Effect of the Administration of Fructose on the Glycogenolytic Action of Glucagon

AN INVESTIGATION OF THE PATHOGENY OF HEREDITARY FRUCTOSE INTOLERANCE

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1. The mechanism by which the administration of fructose to patients with hereditary fructose intolerance makes them unresponsive to the hyperglycaemic action of glucagon was studied. In four patients, a 10-fold increase in the urinary excretion of cyclic AMP was induced by glucagon, but this effect was drastically decreased by the previous administration of fructose (250mg/kg). Further, the intravenous injection of 6-*N*,2'-*O*-dibutyl cyclic AMP did not cause an increase in the blood glucose during fructose-induced hypoglycaemia. 2. The administration of a large dose of fructose (5g/kg) to mice decreased markedly both the concentration of ATP and the increase in the concentration of cyclic AMP caused by glucagon in the liver. Other ATP-depleting agents had a similar effect and a linear correlation could be drawn between the concentration of ATP and the change in cyclic AMP concentration; a half-maximal effect was obtained for a concentration of ATP close to the K_m value of adenylate cyclase. 3. The administration of fructose to mice caused the inactivation of phosphorylase in the liver, but this effect was easily reversed by glucagon. 4. At a concentration of 10mM-fructose 1-phosphate and 1.5mM- P_i , purified liver phosphorylase *a* was inhibited by 70%. This inhibition appears to be a likely explanation for the unresponsiveness to glucagon of patients with hereditary fructose intolerance.

Hereditary fructose intolerance is an inborn error of metabolism (Chambers & Pratt, 1956; Froesch *et al.*, 1957) in which the ability of liver aldolase to split fructose 1-phosphate is almost completely lost (Hers & Joassin, 1961). In patients with this disorder, the ingestion of fructose produces a profound hypoglycaemia. One characteristic of this hypoglycaemia is that it cannot be relieved by the administration of glucagon, even when the patients are well fed and presumably have a normal content of glycogen in their liver (Perheentupa *et al.*, 1962; Froesch, 1962; Cornblath *et al.*, 1963).

In normal animals, the parenteral administration of a large dose of fructose (1 mg/g body wt. or more) causes within a few minutes important changes in the concentration of several metabolites in the liver. Fructose 1-phosphate, which is barely detectable in normal liver, may reach concentrations as high as 10mM (Günther *et al.*, 1967; Heinz & Junghänel, 1969; Burch *et al.*, 1969), whereas the concentrations of ATP and of P_i can fall to one-third of their normal values (Mäenpää *et al.*, 1968). Although no extensive results are available about the concentration of these metabolites in the liver of patients with hereditary fructose intolerance, it is likely that similar changes occur after the ingestion of even a small amount of fructose.

The present study was undertaken to elucidate the mechanism by which fructose metabolism interferes with the glycogenolytic action of glucagon. This hormonal effect involves the following steps, which are described in Scheme 1. (1) Glucagon stimulates liver adenylate cyclase causing a large increase in the concentration of cyclic AMP in the liver (for a review see Robison *et al.*, 1971); this increase in intrahepatic cyclic AMP is reflected in the plasma and the urine (Broadus *et al.*, 1970). (2) Cyclic AMP induces the conversion of phosphorylase *b* into the active form *a* through the successive stimulation of protein kinase and activation of phosphorylase kinase; this sequence has been established in muscle (for a review see Krebs, 1972) and presumably also operates in liver. The action of both kinases is antagonized by that of two specific phosphatases (not shown on Scheme 1) that inactivate active phosphorylase kinase and phosphorylase *a* respectively. (3) Phosphorylase *a* reacts with glycogen and P_i , producing glucose 1-phosphate, allowing the limiting step in the degradation of glycogen to glucose to proceed (Sutherland & Cori, 1951). An indirect, harmless investigation of step (1) was performed in patients by the study of the urinary excretion of cyclic AMP, and of steps (2) and (3) by following the effect of the administration of dibutyl cyclic AMP on the concentration of

Scheme 1. Sequence of events involved in the glycogenolytic mechanism of glucagon

Explanations are given in the text.

blood glucose. More direct information on the influence of fructose on the concentration of metabolites and on the glycogenolytic action of glucagon in the liver, was obtained by experiments in mice *in vivo* and enzyme studies *in vitro*. Part of this study has been presented elsewhere (Van den Berghe, Hue, Corbeel & Hers, Annual Meeting of the European Society for Paediatric Research, Heidelberg, 11–14 September, 1972).

