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The effect of fructose on pyruvate kinase activity in isolated hepatocytes

Inhibition by allantoin and alanine

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1. Incubation of isolated hepatocytes with fructose at concentrations above 3mM resulted in an apparent inhibition of pyruvate kinase assayed in crude extracts at sub-optimal phosphoenolpyruvate concentrations. 2. Fructose at concentrations below 3mM caused an activation of the enzyme. 3. Increases in the hepatocyte contents of the positive effectors fructose 1,6-bisphosphate and fructose 1-phosphate were found at all concentrations of fructose up to 10mM. 4. Removal of the extrahepatocyte medium from the hepatocytes by washing resulted in an activation of the enzyme at all concentrations of fructose examined. 5. Inhibitors of the enzyme were shown to accumulate in the hepatocytes despite the depletion of ATP (a known negative effector) caused by higher concentrations of fructose. Indeed the inhibition of pyruvate kinase appeared to be correlated to the depletion of ATP. 6. Alanine (a known inhibitor) was shown to accumulate in hepatocytes as a consequence of incubation with fructose. 7. Allantoin and uric acid were shown to be inhibitors of a partially purified pyruvate kinase preparation assayed both in the presence and in the absence of fructose 1,6-bisphosphate. 8. Allantoïn, but not uric acid, accumulated in the extrahepatocyte medium as a result of incubation of the cells with 10mM-fructose.

The L-type pyruvate kinase (EC 2.7.1.40) from rat liver is an allosteric enzyme that is inhibited by ATP and alanine (Llorente *et al.*, 1970: Seubert & Schoner, 1971) and is activated by fructose 1,6-bisphosphate (Taylor & Bailey, 1967; Seubert & Schoner, 1971) and fructose 1-phosphate (Eggleston & Woods, 1970). The enzyme is also regulated by a phosphorylation–dephosphorylation mechanism involving a cyclic AMP-dependent protein kinase (Engström, 1978). Pyruvate kinase is regarded as the only regulatory enzyme involved in the glycolysis of fructose to lactate, since the metabolism of the ketose bypasses the other regulatory glycolytic enzymes, glucokinase and phosphofructokinase. Fructose is metabolized to triose phosphate by ketohexokinase, fructose bisphosphate aldolase and triokinase.

Fructose causes lactate acidosis in humans, and it is also known that lactate output by perfused liver and by isolated hepatocytes is severalfold faster from fructose than from glucose. This has been ascribed to several factors, such as the high activity of ketohexokinase compared with glucokinase and the fact that fructolysis bypasses the regulatory phosphofructokinase reaction (Van den Berghe, 1978). Furthermore, it has been reported that when rat liver is perfused with fructose (10mM) there is an accumulation of fructose 1-phosphate and a depletion of adenine nucleotides and P_i in the tissue (Mäenpää *et al.*, 1968; Woods *et al.*, 1970; Van den Berghe *et al.*, 1977).

It was therefore decided to examine the effects of fructose on glycolysis and on pyruvate kinase activity in order to test the hypothesis that accumulation of fructose 1-phosphate would cause a stimulation of the enzyme and that this results in a high rate of glycolysis.

Materials and methods

Animals and diets

Mature male Wistar rats (300–350g) were fed *ad libitum* on a normal laboratory chow diet.

Reagents

All enzymes and substrates were obtained from Sigma Chemical Co., Poole, Dorset, U.K., or Boehringer Corp., Lewes, Sussex, U.K. Substrates were in the form of their sodium salts. Inorganic reagents and solvents were of AnalaR grade from BDH Chemicals, Poole, Dorset, U.K.

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Isolation of hepatocytes

Isolated hepatocytes were prepared from adult male rats by the method described by Berry & Friend (1969) with some modifications (Krebs *et al.*, 1974; Wagle & Ingebretson, 1975). The hepatocytes were suspended in Krebs–Ringer bicarbonate (Krebs & Henseleit, 1932) containing 2.3mM-$CaCl_2$ and 1.5% (w/v) bovine serum albumin (essentially fatty acid-free, fraction V; Sigma Chemical Co.). Hepatocytes prepared this way were viable when examined microscopically for Trypan Blue exclusion.

