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Metabolic effects of D-glyceraldehyde in isolated hepatocytes

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1. The effects of D-glyceraldehyde on the hepatocyte contents of various metabolites were examined and compared with the effects of fructose, glycerol and dihydroxyacetone, which all enter the glycolytic/gluconeogenic pathways at the triose phosphate level. 2. D-Glyceraldehyde (10 mM) caused a substantial depletion of hepatocyte ATP, as did equimolar concentrations of fructose and glycerol. 3. D-Glyceraldehyde and fructose each caused a 2-fold increase in fructose 1,6-bisphosphate and the accumulation of millimolar quantities of fructose 1-phosphate in the cells. 4. D-Glyceraldehyde caused an increase in the glycerol 3-phosphate content and a decrease in the dihydroxyacetone phosphate content, whereas dihydroxyacetone increased the content of both metabolites. 5. The increase in the [glycerol 3-phosphate]/[dihydroxyacetone phosphate] ratio caused by D-glyceraldehyde was not accompanied by a change in the cytoplasmic $[NAD^+]/[NADH]$ ratio, as indicated by the unchanged [lactate]/[pyruvate] ratio. 6. The accumulation of fructose 1-phosphate from D-glyceraldehyde and dihydroxyacetone phosphate in the hepatocyte can account for the depletion of the intracellular content of the latter. Presumably ATP is depleted as the result of the accumulation of millimolar amounts of a phosphorylated intermediate, as is the case with fructose and glycerol. 7. It is suggested that the accumulation of fructose 1-phosphate during hepatic fructose metabolism is the result of a temporary increase in the D-glyceraldehyde concentration because of the high rate of fructose phosphorylation compared with triokinase activity. The equilibrium constant of aldolase favours the formation and thus the accumulation of fructose 1-phosphate.

INTRODUCTION

It is well known that D-fructose metabolism involves its phosphorylation by fructokinase, yielding fructose 1-phosphate, which is in turn cleaved by aldolase to give dihydroxyacetone phosphate and D-glyceraldehyde. The latter is phosphorylated by triokinase to give glyceraldehyde 3-phosphate. The triose phosphates thus formed can then undergo either glycolysis to lactate or gluconeogenesis (for review see Van den Berghe, 1978).

Fructose is rapidly phosphorylated by isolated liver preparations, and fructose 1-phosphate accumulates (Woods *et al.*, 1970; Van den Berghe *et al.*, 1977; Iles *et al.*, 1980; Mapungwana & Davies, 1982). As a consequence there is a concomitant decrease in intracellular P_i, limited ADP phosphorylation, and the hepatic ATP content becomes severely depleted (Woods *et al.*, 1970; Siess & Wieland, 1976; Van den Berghe *et al.*, 1977; Iles *et al.*, 1980). Intravenous administration of fructose also leads to the depletion of hepatic ATP (Mäenpää *et al.*, 1968) and the accumulation of fructose 1-phosphate in millimolar concentrations (Mäenpää *et al.*, 1968; Heinz & Junghänel, 1969). The latter authors also found a D-glyceraldehyde content of 0.56 μmol/g in the liver after a fructose load.

There have been few reports on the metabolic effects of D-glyceraldehyde on the liver, with the exception of the use of the triose as a gluconeogenic precursor to examine the stimulatory effect of glucagon on the process (Veneziale, 1972, 1976) and an investigation of the kinetic properties of triokinase (Frandsen & Grunnet, 1971), the enzyme thought to be the major route of D-glyceraldehyde metabolism in liver (Sillero *et al.*, 1969; Hue & Hers, 1972). Sestoft *et al.* (1972) reported that fructose 1-phosphate did not accumulate when pig liver was perfused with D-glyceraldehyde.

We have therefore investigated the effects of D-glyceraldehyde on the concentrations of some important metabolites in isolated hepatocytes and compared its effects with those of dihydroxyacetone, fructose and glycerol, which are all precursors that enter the glycolysis/gluconeogenesis sequence at the triose phosphate level.

MATERIALS AND METHODS

Animals and diets

Mature male Wistar rats (250–350 g) were fed *ad libitum* on a normal laboratory diet.