Materials and Methods

Chemicals

Glucagon was obtained from Novo Industri A/S, Copenhagen, Denmark. The 6-*N*,2'-*O*-dibutyl-adenosine 3':5' cyclic monophosphate (monosodium salt) was purchased from Boehringer und Soehne G.m.b.H., Mannheim, Germany, and prepared for human use as follows. A solution (5 mg/ml) of the dry powder was made in 0.9% NaCl and adjusted to pH 7.4 with 0.01 M-NaOH. The solution was sterilized by filtration (Millipore Corp., Bedford, Mass., U.S.A.).

D-Fructose, L-sorbose and glycerol were from E. Merck A.G., Darmstadt, Germany. DL-Ethionine, histone (type II-A), glycogen (type II) and D-fructose 1-phosphate (barium salt) were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. The barium salt was converted into the sodium salt by passage through a column of Amberlite IR 120 (Na⁺ form). L-Sorbose 1-phosphate was a gift from Dr. H. A. Lardy, University of Wisconsin, Madison, Wis., U.S.A.

Enzymes

Liver phosphorylase *a* was purified 200-fold from dog liver by the method of Appleman *et al.* (1966) with the following modifications: the liver was perfused *in situ* with 0.1 M-NaF and homogenized in the same medium; the digestion with α -amylase was omitted. Amylo-1,6-glucosidase was purified from rabbit muscle as described by Bueding & Hawkins (1964). Phosphoglucomutase was obtained from Boehringer G.m.b.H. The source of the other enzymes used has been reported (Van den Berghe *et al.*, 1970).

Experimental procedures

Studies on human subjects. These studies were performed in children with hereditary fructose intolerance and in normal controls of approximately the same age. The diagnosis of hereditary fructose intolerance was established by clinical data and confirmed by the assay of liver aldolase. Fructose, when given, was administered intravenously at the dose of 250 mg/kg 20 min before the injection of glucagon. Glucagon was injected intramuscularly at the dose of 0.1 mg/kg. A first urine sample was taken before the injection of glucagon, the bladder was emptied, and the second urine sample was collected during the 30–60 min that followed the injection of glucagon. The urine samples were kept frozen until the determination of cyclic AMP. Dibutyl cyclic AMP was injected intravenously at the dose of 2 mg/kg. Informed consent of the parents of the children was obtained for the latter experiments.

Animal experiments. Animal experiments *in vivo* were performed on normally fed Naval Medical Research Institute mice weighing about 20 g. Fructose, sorbose or glycerol was injected intraperitoneally at the dose of 5 mg/g, as a 25% (w/v) solution, 20 min before the injection of glucagon. Ethionine (1 mg/g) was injected intraperitoneally 4 h before the administration of glucagon. Glucagon (0.1 µg/g) was injected via a tail vein. The mice were killed by decapitation. The liver was quick-frozen as described by Wollenberger *et al.* (1960), and stored at -20°C until processed.

Analytical methods

Determination of metabolites. Liver metabolites were measured in trichloroacetic acid extracts processed as described by Van den Berghe *et al.* (1970). Cyclic AMP was determined by a modification (Van den Berghe *et al.*, 1970) of the method of Breckenridge (1964). ATP was measured as described by Strehler & Totter (1952). The concentrations of free ketose and ketose 1-phosphate were determined by the method of Roe *et al.* (1949). The amount of fructose 1-phosphate and sorbose 1-phosphate was calculated from the difference between the value in the untreated extract and the value obtained after precipitation of the phosphate esters by addition of equimolar amounts of ZnSO_4 and Ba(OH)_2 .