Incubation procedure

Hepatocytes suspended in Krebs–Ringer bicarbonate buffer (containing Ca^{2+} and bovine serum albumin) were preincubated at 37°C for 10min and gassed with O_2/CO_2 (19:1). Samples of the cell suspension were added to an equal volume of the buffer containing either fructose or glucose to give a final concentration of hepatocytes between 48 and 64mg wet wt./ml. The cells were then incubated at 37°C in a shaking water bath and the gassing was continued. After the appropriate time intervals, samples of the incubation mixture were removed and used for enzyme and metabolite assays as described below.

Washing procedure

Samples of the incubated hepatocytes were layered on ice-cold Krebs–Ringer bicarbonate containing Ca^{2+} and bovine serum albumin and rapidly sedimented by centrifugation at 50**g** for 60s. The supernatant was discarded, and the pellet was resuspended in the buffer and rapidly frozen in liquid N_2.

Partial purification of pyruvate kinase

One volume of cell extract was frozen in liquid N_2 and thawed by adding 4vol. of suspending medium containing 100mM-KF, 15mM-EGTA and 50mM-glycylglycine adjusted to pH7.4. Then 1vol. of saturated $(NH_4)_2SO_4$ was added to the cell extract and the mixture was kept in the cold with stirring. After 30min the precipitate was collected by centrifugation at 17000**g** for 20min. The precipitate was resuspended in 50%-satd. $(NH_4)_2SO_4$ and re-centrifuged at 17000**g** for 30min. The resulting pellet was dissolved in suspending medium and kept at −70°C until required for the pyruvate kinase assay.

Analytical methods

(*a*) *Assay for pyruvate kinase.* After the incubation period samples of cell suspension were frozen in liquid N_2 and stored at −70°C until required for enzyme assay. Frozen samples were thawed by shaking with 5vol. of ice-cold suspending medium. The incubation mixture contained 50mM-glycylglycine/NaOH buffer, pH7.4, 100mM-KCl, 10mM-$MgCl_2$, 1.24mM-ADP, 0.15mM-NADH and 2 units (μmol/min) of lactate dehydrogenase (Felíu *et al.*, 1976). Portions (50μl) of homogenate were added to the mixture and preincubated for 10min before the addition of phosphoenolpyruvate to start the reaction. Pyruvate kinase was assayed spectrophotometrically at 25°C. $V_{max.}$ was determined by assaying the enzyme in the presence of 4mM-phosphoenolpyruvate, and v was measured at 0.2mM-phosphoenolpyruvate. All enzyme assays were duplicated.

(*b*) *Determination of metabolites.* The incubation was terminated with 0.05vol. of 4.2M-$HClO_4$. The contents were blended in a vortex mixer and the protein precipitate was removed by centrifugation at 3000**g** for 10min. The supernatant fraction was neutralized with 4.2M-K_2CO_3 and the neutralized extract was used to determine metabolite concentrations.

Fructose 1,6-bisphosphate was assayed by an enzymic method (Michal & Beutler, 1974), and fructose 1-phosphate was determined by the method of Eggleston (1974). Alanine, pyruvate and lactate were measured as described by Williamson (1974), Czok & Lamprecht (1974) and Gutmann & Wahlefeld (1974) respectively. Allantoin and uric acid were assayed by the methods of Vogels & Van der Drift (1970) and Scheibe *et al.* (1974) respectively.

Expression of results

The enzyme activity is expressed as $v/V_{max.}$. Use of this ratio of activities helps to eliminate any slight variations of v and $V_{max.}$ that may be due to non-uniform cell distribution during sampling. The mean value for $V_{max.}$ was 38.6 ± 2.8μmol/min per g of hepatocytes. Metabolite concentrations are expressed as μmol (or nmol)/g of hepatocytes.

