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# Effects of Fructose Concentration on Carbohydrate Metabolism, Heat Production and Substrate Cycling in Isolated Rat Hepatocytes

By Dallas G. CLARK, Owen H. FILSELL and David L. TOPPING
*Division of Human Nutrition, CSIRO, Kintore Avenue, Adelaide 5000, South Australia, Australia*

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1. Hepatocytes from starved rats were incubated with 5mM-glucose, labelled uniformly with $^{14}C$ and specifically with $^{3}H$ at positions 1, 2, 3 or 6, and with fructose at concentrations of 2.5, 7.5 or 25mM. 2. In the absence of other substrates only 1% of the radioactivity initially present in [U-$^{14}C$]glucose appeared in the metabolic products, $CO_2$, lactate, pyruvate, amino acids and glycogen. 3. Fructose at 2.5mM caused a 30% increase in the glucose concentration and a 4-fold increase in the apparent oxidation of [U-$^{14}C$]-glucose. 4. The formation of $^{3}H_2O$ from [1-$^{3}H$]-, [2-$^{3}H$]-, [3-$^{3}H$]- or [6-$^{3}H$]-glucose was 2.4, 4.3, 2.15 or 1.6% respectively in the control incubations and 4.1, 10.4, 7.7 or 5.1% with 2.5mM-fructose. 5. Fructose at 7.5 and 25mM decreased the $^{3}H_2O$ yields to less than the control values, but had no apparent effect on the amount of [U-$^{14}C$]glucose metabolized. 6. In the incubations with 5mM-glucose and 25mM-fructose there were significant decreases in heat production, $O_2$ consumption and in the ratio of $O_2$ uptake to heat output. 7. Fructose at 2.5mM caused a 64% increase in heat output, but only a 43% increase in $O_2$ uptake. 8. The radioisotopic and calorimetric data demonstrate that physiological concentrations of fructose greatly increase metabolism in hepatocytes from starved rats. These data also indicate increased cycling at glucose/glucose 6-phosphate and at fructose 6-phosphate/fructose 1,6-bisphosphate in the presence of 2.5mM-fructose, although the rates of cycling were actually decreased relative to the amount of glucose catabolized. 9. At concentrations of 2.5, 7.5 and 25mM, fructose depressed hepatocyte ATP concentrations by 20, 65 and 80% respectively. Although fructose at 7.5 and 25mM increased glucose and lactate release, $O_2$ consumption, production of heat and formation of $^{3}H_2O$ from [1-$^{3}H$]-, [2-$^{3}H$]-, [3-$^{3}H$]- or [6-$^{3}H$]-glucose were lowered to values equal to, or less than, controls. These effects probably reflect a severe derangement of hepatic metabolism due to excess phosphorylation of fructose when present at high concentrations.

The existence of 'futile' substrate cycles in the glycolytic and gluconeogenic pathways has been demonstrated both *in vitro* (Clark *et al.*, 1973, 1975) and *in vivo* (Clark *et al.*, 1974*a*), and their possible roles in energy expenditure and heat production have been reviewed (Katz & Rognstad, 1976; Newsholme & Crabtree, 1976).

We have shown that either 2.5mM-fructose or 10mM-dihydroxyacetone caused a marked increase in heat production in isolated hepatocytes from food-deprived rats (Jarrett *et al.*, 1979). As there was no corresponding increase in $O_2$ consumption, it was suggested that part of this increase was due to increased energetically wasteful metabolism. Katz *et al.* (1975) have shown that 10mM-dihydroxyacetone increased glucose release and the formation of $^{3}H_2O$ from [2-$^{3}H$]-, [3-$^{3}H$]- and [5-$^{3}H$]-glucose in hepatocytes from fed and starved rats. It is possible therefore that the increased heat production that we have observed with 2.5mM-fructose is partly due to increased substrate cycling at the reactions glucose/glucose 6-phosphate and fructose 6-phosphate/fructose 1,6-bisphosphate.

