CCXXVII. A STUDY OF THE FREE SUGAR CONCENTRATION OF THE LIVERS OF RATS ABSORBING GLUCOSE AND FRUCTOSE AND ITS RELATIONSHIP TO GLYCOGEN SYNTHESIS

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It is a somewhat paradoxical fact that fructose, although absorbed from the intestine considerably more slowly than glucose, gives rise to the greater deposition of glycogen in the liver. It is pertinent to reflect upon the possible fate of ingested fructose. Unsupported by any positive experimental data the suggestion has been made that conversion of fructose into glucose occurs in the lumen of the intestine. Again, it has been stated that this conversion takes place during the actual passage of the sugar through the intestinal mucosa. This was the explanation given by Bollman & Mann [1931] for the fact that, whilst they were able to maintain a liverless animal alive for a long period by infusion of fructose, this sugar was ineffective when the intestines were also removed. Later Griffiths & Waters [1936] showed that in the liverless animal and also in the eviscerated animal fructose is used, apparently directly, and not by preliminary conversion into glucose. This finding does not invalidate the hypothesis that some conversion of fructose into glucose may take place in the intestinal cells, but it does deprive it of its strongest piece of experimental support. Fructose passing from the intestine through the portal system is brought to the liver where conversion into glucose can and to some extent at least normally does take place. Further, accompanying the ingestion of fructose a number of investigators have reported a rise in blood lactic acid, which presumably is derived from some of the fructose.

Cori [1926] showed that previously fasted rats, when fed with fructose, deposited more liver glycogen than those fed with glucose, and this in spite of the facts that fructose was absorbed at less than half the rate of glucose and that the blood sugar was lower in the former animals than in the latter, two prepotent factors determining the availability of sugar for hepatic glycogen synthesis. Cori [1931] concluded that "It is therefore probable that glycogen formation in the liver from fructose does not take place via $\alpha\beta$ -glucose." But it might be argued that fructose may be absorbed from the blood by hepatic cells in greater amount than is glucose; that the avidity for fructose is such as to result in a greater retention of this sugar by the liver than of glucose even when glucose in the blood is present in higher concentration. Thus in spite of the smaller amount of fructose passing to the livers of the fructose-fed rats, in comparison with the amount of extra glucose passing to the livers of the glucose-fed rats, more sugar is withdrawn from the hepatic blood of the former animals, and hence the glycogen formation is greater. It is presupposed that there is a ready hepatic mechanism which rapidly isomerizes fructose to glucose. The rapid conversion of fructose into glucose in these cells might be such as to result in a greater concentration of glucose than in the livers of rats directly absorbing glucose. It was with the object, primarily, of testing this hypothesis that the following

experiments were conducted. As so often happens in biochemical investigations we are attempting to obtain information about a dynamic process by a static method, the analysis of various constituents at one particular instant, which imposes severe restriction on subsequent interpretation of results.

The general plan of the research was to determine the relative rates of absorption of glucose and fructose from the intestines of previously fasted white rats, to determine the extent of hepatic glycogen synthesis from these sugars and to ascertain the concentration of total free sugar not only in the blood but also in the liver, and further to estimate separately the concentrations of fructose and of glucose in the blood and liver of the fructose-fed rats. A higher glucose concentration in the livers of fructose-fed rats than in the livers of glucose-fed rats would, it was considered, be strong evidence in favour of the hypothesis just enunciated.

