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Lactate Production in the Perfused Rat Liver

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1. In aerobic conditions the isolated perfused liver from well-fed rats rapidly formed lactate from endogenous glycogen until the lactate concentration in the perfusion medium reached about 2mM (i.e. the concentration of lactate in blood *in vivo*) and then production ceased. Pyruvate was formed in proportion to the lactate, the [lactate]/[pyruvate] ratio remaining between 8 and 15. 2. The addition of 5mM- or 10mM-glucose did not affect lactate production, but 20mM- and 40mM-glucose greatly increased lactate production. This effect of high glucose concentration can be accounted for by the activity of glucokinase. 3. The perfused liver released glucose into the medium until the concentration was about 6mM. When 5mM- or 10mM-glucose was added to the medium much less glucose was released. 4. At high glucose concentrations (40mM) more glucose was taken up than lactate and pyruvate were produced; the excess of glucose was probably converted into glycogen. 5. In anaerobic conditions, livers of well-fed rats produced lactate at relatively high rates (2.5μmol/min per g wet wt.). Glucose was also rapidly released, at an initial rate of 3.2μmol/min per g wet wt. Both lactate and glucose production ceased when the liver glycogen was depleted. 6. Addition of 20mM-glucose increased the rate of anaerobic production of lactate. 7. D-Fructose also increased anaerobic production of lactate. In the presence of 20mM-fructose some glucose was formed anaerobically from fructose. 8. In the perfused liver from starved rats the rate of lactate formation was very low and the increase after addition of glucose and fructose was slight. 9. The glycolytic capacity of the liver from well-fed rats is equivalent to its capacity for fatty acid synthesis and it is pointed out that hepatic glycolysis (producing acetyl-CoA in aerobic conditions) is not primarily an energy-providing process but part of the mechanism converting carbohydrate into fat.

In liver slices the rates of metabolic activities, e.g. of gluconeogenesis (Ross, Hems & Krebs, 1967) and of ketogenesis (Krebs, Wallace, Hems & Freedland, 1969), are usually much lower than those found in the intact isolated perfused organ. Experiments by Berry & Scheuer (1967) indicate that this may also apply to the glycolytic activity, but so far no detailed systematic investigation of lactate production by the intact liver has been reported. The present work was undertaken to fill this gap and to study the formation of lactate in the perfused liver under a variety of experimental conditions.

From the work on liver slices it is known that the rate of lactate production during anaerobiosis is very low after starvation, when the glycogen content of the liver has fallen to low values, and that addition of glucose does not increase lactate production (Rosenthal, 1930*a*,*b*). Slices of livers from well-fed rats containing glycogen form lactate, but the rates tend to be low by comparison with many other tissues. Rosenthal & Lasnitzki (1928) discovered that aerobic preincubation of slices for 10–15min raises the subsequent anaerobic production of lactate (see also Rosenthal, 1929*a*,*b*; Orr & Stickland, 1941; Negelein & Noll, 1963; Gaja, Bernelli-Zazzera & Sorgato, 1965; Bernelli-Zazzera, Gaja & Ragnotti, 1966; Gaja, Ragnotti, Cajone & Bernelli-Zazzera, 1967). Burk, Woods & Hunter (1967) found that, besides preincubation, addition of pyruvate and NAD^+ also increases anaerobic formation of lactate.

MATERIALS AND METHODS

Animals and diets. Female and male Wistar rats each weighing 170–210g obtained from Carworth (Europe) Ltd., Alconbury, Hunts., U.K., were used and were fed on a standard small-animal diet (Spillers Mills Ltd., Gainsborough, Lincs., U.K.), water being provided *ad libitum*.

Reagents. Diazyme was obtained from Miles Chemical Co., Clifton, N.J., U.S.A., and other substrates, nucleotides, coenzymes and crystalline enzymes from the Boehringer Corp. (London) Ltd., London W.5, U.K.

Liver perfusion. The method of liver perfusion was that described by Hems, Ross, Berry & Krebs (1966), except that the bovine serum albumin was dialysed three times against 3 vol. of bicarbonate-buffered saline (Krebs & Henseleit, 1932) before use: erythrocytes were thoroughly washed as described by Woods, Eggleston & Krebs (1970) to lower their initial lactate content.

Measurements of glycolysis under 'anaerobic' conditions. In experiments where O_2 was to be excluded from the perfusion apparatus and medium, the B14 standard ground-glass joints (see Fig. 1 in Hems *et al.* 1966) of the apparatus were kept closed with ground-glass stoppers except for the moment when samples of the medium were being withdrawn or substrates added. The transparent vinyl tubing no. NT/6 was replaced by nylon tubing (Portex no. 7; Portland Plastics, Hythe, Kent, U.K.), which is less permeable to O_2. Before the perfusions were started the medium was circulated and gassed for 20 min with CO_2+N_2 (5:95) while the top of the glass collecting vessel was closed with a plastic film. Occlusion of the gas outlet in the oxygenator for a few seconds resulted in backflow of the CO_2+N_2 (5:95) down the overflow tube to fill the dead space in the collecting vessel. This vessel has a flanged top on which the animal platform rests, and a ring attached underneath the platform fits closely inside the top of the collecting vessel. The joint was sealed with a strip of plastic adhesive tape (Lassotape; Smith and Nephew Tapes Ltd., Welwyn Garden City, Herts., U.K.).