The concentrations of fructose in blood from the hepatic portal vein of rats absorbing a meal of fructose are in the range 1.5–2.5$\mu$mol/ml of blood (Topping & Mayes, 1971). These concentrations are at least 4-fold less than that commonly employed in studies on hepatic metabolism (Van den Berghe, 1978). Consequently we have examined the effects of fructose, over the concentration range 2.5–25mM, on carbohydrate metabolism in starved rats. In particular, we have compared the formation of $^{3}H_2O$ from glucoses specifically labelled with $^{3}H$ at positions 1, 2, 3 and 6.

## Materials and Methods

Collagenase was obtained from Worthington Corp., Freehold, NJ, U.S.A. [U-$^{14}C$]Glucose, [1-$^{3}H$]glucose, [2-$^{3}H$]glucose, [3-$^{3}H$]glucose and [6-$^{3}H$]glucose (specific radioactivities 0.304, 12.7, 10, 2.2 and 22.5 Ci/mmol respectively) were all purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Albumin (crystalline bovine, fatty acid-poor) was purchased from the Sigma Chemical Co., St.

Louis, MO, U.S.A. All other substrates, coenzymes, enzymes and general reagents were of analytical grade and were obtained from Sigma, BDH Chemicals, Poole, Dorset, U.K. or Boehringer/Mannheim Pty., Mount Waverley, Vic. 3149, Australia.

*Metabolic studies*

Isolated liver parenchymal cells were prepared by a modification of the method of Berry & Friend (1969) from male rats of the Hooded Wistar strain (290–321 g body wt.) that had been deprived of food for 24h. In this modification hyaluronidase was omitted from the perfusion medium, which was $Ca^{2+}$-free Krebs-improved Ringer I, pH7.3, containing 4.9mM-pyruvate (Dawson *et al.*, 1969). The final concentration of collagenase was 0.037% (w/v). The hepatocytes were washed in a Krebs–Henseleit bicarbonate buffer (Dawson *et al.*, 1969) and finally suspended in the same buffer containing 2.5% (w/v) bovine serum albumin at pH7.3. All buffers were gassed with $O_2/CO_2$ (19:1). The hepatocytes (approx. 100mg wet wt./ml of buffer) were incubated for 60min at 37°C in stoppered 50ml glass Erlenmeyer flasks in an orbital shaking water bath (150 oscillations/min). After addition of substrates, radioisotopes and cells the final incubation volume was 5.0ml. The flasks were gassed for 15s with $O_2/CO_2$ (19:1) before being sealed with a Suba-Seal bung containing a small plastic cup for $CO_2$ collection. At the end of the experiment 0.25ml of phenethylamine was injected on to the filter paper wick housed in the plastic cup, and $^{14}CO_2$ was released by injecting 0.5ml of 12% (w/v) $HClO_4$ into the cell suspension. Zero-time incubations were similarly treated to obtain initial metabolite concentrations.

The flask contents were washed into tared graduated glass centrifuge tubes and made to 15ml with 99% (w/v) ethanol. After centrifugation portions of the supernatants were passed through three ion-exchange columns: Amberlite CG-120; Dowex 1 (acetate); Dowex 1 (borate). Water, glucose plus fructose, lactate, pyruvate and amino acids were eluted as described previously (Katz & Wals, 1972; Clark *et al.*, 1975).

The precipitated cell residue was washed with 15ml of 65% (w/v) ethanol, and the lipids were extracted with 2×10ml of chloroform/methanol (2:1, v/v). The extracted pellet was dried, weighed and then digested with 1.0ml of 30% (w/v) KOH at 100°C for 2–3h. Glycogen was precipitated with ethanol, washed and hydrolysed as described previously (Clark *et al.*, 1974). Liberated glucose and glucose in the incubation supernatant, after $HClO_4$ treatment, were measured by a glucose oxidase method (Boehringer/Mannheim Pty.). L(+)-Lactate was determined enzymically by the method of Hohorst (1963). ATP was measured in neutralized $HClO_4$ extracts by a modification of the method of Lamprecht & Trautschold (1963). Thus phosphoglucose isomerase (EC 5.3.1.9) was included in the assay system to convert fructose 6-phosphate to glucose 6-phosphate (Bernt & Bergmeyer, 1974).