Reagents

All enzymes and substrates were obtained from Sigma Chemical Co., Poole, Dorset, U.K., or Boehringer Corp., Lewes, Sussex, U.K. Inorganic reagents were of AnalaR grade from BDH Chemicals, Poole, Dorset, U.K. D-Glyceraldehyde solutions were kept at 20 °C for 24 h to ensure that the triose was in the monomeric form (Lardy, 1957). D-Glyceraldehyde was assayed by the method of Nelson (1944) as modified by Hers (1962); this modification allows for a more specific measurement of trioses in the presence of hexoses.

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Isolation and incubation of hepatocytes

Hepatocytes from fed rats were prepared and then incubated at 37 °C in the presence of D-glyceraldehyde, dihydroxyacetone, glycerol or fructose, or in the absence of these substrates, as described previously (Mapungwana & Davies, 1982).

Determination of metabolites

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

ATP was determined, immediately, by the method of Jaworek *et al.* (1974). Dihydroxyacetone phosphate, glyceraldehyde 3-phosphate and fructose 1,6-bisphosphate were measured by the method of Michal & Beutler (1974). Fructose 1-phosphate and glycerol 3-phosphate were measured as described by Eggleston (1974) and Michal & Lang (1974) respectively. Pyruvate and lactate were assayed by the methods of Czok & Lamprecht (1974) and Gutmann & Wahlefeld (1974) respectively.

Triokinase assay

Triokinase activity was determined in dialysed high-speed supernatants of rat liver homogenates as described by Heinz & Lamprecht (1961).

Statistical methods

Unless otherwise stated, the data are expressed as mean values ± S.E.M. for three different hepatocyte preparations. Statistical significance was determined by Student's *t* test.

RESULTS AND DISCUSSION

When isolated hepatocytes from fed rats were incubated with 10 mM-D-glyceraldehyde, the initial rate of clearance of the triose was 4.91 ± 0.82 μmol/min per g of cells ($n = 3$); this rate was linear for 30 min. Veneziale (1972) reported a clearance rate of 2.27 μmol/min per g for livers from starved rats perfused with 4 mM-D-glyceraldehyde. The difference is presumably related to the type of liver preparation and the concentration of triose used. The rates of D-glyceraldehyde clearance observed in the present study are somewhat higher than the triokinase activity in liver extracts measured with D-glyceraldehyde as substrate. We have found a triokinase activity of 0.99 ± 0.07 μmol/min per g of liver ($n = 8$), measured at 30 °C, which is in agreement with data obtained by others (Adelman *et al.*, 1966; Hers, 1962). The causes of the discrepancy between the rate of D-glyceraldehyde clearance by hepatocytes and the maximal triokinase activity were further investigated by examining some of the metabolic consequences of D-glyceraldehyde loading on hepatocyte metabolism.

Effect of D-glyceraldehyde on the hepatocyte ATP content

Fructose (Woods *et al.*, 1970; Van den Berghe *et al.*, 1977; Clark *et al.*, 1979; Iles *et al.*, 1980) and glycerol (Woods & Krebs, 1973; Des Rosiers *et al.*, 1982), but not dihydroxyacetone (Woods & Krebs, 1973; Siess & Wieland, 1976), are known to cause a severe depletion of the ATP content of liver preparations. In the present study D-glyceraldehyde was also shown to cause a rapid and profound decrease in the ATP content of hepatocytes, followed by a slow recovery, but control values were not attained within a 60 min incubation period (Fig. 1). To our knowledge this is the first demonstration that D-glyceraldehyde depletes ATP in a liver preparation. Veneziale (1972) found no such decrease in the concentration of the nucleotide in liver perfused with 4 mM-D-glyceraldehyde. The results shown in Fig. 2 show that the ATP content was decreased by about 10% at this concentration, but this was not statistically significant. However, concentrations of 6 mM and above did cause a statistically significant decrease in the ATP concentration.