In the urine samples, cyclic AMP was measured as previously described (Van den Berghe *et al.*, 1970), except that no extraction with trichloroacetic acid was performed and that the purification procedure included only the adsorption on ZnSO_4 – Ba(OH)_2 mixture and column chromatography on Dowex 50. Creatinine was determined by the method of Bonsnes & Taussky (1945). Blood glucose was measured as described by Huggett & Nixon (1957).

Enzyme assays. In the animal experiments performed

in vivo, enzyme activities were assayed in homogenates made from the quick-frozen tissue, in cold 50 mM-glycylglycine, adjusted to pH 7.4 with 1 M-HCl. Cyclic 3':5'-AMP phosphodiesterase was assayed by the method of Butcher & Sutherland (1962). The activity of protein kinase was determined by method B of Reimann *et al.* (1971) with histone as substrate. For the assay of phosphorylase kinase, the procedure of Krebs *et al.* (1964) was followed. The activity of phosphorylase *a* in the liver homogenates was measured at 20°C as previously described (Hers, 1964) except that the substrate contained 1 mM-caffeine and no AMP. The activity of purified liver phosphorylase *a* was measured in the phosphorylytic direction by coupling the reaction with an excess of phosphoglucomutase, glucose 6-phosphate dehydrogenase and NADP^+ . The rate of formation of NADPH was measured at 340 nm and 20°C in the presence of 25 mM-Tris adjusted to pH 7.5 with 1 M-HCl, 10 mM-magnesium acetate, 0.5 mM- NADP^+ , 0.5% (w/v) glycogen, 5 mM-histidine, 1.5 units of glucose 6-phosphate dehydrogenase and 1 unit of phosphoglucomutase in a total volume of 1 ml. The activity of amylo-1,6-glucosidase was assayed by the incorporation of radioactive glucose into glycogen by the method of Hers & Van Hoof (1966).

Results

Effects of fructose in children with hereditary fructose intolerance

Urinary excretion of cyclic AMP. Table 1 shows the concentration of cyclic AMP in the urine of control children and of children with hereditary fructose intolerance before and after the injection of glucagon. In both groups of subjects, the basal excretion of cyclic AMP was between 4 and 17 nmol/mg of creatinine, a value significantly higher than that of 2–5 nmol/mg of creatinine, reported by Chase *et al.* (1969) and also currently observed in this laboratory (unpublished results) for normal adults. This difference is presumably explained by the lower excretion of creatinine in children, since it appears less marked when the excretion of cyclic AMP is expressed per kg body wt. After the administration of glucagon, the excretion of cyclic AMP was as a mean increased 10-fold. In control children, this effect of glucagon was not modified by the previous administration of a small dose of fructose (250 mg/kg) whereas in children with hereditary fructose intolerance it was greatly diminished although not always completely suppressed by that treatment.

Hyperglycaemic response to dibutyl cyclic AMP. Fig. 1 shows the effects of an intravenous injection of dibutyl cyclic AMP on the blood glucose concentration of one patient with hereditary fructose

Table 1. *Effect of a fructose load on the stimulation of the urinary excretion of cyclic AMP by glucagon in human subjects*

Results are expressed as nmol of cyclic AMP/mg of creatinine. Values in parentheses are approximations of the excretion of cyclic AMP in nmol/h per kg, calculated from average rates of creatinine excretion in mg/24h per kg, given by O'Brien & Ibbott (1964).

Subject	Age	Cyclic AMP excreted			
		Without fructose		After fructose	
		Before glucagon	After glucagon	Before glucagon	After glucagon
Controls					
VP	3 weeks	—	—	14.7 (8.1)	68.6 (37.7)
VDH	8 weeks	8.7 (4.8)	91.0 (50.0)	11.3 (6.2)	143.1 (78.6)
TA	18 months	11.8 (6.1)	69.3 (36.1)	16.6 (8.6)	81.6 (42.5)
VL	13 years	4.1 (4.3)	26.3 (27.5)	4.6 (4.8)	33.9 (35.4)
Hereditary fructose intolerance					
PN	7 weeks	16.3 (8.9)	41.5 (22.8)	16.8 (9.2)	16.0 (8.8)
TB	4 months	8.1 (4.3)	236.9 (126.3)	12.6 (6.7)	24.7 (13.2)
LJ	5 months	40.2 (5.4)	80.1 (42.7)	12.2 (6.5)	14.4 (7.7)
PT	3 years	6.3 (3.1)	69.5 (34.7)	7.8 (3.9)	20.5 (10.2)