Portions of the eluates from column chromatography, liver glycogen and $^{14}CO_2$ were assayed for radioactivity in a Triton-based liquid-scintillation mixture (Philippidis *et al.*, 1972).

*Hepatocyte heat production and $O_2$ consumption*

The production of heat by isolated hepatocytes was measured in the presence of various substrates by using an LKB model 10700 batch-type microcalorimeter, modified as described previously (Jarrett *et al.*, 1979). $O_2$ consumption was determined simultaneously by transferring 6ml of the cell suspension used for calorimetry to a Yellow Springs Instrument model 53 oxygen electrode assembly immediately after loading of the microcalorimeter. The hepatocytes used for the calorimetry study were from the same preparation of cells that were used in the metabolic studies.

*Measurement of cellular integrity*

This was assessed, before and after the incubations, by determining lactate dehydrogenase (EC 1.1.1.27) activity released into the medium and by staining with fresh Trypan Blue (Jarrett *et al.*, 1979). The rates of $O_2$ consumption and heat production were linear throughout the incubations in the presence or absence of added fructose.

*Statistical methods*

The statistical significance of differences between experimental groups was determined by the analysis of variance (Brownlee, 1949). A $P$ value of 0.05 or less was taken as the criterion of statistical significance.

**Results and Discussion**

During the incubations there was no change in free glucose in the control experiments (Table 1). However, in the presence of 2.5mM-, 7.5mM- and 25mM-fructose, final glucose concentrations rose by 30, 60 and 40% respectively. Similar changes were observed with lactate.

In the control incubations only 1% of initial [U-$^{14}$C]glucose radioactivity appeared in metabolic products. Most was recovered in $CO_2$ and amino acids (Table 1). With 2.5mM-fructose there was an apparent 4-fold stimulation in the incorporation of [U-$^{14}$C]glucose into metabolic products, principally $CO_2$, lactate and pyruvate. At the higher fructose concentrations the overall metabolism of [$^{14}$C]-glucose returned to control values (Table 1).

Table 1. *Effects of fructose concentration on the metabolism of [U-$^{14}$C]glucose by hepatocytes from starved rats*

Isolated hepatocytes were prepared from 24h-starved rats (299±3g body wt.) as described in the Materials and Methods section. The cells were diluted to 33ml with Krebs–Henseleit bicarbonate-buffered saline (Dawson *et al.*, 1969), containing 2.5% (w/v) bovine serum albumin (26.6±2.7mg dry wt. of cells/ml of buffer or $18.9\times10^6\pm1.04\times10^6$ cells/ml of buffer), and incubated for 1h in 5.0ml of the same buffer, 5mM in glucose and 2.5, 7.5 or 25mM in fructose. Glucose was labelled uniformly with $^{14}$C and specifically with $^3$H at positions 1, 2, 3 or 6. Values are the means±S.E.M. for incubations performed in quadruplicate; the numbers of observations are shown in parentheses. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