Statistical methods

Unless otherwise stated the data are expressed as the means ± S.E.M. for at least three different cell preparations. Statistical significance was determined by Student's *t* test.

Results

Effect of fructose on pyruvate kinase activity measured in vitro

The short-term effects of insulin and glucagon (Felíu *et al.*, 1976; Friedrichs, 1976; Van Berkel *et al.*, 1976, 1977*a*,*b*; Claus *et al.*, 1979) and of dihydroxyacetone (Claus *et al.*, 1979) on pyruvate kinase activity have previously been demonstrated with crude hepatocyte extracts. In the present study the effect of fructose on pyruvate kinase was

examined by using a similar technique. Fig. 1 shows that incubation of hepatocytes with 10mM-fructose produced apparently contradictory results depending on whether or not the hepatocytes were washed with fresh medium after the incubation. An inhibition of pyruvate kinase was apparent when unwashed hepatocytes were used as a source of enzyme, whereas with washed hepatocytes the expected stimulation was observed.

The concentration-dependences of these effects are shown in Fig. 2. It is clear that stimulation of pyruvate kinase occurs with washed cells at all concentrations of fructose used, whereas with unwashed cells stimulation was observed at low fructose concentrations and inhibition was found at fructose concentrations of 5 and 10mM. The $v/V_{max.}$ values were somewhat lower in unwashed hepatocyte preparations after glucose treatment than in washed cells, but there were no concentration-dependent changes in enzyme activity with the aldose. The $V_{max.}$ values were not significantly changed in any of the experiments described in the present paper. The $v/V_{max.}$ values were lowered by about 50% when hepatocytes were treated with glucagon (1 μM) in the presence and in the absence of 10mM-fructose (results not shown).

Table 1 shows that the difference in activity ratio ($v/V_{max.}$) between the glucose-treated and fructose-treated hepatocytes is abolished by $(NH_4)_2SO_4$ precipitation of pyruvate kinase before the assay. This suggests that the changes observed are due to alterations in the concentrations of low-molecular-weight effectors and not stable changes involving covalent modification of the enzyme. The activity ratio for the unwashed hepatocytes treated with 10mM-fructose was similar to that of the $(NH_4)_2SO_4$-precipitated enzyme, suggesting that both these treatments resulted in a removal of an activator of the enzyme. This conclusion was also evident in a survey of the kinetics of pyruvate kinase in crude extracts after various treatments, which showed that the $[S]_{0.5}$ values for phosphoenolpyruvate were altered but that the Hill coefficients were not significantly changed. The lowest $[S]_{0.5}$ value was found with the enzyme from fructose-treated cells that were washed before extrac-

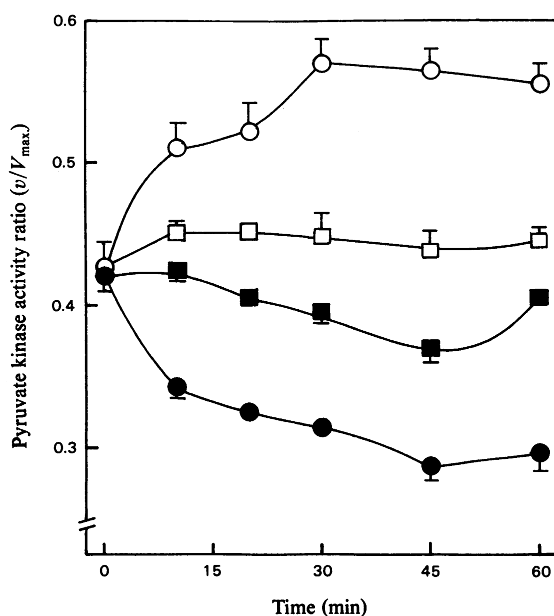

Fig. 1. *Time course of the effects of glucose and fructose on hepatocyte pyruvate kinase activity*

Isolated hepatocytes were incubated with 10mM-glucose (■) or 10mM-fructose (●) and after the appropriate time intervals the reaction was stopped by freezing samples in liquid N_2. At the same time further samples were removed and washed (□, glucose; ○, fructose) by rapidly sedimenting the cells through ice-cold Krebs–Ringer bicarbonate buffer (containing Ca^{2+} and bovine serum albumin). The supernatant fraction was aspirated and the pellet frozen in liquid N_2. Pyruvate kinase activity was assayed with 0.2mM- (v) and 4mM- ($V_{max.}$) phosphoenolpyruvate, and the results plotted are means of the $v/V_{max.}$ values for three different cell preparations. The bars indicate S.E.M., and where these are not shown they lie within the symbol.