Fig. 1. *Blood glucose concentrations after administration of dibutyryl cyclic AMP to a patient with hereditary fructose intolerance*

The tests were performed without prior administration of fructose (○) and after a fructose load (250mg/kg, intravenously) 30min before zero time (●).

intolerance. When the nucleotide was given under control conditions it produced a hyperglycaemia comparable with that observed after the administration of glucagon. This effect of cyclic AMP (Levine, 1968) or of its dibutyryl derivative (Levine, 1970) has been observed in normal human subjects. However, when dibutyryl cyclic AMP was given during the hypoglycaemic phase of a fructose tolerance test, no increase in blood glucose was recorded. This test was performed twice with this subject and once with another patient with the same results. In control children the infusion of the same dose of fructose induced a slight hyperglycaemia and the subsequent injection of dibutyryl cyclic AMP caused a further increase in blood glucose.

Effects of fructose on the action of glucagon in mice

Concentration of cyclic AMP and activity of phosphorylase a in the liver. At 20min after the administration of a large dose of fructose (5mg/g body wt.) to mice, the concentration of cyclic AMP was the same as in the liver of control mice (about $0.6\mu\text{M}$). The concentration of ATP was, however, markedly decreased (Table 2). Within 3min after the injection of glucagon, the concentration of cyclic AMP increased over 100-fold in the liver of control mice, whereas in the liver of mice treated with fructose the increase was only about 30-fold. An unexpected finding in this experiment was the marked decrease in the

Table 2. *Effect of a fructose load on the concentration of ATP and on the action of glucagon in the liver of mice*

Control mice and animals that had received a fructose load (5 mg/g) 20 min before were injected with glucagon at zero time and killed at various times afterwards. Values shown are means $\pm$ S.E.M. and the numbers of animals are given in parentheses.

Treatment	Time (min)	ATP (μ mol/g)	Cyclic AMP (nmol/g)	Phosphorylase <i>a</i> (μ mol/min per g)
None	0 (5)	2.57 $\pm$ 0.12	0.61 $\pm$ 0.05	8.69 $\pm$ 1.01
	1 (5)	2.43 $\pm$ 0.12	27.1 $\pm$ 3.2	12.21 $\pm$ 0.29
	3 (4)	2.26 $\pm$ 0.06	73.3 $\pm$ 13.6	9.42 $\pm$ 0.96
Fructose	0 (5)	1.14 $\pm$ 0.04	0.56 $\pm$ 0.02	2.54 $\pm$ 0.35
	1 (5)	1.27 $\pm$ 0.22	5.9 $\pm$ 2.3	9.78 $\pm$ 1.26
	3 (4)	1.38 $\pm$ 0.11	18.4 $\pm$ 6.4	9.55 $\pm$ 0.37

activity of phosphorylase *a* in the liver of the mice that had received fructose without glucagon. Within the first minute after the injection of glucagon, however, phosphorylase *a* reached the same activity in both groups. It has been verified that treatment of the animals with fructose resulted in intrahepatic concentrations of fructose 1-phosphate around 10 mM.

Mechanism of the effect: investigation with other ATP-depleting agents. One of the most striking effects of fructose administration is to decrease markedly the hepatic concentration of ATP, the substrate of adenylate cyclase (Table 2). It was therefore decided to investigate the effect of other ATP-depleting agents on the ability of the liver to form cyclic AMP on stimulation by glucagon. Glycerol, ethionine, fructose, or a combination of ethionine and fructose, were administered to mice to obtain a wide range of ATP concentrations in the liver. The animals were killed 3 min after the administration of glucagon and the concentrations of ATP and cyclic AMP in the liver were determined. The ATP concentration could not be lowered below 0.8 μ mol/g. However, a linear correlation could be drawn between the concentrations of ATP and of cyclic AMP (Fig. 2).