| [Fructose] (mM) | Glucose formed (μmol) | Lactate formed (μmol) | Apparent incorporation of $^{14}$C from [U-$^{14}$C]glucose (% of initial radioactivity incorporated/h per 27mg dry wt. of cells) into: | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | $CO_2$ | Lactate | Pyruvate | Amino acids | Glycogen | Total |
| 0 (4) | −0.05±0.15 | −0.38±0.20 | 0.48±0.09 | 0.10±0.04 | 0.12±0.04 | 0.24±0.02 | 0.11±0.02 | 1.05±0.21 (4) |
| 2.5 (4) | 7.36±0.16*** | 3.27±0.75 ** | 1.12±0.17* | 1.72±0.74** | 0.57±0.10** | 0.41±0.04** | 0.22±0.05 | 4.04±0.70** (4) |
| 7.5 (4) | 14.70±0.93*** | 8.91±0.93 *** | 0.35±0.01 | 0.29±0.11 | 0.19±0.14 | 0.13±0.04* | 0.22±0.05 | 1.18±0.35 (2) |
| 25.0 (4) | 9.92±0.83*** | 6.66±1.19 ** | 0.35±0.06 | 0.24±0.03 | 0.11±0.08 | 0.13±0.05 | 0.12±0.04 | 0.95±0.26 (2) |

In order to compare the metabolism of glucose and fructose some of the hepatocytes were incubated with [U-$^{14}$C]fructose at 2.5, 7.5 or 25mM and 5mM-glucose (Table 2). To make a more direct comparison the data are expressed as μg-atom of C incorporated into metabolic products/h per flask contents. In the incubations with 5mM-glucose and 2.5mM-fructose 3 times as much carbon from the ketose was incorporated into metabolic products (Table 2). With 7.5mM-fructose there was increased synthesis of lactate and pyruvate, but $CO_2$ production was not increased above that obtained with 2.5mM-fructose. Fructose at 25mM had no further effect on the apparent production of these metabolites (Table 2).

Simultaneously with the studies on the metabolism of [U-$^{14}$C]glucose and [U-$^{14}$C]fructose the effects of fructose concentration on the metabolism of [1-$^3$H]-, [2-$^3$H]-, [3-$^3$H]- or [6-$^3$H]-glucose were also examined (Table 3). The pattern of formation of $^3H_2O$ from the isotopically labelled glucoses conform to the previously published data in hepatocytes from food-deprived rats (Clark *et al.*, 1975). Thus the yield of $^3H_2O$ from [3-$^3$H]glucose was intermediate between that obtained with [2-$^3$H]- and [6-$^3$H]-glucose. This may be interpreted as indicating the presence of some cycling at glucose/glucose 6-phosphate and at fructose 6-phosphate/fructose 1,6-bisphosphate. With 5mM-glucose and 2.5mM-fructose there was a 2–4-fold increase in $^3H_2O$ production from the various $^3$H-labelled glucoses. As the yield of $^3H_2O$ from [3-$^3$H]glucose relative to that from [6-$^3$H]-glucose increased by more than 60% in the presence of 2.5mM-fructose (Table 3), this indicates increased substrate cycling at fructose 6-phosphate/fructose 1,6-bisphosphate. With the higher concentrations of fructose the yields of $^3H_2O$ from all $^3$H-labelled glucoses were depressed.

By using the analytical data (Table 1) and also the radioisotope data (Tables 1 and 3) it is possible to calculate the apparent rates of glucose phosphorylation, glycolysis and the dephosphorylation of glucose 6-phosphate (Clark *et al.*, 1973). As the concentration of glucose increased considerably in the incubations with added fructose, we have used the average concentrations of glucose in these calculations. The apparent rates of glycolysis were also determined by using $^3H_2O$ formation from [6-$^3$H]glucose. Values obtained by this method for rates of glycolysis are about 30% higher than those obtained by using $^{14}$C yields. This is not unexpected as most of the label from [$^3$H]lactate is lost when this compound is metabolized in the citric acid cycle and the gluconeogenic pathway (Rognstad & Wals, 1976). The data (Table 4) indicate that 1.1μmol of glucose was phosphorylated/h per 27mg wet wt. of cells; 30% of this glucose 6-phosphate was metabolized in the glycolytic pathway and 0.75μmol (70%) was dephosphorylated and returned to the incubation medium.