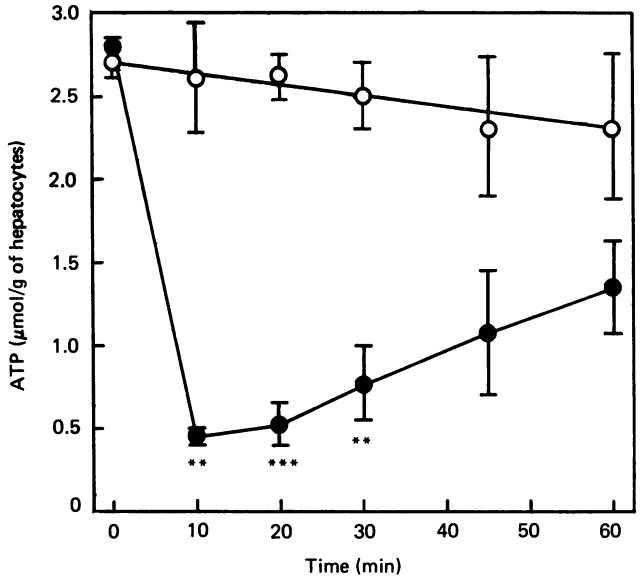

Fig. 1. Time-course experiment showing the effect of 10 mM-D-glyceraldehyde on the ATP content of isolated hepatocytes from fed rats

Isolated hepatocytes were prepared as described in the Materials and methods section. The cells were suspended in Krebs–Ringer bicarbonate buffer containing 2.3 mM-$CaCl_2$ (Krebs & Henseleit, 1932), preincubated for 15 min, and then an equal volume of D-glyceraldehyde dissolved in the same buffer was added. The incubation was terminated by the addition of $HClO_4$ after the appropriate time interval and the ATP was determined as described in the text. ○, Control; ●, + 10 mM-D-glyceraldehyde. The values are shown as means ± S.E.M.; where no error bars are shown these lie within the symbol. The differences from the control values at the appropriate time intervals were significant at: $^{**}P < 0.01$; $^{***}P < 0.001$.

The extent of the depletion in the ATP content of hepatocytes caused by various substrates is shown in Table 1; D-glyceraldehyde (10 mM) was at least as effective as equimolar concentrations of either fructose or glycerol. Fructose has previously been shown to decrease the ATP content of isolated hepatocytes (Siess & Wieland, 1976; Van de Werve & Hers, 1979; Clark *et al.*, 1979; Van den Berghe *et al.*, 1980), as has glycerol (Siess & Wieland, 1976; Des Rosiers *et al.*, 1982). The explanation given by these authors for the rapid

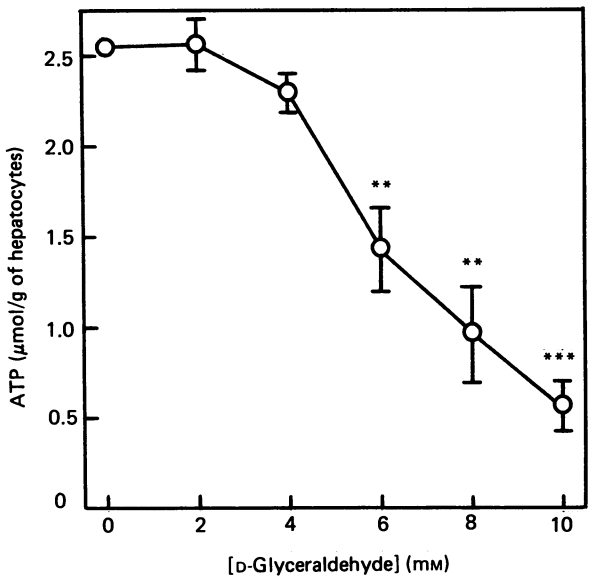

Fig. 2. Effect of incubation for 20 min with various concentrations of D-glyceraldehyde on the ATP content of isolated hepatocytes

For method and other details, see the legend to Fig. 1. The differences between control and D-glyceraldehyde-treated hepatocytes were statistically significant where indicated: $**P < 0.001$; $***P < 0.001$.

depletion of ATP by fructose and glycerol is that both are rapidly phosphorylated to give fructose 1-phosphate and glycerol 3-phosphate respectively. Both phosphorylated intermediates accumulate in millimolar concentrations (Table 1) because their rate of synthesis exceeds that of their breakdown, and as a result intracellular P_i is sequestered and there is a decreased mitochondrial phosphorylation of ADP.