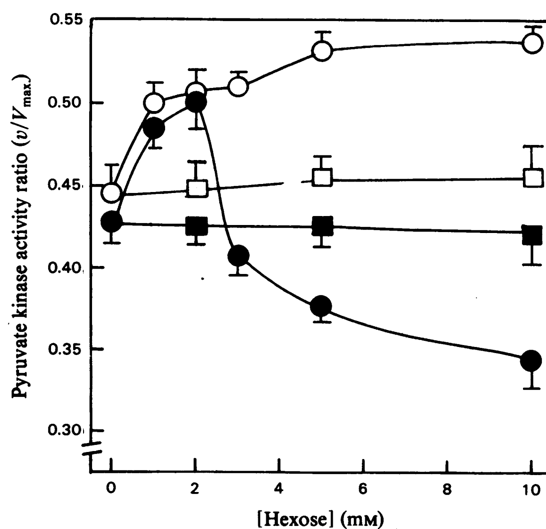

Fig. 2. *Effects of glucose and fructose concentrations on hepatocyte pyruvate kinase activity*

Isolated hepatocytes were incubated with glucose or fructose for 20min. See Fig. 1 for other details, including key to symbols.

Table 1. *Effects of fructose on hepatocyte pyruvate kinase activity before and after $(NH_4)_2SO_4$ precipitation*
Hepatocytes were incubated for 20 min with or without added hexose, and samples were then removed, frozen in liquid N_2 and assayed directly or after precipitation with 50%-satd. $(NH_4)_2SO_4$ as described in the Materials and methods section. $V_{max.}$ and v values were determined at 4 mM- and 0.2 mM-phosphoenolpyruvate for at least three different preparations of hepatocyte. The values of $v/V_{max.}$ are expressed as means ± S.E.M. The $[S]_{0.5}$ and h values for phosphoenolpyruvate were determined by using data obtained with one preparation of cells.

	$v/V_{max.}$	$[S]_{0.5}$ (mM-phosphoenolpyruvate)	h
Before $(NH_4)_2SO_4$ treatment			
Control	0.42 ± 0.02	0.25	1.0
10 mM-Glucose	0.40 ± 0.03	0.25	1.0
10 mM-Fructose	0.33 ± 0.03	0.50	0.9
10 mM-Fructose (washed*)	0.58 ± 0.02	0.15	1.1
After $(NH_4)_2SO_4$ treatment			
Control	0.34 ± 0.01	0.59	1.1
10 mM-Glucose	0.35 ± 0.01	0.59	1.1
10 mM-Fructose	0.34 ± 0.02	0.58	1.1

* Hepatocytes were washed after incubation with 10 mM-fructose as described in Fig. 1.

tion, whereas with the unwashed cells the value was approaching that found with the $(NH_4)_2SO_4$-precipitated enzyme. The latter treatment also abolished the differences in $[S]_{0.5}$ values for the enzyme from glucose-treated and fructose-treated hepatocytes. Removal of the $(NH_4)_2SO_4$ by gel filtration of the $(NH_4)_2SO_4$-precipitated enzyme on Sephadex G-25 resulted in no further change in $v/V_{max.}$ values.

The fructose-treated hepatocytes, together with the incubation medium, were extracted with $HClO_4$, and the neutralized extracts were found to contain an effector that caused the inhibition of a partially purified pyruvate kinase preparation. However, this inhibition was considerably less marked than the inhibition observed with the crude enzyme from fructose-treated hepatocytes (results not shown).