A time-course of the changes in the concentration of cyclic AMP and the activity of phosphorylase *a* after the administration of glucagon to mice treated with ethionine and fructose is shown in Fig. 3. In this experiment the ATP concentration in the liver was as low as 0.96 $\pm$ 0.05 μ mol/g and the increase in cyclic AMP was several times lower than in the experiment shown in Table 2. Again it was found that phosphorylase was markedly inactivated before the administration of glucagon in the treated animals, and reached the same activation as in the control animals after the injection of the hormone.

The activities of phosphodiesterase, protein kinase and phosphorylase kinase in the liver were not modified by the treatment of the animals with fructose.

Fig. 2. *Correlation between the contents of ATP and of cyclic AMP in the liver of mice 3 min after the administration of glucagon*

ATP and cyclic AMP were measured in control mice ($\circ$) and in mice treated with glycerol ($\square$), ethionine (Δ), fructose ($\bullet$) or ethionine and fructose ($\blacktriangle$). Regression coefficient = 0.838 ($P < 0.001$).

Effects of fructose 1-phosphate on cell-free systems and purified enzymes

From the animal experiments reported, it appears that a fructose load, even when drastically decreasing the intrahepatic concentration of ATP, does not prevent glucagon achieving the activation of liver phosphorylase. One can reasonably assume that in a patient with hereditary fructose intolerance, after a fructose load, a similar activation of phosphorylase occurs when glucagon or dibutyryl cyclic AMP are injected. To find an explanation for the absence of a hyperglycaemic response in these circumstances, the

Fig. 3. Action of glucagon on mice treated with fructose and ethionine

Phosphorylase *a* activity (a) and cyclic AMP (b) were measured in control mice (○) and in animals treated with ethionine and fructose (▲). There were five animals per experimental point. Vertical bars represent $\pm$ S.E.M.

effect of fructose 1-phosphate on liver phosphorylase *a* was studied. The effect of sorbose 1-phosphate was also studied, since it has been reported that sorbose does not provoke hypoglycaemia in patients with hereditary fructose intolerance (Wolf *et al.*, 1959).

As shown in Fig. 4 the activity of purified liver phosphorylase *a* in the presence of P_i is competitively inhibited by fructose 1-phosphate and sorbose 1-phosphate; K_i ranged from 2.7 to 4.0 mM and from 1.8 to 3.0 mM respectively; K_m for P_i ranged from 0.7 to 1.5 mM. From these values one can calculate that at a concentration of 10 mM-fructose 1-phosphate and 1.5 mM- P_i , which are the conditions prevailing after a large fructose load in the experimental animal, the inhibition reaches 70%. With 10 mM-sorbose 1-phosphate, this inhibition would be 75%, but even after the injection of a very large dose of sorbose (5 mg/g body wt.) to the animal, the concentration of sorbose 1-phosphate did not reach 4 mM in the liver. Fructose 1-phosphate had no effect on the

Fig. 4. Double-reciprocal plots showing the effects of fructose 1-phosphate and sorbose 1-phosphate on the rate of purified liver phosphorylase *a*

The reaction was performed in the absence (○) and in the presence of 5 mM-fructose 1-phosphate (●) or 1 mM-sorbose 1-phosphate (■).

activity of purified amylo-1,6-glucosidase. To provide an explanation for the inactivation of phosphorylase observed after a fructose load, the effect of fructose 1-phosphate was also studied on the activity of protein kinase and phosphorylase kinase in mouse liver homogenates. Our attempts to demonstrate an inhibition of these kinases by the fructose ester have given negative results.

Discussion

The sequence of events by which glucagon is believed to promote glycogenolysis in the liver is summarized in Scheme 1. To clarify the mechanism by which the administration of fructose to patients with hereditary fructose intolerance prevents the glucagon effect, we have studied not only the patients themselves but also experimental models in mice and the properties of some enzymes.