The results in Table 1 also show that dihydroxyacetone (10 mM) causes a small (approx. 20%) but significant ($P < 0.05$) depletion of hepatocyte ATP. Siess & Wieland (1976) observed a 10% decrease in the cytosolic ATP content of hepatocytes incubated with the triose, but this was not statistically significant. In the present study the decreased ATP content found on incubation of the cells with dihydroxyacetone was only transitory and returned to normal values after 45 min incubation (results not shown). The relative lack of ATP depletion by dihydroxyacetone is probably due to the fact that, in contrast with the other substrates examined, a millimolar quantity of a phosphorylated intermediate does not accumulate.

Effect of D-glyceraldehyde on the fructose 1,6-bisphosphate and fructose 1-phosphate contents of hepatocytes

The possibility that the depletion of ATP is associated with the accumulation of a phosphorylated intermediate was investigated by examining the changes in the hepatocyte contents of various metabolites which occur as the result of incubation with D-glyceraldehyde. The data in Fig. 3 show the fructose 1,6-bisphosphate content of hepatocytes after incubation with either dihydroxyacetone or D-glyceraldehyde. The former caused an 11-fold increase in the bisphosphate, which contrasted with a relatively modest 2-fold increase caused by the latter. The difference presumably reflects the lower dihydroxyacetone phosphate content of the cells incubated with the aldotriose (Table 2). Fructose (10 mM) increased fructose 1,6-bisphosphate to approximately the same extent as did D-glyceraldehyde, but glycerol was without effect (Table 1). Claus *et al.* (1979) have previously reported a 4-fold increase in the bisphosphate in isolated hepatocytes incubated with 5 mM-dihydroxyacetone, but in the perfused liver (Woods & Krebs, 1973) such an effect was not found. Woods *et al.* (1970) have found a 5-fold increase in the fructose 1,6-bisphosphate content of liver after perfusion with fructose, but we can find no reports in the literature of a change in the bisphosphate content after D-glyceraldehyde treatment of hepatic preparations.

D-Glyceraldehyde caused a substantial accumulation of fructose 1-phosphate (Table 2), especially at higher concentrations of the aldotriose. The fructose 1-phosphate content of the hepatocyte observed after 20 min incubation with 10 mM-D-glyceraldehyde was similar to that found with 3 mM-fructose (3.71 ± 0.60 and 4.00 ± 0.09 μmol/g of cells respectively; $n = 3$). An initial rate of fructose 1-phosphate accumulation of 0.49 ± 0.08 μmol/min per g of cells ($n = 3$) was found for hepatocytes incubated with D-glyceraldehyde (10 mM); this rate was linear for at least 10 min. In contrast, the initial rate of fructose 1-phosphate accumulation with

Table 1. Effects of incubation of isolated hepatocytes with various substrates on the metabolite contents of the cells

Metabolite concentrations are expressed in μmol/g of cells, after incubation of the hepatocytes for 20 min in the presence of 10 mM substrate. For other details see legends for Figs 2 and 3. Results are given as mean values ± S.E.M. for three different cell preparations, except where indicated by †, where $n = 2$. The differences from the control values, where significant, are indicated: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Substrate	Metabolite content (μmol/g)				
	ATP	Dihydroxyacetone phosphate	Glycerol 3-phosphate	Fructose 1,6-bisphosphate	Fructose 1-phosphate
None	2.49 ± 0.11	0.042 ± 0.005	0.196 ± 0.04	0.016 ± 0.001	0.22 ± 0.01
D-Glyceraldehyde	0.56 ± 0.06***	0.012 ± 0.002**	0.320 ± 0.08	0.032 ± 0.008	3.71 ± 0.60**
D-Fructose	0.95 ± 0.01***	0.043 ± 0.002	0.430 ± 0.06*	0.036 ± 0.006*	6.87 ± 0.60***
Dihydroxyacetone	2.00 ± 0.09*	0.140 ± 0.009***	0.710 ± 0.03***	0.180 ± 0.030***	0.25 ± 0.04
Glycerol	0.80 ± 0.06***	0.044 ± 0.002	5.30 ± 0.70**	0.016†	0.22†