These results are consistent with the observation that fructose causes an accumulation of fructose 1,6-bisphosphate and fructose 1-phosphate, which can stimulate pyruvate kinase activity. Incubation of isolated hepatocytes with 10 mM-fructose for 20 min resulted in an increase in fructose 1,6-bisphosphate concentration from 16.6 ± 2.4 to 36.3 ± 4.5 nmol/g of cells ($n = 7$) and an increase in fructose 1-phosphate from 0.22 ± 0.01 to 6.87 ± 0.6 μmol/g of cells ($n = 7$). However, it is also evident that in the unwashed hepatocytes fructose causes the accumulation of an inhibitor that prevents stimulation of the enzyme. Inhibition of pyruvate kinase by fructose has also been reported briefly by Van Berkel *et al.* (1977*b*).

Effects of fructose on the concentrations of ATP and alanine in hepatocytes

The nature of the inhibition of pyruvate kinase by fructose was further examined. ATP, a well-known allosteric inhibitor of the enzyme (Seubert & Schoner, 1971; Imamura *et al.*, 1972), was ruled out as a possible candidate in this case because the concentration of the triphosphate in isolated hepatocytes is known to decrease in the presence of fructose (Van den Berghe *et al.*, 1980). The depletion of ATP at fructose concentrations above 2 mM was confirmed during the present study (results not shown).

Another potential allosteric inhibitor of pyruvate kinase that may be affected by fructose is alanine. A study of alanine production by isolated hepatocytes showed that alanine output was stimulated at 5 mM- and 10 mM-fructose, but not at lower concentrations of the ketose (Fig. 3). The rate of output of alanine was 0.31 ± 0.08 μmol/g per 20 min in the control cells ($n = 7$) and 1.32 ± 0.12 μmol/g per 20 min in the fructose (10 mM)-treated cells ($n = 7$). After 20 min the alanine concentration in the medium with the control cells was 24 μM, and 64 μM with the fructose-treated cells. The appearance of alanine paralleled the change in pyruvate concentration (Fig. 3). The hepatic synthesis of alanine from pyruvate involves a transamination from glutamate catalysed by alanine aminotransferase.

The transaminase inhibitor amino-oxyacetate (Hopper & Segal, 1962; Blackshear *et al.*, 1975; John *et al.*, 1978), at a concentration of 0.5 mM, substantially inhibited alanine output by isolated hepatocytes incubated with 10 mM-fructose. There was a concomitant reversal of the apparent inhibition of pyruvate kinase (results not shown), but there was no stimulation of pyruvate kinase. There was no substantial difference between the alanine concentrations in the medium for control and fructose-treated cells after amino-oxyacetate treatment. These

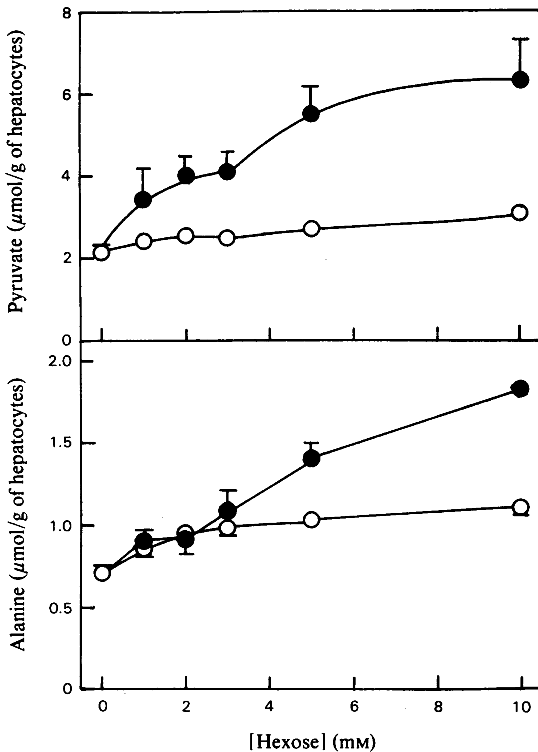

Fig. 3. *Effect of fructose and glucose on alanine and pyruvate output by isolated hepatocytes*

