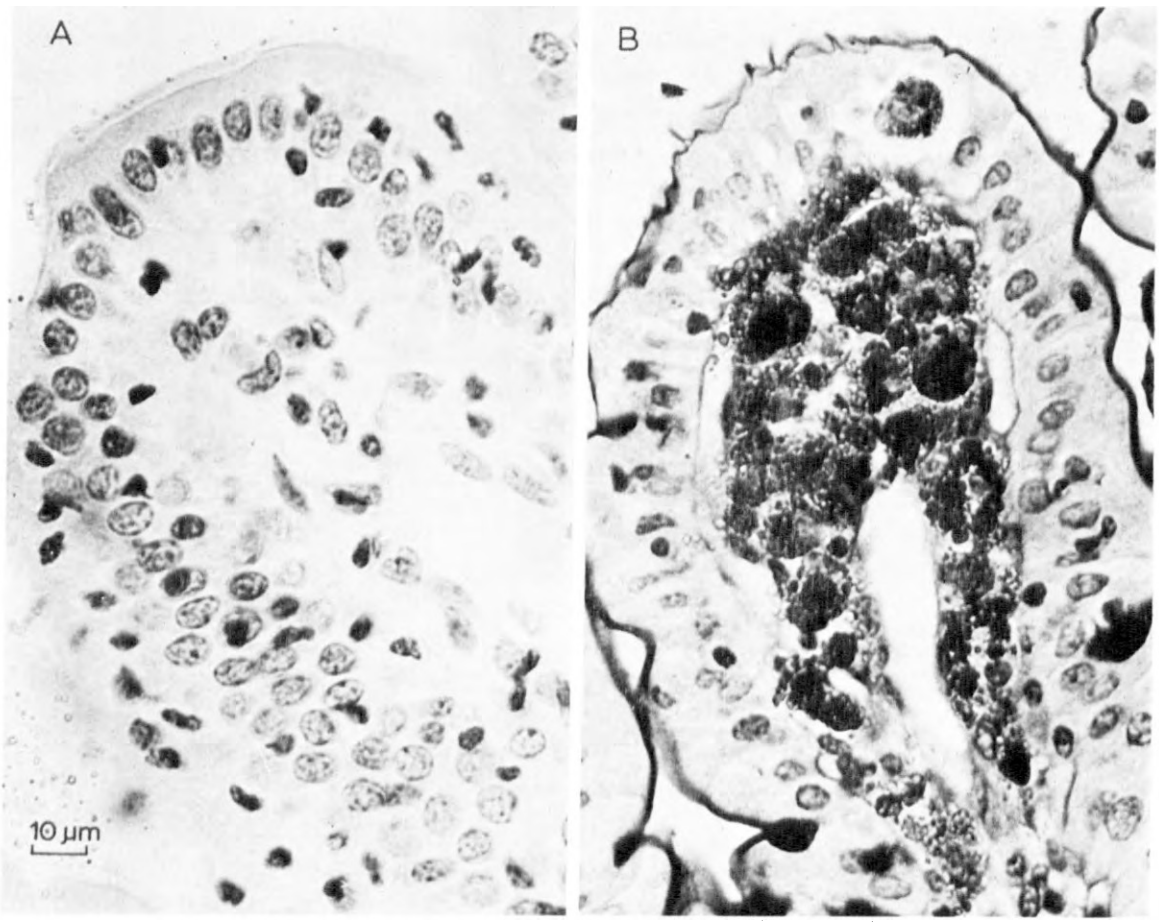

Fig. 4. (A) Jejunum from guinea pig fed the control diet. The section of jejunum from control intestine was stained with haemotoxylin and eosin. (B) Jejunum from guinea pig fed on a high fructose diet for 20 days. This section of jejunum was stained with haemotoxylin and periodic acid–Schiff reagent. Note the marked accumulation of periodic acid–Schiff-positive material in the sub-epithelial region. Preliminary evidence indicates that the material is a protein containing a carbohydrate component.