Our observations of patients suggest that more than one mechanism is involved in their inability to respond to glucagon when they have received fructose. Not only do they have a lower capacity to form cyclic AMP in their liver but also the cyclic AMP that is formed appears unable to cause hyperglycaemia. These two aspects of the problem will be discussed separately.

Effect of fructose on the formation of cyclic AMP

Origin of the cyclic AMP in urine after glucagon. The administration of a small dose of fructose to patients with hereditary fructose intolerance decreases

ed drastically the urinary excretion of the cyclic nucleotide that follows the administration of the hormone (Table 1). Broadus *et al.* (1970) reported that an infusion of glucagon in a hepatectomized dog produced no increase in cyclic AMP concentrations in plasma or urine. There is thus little doubt that the diminished urinary excretion that we observe reflects a decreased production of cyclic AMP in the liver. This conclusion is confirmed by our findings in mice, where a decreased hepatic production of the cyclic nucleotide after the injection of glucagon was seen in fructose-treated animals.

Lowering of ATP concentration in the liver. The important fall that we observed in the concentration of ATP in the liver after administration of fructose to mice confirms similar observations made by Mäenpää *et al.* (1968). This effect requires the intravenous administration of a relatively large amount of fructose and is dose-dependent (Raivio *et al.*, 1969). It has also been observed in normal man with a dose of 0.7 g of fructose/kg body wt. (Bode *et al.*, 1971) and is presumably obtained at a much smaller dose in patients with hereditary fructose intolerance. In these subjects, a dose of 125 mg/kg body wt. is indeed sufficient to cause a fall in the concentration of P_i in blood whereas this amount is ineffective in normal human subjects (Froesch *et al.*, 1959, 1963). Small doses of fructose (250 mg/kg) caused an increase in the concentration of methionine in the serum of one patient but not of control children (L. Corbeel, unpublished work). Since the K_m of the methionine-activating enzyme is 2.2 mM (Mudd & Cantoni, 1958), the increase in methionine suggests that ATP concentration in the liver has fallen below this value.

Mechanism by which fructose prevents the formation of cyclic AMP in the liver. As shown by other authors, the administration of glycerol (Burch *et al.*, 1970) or of ethionine (Shull, 1962) can also lower the concentration of ATP in the liver. The mechanism of their action is different, since ethionine traps the adenosine residue (Farber *et al.*, 1964), whereas glycerol, like fructose, binds to phosphate. However, all these ATP-depleting agents inhibit the effect of glucagon on the formation of cyclic AMP and a linear correlation between the concentration of ATP and that of cyclic AMP in the liver has been observed, a half-maximal effect of glucagon being obtained at a concentration *in vivo* of 1.8 mM-ATP (Fig. 2). This value is in close agreement with the K_m of about 1.5 mM-ATP reported for the glucagon-stimulated adenylate cyclase present in plasma membranes of rat liver (Pohl *et al.*, 1971). It is therefore reasonable to assume that, both in human subjects and in mice, the unresponsiveness of adenylate cyclase to glucagon after a load of fructose is secondary to a lowering of the intrahepatic concentration of ATP.

The potential toxicity of high doses of fructose to

normal subjects has been stressed by several authors (Hers, 1970; Woods & Alberti, 1972). As concluded by Perheentupa *et al.* (1972) many metabolic effects of fructose in hereditary fructose intolerance are marked exaggerations of events taking place in normal individuals. This is why we suspect that in patients with hexose diphosphatase deficiency who display a partial sensitivity to fructose (Baker & Winegrad, 1970; Baerlocher *et al.*, 1971) intermediate doses of fructose could provoke a diminution of the excretion of cyclic AMP after the administration of glucagon. Unfortunately, we have had no opportunity to check this hypothesis.