Isolated hepatocytes were incubated with either glucose (○) or fructose (●) for 20 min. The reaction was stopped with $HClO_4$ and the neutralized extracts were used to determine metabolite concentrations as described in the Materials and methods section. The results represent means of values obtained from the three different cell preparations, except for pyruvate assays performed with glucose-treated cells, which were obtained with two cell preparations. The error bars represent S.E.M.

results suggest that the observed inhibition of pyruvate kinase at high fructose concentration may be partially, but not wholly, due to alanine accumulation in the extrahepatocyte medium.

Effects of products of AMP catabolism on pyruvate kinase

The concentration-dependence of the effect of fructose on hepatocyte pyruvate kinase suggests that the inhibition observed at the higher concentrations of fructose may be the result of ATP depletion, which results in an increased catabolism of AMP. Table 2 shows the effects of some of the products of AMP catabolism on pyruvate kinase activity. Of all the possible effectors tested, only allantoin and uric acid were inhibitory. This suggested that the inhibition of pyruvate kinase was the result of the accumulation of these end products in response to high concentrations of fructose.

Table 2. *Effects of the products of AMP catabolism on partially purified hepatocyte pyruvate kinase assayed with 0.2 mM-phosphoenolpyruvate*

The results are expressed as percentages of the control $v/V_{max.}$ value. The values obtained for control, uric acid and allantoin represent the means ± S.E.M. for seven different hepatocyte extracts. All other values are means obtained with two different extracts.

Metabolite	Concentration (mM)	Enzyme activity (%)
Control	—	100
Adenosine	1.0	97
AMP	1.0	98
Allantoin	1.0	69 ± 2.4 (7)
Hypoxanthine	0.5	97
Uric acid	0.5	78 ± 4.0 (7)
Xanthine	0.5	98

The output of allantoin, but not of uric acid, by isolated hepatocytes has been shown to increase in response to fructose (Van den Berghe *et al.*, 1980). This was confirmed in the present study. The output of allantoin by control cells was 1.4 μmol/g per 20 min, and in fructose (10 mM)-treated cells was 3.5 μmol/g per 20 min. The concentration of allantoin in the extrahepatocyte medium reached approx. 0.4 mM after 20 min of incubation; only a small proportion of the allantoin was found to be associated with the hepatocytes, suggesting that the plasma membrane is freely permeable to allantoin. Uric acid could not be detected in any of the experiments presumably because of the high uricase activity in oxygenated hepatocytes.

Allopurinol, a xanthine oxidase inhibitor, is a potent inhibitor of AMP catabolism and allantoin accumulation (Fain & Shepherd, 1977). Fig. 4 shows the effect of allopurinol on the inhibition of pyruvate kinase by fructose. It was clear that at low fructose concentrations there was no effect of the xanthine oxidase inhibitor, yet at high fructose concentrations the inhibition was abolished but the stimulation observed at low concentrations was not found. This was further evidence to suggest that the inhibition of pyruvate kinase was at least partly related to the accumulation of allantoin in the extrahepatocyte medium.

The effect of allantoin on a partially purified pyruvate kinase preparation is shown in Fig. 5. It is clear that allantoin exerts an inhibitory effect on the enzyme at subsaturating phosphoenolpyruvate concentrations. Allantoin inhibited the enzyme assayed in the absence of other effectors; the inhibition resulted in a change in the $[S]_{0.5}$ values for

phosphoenolpyruvate from 580 μM to 930 μM, a change that is very similar to that observed with 1 mM-alanine (results not shown). These values changed to 45 μM and 370 μM respectively in the presence of 50 μM-fructose 1,6-bisphosphate. Similar inhibition was found with 0.5 mM-uric acid (results not shown).

