*Biochem. J.* (1973) **134**, 143–156
*Printed in Great Britain*



# Induction and Suppression of the Key Enzymes of Glycolysis and Gluconeogenesis in Isolated Perfused Rat Liver in Response to Glucose, Fructose and Lactate

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(*Received* 3 *October* 1972)

1. Measurements were made of the activities of the four key enzymes involved in gluconeogenesis, pyruvate carboxylase (EC 6.4.1.1), phosphoenolpyruvate carboxylase (EC 4.1.1.32), fructose 1,6-diphosphatase (EC 3.1.3.11) and glucose 6-phosphatase (EC 3.1.3.9), of serine dehydratase (EC 4.2.1.13) and of the four enzymes unique to glycolysis, glucokinase (EC 2.7.1.2), hexokinase (EC 2.7.1.1), phosphofructokinase (EC 2.7.1.11) and pyruvate kinase (EC 2.7.1.40), in livers from starved rats perfused with glucose, fructose or lactate. Changes in perfusate concentrations of glucose, fructose, lactate, pyruvate, urea and amino acid were monitored for each perfusion. 2. Addition of 15mM-glucose at the start of perfusion decreased the activity of pyruvate carboxylase. Constant infusion of glucose to maintain the concentration also decreased the activities of phosphoenolpyruvate carboxylase, fructose 1,6-diphosphatase and serine dehydratase. Addition of 2.2mM-glucose initially to give a perfusate sugar concentration similar to the blood sugar concentration of starved animals had no effect on the activities of the enzymes compared with zero-time controls. 3. Addition of 15mM-fructose initially decreased glucokinase activity. Constant infusion of fructose decreased activities of glucokinase, phosphofructokinase, pyruvate carboxylase, phosphoenolpyruvate carboxylase, glucose 6-phosphatase and serine dehydratase. 4. Addition of 7mM-lactate initially elevated the activity of pyruvate carboxylase, as also did constant infusion; maintenance of a perfusate lactate concentration of 18mM induced both pyruvate carboxylase and phosphoenolpyruvate carboxylase activities. 5. Addition of cycloheximide had no effect on the activities of the enzymes after 4h of perfusion at either low or high concentrations of glucose or at high lactate concentration. Cycloheximide also prevented the loss or induction of pyruvate carboxylase and phosphoenolpyruvate carboxylase activities with high substrate concentrations. 6. Significant amounts of glycogen were deposited in all perfusions, except for those containing cycloheximide at the lowest glucose concentration. Lipid was found to increase only in the experiments with high fructose concentrations. 7. Perfusion with either fructose or glucose decreased the rates of ureogenesis; addition of cycloheximide increased urea efflux from the liver.

The activities of many hepatic enzymes are known to vary in response to a variety of nutritional and endocrine changes. In particular, on starvation and in response to glucocorticoids there is an increase in the activity of several enzymes associated with gluconeogenesis. Also, ingestion of carbohydrate increases activities of certain glycolytic enzymes, which also respond to administration of insulin. Work with intact and alloxan-diabetic animals leads to the general conclusion that nutritionally induced changes in enzyme activity are mediated through stimulated or suppressed endocrine secretion.

There are, however, few observations of change in enzyme activity brought about in the perfused liver by substrate or change in concentration of metabolic intermediates alone under conditions in which hormones must be playing a minimal part (Veneziale *et al.*, 1967; Buschiazzo *et al.*, 1970). Wimhurst & Manchester (1970*a*) showed that addition of glucose appears to diminish activity of pyruvate carboxylase. The present report is a more systematic investigation of the extent to which addition of various metabolites to the perfused liver system affects the activities of the key enzymes of glycolysis and gluconeogenesis, namely glucokinase (EC 2.7.1.2), hexokinase (EC 2.7.1.1), phosphofructokinase (EC 2.7.1.11), pyruvate

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kinase (EC 2.7.1.40), pyruvate carboxylase (EC 6.4.1.1), phosphoenolpyruvate carboxylase (EC 4.1.1.32), fructose 1,6-diphosphatase (EC 3.1.3.11), glucose 6-phosphatase (EC 3.1.3.9), and of serine dehydratase (EC 4.2.1.13).

## Materials and Methods

### *Materials*

*Chemicals.* ITP (disodium salt), glucose 6-phosphate (sodium salt), fructose 6-phosphate (sodium salt), fructose 1,6-diphosphate (sodium salt), glutathione, sodium pyruvate, CoA, $NADP^+$, $NAD^+$, NADH, acetylphosphate (potassium lithium salt), oxaloacetate, phosphoenolpyruvate (potassium salt), ADP, AMP, phosphotransacetylase, citrate synthase, phosphoglucose isomerase, glucose 6-phosphate dehydrogenase, lactate dehydrogenase, aldolase, triose phosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase and total lipid assay kit were purchased from C.F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. Tris (Trizma base) and L(+)-lactic acid were purchased from Sigma (London) Chemical Co., London S.W.6, U.K. Pyridoxal phosphate and serine were obtained from British Drug Houses Ltd., Poole, Dorset, U.K., and crystalline bovine plasma albumin came from Armour Pharmaceutical Co., Eastbourne, Sussex, U.K. $NaH^{14}CO_3$ was purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and Halothane is a product of I.C.I. Ltd., Pharmaceuticals Division, Macclesfield, Cheshire, U.K.

*Animals.* Rats used were male albino Wistars, weight 150–200g for liver donors, and 300g or more for blood donors. They were mostly supplied from A. Tuck and Sons, Rayleigh, Essex, U.K.

### *Methods*

Livers from 150–200g male rats starved for 18h were perfused in the recycling circuit described by Mortimore & Tietze (1959). Infusion of substrate and sampling of perfusate was via the oxygenator reservoir drum. The perfusate (75–80ml) consisted of fresh blood taken from starved male rats under Halothane anaesthesia and diluted to 40% with Krebs–Ringer bicarbonate buffer (Krebs & Henseleit, 1932), haematocrit was 20% and a final heparin concentration of 125 units/ml was used. Flow rate was 6ml/min. Substrates were added as indicated. Blood glycolysis was measured either by using a second circuit without a liver, or by sampling the perfusate over a period before cannulation of the organ. Organ donors were anaesthetized with Halothane and the liver was cannulated by the technique of Miller *et al.* (1951). Choice of Halothane for anaesthesia was based on the findings by Bloxam (1967) of its minimal toxicity. Any effects appear to be readily reversible (Biebuyck *et al.*, 1972). The period of hypoxia was decreased to approximately 30s by inserting the cannula into the hepatic portal vein with a slow flow rate during the manipulations. After perfusion for 4h at 37°C livers were cleared of blood by perfusion with 10ml of cold 0.9% NaCl, complete removal of blood cells being used as a criterion of preparation viability. The liver was frozen rapidly to −80°C, weighed and stored at −80°C until pyruvate carboxylase was assayed, which was within 24h of perfusion. It was then stored at −30°C. All enzymes were assayed within 7 days of perfusion. Control perfusions for the assay of enzyme activities at zero time were operated on as described above, but were flushed directly with NaCl without connection to the perfusion circuit. Increase or decrease in activity of a particular enzyme as a result of perfusion was assessed by comparison with activity of the appropriate controls.

Enzymes were assayed at 30°C as described previously (Wimhurst & Manchester, 1970*c*, 1972). Serine dehydratase was extracted by homogenization of a portion of liver in 9vol. of 0.1M-$K_2HPO_4$, pH8.6, containing 0.05mM-pyridoxal phosphate. Activity in the supernatant, after centrifugation for 1h at 100000*g*, was assayed in a total volume of 1ml containing 50mM-Tris–HCl, pH8.6, 112mM-KCl, 0.05mM-pyridoxal phosphate, 0.25mM-NADH, 100mM-serine. The reaction was started by addition of 20$\mu$l of enzyme extract and the rate of loss of NADH at 30°C was followed with a Unicam SP.800 recording spectrophotometer. The rate of disappearance of NADH in the absence of serine was subtracted from these values.