DISCUSSION

The results presented in this paper confirm the rapid metabolism of fructose reported by other authors[1-3] in the small intestine of the guinea pig. The effect of fructose feeding was to markedly increase the rate of fructose metabolism, particularly after 10 days on the fructose diet. This contrasted with the situation in rat intestine where fructose feeding had no effect on fructose metabolism[4]. The increased rate of fructose metabolism was associated with a decrease in the DNA content/unit weight

of the epithelial cell preparation. Hence, although the number of cells in incubations from animals fed the fructose diet for 10 days or longer was considerably decreased, the capacity of these cells to metabolize fructose was greatly enhanced.

The activities of fructokinase and fructose-1-phosphate aldolase in the intestine of control animals (Table I) were twice the values reported by Heinz and Lamprecht[5]. This may partly be explained by the difference in the intestinal preparations, the difference in the method of assay for fructokinase, and to the fact that the assays of Heinz and Lamprecht[5] were carried out at 25 °C. The activities of these two enzymes were approx. 2-fold greater in the guinea pig intestine than in the rat intestine[4]. These results contrast with the approx. 10-fold difference for fructokinase and 50-fold difference for fructose-1-phosphate aldolase observed by Heinz and Lamprecht[5]. Fructose feeding for 3 days resulted in no change in the activities of fructokinase and fructose-1-phosphate aldolase in guinea pig intestinal epithelial cells. This is in marked contrast to rat where there was a significant increase in the activities of these two enzymes in the epithelial cell preparation following feeding a high fructose diet for 3 days[4,6].

Fructose uptake by isolated surviving guinea pig intestine was considered to be by simple diffusion[7]. Such a process may be facilitated by reducing the intracellular fructose concentration by the rapid conversion of fructose to glucose which occurs in guinea pig intestine. These authors[8,9] report that the proportion of glucose to fructose appearing on the serosal side of their preparation was dependent on the mucosal concentration of fructose being high in preparations incubated with low fructose concentrations and low in preparations incubated with high fructose concentrations (>10 mM). However, since fructose is rapidly converted to glucose by the guinea pig intestine, studies of net transport across the lumen or accumulation by segments of intestine *in vitro* over long time periods are unsuited for the investigation of fructose transport *per se*. For this reason very much shorter incubation times were employed in this study for the measurement of fructose uptake in the guinea pig small intestine. This method minimizes the influence of intracellular metabolism, provided that the metabolic products are retained within the intestinal cells.

The rate of fructose uptake observed in segments of intestine prepared from guinea pigs fed the control diet (Fig. 2) was found to be greater (2-fold) than that measured in the rat small intestine[4]. The K_m for fructose transport in guinea pig intestine (Fig. 3) was greater than that found in the rat intestine by Gracey *et al.*[10].

Darlington and Quastel[7] found dinitrophenol ($1 \cdot 10^{-4}$ M) inhibited fructose to glucose conversions in the guinea pig intestine, but it had no effect on the rate of absorption of fructose. This is in contrast with the findings presented in this paper where dinitrophenol ($5 \cdot 10^{-4}$ M) was found to markedly inhibit fructose uptake.

The appearance of periodic acid–Schiff-positive material in macrophages at the tips of the villi of guinea pig intestine has been described previously by Clarke and Hardy[11]. These authors found a very similar picture to that shown in fructose-fed animals (Fig. 4B) in the small intestine of suckled new born guinea pigs. They further showed that macrophages containing this material migrate into the villus epithelium from which they are presumably lost into the lumen of the intestine. The section presented in Fig. 4B also shows a macrophage containing the periodic acid–Schiff-positive material which is between the epithelial cells.

The presence of periodic acid–Schiff-positive material in macrophages has been observed not only in newborn guinea pigs[11] but also in late foetal guinea pig

small intestine[12]. The phenomenon was not observed in other species studied[11,12].

The periodic acid – Schiff-positive material in macrophages in the small intestine does not normally occur in adult guinea pigs. It is interesting that long term feeding on the fructose diet resulted in a reappearance of this phenomenon. It is not known if the presence of the material is specific to this diet, or if it would occur with other synthetic diets.

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