Table 2. *Metabolism of [U-$^{14}$C]glucose or [U-$^{14}$C]fructose by hepatocytes from starved rats*
The data with [U-$^{14}$C]glucose are derived from two of the experiments of Table 1. In some of the incubations with fructose at 2.5, 7.5 or 25mM, [U-$^{14}$C]glucose was replaced with [U-$^{14}$C]fructose. Results were calculated as incorporation of $\mu$g-atoms of $^{14}$C per flask contents in products. Values are means of results from incubations performed in quadruplicate.

| [Fructose] (mM) | [U-$^{14}$C]-Glucose | [U-$^{14}$C]-Fructose | Glucose formed ($\mu$mol) | Lactate formed ($\mu$mol) | Apparent incorporation of $^{14}$C from [U-$^{14}$C]glucose or [U-$^{14}$C]fructose ($\mu$g-atom of $^{14}$C/h per 27mg dry wt. of cells) into: $CO_2$ | Lactate | Pyruvate | Amino acids | Glycogen | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | + | — | −0.31 | −0.01 | 0.66 | 0.32 | 0.25 | 0.43 | 0.22 | 1.88 |
| 2.5 | + | — | 7.26 | 3.03 | 1.72 | 2.74 | 0.94 | 0.71 | 0.53 | 6.64 |
| 2.5 | — | + | 7.26 | 3.03 | 6.06 | 6.56 | 2.68 | 1.26 | 0.76 | 17.32 |
| 7.5 | — | + | 12.12 | 9.49 | 6.27 | 27.89 | 13.41 | 2.98 | 0.83 | 51.38 |
| 25.0 | — | + | 9.66 | 7.73 | 4.10 | 24.06 | 15.94 | 4.13 | 0.55 | 48.77 |
| 0 | + | — | −0.73 | −0.70 | 0.40 | 0.10 | 0.00 | 0.32 | 0.11 | 0.93 |
| 2.5 | + | — | 8.28 | 1.57 | 1.32 | 1.64 | 0.38 | 0.48 | 1.27 | 4.09 |
| 2.5 | — | + | 8.72 | 1.57 | 6.46 | 4.23 | 2.60 | 0.92 | 0.35 | 14.56 |
| 7.5 | — | + | 15.39 | 9.32 | 6.22 | 20.97 | 10.89 | 1.64 | 0.57 | 40.29 |
| 25.0 | — | + | 9.60 | 7.45 | 4.09 | 14.20 | 10.32 | 0.83 | 0.41 | 29.85 |

Table 3. *Effect of fructose on the metabolism of [1-$^3H$]-, [2-$^3H$]-, [3-$^3H$]- or [6-$^3H$]-glucose by hepatocytes from starved rats*
The data are from the same experiments as reported in Tables 1 and 2. Values are means ± S.E.M. for four independent experiments. *$P$ <0.05; **$P$ <0.01; ***$P$ <0.001.

| [Fructose] (mM) | Glucose formed ($\mu$mol) | Incorporation of $^3H$ into $^3H_2O$ (% of initial radioactivity) from: [1-$^3$H]Glucose | [2-$^3$H]Glucose | [3-$^3$H]Glucose | [6-$^3$H]Glucose |
|---|---|---|---|---|---|
| 0 | −0.05 | 2.40 ± 0.16 | 4.30 ± 0.25 | 2.15 ± 0.32 | 1.63 ± 0.22 |
| 2.5 | 7.36 | 4.13 ± 0.48* | 10.35 ± 1.46** | 7.65 ± 1.12** | 5.05 ± 0.48*** |
| 7.5 | 14.70 | 1.38 ± 0.25* | 2.75 ± 0.33** | 1.95 ± 0.30 | 1.50 ± 0.27 |
| 25.0 | 9.92 | 0.53 ± 0.13*** | 1.55 ± 0.05*** | 1.55 ± 0.31 | 1.25 ± 0.48 |