METHOD

Blood glucose determinations were made by the method of Hagedorn & Jensen, employing 0.1 ml. blood. The non-fermentable reducing substances of rat blood by this method gave us an average value of 17 mg. per 100 ml., calculated as glucose. As there was little variation in this value in the animals tested this figure has been used throughout in calculating true blood sugar values from total reduction values. The estimation of blood fructose has been done as described by Griffiths & Waters [1936] by the method of Harding & Nicholson [1933] in which use is made of the selective removal of glucose in the presence of fructose by their particular strain of *Proteus vulgaris*.¹

The free sugar of the liver has been estimated by the method described by Cuenca [1927] for muscle sugar estimation, and used by Molitor & Pollak [1931] for the determination of free sugar in liver. After freezing some of the liver in liquid air, pieces of about 150–200 mg. weight were rapidly weighed and then ground up with sand in ice-cold 94% alcohol. After thorough extraction the extracts were separated on the centrifuge and concentrated on the steam-bath, the last 2 ml. being removed at room temperature with an electric fan. The residue was taken up in 5 ml. 0.45% ZnSO_4 and 1 ml. 0.1 N NaOH added and the solution heated. The reducing material was estimated in the zinc hydroxide filtrate by the Hagedorn & Jensen method. Duplicate analyses were in excellent agreement. In the experiments quoted below two samples of each liver were always taken; the final zinc hydroxide filtrate of one sample was fermented with yeast, the residual reduction determined, and in the other the total reduction, hence by difference the true sugar value was obtained. It was necessary to determine the non-sugar fraction in these extracts every time, for the value varied considerably from liver to liver. For the estimation of the fructose content of the liver four samples were weighed out and extracted separately in the manner described above. The extracts were combined in pairs and finally the two zinc hydroxide filtrates were combined, and the volume made up to 25 ml. 12 ml. were incubated for 10 min. at 37° with 0.25 ml. of well-washed baker's yeast, and 12 ml. incubated with 1 g. well-washed *P. vulgaris* for 30 min. at 37°. The solutions were centrifuged, in the case of the *Proteus* tube on an angle centrifuge, the supernatants treated with a little Lloyd's reagent and the reducing powers of the final water-clear filtrates estimated by the procedure of Shaffer & Somogyi [1933], employing the 0.1% potassium iodide reagent. The difference in reducing values between the two solutions gave the value for the fructose present.

¹ Our thanks are due to Dr D. T. Fraser, Department of Preventive Medicine, of this University, for culturing and providing us with generous supplies of this organism.

Liver glycogens were estimated by the modification of Pflüger's method introduced by Good *et al.* [1933]. We have found that a small amount of material, apparently glycogen, remains unprecipitated by this method, but the amount is so small as to be negligible in this work, being approximately about 2% of the total glycogen precipitated. Using this method, we found in rats in the post-absorptive state some variation in the distribution of glycogen in different lobes, amounting to as much as 10% in one animal, whereas in the same lobe the variation obtained was hardly significant.

EXPERIMENTAL

The experiments reported in this paper were conducted on white rats from the Connaught Laboratory Farm which were of the Wistar strain. The weights of the rats averaged from 120 to 160 g., unless otherwise stated. Previous to the experiment the rats were fasted for 18 hr. and then allowed to feed for 2 hr. on the stock diet, chiefly mixed grain. Food was then removed and the rats fasted for 24 hr. At the end of that time the rats were fed, by stomach tube, according to the Cori technique, solutions of glucose (Merk's dextrose) or fructose (Eastman Kodak Laboratory). Finally the animals were rapidly anaesthetized by an intraperitoneal injection of amytal (7 mg. per 100 g. of body weight). Control experiments were carried out simultaneously on 24 hr. fasted rats for the determination of liver glycogen, intestinal sugar and blood sugar values.

The absorption coefficients (mg. of sugar absorbed per 100 g. body weight of rat per hr.) in the case of rats ranging in weight from 120 to 160 g., after a 24 hr. fast, were:

Glucose: 297, 253, 196, 216, 276, 242, 226, 210, 335. Average = 250.

Fructose: 169, 130, 231, 74, 128, 268, 99, 147, 170, 200. Average = 160.

Calculating the absorption coefficients for 24 hr.-fasted rats from data provided by Cori [1927] and figures given for 48 hr.-fasted rats by Cori [1925], one obtains for glucose 266 and for fructose 135.