Perfusate glucose, fructose, lactate, pyruvate, urea and amino acids and liver $K^+$, glycogen, water, lipid, protein, DNA and RNA were assayed by the methods of Wimhurst & Manchester (1970*a*).

## Results

The purpose of the investigation was to study changes in enzymic activity of liver after perfusion with a variety of substrates. In particular we studied the extent to which activities enhanced by starvation decline when fed conditions are simulated in contrast with any increase in activity of enzymes normally repressed in the starved state. Activities of enzymes are expressed first as concentration [units/g of liver (specific activity)] and secondly as content [units/100$\mu$g of DNA P (total activity)], to give two reference points so that change in activity caused by change in organ weight is distinguished from increase or decrease in activity of the liver as a whole.

Many non-enzymic parameters of the liver and perfusate were also measured. These are contained in Table 1, to which reference is to be made in relation to the other tables. The DNA content of liver from

Table 1. *Protein, lipid, nucleic acid, glycogen, $K^+$ and amino acid content of livers in perfusion conditions described in subsequent tables, and amounts of pyruvate, urea and amino acid efflux*

Each value is the mean ±S.E.M. of the number of observations indicated (*n*). Perfusions were performed in three groups (see the text), columns 1–8 being the perfusion conditions of Tables 2, 4, 6 and 7 sequentially whose legends state full details of additions to the perfusate; columns 9–11 refer to Table 8; and columns 12–16 refer to Tables 3 and 5. * Indicates significant difference (*P* at least <0.05) from appropriate zero-time control.

| | (1) Starved control rats | (2) Fed control rats | (3) Glucose added initially | (4) Glucose infused | (5) Fructose added initially | (6) Fructose infused | (7) Lactate added initially | (8) Lactate infused |
|---|---|---|---|---|---|---|---|---|
| *n* ... | (6) | (6) | (4) | (4) | (4) | (4) | (4) | (4) |
| Total protein (mg/g of liver) | 233±6.5 | 233±2.6 | 242±9.4 | 228±9.1 | 231±10.4 | 221±10.5 | 224±4.2 | 215±10.6 |
| Total lipid (mg/g of liver) | 59±2.8 | 79±3.9 | 59±4.4 | 51±4.9 | 45±5.0 | 75±3.3* | 61±4.7 | 53±5.0 |
| Water/solid | 2.73±0.09 | 2.84±0.08 | 2.83±0.06 | 2.85±0.11 | 2.98±0.04 | 2.93±0.05 | 2.85±0.08 | 2.89±0.08 |
| $K^+$ (μmol/g of liver) | 93±3.1 | 90±2.1 | 91±3.2 | 94±2.4 | 94±3.3 | 90±3.8 | 95±3.5 | 96±2.6 |
| DNA (μg of P/g of liver) | 238±4.6 | 153±6.8* | 238±7.1 | 240±5.1 | 236±3.3 | 238±2.5 | 254±7.1 | 237±4.4 |
| RNA (μg of P/g of liver) | 985±36 | 846±49 | 998±45 | 949±41 | 953±15 | 884±27 | 1022±26 | 959±30 |
| RNA/DNA | 4.2±0.13 | 5.5±0.22* | 4.2±0.10 | 4.0±0.14 | 4.0±0.06 | 3.7±0.08* | 4.0±0.09 | 4.1±0.18 |
| Glycogen (mg/g of liver) | 0.26±0.03 | 38.8±7.1* | 2.0±0.53* | 3.7±0.87* | 4.7±1.2* | 14.6±3.5* | 0.82±0.13* | 1.2±0.34* |
| Hepatic amino acid (mg of amino N/g of liver) | 0.25±0.03 | 0.28±0.05 | 0.29±0.04 | 0.24±0.02 | 0.23±0.03 | 0.23±0.03 | 0.36±0.02 | 0.36±0.06 |
| Amino acid efflux (mg of amino N/4h per g of liver) | | | 0.28±0.07 | 0.23±0.04 | 0.23±0.02 | 0.27±0.04 | 0.32±0.01 | 0.33±0.06 |
| Pyruvate efflux (μmol/4h per g of liver) | | | 1.29±0.17 | 2.24±0.45 | −0.19±0.29 | 2.95±0.93 | −0.52±0.45 | 5.24±1.16 |
| Urea synthesis (mg of N/4h per g of liver) | | | 0.71±0.01* | 0.58±0.03* | 0.48±0.03 | 0.12±0.04* | 1.02±0.06 | 0.96±0.06 |

| | (9) Lactate infused | (10) Lactate infused+ cycloheximide | (11) Starved control rats | (12) Glucose added initially | (13) Glucose added initially+ cycloheximide | (14) Starved control rats | (15) Glucose infused+ cycloheximide | (16) Starved control rats |
|---|---|---|---|---|---|---|---|---|
| *n* ... | (4) | (4) | (8) | (4) | (4) | (4) | (4) | (4) |
| Total protein (mg/g of liver) | 236±5.0 | 253±10.5 | 253±6.6 | 275±9.1 | 273±11.6 | 272±13.8 | 247±12 | 252±11 |
| Total lipid (mg/g of liver) | 26±3.4 | 30±2.3 | 30±1.4 | 25±2.6 | 25±2.2 | 30±4.1 | 42±1.8 | 41±4.7 |
| Water/solid | 3.05±0.03 | 2.91±0.05 | 2.81±0.09 | 2.82±0.08 | 2.88±0.14 | 2.61±0.09 | 2.70±0.12 | 2.50±0.11 |
| $K^+$ (μmol/g of liver) | 92±6.4 | 97±4.3 | 94±3.4 | 101±2.0 | 96±5.5 | 106±3.8 | 96±3.1 | 101±2.6 |
| DNA (μg of P/g of liver) | 334±4.2 | 347±12.4 | 353±8.9 | 376±6.1 | 366±10.5 | 382±10.0 | 370±12 | 371±6 |
| RNA (μg of P/g of liver) | 905±22 | 929±26 | 961±32 | 996±17* | 994±25 | 1063±17 | 1173±31 | 1258±17 |
| RNA/DNA | 2.6±0.06 | 2.7±0.03 | 2.7±0.06 | 2.7±0.04 | 2.7±0.07 | 2.8±0.10 | 3.20±0.11 | 3.38±0.03 |
| Glycogen (mg/g of liver) | 1.7±0.54* | 1.7±0.34* | 0.26±0.09 | 1.6±0.35* | 0.81±0.19 | 0.32±0.16 | 3.42±0.48* | 0.33±0.01 |
| Hepatic amino acid (mg of amino N/g of liver) | 0.25±0.04 | 0.18±0.03 | 0.24±0.02 | 0.26±0.02 | 0.30±0.03 | 0.32±0.03 | 0.44±0.04 | 0.41±0.03 |
| Amino acid efflux (mg of amino N/4h per g of liver) | 0.41±0.04 | 0.33±0.02 | | 0.37±0.05 | 0.65±0.05* | | 0.50±0.06 | |
| Pyruvate efflux (μmol/4h per g of liver) | 8.94±1.23 | 9.64±1.88 | | 0.32±0.62 | 0.03±0.23 | | 2.71±0.48 | |
| Urea synthesis (mg of N/4h per g of liver) | 0.84±0.07 | 1.33±0.07 | | 1.08±0.07 | 1.92±0.11* | | 1.64±0.07* | |

Table 2. *Comparison of the concentration and content of the hepatic glycolytic and gluconeogenic enzymes in livers from fed rats and rats starved for* 18 *h*

Each value is the mean±S.E.M. of six observations. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, with respect to fed animals. For further details see Table 1 and the text.