Table 4. *Utilization of glucose by hepatocytes from starved rats*
The average total glucose was determined by analysis of the medium. Apparent glucose phosphorylation was the average total glucose multiplied by the percentage yield of $^3H_2O$ from [2-$^3$H]glucose. Apparent glycolysis was calculated as for glucose phosphorylation, but either (A) by using the total yield of $^{14}$C from [U-$^{14}$C]glucose in the products or (B) by using the $^3H_2O$ yield from [6-$^3$H]glucose.

| Glucose formed ($\mu$mol) | [Fructose] (mM) | Average total glucose ($\mu$mol) | Apparent glucose phosphorylation ($\mu$mol/h per 27mg dry wt. of cells) | Apparent glycolysis ($\mu$mol/h per 27mg dry wt. of cells) (A) | (B) |
|---|---|---|---|---|---|
| −0.05 | 0 | 24.98 | 1.07 | 0.26 | 0.41 |
| 7.36 | 2.5 | 28.68 | 2.97 | 1.16 | 1.45 |
| 14.70 | 7.5 | 32.35 | 0.89 | 0.34 | 0.49 |
| 9.92 | 25.0 | 29.96 | 0.46 | 0.27 | 0.38 |

The same calculations with 5mM-glucose and 2.5mM-fructose suggest that 3$\mu$mol of glucose was phosphorylated by the cells. Over 40% of this was catabolized in the Emden–Meyerhof pathway, and 1.7$\mu$mol (57%) dephosphorylated. These data indicate a more than 2-fold increase in 'futile' cycling at glucose/glucose 6-phosphate. This result is an underestimate, as the glucose formed from fructose during these incubations lowers the specific radioactivity of the [2-$^3$H,U-$^{14}$C]glucose pool. Katz *et al.* (1978) have shown that calculations of glucose phosphorylation by $^3$H yields in water from [2-$^3$H]glucose underestimate the true rate by 25–35%. In addition these workers demonstrated that the error may be considerably higher when hepatocytes are incubated with fructose. It should be noted that

the rate of cycling at glucose/glucose 6-phosphate relative to the rate of glycolysis (as determined by the yields of $^{14}C$ from [U-$^{14}C$]glucose in metabolic products) decreased from a value of 3 in the control incubations to 1.5 in the presence of 2.5mM-fructose (Table 4).

There are insufficient data in these experiments to estimate the rate of cycling at fructose 6-phosphate/fructose 1,6-bisphosphate, but the increase in $^3H_2O$ production from [3-$^3$H]glucose relative to that from [6-$^3$H]glucose (Table 3) would suggest that cycling at fructose phosphate was also increased in the presence of 2.5mM-fructose.

The hepatocytes used in this investigation were also employed to measure heat output and $O_2$ consumption in the presence and absence of added fructose (Table 5). The addition of 25mM-fructose to 5mM-glucose caused a 37% decrease in $O_2$ uptake and a 24% decrease in heat production in these hepatocytes. Conversely 2.5mM-fructose caused a 43% increase in $O_2$ consumption and a 64% increase in heat output. Both these latter changes were highly significant statistically ($P<0.001$). As $O_2$ uptake and hence substrate catabolism and ATP production were stimulated to a much smaller extent than the heat output, the data obtained with 2.5mM-fructose indicate less efficient metabolism in these cells (Jarrett *et al.*, 1979). These results, taken with the increased formation of $^3H_2O$ from [2-$^3$H]- and [3-$^3$H]-glucose, must be interpreted as increased cycling.