The coefficient of glucose obtained by us is in good agreement with that of Cori. The coefficient of absorption of fructose in our experiments obtained during the spring is slightly higher than Cori's figure. Meyer [1934] found no difference due to length of preliminary starvation in the rate of absorption of fructose by the rat, but did observe a seasonal variation, the values being 200 mg. per 100 g. per hr. in winter and in summer 131 mg. per 100 g. per hr. With young rats, about 1 month old and ranging in weight from 60 to 80 g., we found a much higher coefficient of absorption of glucose than in older animals, while the value for fructose was essentially the same.

Glucose absorption (mg. per 100 g. body weight per hr.):

313, 250, 315, 379, 468, 353, 357, 366, 422. Average = 358.

Fructose absorption:

146, 131, 133, 120, 127, 148, 243, 160. Average = 151.

The average rate of absorption of sugars from the intestine of the rat is susceptible to a number of influences quite apart from any variations inherent in different strains of these animals.

Table I shows the values of true blood sugar, fermentable sugar of liver and liver glycogen, after feeding 24 hr.-fasted rats with glucose or fructose for various times. In the interests of brevity the results of different experiments are averaged but the numbers of animals used are indicated. Control fasted

animals gave a low liver glycogen content, rarely above 50 mg. per 100 g. When anaesthetized, usually about 5–6 min. after injection of amytal, the jugular vein was slit and about 0.25 ml. blood collected in a small tube containing oxalate. The bleeding was stopped with a bulldog clamp.

Table I

Exp.	Sugar fed	Absorption time min.	No. of exps.	Av. blood sugar mg. per 100 ml.	Av. liver sugar mg. per 100 g.	Av. liver glycogen g. per 100 g.
A	Glucose	100	3	—	—	1.67
	Fructose	110	3	—	—	2.04
B	Glucose	120	3	—	—	1.00
	Fructose	128	3	—	—	1.72
C	Glucose	150	3	—	—	2.51
	Fructose	170	3	—	—	3.08
D	Glucose	80	2	116	152	1.25
	Fructose	80	3	114	126	1.35
E	Glucose	80	3	125	154	1.09
	Fructose	80	3	133	123	1.31
F	Glucose	120	4	130	128	1.38
	Fructose	120	4	138	143	1.22
G	Glucose	105	4	143	165	1.63
	Fructose	105	4	141	171	1.92

In the first place there is a slightly greater deposition of glycogen in the rats fed fructose. Secondly, the blood sugar values of the two sets of animals in the different experiments are substantially the same. Thirdly, the concentrations of free sugar of the liver of rats receiving glucose show no consistent difference from the livers of those receiving fructose. Perusal of the individual results leads to the same conclusions. We find the values of the blood sugar in these two groups of rats to be essentially the same, while Cori found the average figure for the glucose-fed rats to be 207 mg. per 100 ml. and for the fructose-fed rats 155 mg. per 100 ml. It may be that this discrepancy can be accounted for by the difference in absorption ratios; in Cori's experiments fructose was absorbed at about 50 % of the speed of glucose, while we obtained a value of 60 %.

The fructose content of the blood of rats absorbing fructose (Table II) was determined on jugular vein samples and again under amytal anaesthesia. As about 2.5–3 ml. blood were withdrawn no other investigations were carried out on the animals. Zinc hydroxide filtrates were prepared from 1 ml. samples of blood. One filtrate was incubated with yeast, the other with *P. vulgaris*. The difference in reducing power gives the fructose content of the blood. After yeast the reducing value was about 1.5 mg. per 100 ml., calculated as glucose.

Table II

Blood fructose mg. per 100 ml.		Liver fructose mg. per 100 g.	
Absorption time 1 hr.	Absorption time 2 hr.	Absorption time 1 hr.	
2.8	3.4	3.6	4.8
2.0	3.4	5.2	0.0
2.4	3.4	4.8	1.9
4.4	—	2.7	—
2.6	—	2.5	—
5.0	—	—	—

Considerable care was taken in the estimation of liver fructose by the method outlined above. Control tests of adding fructose to the frozen livers of fasting rats gave excellent recovery values for fructose. It will be seen that the fructose is present in the liver in negligible amount. In fact the fructose values for both blood and liver tissue in the rats are just slightly higher than the limits of accuracy of the method of estimation.