Rates of anaerobic glycolysis were determined under three separate experimental conditions. In the first group the perfusion medium, containing bicarbonate-buffered saline, bovine serum albumin and washed human erythrocytes, was gassed during circulation with CO_2+N_2 (5:95), which was passed up the 'oxygenator' at a rate of 1 litre/min (as measured by a Rotameter flowmeter). In the second group the perfusions were performed in the presence of cyanide, which was added as a 0.2 M solution of KCN neutralized with HCl to give a final concentration of 1 mM in the medium. Further additions were made every 0.5 h to replace the HCN blown off in the 'oxygenator'. In a few experiments 1 mM-NaN_3 was added instead of HCN because it is not volatile at pH 7.3. In the third group the erythrocytes were omitted from the perfusion medium and cyanide was added as described above. The first 4 ml of perfusate leaving the liver was collected in a graduated glass tube and discarded. This removed most of the blood present in the liver and thus lowered to a minimum the number of rat erythrocytes released into the medium; also lactate, which may have accumulated in the liver during the anoxic period of liver preparation (usually about 1 min), was flushed out and not added to the circulating medium.

These procedures did not completely eliminate O_2 from the medium. When erythrocytes were present the circulating medium gassed with CO_2+N_2 was found to contain up to 0.3 μmol of O_2/ml (method of Van Slyke & Neill, 1924). When erythrocytes were omitted the O_2 content was about 0.15 μmol/ml. The gas mixture analysed by the method of Warburg & Kubowitz (1928) contained less than 0.02% (v/v) O_2. A possible source of the O_2 in the perfusion medium was diffusion of air through the nylon tubing. However, the presence of HCN or NaN_3 eliminated mitochondrial respiration.

Sampling of perfusion medium and liver. Samples of the medium (0.5 ml) were taken from the collecting vessel and pipetted directly into 4 ml of 2% (w/v) $HClO_4$. The precipitate was centrifuged off after 15 min and the supernatant was neutralized with 20% (w/v) KOH to pH 7, with BDH Universal Indicator. The samples were cooled in ice and the supernatant obtained on centrifugation was used for analysis.

Liver samples were rapidly frozen *in vivo* or during perfusion and extracted as described previously (Woods *et al.* 1970). For the determination of the liver glycogen content a liver sample (weighing about 0.15 g) was obtained by placing a ligature of 3/0 silk (Ethicon Ltd., Bankhead Ave., Edinburgh 11, U.K.) round the base of a small lobe, which was severed at a point distal to the ligature. The sample was weighed and homogenized with 4 ml of 2% (w/v) $HClO_4$ in a thick-walled glass test tube. The tube was sealed and left overnight at room temperature to permit extraction of the glycogen.

Analytical methods. Glycogen was determined as glucose after treatment of the liver extract with Diazyme (Krebs, Dierks & Gascoyne, 1964). Glucose was determined by the hexokinase–glucose 6-phosphate dehydrogenase method (Slein, 1963). The other analytical methods have been described previously (Woods *et al.* 1970). The O_2 consumption by the liver was determined by estimating the total O_2 content of the perfusion medium on the 'arterial' and venous side of the liver during perfusion. Samples of perfusion medium were drawn directly into an Ostwald–Van Slyke pipette through rubber links placed in the perfusion circuit. The total O_2 content of the medium was determined by the method of Van Slyke & Neill (1924). The efflux from the liver was directed into a glass siphon (volume 10 ml) and the time of emptying determined at intervals throughout the perfusion. The O_2 consumption was calculated from 'arterio'-venous difference, the rate of flow of medium and the liver weight.

RESULTS

Lactate and glucose production during aerobic perfusion. When the livers of well-fed rats were perfused with the basal medium (no added substrate and a low initial lactate content) there was a rapid initial rise in the lactate, pyruvate and glucose contents of the medium. After about 30 min the production of these metabolites decreased and eventually ceased (Table 1). During the first 15 min the mean rate of lactate production was 0.78 μmol/min per g wet wt. It fell to about half this rate within 45 min and then became negligible. The pyruvate concentration in the medium rose parallel with that of lactate so that the [lactate]/[pyruvate] ratio was maintained at a value of about 10 (Table 1). The rate of glucose formation was 2.97 μmol/min per g wet wt. during the first 15 min and decreased to about one-third of this rate within 45 min. It was very low after 1 h (Table 1).

The reason for the decline in the glucose and lactate production was not a depletion of the glycogen store. The mean sum of glucose, lactate

Table 1. *Time-course of the production of lactate, pyruvate and glucose by perfused livers from well-fed rats under aerobic conditions (no substrate added)*

The initial amounts of lactate, pyruvate and glucose came from the washed erythrocytes. The livers had a mean wet weight of 7.30g. The results are means ± S.E.M. for four experiments. The initial volume of the medium was 150ml; it decreased every 15min by 0.5ml (removed for analyses). The metabolic rates refer to successive 15min periods.