In the experiments with fructose at higher concentrations both glucose and lactate synthesis were increased, but $O_2$ uptake, heat output and the production of $^3H_2O$ from [1-$^3$H]-, [2-$^3$H], [3-$^3$H]- or [6-$^3$H]-glucose were decreased to less than the control values. These effects could result from the fall in liver ATP concentration due to excess phosphorylation of fructose when present at high concentrations (Maënpää *et al.*, 1968; Van den Berghe, 1978). In order to test this hypothesis the effect of different concentrations of fructose on ATP concentrations in hepatocytes from 24h-starved rats was determined. At 2.5mM-fructose the ATP concentration was decreased by 20%, but at 7.5mM- and 25mM-fructose the concentrations of the nucleotide were lowered by 65 and 80% respectively (Fig. 1). In contrast with this, van de Werve & Hers (1979) have reported that fructose at concentrations between 2.5 and 28mM lowered hepatocyte ATP concentration by over 80%. The reason for this discrepancy is not

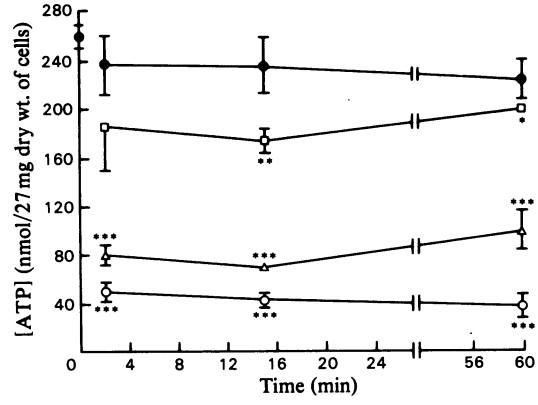


Fig. 1. *Effects of various concentrations of fructose on the concentration of ATP in hepatocytes from starved rats*
Isolated hepatocytes were prepared from three 24h-starved rats (314±7g body wt.) as described in the Materials and Methods section. The cells were diluted to 33ml with Krebs–Henseleit bicarbonate-buffered saline (Dawson *et al.*, 1969), containing 2.5% (w/v) bovine serum albumin (28.7±3.6mg dry wt. of cells/ml of buffer or $19.3\times10^6\pm1.3\times10^6$ cells/ml of buffer) and incubated in the same buffer for 0, 2, 15 or 60min. ATP was determined as described in the text and is expressed as nmol/27mg dry wt. of cells. ●, Control; □, +2.5mM-fructose; △, +7.5mM-fructose; ○, +25mM-fructose. Values are shown as means±S.E.M. The differences from the control values at appropriate time intervals were significant at: $*P<0.05$; $**P<0.01$; $***P<0.001$.

Table 5. *Effect of fructose on $O_2$ uptake and heat output by hepatocytes from starved rats*
Isolated hepatocytes were prepared from 24h-starved rats (299±3g body wt.) as described in the Materials and Methods section. The cells were diluted in Krebs–Henseleit bicarbonate-buffered saline (Dawson *et al.*, 1969), containing 2.5% (w/v) bovine serum albumin to give approx. $10^6$ cells/ml. After pre-equilibration of the cell suspension at 37°C with the appropriate substrates for 6min, 12ml was transferred to the microcalorimeter and 6ml to the oxygen electrode assembly. Values are shown as means±S.E.M. and for four observations. $*P<0.05$; $**P<0.01$; $***P<0.001$.

| Additions to medium | $O_2$ uptake (μmol/min per g dry wt.) | Heat output (J/min per g dry wt.) | $O_2$ uptake / heat output (μmol/J) |
|---|---|---|---|
| None | 8.86±0.33 | 4.97±0.25 | 1.79±0.07 |
| 2.5mM-Fructose | 12.7±0.53*** | 8.17±0.39*** | 1.56±0.05* |
| 7.5mM-Fructose | 7.65±0.35 | 4.95±0.36 | 1.55±0.04* |
| 25mM-Fructose | 5.57±0.19** | 3.76±0.13** | 1.49±0.09* |

clear, as other studies have shown that 2mM-fructose has no effect on the concentration of ATP in hepatocytes from fed animals (Clark *et al.*, 1977) or in the perfused rat liver (Topping & Mayes, 1977). A partial explanation for this paradox is found when it is realized that most determinations of ATP concentration by the method of Lamprecht & Trautschold (1963) do not allow for the formation of fructose 6-phosphate when fructose is present in the neutralized cellular extracts (Bernt & Bergmeyer, 1974).