| | Enzyme concentration and content in rat livers | | | |
|---|---|---|---|---|
| | Fed rats | | Starved rats | |
| Enzyme | (μmol/min per g of liver) | (μmol/min per 100 μg of DNA P) | (μmol/min per g of liver) | (μmol/min per 100 μg of DNA P) |
| Glucokinase | 2.54±0.27 | 1.67±0.19 | 2.89±0.31 | 1.22±0.13 |
| Hexokinase | 0.59±0.08 | 0.40±0.07 | 0.91±0.01** | 0.38±0.04 |
| Phosphofructokinase | 7.63±0.31 | 5.02±0.25 | 6.98±0.26 | 2.97±0.10*** |
| Pyruvate kinase | 86 ±7.7 | 55 ±5.5 | 62 ±4.0* | 26 ±1.2*** |
| Pyruvate carboxylase | 2.99±0.22 | 1.96±0.12 | 6.05±0.10*** | 2.53±0.08** |
| Phosphoenolpyruvate carboxylase | 3.19±0.21 | 2.08±0.09 | 6.40±0.07*** | 2.71±0.06*** |
| Fructose 1,6-diphosphatase | 11.9 ±0.92 | 7.86±0.66 | 12.8 ±0.76 | 5.42±0.35** |
| Glucose 6-phosphatase | 10.6 ±0.74 | 6.9 ±0.41 | 20.4 ±0.50*** | 8.6 ±0.33** |
| Serine dehydratase | 0.66±0.10 | 0.43±0.06 | 1.78±0.12*** | 0.76±0.05** |

starved rats in groups 1–8 was fairly constant but substantially different from that in groups 9–16. The reason for this is not certain, but has necessitated the use of three different groups of control animals. Variability in enzyme activities in different batches of animals was encountered, as also noted by others (Bartley *et al.*, 1967; Eggleston & Krebs, 1969; Garfinkel, 1971; Berdanier *et al.*, 1971). Hepatic concentrations of $K^+$ and water, used to assess the viability of the perfused-liver preparation (Bloxam, 1967), were unchanged relative to zero-time controls (Table 1). Protein and amino acid concentrations were also uniform. All perfusions, except those at low glucose concentrations in the presence of cycloheximide, showed small but significant glycogen depositions in contrast to the work of Ross *et al.* (1967) in which the presence of a minimum perfusate glucose concentration of 10mM was required before glycogen increased. Most glycogen was produced with fructose as substrate; significant lipid deposition was found only in the presence of high concentrations of fructose.

*Effects of starvation*

Table 2 compares the activities of the enzymes unique to gluconeogenesis and glycolysis and serine dehydratase in livers from fed rats and control rats starved overnight. The increase in the tissue content of pyruvate carboxylase, phosphoenolpyruvate carboxylase and glucose 6-phosphatase and the decrease in fructose 1,6-diphosphatase in livers from starved rats confirm earlier results (Wimhurst & Manchester, 1970*c*). Of the glycolytic enzymes, the decrease in pyruvate kinase (to 47%) was most pronounced and similar to published data (Krebs & Eggleston, 1965; Tanaka *et al.*, 1967). Phosphofructokinase decreased to 59% of the concentration found in livers from fed rats (as in studies of Weber *et al.*, 1966). The increase in concentration of hexokinase after starvation resulted from loss of cell constituents, since the tissue DNA increased from 153 to 238 μg of DNA P/g of liver (Table 1). The lack of change in content of hepatic hexokinase is consistent with it being located exclusively in non-parenchymal cells (Sapag-Hagar *et al.*, 1969). Although the hepatic content of glucokinase decreased by 25%, the decrease is not significant and compares with the small loss of this enzyme after short periods of starvation, as found by Salas *et al.* (1963) and Sharma *et al.* (1963), though less than that found by Walker & Rao (1964) and Weber *et al.* (1966). Serine dehydratase showed an increase in both content and concentration similar to the results of Bojanowska & Williamson (1968).

Comparison of the ratio of the glycolytic to gluconeogenic enzymes at each of the potential control sites showed significant decreases, the ratio pyruvate kinase/pyruvate carboxylase and pyruvate kinase/phosphoenolpyruvate carboxylase declining from 29 to 10 and from 27 to 10 respectively in livers from rats starved overnight. Although the ratios phosphofructokinase/fructose 1,6-diphosphatase and glucokinase plus hexokinase/glucose 6-phosphatase increased from 0.19 to 0.29 and 0.54 to 0.64, neither

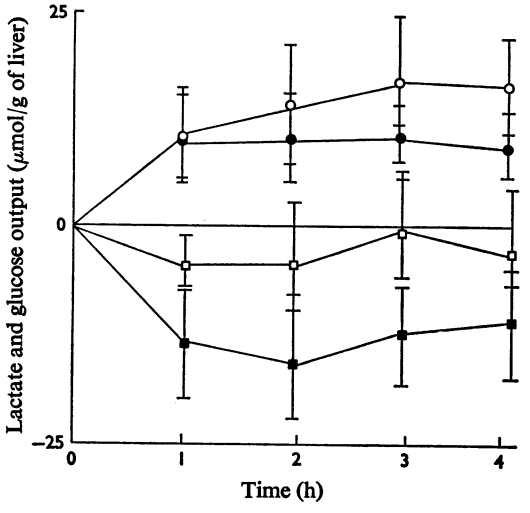


Fig. 1. *Alterations in perfusate lactate and glucose in experiments where 2.2 mM-glucose was given initially either with or without cycloheximide at 25 μg/ml*

The results are from the perfusions reported in Table 3. Bars on points are ±1 S.E.M. ($n = 4$). ■, Lactate uptake and ●, glucose output in the absence of cycloheximide. □, Lactate uptake and ○, glucose output with 25 μg of cycloheximide/ml.

change was statistically significant for 18 h starvation. Söling *et al.* (1971) found large increases in these ratios after 48 h starvation.

*Perfusions with glucose*

Output and uptake of glucose and lactate in perfusions at three different initial concentrations of perfusate glucose are shown in Figs. 1 and 2. Addition initially of 2.2 mM-glucose maintained a steady glucose concentration of 80–90 mg% in the perfusing medium. There were no significant differences in enzyme activities between livers perfused for 4 h at this glucose concentration and those found in zero-time controls (Table 3). Table 3 also shows experiments in which cycloheximide was included in the perfusate. With the rather surprising exception of hexokinase, no significant changes were seen in these perfusions either with respect to the zero-time controls or the full-term perfusions. Cycloheximide decreased lactate uptake ($P < 0.05$) and possibly enhanced ($P > 0.05$) glucose output (Fig. 1).