Consequences of decreased formation of cyclic AMP. Although the administration of fructose provokes an important decrease in the capacity of the liver to form cyclic AMP, the concentration of cyclic nucleotide obtained after the injection of a pharmacological amount of glucagon to mice treated with fructose is still large enough to cause the activation of liver phosphorylase (Table 2, Fig. 3). Indeed, the amount required to stimulate glycogenolysis is very small (Robison *et al.*, 1971). The same conclusion presumably applies to patients with hereditary fructose intolerance, since in some of them, the injection of glucagon during a fructose tolerance test still caused a slight increase in the urinary excretion of the cyclic nucleotide.

It seems, however, that in the presence of a physiological amount of glucagon the negative influence of fructose administration on the formation of cyclic AMP may have important consequences. The concentration of free cyclic AMP in the unstimulated liver is probably below the K_m of protein kinase (3×10^{-8} M; Krebs, 1972) and thus more than an order of magnitude below the total amount of cyclic AMP measured. As discussed by Jefferson *et al.* (1968), a large proportion of the cyclic nucleotide is presumably bound to cell structures or proteins, so that it does not reach its target enzymes. The fact that we have observed no change in the total amount of cyclic AMP in the liver after administration of fructose (Table 2) may therefore be inconclusive owing to the difficulty of measuring small variations of free cyclic AMP against the large background of bound cyclic nucleotide. Assuming that the expected decrease occurs, the result would be to cause an imbalance in the antagonistic effects of phosphorylase kinase and phosphorylase phosphatase, in favour of the latter enzyme. This would be a simple explanation for the inactivation of liver phosphorylase brought about in normal mice 20 min after the administration of fructose (Table 2).

Effect of fructose on the response to cyclic AMP

As indicated in Scheme 1, several steps are involved in the glycogenolytic effect of cyclic AMP and

could either be altered by the decreased ATP concentration or be inhibited by fructose 1-phosphate. In contrast with glucagon-stimulated adenylate cyclase, protein kinase (Krebs, 1972) and phosphorylase kinase (Krebs *et al.*, 1964) have a K_m for ATP which is at least one order of magnitude below the actual concentration of the nucleotide in the liver. It thus appears unlikely that the activity of these two enzymes could be affected by the observed change in the concentration of ATP. We have also verified that protein kinase and phosphorylase kinase are not inhibited by fructose 1-phosphate at the concentration present in the liver after a load of fructose. The normal activation of phosphorylase in the liver of mice treated with fructose and glucagon are also indicative of normal function of the two kinases.

The fact that in patients with hereditary fructose intolerance blood glucose can be increased by the administration of galactose during a fructose-induced hypoglycaemia (Cornblath *et al.*, 1963) indicates that the conversion of glucose 1-phosphate into glucose 6-phosphate and free glucose is not impaired. The marked inhibition of liver phosphorylase *a* by fructose 1-phosphate, together with the decreased concentration of P_i , is thus the best explanation that we can presently propose for the inhibitory effect of a fructose load on glycogenolysis. At a concentration of 10 mM-fructose 1-phosphate and 1.5 mM- P_i , which are the conditions prevailing in the liver of animals after a large fructose load, this inhibition reaches 70%. In this respect, our results confirm previous observations by Maddaiah & Madsen (1966). It is difficult to be certain whether the 70% inhibition of liver phosphorylase is a satisfactory explanation for the unresponsiveness of the patients to glucagon. Studies of the correlation between the activity of phosphorylase *a* and the production of glucose by the perfused liver have shown that the amount of glucose released is always much lower than expected from the measurement of the activity of the enzyme, and that even the unstimulated liver contains an appreciable amount of phosphorylase *a* (Weintraub *et al.*, 1969).

The fact that sorbose 1-phosphate is as inhibitory as fructose 1-phosphate might appear disturbing, since the administration of sorbose to patients with hereditary fructose intolerance does not induce hypoglycaemia (Wolf *et al.*, 1959). This could be due to a lower rate of phosphorylation of sorbose (Sanchez *et al.*, 1971). This hypothesis is sustained by the fact that in mice the intrahepatic accumulation of ketose 1-phosphate was smaller after sorbose than after fructose, even when very large doses (5 mg/g body wt.) were injected.

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