One of the major effects of 2.5mM-fructose in this investigation was to stimulate greatly the catabolism of [U-$^{14}$C]- and [6-$^{3}$H]-glucose. This would increase ATP consumption at hexokinase (EC 2.7.1.1) and at phosphofructokinase (EC 2.7.1.11), but it would also lead to a concomitant increase in ATP production in the glycolytic pathway and in the citric acid cycle. These effects help to explain the increase in hepatocyte $O_2$ consumption and heat production, but they do not account for the fall in the ratio $O_2$ uptake/heat output (Table 5). As gluconeogenesis from fructose requires 2mol of ATP/mol of glucose formed, the production and hydrolysis of this high-energy intermediate for glucose synthesis could account for some of the enhanced heat production and $O_2$ consumption observed with 2.5mM-fructose. This explanation is untenable, however, as at higher concentrations of fructose (7.5mM), where glucose production was greatly stimulated (Table 1), neither $O_2$ uptake nor heat production differed from control values (Table 5). The most reasonable conclusion is that increased substrate cycling was responsible for the fall in the ratio $O_2$ uptake/heat output, and the data of Tables 3 and 4 indicate that the cycles glucose/glucose 6-phosphate and fructose 6-phosphate/fructose 1,6-bisphosphate were increased.

Other investigators have studied some of the effects of fructose and dihydroxyacetone on glucose metabolism in isolated hepatocytes. Clark *et al.* (1974*b*) demonstrated increased rates of glucose production with these substrates at 2.5, 5.0 and 10mM. At all concentrations dihydroxyacetone increased the loss of $^{3}$H from [5-$^{3}$H]glucose by 15–30%. Fructose at 2.5mM increased the detritiation of [5-$^{3}$H]glucose by 40%, but at the higher concentrations $^{3}H_2O$ formation was depressed by 15–40%. Although these changes are similar to those observed in the present experiments with [3-$^{3}$H]glucose, the increased detritiation of [5-$^{3}$H]glucose in the presence of 2.5mM-fructose was considerably smaller than that observed with [3-$^{3}$H]glucose (Table 3). This may be because $^{3}$H from [5-$^{3}$H]glucose is lost both at triose phosphate isomerase (EC 5.3.1.1) and in an exchange reaction catalysed by transaldolase (EC 2.2.1.2) (Katz *et al.*, 1975). In some similar studies Katz *et al.* (1975) showed that fructose, at 10$\mu$mol/ml, inhibited the loss of $^{3}$H from [2-$^{3}$H]-, [3-$^{3}$H]- or [5-$^{3}$H]-glucose in isolated rat hepatocytes, whereas 10mM-dihydroxyacetone stimulated $^{3}H_2O$ production from these glucoses by 5–10%. These workers also studied the effects of these and other substrates on the metabolism of [U-$^{14}$C]glucose. Dihydroxyacetone was the only substrate that did not depress the utilization of [$^{14}$C]glucose. The physiological significance of this latter finding is obscure, as this substrate was used at a concentration vastly in excess of that found *in vivo* (Topping & Mayes, 1977).

To our knowledge this is the first study in which a naturally occurring substrate, at physiological concentrations, has been shown to increase the cycles glucose/glucose 6-phosphate and fructose 6-phosphate/fructose 1,6-bisphosphate. Although isolated hepatocytes provide a very useful method for preliminary detailed studies of acute effects on liver metabolism, it should be realized that these cells do not generally receive either the constant supply of lactate that is obtained *in vivo* (Topping & Mayes, 1971) or of $O_2$, fatty acids or amino acids. Consequently, further studies on the effects of fructose on glucose metabolism and substrate cycling in livers from fed and starved rats perfused with whole rat blood are required.

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