It is highly unlikely that the inhibition of pyruvate kinase observed in the present study is the result of direct inhibition by either alanine and/or allantoin, since there is a 120-fold dilution of the hepatocyte medium in the assay procedure. It also appears that the inhibition is due to the appearance of the inhibitor(s) in the extrahepatocyte medium. It is therefore possible that the inhibition observed is the

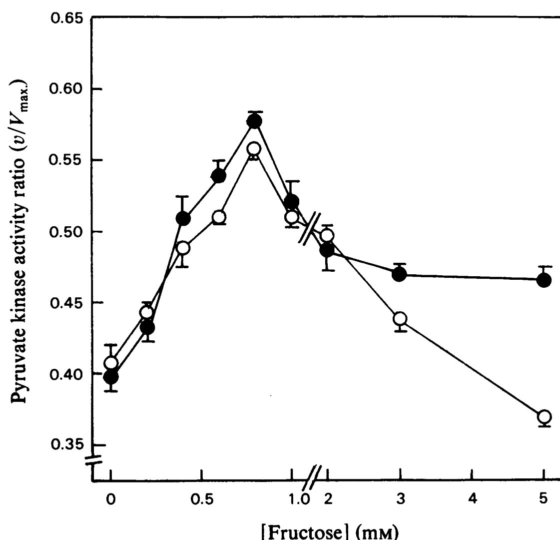

Fig. 4. *Effect of incubating hepatocytes with 20 μM-allopurinol on the fructose-mediated changes in pyruvate kinase activity*

Isolated hepatocytes were incubated for 15 min with 10 mM-fructose in the presence (●) and in the absence (○) of 20 μM-allopurinol. For full experimental details see the text. The results shown are the means of values for four different cell preparations. Error bars represent S.E.M.

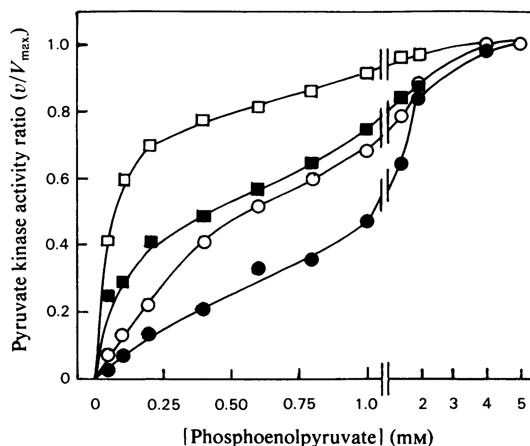

Fig. 5. *Effect of 1 mM-allantoin on the phosphoenolpyruvate saturation curve of partially purified pyruvate kinase in the presence and in the absence of fructose 1,6-bisphosphate*

The enzyme was partially purified and assayed in the presence of 50 μM-fructose 1,6-bisphosphate (□), 50 μM-fructose 1,6-bisphosphate + 1 mM-allantoin (■), 1 mM-allantoin (●) or no added effectors (○). For full experimental details see the text.

Table 3. *Hepatocyte pyruvate kinase activity after treatment with allantoin and alanine*

The cells were incubated for 20 min in the presence or in the absence of 10 mM-fructose. After the incubation period a sample was frozen in liquid N_2 to stop the reaction and at the same time samples were removed and rapidly sedimented through Krebs–Ringer bicarbonate buffer (containing Ca^{2+} and bovine serum albumin). After the washing step, the cells were resuspended in the same buffer (containing Ca^{2+} and bovine serum albumin) containing allantoin and/or alanine and then frozen in liquid N_2 before use for enzyme assay.