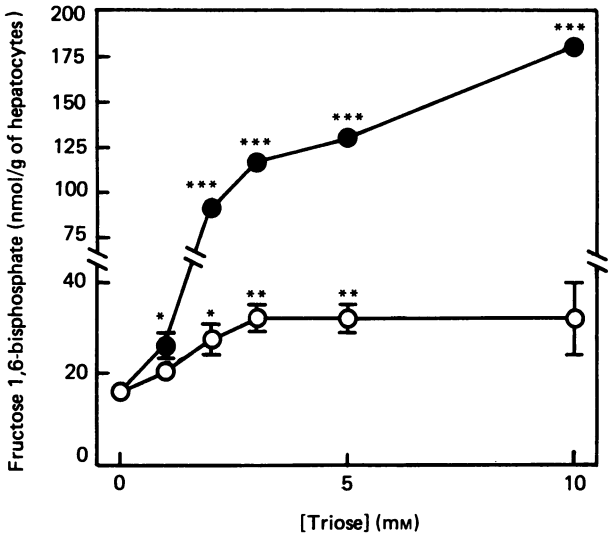

Fig. 3. Effect of D-glyceraldehyde (○) and dihydroxyacetone (●) concentrations on the fructose 1,6-bisphosphate content of isolated hepatocytes after 20 min incubation

Details were as for Fig. 1, except that 1.5% (w/v) bovine serum albumin (essentially fatty acid-free, fraction V; Sigma Chemical Co.) was added to the Krebs–Ringer bicarbonate buffer. Fructose 1,6-bisphosphate was assayed as described in the text. Differences between control and triose-treated cells are indicated: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

fructose (10 mM) was 8.62 ± 0.90 μmol/min per g of cells ($n = 3$) and was linear for only 1 min. In both cases, the accumulation of fructose 1-phosphate, presumably resulting in the sequestration of intracellular P_i, must in turn result in the depletion of ATP content of the hepatocytes.

The accumulation of fructose 1-phosphate caused by D-glyceraldehyde is presumably the result of the reversal of the fructose 1-phosphate aldolase reaction. In hepatic fructose metabolism there is a net flux of carbon in the direction of triose phosphate production, although liver aldolase is also able to catalyse both the synthesis and the cleavage of fructose 1,6-bisphosphate, since the liver is capable of net glycolysis or gluconeogenesis, depending on the dietary state of the animal (Hers & Hue, 1983). In the present study we have shown for the first time that in isolated hepatocytes incubated with D-glyceraldehyde there is a net reversal of the aldolase reaction, resulting in the formation of fructose 1-phosphate from exogenously supplied D-glyceraldehyde and intracellular dihydroxyacetone phosphate. Lehninger *et al.* (1955) have calculated that the equilibrium constant for the cleavage of fructose 1-phosphate by aldolase at 37 °C is 2.8×10^{-6} M, and from the hepatocyte contents of the product and reactants after 20 min incubation with D-glyceraldehyde it is possible to calculate a mass-action ratio of approx. 9×10^{-6} M, suggesting that the system is approaching thermodynamic equilibrium. The latter value is calculated from the data of further experiments in which we found fructose 1-phosphate and dihydroxyacetone phosphate contents of 6.74 ± 0.52 and 0.012 ± 0.02 μmol/g of cells, respectively, at a D-glyceraldehyde concentration of 5.3 ± 0.8 mM after 20 min incubation ($n = 3$). An even distribution of product and reactants within the cells is assumed, and the problems of compartmentalization of the phosphorylated intermediates are ignored, as are the contributions of fructose 1,6-bisphosphate and fructose 1,6-bisphosphate aldolase activities. Hers (1962) has found that crude liver extracts containing triokinase, triose phosphate isomerase and aldolase are capable of converting D-glyceraldehyde into fructose 1-phosphate in the presence of ATPMg and that the equilibrium of these reactions is

Table 2. Effects of D-glyceraldehyde and dihydroxyacetone on the metabolite contents of isolated hepatocytes