The activities of the enzymes corresponding to the perfusions in Fig. 2 are shown in Table 4. Initial addition of 14 mM-glucose resulted in a 40% increase

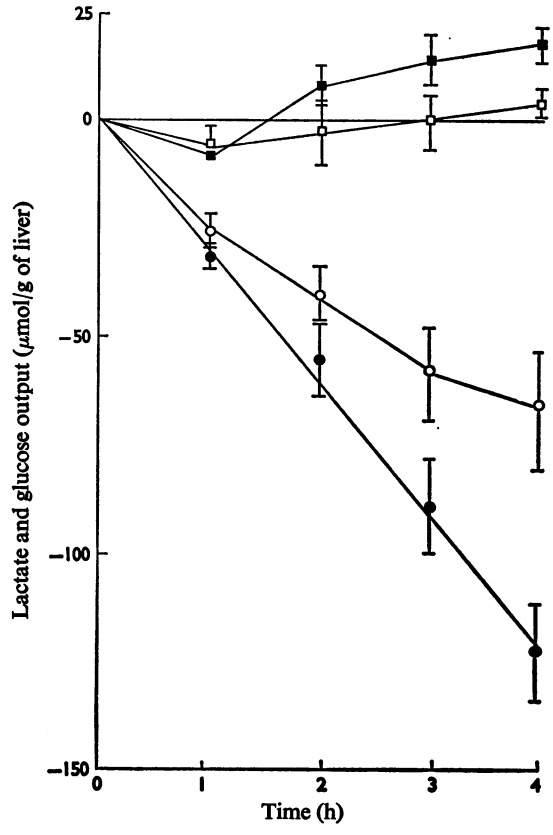


Fig. 2. *Uptake of glucose and output of lactate from livers perfused with glucose*

The results are from the perfusions reported in Table 4. Bars on points are ±1 S.E.M. ($n = 4$). □, Lactate output and ○, glucose uptake from perfusate containing 14 mM-glucose added initially. ■, Lactate output and ●, glucose uptake from perfusate containing 14 mM-glucose given initially plus a continuous infusion of 3.5 μmol of glucose/min to balance the rate of glucose uptake.

in the activity of pyruvate kinase and an 18% decrease in pyruvate carboxylase activity. A similar decrease in pyruvate carboxylase was found with glucose infusion, coupled with a 25% decrease in phosphoenolpyruvate carboxylase. The net result was a statistically significant increase in the ratio pyruvate kinase/pyruvate carboxylase from 10.2 in the controls to 17.8 and 14.5 with initial and infused dosages, and similar increases from 9.7 to 14.8 and 15.3 for the ratio pyruvate kinase/phosphoenolpyruvate carboxylase. Significant decreases in content and concentration of serine dehydratase and fructose

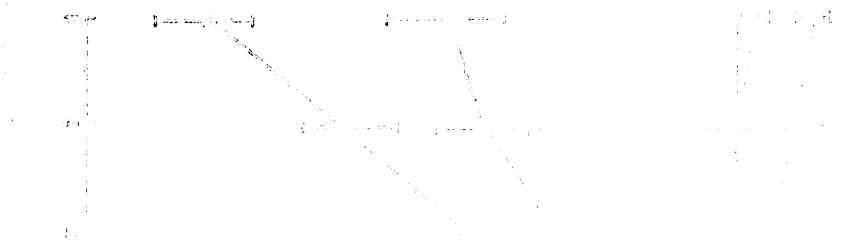

Table 3. *Comparison of the concentration and content of the glycolytic and gluconeogenic enzymes in livers perfused with a low concentration of glucose and with cycloheximide*

Each value is the mean ±S.E.M. of four observations: **P*<0.01 with respect to glucose perfusion. Glucose was added to the perfusate initially to 2.2 mM. Cycloheximide where present was at 25 μg/ml. Control values are from livers of starved animals set up for perfusion but where the perfusion was stopped at zero time. For further details see Table 1 and the text.

| | Enzyme concentration and content in rat livers | | | | | |
|---|---|---|---|---|---|---|
| | Perfused with added glucose | | Cycloheximide added | | Control values | |
| Enzyme | (μmol/min per g of liver) | (μmol/min per 100 μg of DNA P) | (μmol/min per g of liver) | (μmol/min per 100 μg of DNA P) | (μmol/min per g of liver) | (μmol/min per 100 μg of DNA P) |
| Glucokinase | 2.65±0.06 | 0.71±0.02 | 3.05±0.44 | 0.84±0.13 | 2.92±0.35 | 0.78±0.10 |
| Hexokinase | 0.69±0.05 | 0.19±0.01 | 0.45±0.02 | 0.12±0.00* | 0.68±0.12 | 0.18±0.03 |
| Phosphofructokinase | 7.40±0.25 | 1.98±0.07 | 6.70±0.27 | 1.84±0.09 | 7.45±0.16 | 1.97±0.01 |
| Pyruvate kinase | 59 ±4.7 | 15.7 ±1.4 | 60 ±3.1 | 16.4 ±1.2 | 61 ±3.8 | 16.5 ±1.3 |
| Pyruvate carboxylase | 5.85±0.13 | 1.56±0.05 | 6.00±0.11 | 1.65±0.06 | 5.80±0.19 | 1.53±0.08 |
| Phosphoenolpyruvate carboxylase | 11.6 ±0.11 | 3.09±0.77 | 11.4 ±0.19 | 3.15±0.06 | 11.8 ±0.24 | 3.12±0.11 |
| Fructose 1,6-diphosphatase | 10.5 ±0.4 | 2.71±0.17 | 9.6 ±0.3 | 2.65±0.06 | 11.1 ±0.5 | 2.91±0.17 |
| Glucose 6-phosphatase | 19.8 ±1.1 | 5.30±0.35 | 19.5 ±0.6 | 5.36±0.23 | 21.5 ±1.2 | 5.69±0.23 |
| Serine dehydratase | 2.76±0.55 | 0.77±0.13 | 3.61±1.12 | 1.00±0.32 | 2.72±0.21 | 0.72±0.04 |

Table 4. *Comparison of the concentration and content of the glycolytic and gluconeogenic enzymes in livers perfused with different amounts of glucose*

Each value is the mean ±S.E.M. of four observations: *$P<0.05$, **$P<0.01$, between the two perfusions; †$P<0.05$, ††$P<0.01$, †††$P<0.001$ with respect to starved rats (Table 2). Glucose was added initially to the perfusate to 14mM, followed where indicated by constant infusion of 3.5μmol of glucose/min. For further details see Table 1 and the text.

| | Enzyme concentration and content in rat livers | | | |
|---|---|---|---|---|
| | Glucose added initially | | Glucose added initially and by constant infusion | |
| Enzyme | (μmol/min per g of liver) | (μmol/min per 100μg of DNA P) | (μmol/min per g of liver) | (μmol/min per 100μg of DNA P) |
| Glucokinase | 2.35±0.22 | 1.01±0.10 | 2.56±0.06 | 1.06±0.03 |
| Hexokinase | 0.66±0.08 | 0.29±0.03 | 0.64±0.04 | 0.27±0.02 |
| Phosphofructokinase | 6.33±0.39 | 2.73±0.18 | 6.65±0.33 | 2.77±0.08 |
| Pyruvate kinase | 88 ±6.6†† | 38 ±2.75†† | 66 ±3.2 | 28 ±1.9 |
| Pyruvate carboxylase | 4.95±0.20†† | 2.11±0.11†† | 4.57±0.19††† | 1.90±0.12†† |
| Phosphoenolpyruvate carboxylase | 5.95±0.38 | 2.57±0.15 | 4.45±0.29*††† | 1.84±0.11**††† |
| Fructose 1,6-diphosphatase | 12.2 ±0.7 | 5.26±0.34 | 9.9 ±0.35*† | 4.11±0.14*† |
| Glucose 6-phosphatase | 20.9 ±1.6 | 9.03±0.69 | 18.8 ±0.55 | 7.86±0.14 |
| Serine dehydratase | 2.17±0.26 | 0.95±0.13 | 1.28±0.12*† | 0.53±0.04*† |

Table 5. *Comparison of the concentration and content of the glycolytic and gluconeogenic enzymes in livers perfused with a high concentration of glucose and with cycloheximide*

Each value is the mean ±S.E.M. of four observations. *$P<0.01$, **$P<0.001$ with respect to zero-time control perfusion. Glucose was added to the perfusate initially at 14mM followed by a constant infusion of 3.5μmol of glucose/min; cycloheximide was present at 25μg/ml. For further details see the text and Table 1.