In all experiments amytal anaesthesia has been used. What the effect, if any, is on either the sugar value of the blood or of the liver, is by no means easy to ascertain. Certainly fasting animals have been anaesthetized in the customary way, and sugar determinations made on blood samples taken immediately anaesthesia is complete (usually within 6 or 7 min.) and after a further 7 or 8 min., and no change whatever noted in the concentration of sugar. It might well be that there was an initial rise in the value above the fasting level, but to judge from the low values obtained this is doubtful.

Table III shows the values of the ratio liver sugar/blood sugar in a number of different conditions.

Table III

Treatment	No. of exps.	Liver sugar/Blood sugar
Absorbing glucose (given amytal)	13	1.15
Absorbing fructose (given amytal)	14	1.08
Fasting (given amytal)	4	1.07
Fasting (stunned)	3	1.69
Absorbing glucose (stunned)	3	1.68

We do not regard the slight difference in the ratio between the rats fed with glucose, average value 1.15, and the rats fed with fructose, average 1.08, as of any significance. Further, these values are essentially the same as those found in fasting animals under amytal anaesthesia, average value, 1.07. We have also investigated the effect of stunning on the ratio liver sugar/blood sugar. There is a small increase in blood sugar but a very appreciable increase in liver sugar, giving a much enhanced ratio value. This is as one might anticipate in view of the result of hepatic glycogenolysis. Yet it is at variance with the results of Power & Clawson [1928] who found a higher liver sugar concentration in rabbits anaesthetized with amytal than in rabbits suddenly killed—presumably stunned.

The effect of insulin. Using large doses of insulin (15 units per 100 g. body weight) Cori [1926] demonstrated a marked inhibition of hepatic glycogen

Table IV

Fed with fructose—3 ml. 40% sol.				Fed with glucose—3 ml. 40% sol.			
Insulin injected* Absorption for 118 min.		Saline injected* Absorption for 112 min.		Insulin injected* Absorption for 100 min.		Saline injected* Absorption for 99 min.	
Wt. rat g.	Liver glycogen g. per 100 g.	Wt. rat g.	Liver glycogen g. per 100 g.	Wt. rat g.	Liver glycogen g. per 100 g.	Wt. rat g.	Liver glycogen g. per 100 g.
232	1.20	392	1.90	361	1.29	354	2.90
322	0.36	370	1.80	332	2.12	325	1.21
274	0.35	250	1.70	340	1.80	437	1.70
274	0.37	400	2.10	391	1.55	323	1.45
340	2.20	352	2.50	—	—	357	1.77
268	0.54	240	2.10	—	—	—	—
Av. 0.8		2.0		1.7		1.8	

* 1 ml. of insulin solution, containing $\frac{1}{2}$ unit insulin, or 1 ml. normal saline, injected $\frac{1}{2}$ hr. after feeding the sugar.

synthesis from absorbed glucose and from fructose. We have modified this experiment in the following way. After feeding the sugar the control animals were injected intraperitoneally half an hour later with saline, the other animals similarly with a very small dose of insulin, namely, $\frac{1}{4}$ unit. It was hoped that by this procedure there would be no very marked effect on the height of the blood sugar. However, no blood sugar determinations were made on these animals. It will be observed from Table IV that while this amount of insulin is without effect on glycogen synthesis from glucose, in a number of rats absorbing fructose there is a very pronounced decrease in hepatic glycogen deposition.

DISCUSSION

It will be seen from the above results that we have been unable to detect any appreciable difference in the free sugar content of the livers of rats absorbing glucose from those absorbing fructose. It will also be noticed that the liver tissue, as well as the venous blood, of the rats absorbing fructose contains only traces of this sugar. The utilization of this sugar, either by conversion into glucose or glycogen, or by oxidation, takes place at least as rapidly as the fructose is absorbed from the intestinal tract. Further, it should be noted that the increase in blood sugar during absorption of fructose is almost entirely due to glucose, at least as far as venous blood analyses are concerned.