Hepatocytes were incubated for 20 min in the presence of the indicated concentration of the triose. For other details see legends to Figs. 1 and 3. Metabolite concentrations are expressed as mean values ± S.E.M. for three observations: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Substrate (mM)	Glycerol 3-phosphate (nmol/g of cells)	Dihydroxyacetone phosphate (nmol/g of cells)	Glycerol 3-phosphate/dihydroxyacetone phosphate ratio	Lactate plus pyruvate (μmol/g of cells)	Lactate/pyruvate ratio	Fructose 1-phosphate (μmol/g cells)
	D-Glyceraldehyde					
0	196±40	42.3±4.6	4.6±0.6	23.9±1.0	9.5±0.3	0.22±0.01
1	197±40	41.9±5.0	4.7±1.6	28.8±1.1	9.1±0.9	0.36±0.02**
2	213±30	44.3±5.3	4.8±1.0	37.7±0.5	9.8±1.2	0.59±0.06**
3	185±20	46.3±3.8	4.0±0.9	41.5±2.5	9.9±0.8	0.79±0.10**
5	365±70	23.1±4.1*	15.8±3.5*	51.0±0.9	10.1±0.8	1.25±0.15**
10	320±80	11.7±2.1**	27.3±4.8**	54.6±1.7	10.7±0.8	3.71±0.60**
	Dihydroxyacetone					
0	196±40	42.3±4.6	4.6±0.6	23.9±1.0	9.5±1.0	0.22±0.01
1	240±50	40.0±3.0	6.0±1.0	32.7±4.2	9.3±0.3	0.22±0.01
2	360±50	47.0±3.2	7.4±1.8	37.4±3.8	10.7±1.3	0.25±0.01
3	590±100*	96.0±3.0***	6.1±1.4	39.5±3.5	8.6±0.7	0.22±0.02
5	610±70**	115.5±5.0***	5.3±1.8	47.0±4.2	10.2±1.4	0.27±0.04
10	710±30***	139.5±9.3***	5.1±0.9	57.0±4.2	11.7±1.9	0.25±0.04

such that D-glyceraldehyde can be almost totally converted into the ketose phosphate.

The data shown in the present study give further support to the proposal by Van den Berghe (1978) that the accumulation of fructose 1-phosphate during fructose metabolism is due to the relatively slow combined rates of glycolysis and gluconeogenesis from triose phosphates, compared with the potential activity of fructokinase. From the work in the present study, it appears that the initial rate of fructose phosphorylation exceeds that of D-glyceraldehyde clearance, and therefore we propose that fructose 1-phosphate accumulates because there may be sufficient D-glyceraldehyde produced temporarily during fructose metabolism to prevent a high flux of carbon through aldolase in the direction of the triose phosphates.

Effect of D-glyceraldehyde on hepatocyte triose phosphate content

Glyceraldehyde 3-phosphate is always present in low amounts in liver tissue compared with other glycolytic intermediates, such as dihydroxyacetone phosphate and fructose 1,6-bisphosphate (Woods *et al.*, 1970), and in the present study no significant accumulation of the aldotriose phosphate was found with any of the substrates tested (results not shown).

The effect of D-glyceraldehyde on the hepatocyte dihydroxyacetone phosphate content is shown in Table 2. At low concentrations of the substrate the triose phosphate content was unaffected, but at 5 mM and 10 mM substrate there was an unexpected decrease, to about 28% of the control value with the latter concentration. We can find no report in the literature of this effect of D-glyceraldehyde on liver preparations. In contrast, dihydroxyacetone at the higher concentrations caused an accumulation of the triose phosphate; this result is in agreement with the findings of others (Woods & Krebs, 1973; Pilkis *et al.*, 1976). The explanation for this difference is not immediately clear, since both D-glyceraldehyde and dihydroxyacetone are good substrates for triokinase, with K_m values of 19 μM and 6 μM respectively. However, the relative $V_{max.}$ with the latter substrate is 2.5-fold greater than with the former (Frandsen & Grunnet, 1971). The major difference between the two substrates is that with the ketotriose the immediate product is dihydroxyacetone phosphate, whereas with the aldotriose glyceraldehyde 3-phosphate is formed. Since the equilibrium of the triose phosphate isomerase favours the formation of the ketose phosphate (Veech *et al.*, 1969), D-glyceraldehyde might also be expected to increase the dihydroxyacetone phosphate content of the cells. The formation of fructose 1-phosphate in the presence of D-glyceraldehyde found in the present study provides a possible explanation for the depletion of dihydroxyacetone phosphate. The availability of the ketotriose phosphate must be the rate-limiting factor which governs the rate of formation of fructose 1-phosphate.