| | Enzyme concentration and content in perfused rat liver | | | |
|---|---|---|---|---|
| | Glucose added initially and by constant infusion+cycloheximide | | Zero-time perfusions | |
| Enzyme | (μmol/min per g of liver) | (μmol/min per 100μg of DNA P) | (μmol/min per g of liver) | (μmol/min per 100μg of DNA P) |
| Glucokinase | 2.65±0.22 | 0.71±0.04 | 2.61±0.34 | 0.71±0.10 |
| Hexokinase | 0.46±0.04* | 0.13±0.01* | 0.92±0.07 | 0.25±0.02 |
| Phosphofructokinase | 8.80±0.28 | 2.39±0.14 | 8.40±0.48 | 2.27±0.10 |
| Pyruvate kinase | 87 ±7.1 | 23 ±2.21 | 74 ±6.64 | 20 ±1.97 |
| Pyruvate carboxylase | 6.55±0.26 | 1.78±0.10 | 6.34±0.19 | 1.71±0.05 |
| Phosphoenolpyruvate carboxylase | 13.6 ±0.27 | 3.71±0.18 | 14.3 ±0.38 | 3.86±0.09 |
| Fructose 1,6-diphosphatase | 9.36±0.33** | 2.54±0.13* | 12.31±0.13 | 3.33±0.06 |
| Glucose 6-phosphatase | 20.3 ±0.35 | 5.55±0.08 | 21.7 ±0.42 | 5.88±0.21 |
| Serine dehydratase | 1.87±0.27 | 0.51±0.08 | 2.98±0.43 | 0.81±0.12 |

1,6-diphosphatase were also found in the higher-substrate experiment, with a significant increase in the phosphofructokinase/fructose 1,6-diphosphatase ratio to 0.68 from 0.52 as a result of glucose infusion.

Addition of cycloheximide in these circumstances prevented the decreases in pyruvate carboxylase and phosphoenolpyruvate carboxylase (Table 5), and was coupled with a significantly lower overall rate of

hepatic glucose uptake. Fructose 1,6-diphosphatase remained at the depressed concentration shown in similar perfusions in the absence of the inhibitor, whereas the decline in serine dehydratase was no longer significant. As at the lowest concentration of glucose (Table 3), cycloheximide decreased hexokinase activity.

*Perfusions with fructose*

Conversion of fructose into glucose and lactate is shown in Fig. 3; either 14mM-fructose was present initially or was present initially with, in addition, a continuous infusion of 2.3mg/min, the approximate rate of uptake. Over 80% of the initial dose was absorbed within 1h, giving a final perfusate glucose concentration of 10.5±0.12mM after 4h perfusion. With infusion, perfusate fructose was within the range 4.6mM initially to 1.55mM after 4h, and was accompanied by an increase in glucose concentration from 2.3 to 17mM and in lactate concentration from 2.2 to 5.5mM.

Table 6 shows the activities of the enzymes in livers perfused with fructose. With an initial addition only, glucokinase activity dropped to 50% of the control. At higher ketose concentrations, most of the enzymes showed changes in activity. As with high glucose concentrations pyruvate carboxylase, phosphoenolpyruvate carboxylase and serine dehydratase showed statistically significant decreases, but there was no change in fructose 1,6-diphosphatase. As with perfusions with low fructose concentrations glucokinase was decreased 40%; both phosphofructokinase and glucose 6-phosphatase were decreased by 15–20%.

*Perfusions with lactate*

Uptake of different concentrations of lactate from the perfusate and the production of glucose are shown in Figs. 4 and 5. As with glucose and fructose, an initial experiment was carried out to ascertain the rate of lactate removal, subsequent perfusions having a constant infusion at the same rate to maintain the initial concentration. Some 57% of the initial concentration of 6.6mM was utilized in 1h, giving a final concentration at 4h of 1.55mM. With infusion, initial and final lactate concentrations of 8.65 and 8.60mM and of 17.6 and 14.0mM were observed for experiments of Figs. 4 and 5 respectively.

When lactate was added initially to a concentration of 7mM or was maintained at this concentration by infusion (Table 7), activity of pyruvate carboxylase rose 20%. An increase of 60% occurred when the lactate was maintained at about 15mM (Table 8) and was accompanied by a 20% increase in phosphoenolpyruvate carboxylase activity. These increases in activity were sensitive to cycloheximide, indicating the involvement of protein synthesis. The increase in activity of pyruvate carboxylase is in contrast with the findings of Struck *et al.* (1966), who found no change in enzymic activity after 3h in perfusions with lactate either with or without glucagon. Wimhurst &

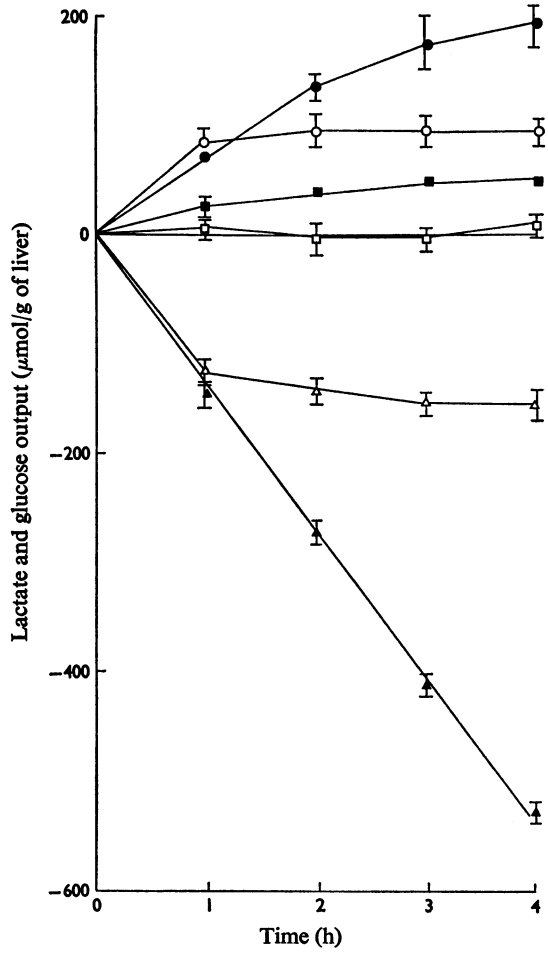


Fig. 3. *Uptake of fructose and output of glucose and lactate from livers perfused with fructose*

The results are from the perfusions reported in Table 6. Bars on points are ±1 S.E.M. ($n = 4$). △, Fructose uptake, ○, glucose output and □, lactate output from liver perfused with 14mM-fructose given as a single initial dose. ▲, Fructose uptake, ●, glucose output, and ■, lactate output from livers perfused with 5.5mM-fructose given initially followed by a continuous infusion of 1.3μmol of fructose/min to maintain fructose concentration in the perfusate.

Table 6. *Comparison of the concentration and content of the glycolytic and gluconeogenic enzymes in livers perfused with different amounts of fructose*

Each value is the mean ±S.E.M. of four observations. *$P<0.05$, **$P<0.01$, ***$P<0.001$ with respect to fructose-only initial perfusions. †$P<0.05$, ††$P<0.01$, †††$P<0.001$ with respect to starved rats (Table 2). Fructose was added initially to the perfusate to 14mM, followed where indicated by constant infusion of 1.3μmol of fructose/min. For further details see the text and Table 1.

| | Enzyme concentration and content in rat liver | | | |
|---|---|---|---|---|
| | Fructose added initially | | Fructose added initially and by constant infusion | |
| Enzyme | (μmol/min per g of liver) | (μmol/min per 100μg of DNA P) | (μmol/min per g of liver) | (μmol/min per 100μg of DNA P) |
| Glucokinase | 1.62±0.15† | 0.68±0.06† | 1.78±0.10† | 0.75±0.10† |
| Hexokinase | 0.67±0.08† | 0.29±0.03 | 0.83±0.08 | 0.35±0.03 |
| Phosphofructokinase | 7.55±0.31 | 3.21±0.11† | 5.98±0.22**† | 2.50±0.10**† |
| Pyruvate kinase | 74 ±6.6 | 32 ±3.0† | 71 ±6.1 | 30 ±2.4 |
| Pyruvate carboxylase | 6.08±0.23 | 2.59±0.13 | 4.60±0.09***††† | 1.93±0.05***††† |
| Phosphoenolpyruvate carboxylase | 6.20±0.23 | 2.63±0.08 | 4.60±0.19**††† | 1.93±0.07***††† |
| Fructose 1,6-diphosphatase | 13.2 ±0.76 | 5.61±0.32 | 11.8 ±0.76 | 4.89±0.29 |
| Glucose 6-phosphatase | 20.6 ±0.94 | 8.8 ±0.47 | 17.2 ±0.76*†† | 7.2 ±0.36*†† |
| Serine dehydratase | 1.48±0.17 | 0.64±0.07 | 0.80±0.07**††† | 0.33±0.03**††† |

Manchester (1973) showed that addition to the perfusate of $MnCl_2$ (50μM), a potent activator of both pyruvate carboxylase and phosphoenolpyruvate carboxylase, prevents the increase in their activities in response to lactate.