	Pyruvate kinase ($v/V_{max.}$)	
	No substrate	Fructose (10 mM)
Unwashed hepatocytes		
Control	0.44	0.36
Washed hepatocytes		
Control	0.47	0.60
1 mM-Allantoin	0.39	0.38
1.0 mM-Alanine	0.38	0.38
1.0 mM-Alanine + 1.0 mM-allantoin	0.36	0.33
0.1 mM-Alanine + 1.0 mM-allantoin	0.39	0.41

result of an artifact of the procedure used. In an experiment in which fructose-treated hepatocytes were washed and resuspended in fresh medium before extraction and assay, the inhibition was not apparent (Table 3). However, if alanine or allantoin was added to the resuspending medium before the freeze–thaw treatment, the apparent stimulation was clearly reversed and inhibition was observed. The effects of alanine and allantoin were additive. Thus it appears that the changes in pyruvate kinase activity observed with fructose are the result of the displacement of fructose 1,6-bisphosphate and fructose 1-phosphate from the enzyme during the extraction procedure.

Discussion

It is well known that the liver is capable of high rates of fructolysis to lactate as compared with rates of glycolysis from glucose. Several explanations have been proposed, for example the higher capacity of the liver to phosphorylate fructose, the fact that fructose metabolism to lactate bypasses the regulatory phosphofructokinase step of glycolysis and the stimulation of pyruvate kinase by fructose 1-phosphate, which accumulated in the presence of fructose (Van den Berghe, 1978).

The activation of pyruvate kinase observed at low fructose concentrations and at high fructose concentrations in washed hepatocytes is most probably due to the accumulation of fructose 1,6-bisphosphate, since this effector has a high affinity for pyruvate kinase (Irving & Williams, 1973) and is more likely to remain bound to pyruvate kinase during the extraction and assay procedure. Some contribution by fructose 1-phosphate cannot be ruled out, but the affinity of pyruvate kinase for the ketose phosphate is such that it is unlikely that there is a substantial carry-over of the activator despite the high concentrations within the hepatocytes after a fructose load. The persistence of the activation of the enzyme by fructrose 1,6-bisphosphate has been described by Claus *et al.* (1979), who demonstrated a stimulatory effect of dihydroxyacetone on pyruvate kinase. In that study, the stimulation of the enzyme by fructose 1,6-bisphosphate was shown to be resistant to dialysis and to gel filtration, but was abolished by $(NH_4)_2SO_4$ precipitation of the enzyme.

It is postulated in the present study that an inhibitor accumulates in the extrahepatocyte medium that enhances the dissociation of the fructose 1,6-bisphosphate–pyruvate kinase complex, resulting in an apparent inhibition of the enzyme. One possible candidate for the role is alanine, a known inhibitor of pyruvate kinase (Seubert & Schoner, 1971), which is accumulated by hepatocytes in response to fructose. Amino-oxyacetate inhibits alanine output and, at the same time, partially reverses the inhibition of pyruvate kinase. However, the alanine accumulating in the extrahepatocyte medium is not sufficient to explain the almost complete removal of fructose 1,6-bisphosphate from the pyruvate kinase that occurs with the hepatocytes incubated in the presence of high fructose concentrations, and in addition alanine accumulation was increased at all concentrations of fructose used, whereas inhibition of pyruvate kinase was not apparent at lower concentrations of the ketose. The latter observation suggested that the major inhibitory effect of fructose was a result of the depletion of ATP, which was significant only at higher concentrations.

There are a number of consequences of hepatic ATP depletion caused by fructose that result from the accelerated degradation of AMP (Van den Berghe, 1978). Of these, only the accumulation of allantoin (Van den Berghe *et al.*, 1980) could cause the inhibition of pyruvate kinase described in the present paper.

To our knowledge there is no report in the literature on the inhibition of pyruvate kinase by allantoin or by urate. Under normal circumstances it is difficult to envisage a physiological role for such inhibition because of the relatively low concentrations of urate and allantoin present in the blood. However, intravenous administration of fructose to rats results in an increase in plasma concentrations of allantoin and of urate to 0.5 mM and 0.22 mM respectively (Mäenpää *et al.*, 1968). These concentrations could influence the activity of pyruvate kinase. *In vivo* pyruvate kinase is normally inhibited by the concentration of ATP in the cytosol, and this would limit glycolysis. Depletion of ATP by fructose would therefore result in a stimulation of pyruvate kinase, but this would be counteracted, at least to some extent, by the increase in plasma allantoin and urate.

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