To account for the greater hepatic glycogenic power of fructose over glucose a number of possible explanations may be given. Rose *et al.* [1930] showed that absorption of fructose from the small intestine of the dog resulted in a marked increase, sometimes double the resting value, of the lactic acid of the portal vein blood, while the hepatic vein showed only a very slight increase. During the absorption of glucose there was no increase in the lactic acid of the blood of the portal vein. Cori & Cori [1929] have demonstrated the marked ability of the liver, in the instance of the rat, to convert lactic acid into glycogen. The synthesis to glycogen in the liver of the lactic acid formed from the fructose during absorption might well be responsible for an appreciable amount of the total glycogen formed in these animals. Again, the relatively rapid accumulation of hepatic glycogen during fructose ingestion may be due to preferential oxidation of this sugar (rather than glucose) by peripheral tissue, thus sparing blood glucose, with a consequent sparing of liver glycogen originating from non-carbohydrate sources, or it may be due to the rapid conversion of the sugar into glucose in the liver. Further, it may be that fructose exerts a catalytic effect on the glycogen synthesis mechanism of the liver cells. Experiments to support this hypothesis are now being conducted and will be reported later.

Hepatic glycogen synthesis in the rat from both fructose and glucose is markedly inhibited by administration of insulin as shown by Cori [1926]. In his experiments Cori used relatively large amounts of insulin—15 units per 100 g. body weight. In our experiments very small amounts of insulin were given— $\frac{1}{4}$ unit to rats weighing approximately 300 g. While such an amount of insulin had no detectable effect on glycogen formation in rats absorbing glucose there was frequently a decided inhibitory effect in animals absorbing fructose. This suggests a synthesis normally of some of the fructose to glycogen but not through an intermediate glucose stage, unless it be agreed that insulin influences the rate of conversion of fructose into glucose, i.e. inhibits gluconeogenesis from fructose.

It is unfortunate that in the experiments in which the free sugar of the livers has been determined there should be such small differences between the liver

glycogen synthesis in the rats fed glucose and the rats fed fructose. Although we have found similar sugar values in the liver, irrespective of the sugar fed, the view that most of the glycogen is formed from fructose via glucose is not rendered untenable. We may consider the fructose to be rapidly taken up by the hepatic cells, very quickly converted to glucose, some of which is converted by the cells' enzyme system into glycogen, while some readily passes to the blood, tending to maintain the customary equilibrium value liver sugar/blood sugar. Hence the increase in blood glucose during the ingestion of fructose would arise from the isomerization of fructose to glucose in the liver, over and above the amount of glucose which can be handled by the glycogenesis mechanism. So in times of rapid glycogen synthesis it seems erroneous to expect a greater value for the ratio liver sugar/blood sugar. This is what obtains during rapid glycogenolysis, as in the experiments on the stunned animals. Indeed it would be more logical therefore to expect to find a lower value for this ratio. In a number of individual instances in our experiments in which marked glycogen synthesis was occurring this result was obtained. But we do not wish to stress these particular results, and certainly in averaging all the values, as mentioned earlier no substantial difference from that of the fasting animal is found.

SUMMARY

1. The absorption coefficients of glucose and fructose in the 24 hr.-fasted rats are approximately 250 and 160 mg. per 100 g. per hr. respectively, while for young rats (60 g.) similarly fasted, the values are 360 and 150.

2. Very small amounts of insulin, having no effect on hepatic glycogen synthesis in rats absorbing glucose, often markedly inhibit this synthesis in animals absorbing fructose.

3. The liver and also the venous blood of rats absorbing fructose contain only traces of fructose.

4. The ratio liver sugar/blood sugar is essentially the same in fasting rats and in rats absorbing glucose and fructose, and is about 1.1.

5. It has been confirmed that fructose is a better hepatic glycogen precursor than glucose. The possible reasons for this result are discussed.

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