## Discussion

The concept that the activity of enzymes of various organs, particularly of liver and adipose tissue, change under different nutritional conditions is well established. Changes occur in starvation or in alteration of the relative content of protein, carbohydrate and fat in the diet. The mechanisms by which these changes are brought about are not always clear. The fact that glucose does not induce hepatic glucokinase activity in the diabetic rat (Preis *et al.*, 1964; Niemeyer *et al.*, 1967) suggests, for example, that dietary influence on insulin secretion and availability of insulin may be of primary importance. Lack of induction of glucokinase in the perfused liver (Table 4) agrees with this. Ruderman *et al.* (1967) found that insulin was necessary to maintain the concentration of glucokinase in perfused livers from fed rats. The results of Young *et al.* (1964), showing that although glucose feeding decreased phosphoenolpyruvate carboxylase activity in normal rats it did not do so with diabetic animals, again imply the intermediacy of insulin. Our present results (Table 4), however, suggest modes of regulation by glucose other than via the control of availability of insulin.

Whether in the present study the non-endocrine factors that can influence enzyme activities are glucose, fructose and lactate themselves or intermediates produced by their metabolism it is not possible to state. There is a very significant linear correlation between the assayed activity of pyruvate carboxylase and the ratio of the perfusate concentration of lactate/glucose (Fig. 6). Loss of pyruvate carboxylase activity seems to occur with glucose alone and not with fructose (Table 6) except when large amounts of glucose are synthesized. Glucose itself does not inhibit pyruvate carboxylase activity (Wimhurst & Manchester, 1970*b*) and the mitochondrial location of the enzyme makes this an unlikely possibility, though glucose can apparently activate enzymes involved in glycogen metabolism (Holmes & Mansour, 1968; Buschiazzo *et al.*, 1970). In the developing tadpole it has been suggested that the increase in activity of certain enzymes results in part from activation of inactive precursor molecules (Shambaugh *et al.*, 1969; Balinsky *et al.*, 1970). However, although pyruvate carboxylase is a highly complex multi-subunit enzyme dependent on structural integrity for activity (Utter & Scrutton, 1969), there is as yet no evidence of

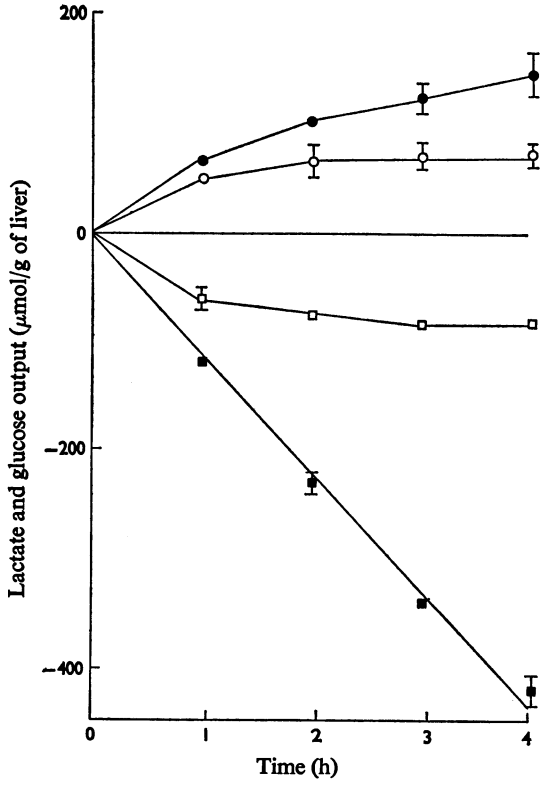


Fig. 4. *Uptake and output of glucose from livers perfused with lactate*

The results are from the perfusions reported in Table 7. Bars on points are ±1 S.E.M. ($n = 4$). □, Lactate uptake and ○, glucose output from livers perfused with 7 mM-lactate given initially. ■, Lactate uptake and ●, glucose output from livers perfused with 7 mM-lactate given initially followed by a continuous infusion of 10.3 μmol of lactate/min.

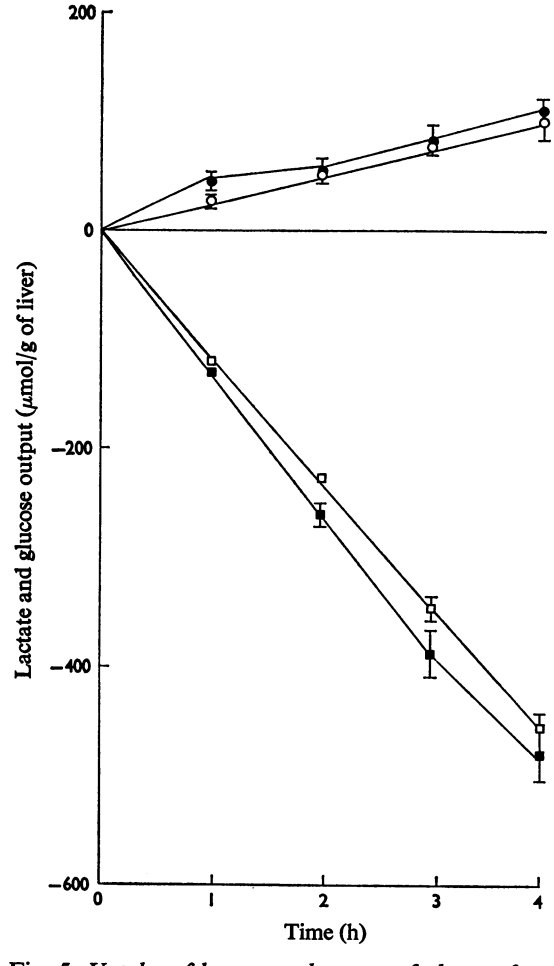


Fig. 5. *Uptake of lactate and output of glucose from livers infused with lactate in the presence or absence of cycloheximide*

The results are from the perfusions reported in Table 8. Bars on points are ±1 S.E.M. ($n = 4$). ■, Lactate uptake and ●, glucose output from livers perfused with 18 mM-lactate. □, Lactate uptake and ○, glucose output from livers perfused with 18 mM-lactate in the presence of cycloheximide at 25 μg/ml.

activation of the vertebrate enzyme protein by any holoenzyme synthetase as is necessary for the analogous enzyme in a thermophilic bacillus (Cazzulo *et al.*, 1970). Pyruvate has been implicated as essential for the maintenance of pyruvate carboxylase in kidney slices (L'age *et al.*, 1968), but pyruvate concentrations in livers perfused with 20 mM-fructose or 20 mM-lactate are similar (Exton & Park, 1969), conditions that would produce enzyme loss or induction (Tables 6 and 7). A correlation, though less precise, between the perfusate [lactate]/[glucose] ratio and phosphoenolpyruvate carboxylase activity is also seen, and between serine dehydratase activity and hepatic glycogen concentration ($r = 0.5$, $P < 0.01$) at zero time, together with an inverse correlation between activity and glycogen deposition in 4 h ($r = 0.44$, $P < 0.02$).