Glycerol depletes cellular ATP as the result of an accumulation of glycerol 3-phosphate (Table 2) and a consequent decrease in the cytosolic [NAD^+]/[NADH] ratio, as evidenced by an increase in the [lactate]/[pyruvate] ratio from 9.5 ± 0.3 to 31.7 ± 7.0 ($n = 3$) after 20 min incubation of the hepatocytes in the absence and presence of 10 mM-glycerol respectively. The accumulation of glycerol 3-phosphate is presumably due to the limited availability of NAD^+ in the cytoplasmic compartment, which results in glycerol-3-phosphate dehydrogenase becoming a rate-limiting enzyme in glycerol metabolism.

An interesting feature of the results shown in Table 2 is the significant increase in the [glycerol 3-phosphate]/[dihydroxyacetone phosphate] ratio in cells incubated with 5 mM- and 10 mM-D-glyceraldehyde. Such changes are normally associated with a decrease in the cytosolic [NAD^+]/[NADH] ratio and are accompanied by an increase in the [lactate]/[pyruvate] ratio, but this does not occur with D-glyceraldehyde. In the present study significant changes in the [lactate]/[pyruvate] ratios were only observed when hepatocytes were incubated with reduced substrates such as glycerol, sorbitol or xylitol, and these were accompanied by concomitant changes in the [glycerol 3-phosphate]/[dihydroxyacetone phosphate] ratios (results not shown). The reason for the discrepancy between the [lactate]/[pyruvate] and the [glycerol 3-phosphate]/[dihydroxyacetone phosphate] ratios found with D-glyceraldehyde is not clear. Berry (1980) has suggested that there may be more than one pool of cytoplasmic NADH which interact with different dehydrogenases, and it is possible that D-glyceraldehyde may deplete NAD^+ in the pool associated with glycerol 3-phosphate dehydrogenase, but not in the lactate dehydrogenase pool. Another possibility is that glycerol-3-phosphate dehydrogenase is not sufficiently active to restore the equilibrium between dihydroxyacetone phosphate and glycerol 3-phosphate after removal of the latter by the fructose 1-phosphate aldolase reaction. This is unlikely, bearing in mind the relatively low rate of fructose 1-phosphate formation found in the present study and the high activity of glycerol-3-phosphate dehydrogenase found in liver extracts (Pridham & Davies, 1978).

It has been suggested (Wolf & Leuthardt, 1953) that D-glyceraldehyde may be converted into glycerol by NADH-dependent alcohol dehydrogenase and may thus be metabolized via glycerol 3-phosphate. The glycerol 3-phosphate content of the hepatocytes was somewhat raised by D-glyceraldehyde at 5 mM and 10 mM, but the increase with dihydroxyacetone was greater and occurred at lower concentrations of the triose (Table 2). In both cases the glycerol 3-phosphate content was much lower than that found with glycerol (Table 1). In addition, the pattern of lactate plus pyruvate output by cells incubated with D-glyceraldehyde is similar to that seen with dihydroxyacetone (Table 2), whereas glycerol (at similar concentrations) decreases the output to below basal values (results not shown), suggesting that the NAD^+ content is not a limiting factor in the metabolism of the aldotriose. The increase in glycerol 3-phosphate and the concomitant decrease in dihydroxyacetone phosphate at the higher concentrations of D-glyceraldehyde suggest the involvement of the high-K_m (11 mM, according to Sillero *et al.*, 1969) alcohol dehydrogenase in the metabolism of the triose. This pathway does not involve a net utilization of NAD^+, and hence there is no inhibition of glycolysis comparable with that seen with glycerol.

It is clear that the cause of the apparent discrepancy between the triokinase activity measured in liver extracts and the rate of clearance of D-glyceraldehyde can be attributed to the activity of alcohol dehydrogenase and to the synthesis of fructose 1-phosphate by aldolase. The

contribution of the latter should be assessed by investigating the K_m and $V_{max.}$ values for aldolase using D-glyceraldehyde as substrate.

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