At the present time the limiting steps in gluconeogenesis are believed to be most likely in the area of phosphoenolpyruvate carboxylase and pyruvate carboxylase. The greater lability of these enzymes than of most of the others studied suggests that their activity can change critically. Counteracting their

Table 7. *Comparison of the concentration and content of the glycolytic and gluconeogenic enzymes in livers perfused with different amounts of lactate*

Each value is the mean ±S.E.M. of four observations. *$P<0.05$, **$P<0.01$, ***$P<0.001$ with respect to starved rats (Table 2), †$P<0.05$ between the two perfusions. Lactate was added initially to the perfusate to 7 mM, followed where indicated by constant infusion of 10.3 μmol of lactate/min. For further details see Table 1 and the text.

| | Enzyme concentration and content of rat livers | | | |
|---|---|---|---|---|
| | Lactate added initially | | Lactate added initially and by constant infusion | |
| Enzyme | (μmol/min per g of liver) | (μmol/min per 100 μg of DNA P) | (μmol/min per g of liver) | (μmol/min per 100 μg of DNA P) |
| Glucokinase | 2.72±0.37 | 1.10±0.16 | 3.03±0.14 | 1.28±0.06 |
| Hexokinase | 0.86±0.15 | 0.34±0.06 | 0.73±0.13 | 0.31±0.05 |
| Phosphofructokinase | 6.95±0.35 | 2.76±0.18 | 6.25±0.28† | 2.62±0.13 |
| Pyruvate kinase | 75 ±7.4 | 30 ±2.5 | 66 ±5.2 | 28 ±1.8 |
| Pyruvate carboxylase | 7.37±0.33** | 2.90±0.06** | 7.39±0.37** | 3.13±0.16** |
| Phosphoenolpyruvate carboxylase | 6.23±0.36 | 2.48±0.13 | 6.01±0.19 | 2.53±0.07 |
| Fructose 1,6-diphosphatase | 12.6 ±0.6 | 4.98±0.33 | 12.3 ±0.7 | 5.17±0.33 |
| Glucose 6-phosphatase | 19.2 ±1.1 | 7.6 ±0.29 | 20.8 ±1.6 | 8.8 ±0.88 |
| Serine dehydratase | 3.60±0.67* | 1.42±0.26* | 2.59±0.43 | 1.10±0.21 |

activities is pyruvate kinase. Its capacity for producing extensive futile cycling of pyruvate (Friedman *et al.*, 1971) makes it desirable that its activity be decreased under conditions of starvation. In our experiments, however, change in assayable activity of glycolytic enzymes did not show marked response to the presence of glycolysable substrates (Tables 4 and 6), and the increase in pyruvate kinase in response to glucose that might have been expected from the work of Gerschenson & Andersson (1971) was not seen.

There are several differences in the response of enzymes in perfused livers to added substrates by comparison with changes found in intact animals fed with high-carbohydrate diets. For example fructose feeding induces glucokinase activity (Preis *et al.*, 1964), but produces a decrease in activity in the perfused organ (Table 6). Pyruvate kinase activity is also induced by injection of fructose (Weber, 1969) even in the diabetic animal. Fructose feeding in addition enhances the activities of fructose 1,6-diphosphatase and glucose 6-phosphatase by a mechanism that, by contrast with activities induced by glucocorticoids, is not suppressed by insulin (Freedland *et al.*, 1966). These effects of fructose, however, were not reproduced with the perfused organ (Table 6). Large amounts of fructose (10 mM) result in depletion of adenine nucleotides in the liver (Woods *et al.*, 1970), but it is not clear from the literature (cf. Exton & Park, 1969) how serious an effect the concentrations of fructose used in the present work might have had. Water content, $K^+$, free amino acids and bile production in the perfusions with fructose were normal but urea synthesis was depressed (Table 1).

Extractable tissue activities of specific enzymes will be controlled, *inter alia*, by rates of synthesis and turnover of enzyme protein and by irreversible or only slowly reversible activation or inhibition. That cycloheximide can prevent induction of pyruvate carboxylase and phosphoenolpyruvate carboxylase in the lactate perfusions (Table 8) implies new protein synthesis. It is surprising, therefore, that the drug does not decrease activities in the perfusions of low concentrations of glucose (Table 3). There is evidence, however, that cycloheximide also inhibits protein degradation (Kenney, 1965; Shambaugh *et al.*, 1969; Barker *et al.*, 1971). The lack of effect of glucose in the presence of cycloheximide to suppress activities of pyruvate carboxylase and phosphoenolpyruvate carboxylase (Table 5) is consistent with this.

Functional activity of many enzymes is regulated intracellularly by the availability of their substrates and by the presence of various low-molecular-weight activators and inhibitors. Adaptive changes in total assayable activity is a common property of hepatic enzymes. The present work emphasizes the suggestion made by Buschiazzo *et al.* (1970) that substrates as well as hormones may be potent initiators of adaptation.

Table 8. *Comparison of the concentration and content of the glycolytic and gluconeogenic enzymes in livers perfused with lactate in the presence and absence of cycloheximide*

Each value is the mean ±S.E.M. of the numbers in parentheses. **$P<0.01$, ***$P<0.001$ with respect to zero-time perfusions. †$P<0.05$, ††$P<0.01$, †††$P<0.001$ for the effect of cycloheximide. Lactate was added initially to the perfusate to 18 mM, followed by a constant infusion of 10.3 μmol of lactate/min. Cycloheximide where indicated was added at a concentration of 25 μg/ml. For further details see Table 1 and the text.

| | Enzyme concentration and content in rat liver | | | | | |
|---|---|---|---|---|---|---|
| | Perfused with lactate | | Perfused with lactate+cycloheximide | | Zero-time perfusions | |
| Enzyme | (μmol/min per g of liver) | (μmol/min per 100 μg of DNA P) | (μmol/min per g of liver) | (μmol/min per 100 μg of DNA P) | (μmol/min per g of liver) | (μmol/min per 100 μg of DNA P) |
| Glucokinase | 3.10±0.32 (4) | 0.91±0.10 (4) | 3.94±0.41 (4) | 1.12±0.13 (4) | 3.51±0.29 (8) | 0.99±0.09 (8) |
| Hexokinase | 0.48±0.02 | 0.14±0.01 | 0.45±0.01 | 0.13±0.01 | 0.49±0.02 | 0.14±0.01 |
| Phosphofructokinase | 6.65±0.41 | 1.94±0.12 | 7.05±0.32 | 2.03±0.03 | 7.15±0.28 | 2.00±0.08 |
| Pyruvate kinase | 73 ±9.1 | 22 ±2.8 | 65 ±1.9 | 19 ±0.3 | 74 ±4.6 | 21 ±1.3 |
| Pyruvate carboxylase | 9.43±0.45*** | 2.75±0.16*** | 6.35±0.07††† | 1.83±0.07†† | 6.12±0.18 | 1.71±0.07 |
| Phosphoenolpyruvate carboxylase | 11.4 ±0.56** | 3.32±0.18** | 9.4 ±0.24† | 2.71±0.09† | 9.74±0.22 | 2.72±0.07 |
| Fructose 1,6-diphosphatase | 10.6 ±0.64 | 3.10±0.17 | 10.9 ±0.81 | 3.15±0.26 | 12.2 ±0.55 | 3.37±0.11 |
| Glucose 6-phosphatase | 15.6 ±0.71 | 4.55±0.20 | 18.2 ±0.74 | 5.30±0.42 | 19.3 ±1.22 | 5.39±0.35 |
| Serine dehydratase | 2.06±0.45 | 0.56±0.13 | 3.06±0.70 | 0.88±0.19 | 2.60±0.39 | 0.72±0.10 |

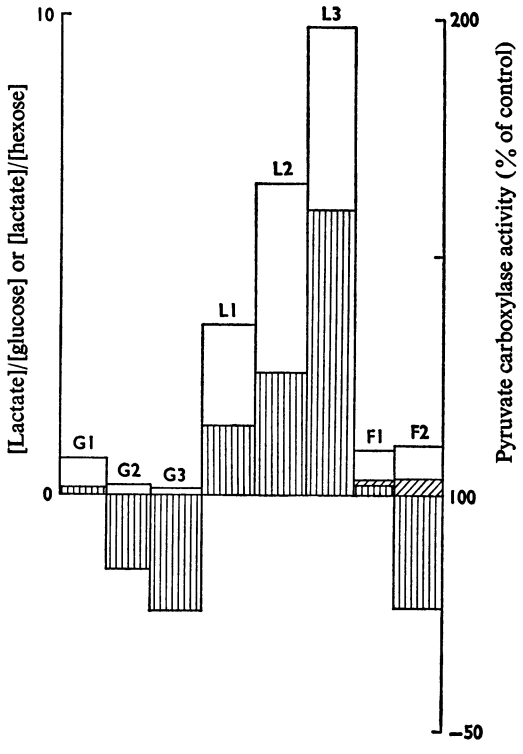


Fig. 6. *Relationship between the perfusate [lactate]/[glucose] ratio and the percentage change in pyruvate carboxylase activity relative to zero-time control*

□, [Lactate]/[glucose] ratio; ▩, percentage change in pyruvate carboxylase activity; ■, lactate/total hexose concentration for fructose perfusions. G1, G2 and G3, glucose perfusions at 2.2mM- and at 14mM-glucose given initially and 14mM initial dose plus infusion of glucose respectively. F1 and F2, perfusions with fructose, 14mM given initially and 5.5mM initially plus infusion respectively. L1, L2 and L3, perfusions with lactate, 7mM given initially and 7 and 18mM initial dose plus infusion respectively. For further details see legends of Figs. 1–5. Concentrations are expressed as μmol/g of liver.

We are grateful to the Medical Research Council for support for this work.

## References

Balinsky, J. B., Shambaugh, G. E. & Cohen, P. P. (1970) *J. Biol. Chem.* **245**, 128–137

Barker, K. L., Lee, K.-L. & Kenney, F. T. (1971) *Biochem. Biophys. Res. Commun.* **43**, 1132–1138

Bartley, W., Dean, B., Taylor, C. B. & Bailey, E. (1967) *Biochem. J.* **103**, 550–555

Berdanier, C. D., Szepesi, B., Moser, P. & Diachenko, S. (1971) *Proc. Soc. Exp. Biol. Med.* **137**, 668–673

Biebuyck, J. F., Lund, P. & Krebs, H. A. (1972) *Biochem. J.* **128**, 711–720

Bloxam, D. L. (1967) *Biochem. Pharmacol.* **16**, 283–294

Bojanowska, K. & Williamson, D. H. (1968) *Biochim. Biophys. Acta* **159**, 560–563

Buschiazzo, H., Exton, J. H. & Park, C. R. (1970) *Proc. Nat. Acad. Sci. U.S.* **65**, 383–387

Cazzulo, J. J., Sundaram, T. K. & Kornberg, H. L. (1970) *Nature (London)* **227**, 1103–1105

Eggleston, L. V. & Krebs, H. A. (1969) *Biochem. J.* **114**, 877–879

Exton, J. H. & Park, C. R. (1969) *J. Biol. Chem.* **244**, 1424–1433

Freedland, R. A., Cunliffe, T. L. & Zinkl, J. G. (1966) *J. Biol. Chem.* **241**, 5448–5451

Friedman, B., Goodman, E. H., Saunders, H. L., Kostos, V. & Weinhouse, S. (1971) *Metab. Clin. Exp.* **20**, 2–12

Garfinkel, D. (1971) *Biochem. Biophys. Res. Commun.* **42**, 621–626

Gerschenson, L. E. & Andersson, M. (1971) *Biochem. Biophys. Res. Commun.* **43**, 1211–1218

Holmes, P. A. & Mansour, T. E. (1968) *Biochim. Biophys. Acta* **156**, 275–284

Kenney, F. T. (1965) *Science* **156**, 525–527

Krebs, H. A. & Eggelston, L. V. (1965) *Biochem. J.* **94**, 3c–4c

Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–43

L'age, M., Henning, H. V., Ohly, B. Seubert, W. (1968) *Biochem. Biophys. Res. Commun.* **31**, 241–246

Miller, L. L., Bly, C. G., Watson, M. L. & Bale, W. F. (1951) *J. Exp. Med.* **94**, 431–453

Mortimore, G. E. & Tietze, F. (1959) *Ann. N.Y. Acad. Sci.* **82**, 329–337

Niemeyer, H., Perez, N. & Codoceo, R. (1967) *J. Biol. Chem.* **242**, 860–864

Preis, N., Kennan, A. L., Pitot, H. C. & Wolf, R. C. (1964) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **23**, 409

Ross, B. D., Hems, R., Freedland, R. A. & Krebs, H. A. (1967) *Biochem. J.* **105**, 869–875

Ruderman, N. C., Laurie, V. & Herrera, M. C. (1967) *Amer. J. Physiol.* **212**, 1169–1173

Salas, M., Vinuela, E. & Sols, A. (1963) *J. Biol. Chem.* **238**, 3535–3538

Sapag-Hagar, M., Marco, R. & Sols, A. (1969) *FEBS Lett.* **3**, 68–71

Shambaugh, G. E., Balinsky, J. B. & Cohen, P. P. (1969) *J. Biol. Chem.* **244**, 5295–5308

Sharma, C., Manjeshwar, R. & Weinhouse, S. (1963) *J. Biol. Chem.* **238**, 3840–3845

Söling, H. D., Willms, B. & Kleineke, J. (1971) in *Regulation of Gluconeogenesis* (Söling, H. D. & Willms, B., eds.), pp. 210–225, Academic Press, New York and London

Struck, E., Ashmore, J. & Wieland, O. (1966) *Advan. Enzyme Regul.* **4**, 219–224

Tanaka, T., Harano, Y., Sue, F. & Morimura, H. (1967) *J. Biochem. (Tokyo)* **62**, 71–97

Utter, M. F. & Scrutton, M. C. (1969) *Curr. Top. Cell. Regul.* **1**, 253–296

Veneziale, C. M., Walter, P., Kneer, N. & Lardy, H. A. (1967) *Biochemistry* **6**, 2129–2138
Walker, D. G. & Rao, S. (1964) *Biochem. J.* **90**, 360–368
Weber, G. (1969) *Advan. Enzyme Regul.* **7**, 15–40
Weber, G., Singhal, R. L., Stamm, N. B., Lea, M. A. & Fisher, E. A. (1966) *Advan. Enzyme Regul.* **4**, 59–81
Wimhurst, J. M. & Manchester, K. L. (1970*a*) *FEBS Lett.* **8**, 91–94
Wimhurst, J. M. & Manchester, K. L. (1970*b*) *Biochem. J.* **120**, 79–93
Wimhurst, J. M. & Manchester, K. L. (1970*c*) *Biochem. J.* **120**, 95–103
Wimhurst, J. M. & Manchester, K. L. (1972) *FEBS Lett.* **27**, 321–326
Wimhurst, J. M. & Manchester, K. L. (1973) *FEBS Lett.* in the press
Woods, H. F., Eggleston, L. V. & Krebs, H. A. (1970) *Biochem. J.* **119**, 501–510
Young, J. W., Shrago, E. & Lardy, H. A. (1964) *Biochemistry* **3**, 1687–1692