Time ...	0min	15min	30min	45min	60min	75min	90min	105min	120min	135min
Concn. of metabolite in medium (μmol/ml)										
Lactate	0.26±0.02	0.83±0.08	1.30±0.04	1.59±0.06	1.87±0.05	1.94±0.17	1.91±0.16	1.92±0.20	2.00±0.24	2.11±0.18
Pyruvate	0.06±0.01	0.13±0.05	0.15±0.06	0.15±0.08	0.19±0.08	0.22±0.10	0.22±0.12	0.22±0.11	0.25±0.12	0.26±0.11
Glucose	0.27±0.26	2.44±0.26	3.44±0.27	4.08±0.34	4.85±0.36	4.80±0.20	5.18±0.30	4.95±0.38	5.91±0.60	6.00±0.44
[Lactate]/[pyruvate]		6.38	8.67	10.60	9.84	8.82	8.68	8.75	8.00	8.12
Metabolic rates (μmol/min per g wet wt.)										
		0.78	0.64	0.40	0.38	0.11	−0.04	0.01	0.11	0.15
Lactate formation		2.97	1.37	0.87	1.04	−0.07	0.51	−0.31	1.28	0.12
Glucose formation										

and pyruvate (expressed as glucose) formed by the liver after 1h was 807μmol for 7.30g of liver. Since the mean glycogen content of this amount of liver (expressed as glucose) was found to be about 1800μmol (see also Kalkhoff, Hornbrook, Burch & Kipnis, 1966; Start & Newsholme, 1968; Rawat, 1968), the production of glucose and lactate ceased when there was still a substantial amount of glycogen in the liver. Nor can the cessation of the glucose and lactate production be attributed to a physiological deterioration of the perfused liver. The ATP content after 80min was 1.92μmol/g wet wt., i.e. 87% of that found in livers freeze-clamped *in vivo*. The O_2 uptake was maintained throughout the perfusion at about 2μmol of O_2/min per g wet wt. This is the same rate as that reported by Forsander, Räihä, Salaspuro & Mäenpää (1965) for perfused liver of fed rats and by Hems *et al.* (1966) for livers from 48h-starved rats. Bile production during the first hour of perfusion was 101 (S.E.M. ±7; four observations) mg/h per g wet wt. and 105 (S.E.M. ±12; four observations) mg/h per g wet wt. during the second hour of perfusion.

On the other hand, the concentrations of glucose, lactate and pyruvate reached in the medium are remarkably similar to those found *in vivo* in blood plasma. This suggests that the liver regulates the concentrations of the three constituents in the circulating fluid and that the cessation of the production of these constituents is due to feedback control mechanisms.

Effect of glucose concentration of the medium on the production of glucose, lactate and pyruvate by the liver from well-fed rats. To test the above assumption, glucose was added to the perfusion medium at initial concentrations of 5, 10, 20 or 40mM. The results are shown in Tables 2, 3 and 4. Glucose at 5 and 10mM did not prevent a production of glucose by the liver, but substantially decreased it. Again there was relatively rapid initial output during the first 30min. Subsequently the concentrations of glucose in the medium changed very little. With initial concentrations of 20mM- and 40mM-glucose there was a continuous uptake of glucose by the liver.

Addition of 5mM- or 10mM-glucose had no major effect on lactate production, and the final lactate concentration reached was the same order as in the absence of added glucose. With 20mM-glucose lactate production was strikingly increased, and during the first hour of perfusion the rates of lactate production were 2.3μmol/min per g wet wt., i.e. almost the same as under anaerobic conditions (see below). At 20mM-glucose the amounts of lactate produced were almost equivalent to those of glucose taken up. At 40mM-glucose there was a large uptake of glucose during the first 90min and the rate of lactate production was somewhat smaller

Table 2. *Effect of added glucose on the production of glucose by the perfused livers from well-fed rats*

The conditions were aerobic. The results are given as μmol of glucose in total medium (means). The initial volume of the medium was 150 ml.

Concn. of added glucose (mM)	Mean liver wet wt. (g)	No. of experiments	Change in glucose (μmol)								
			15 min	30 min	45 min	60 min	75 min	90 min	105 min	120 min	135 min
0	7.30	4	+324	+472	+566	+678	+668	+720	+686	+823	+835
5	7.32	3	+253	+331	+331	+361	+431	+447	+464	+457	+431
10	7.44	3	+289	+262	+209	+364	+388	+394	+312	+355	—
20	7.91	3	+8	−81	−166	−363	−444	−554	−631	−768	−835
40	8.79	3	−310	−441	−653	−873	−1470	−1690	−1740	−1820	—

Table 3. *Effect of added glucose on the rates of glucose and lactate output by perfused livers from well-fed rats*

The conditions were aerobic. The rates were calculated for consecutive 30 min periods. Negative rates denote uptake of metabolite from the medium. The results are given as μmol/min per g wet wt. (means ± S.E.M.); number of observations was three except for value for 0 mM-glucose where it was four.

Concn. of added glucose ...	Rates of metabolic change (μmol/min per g wet wt.)									
	0 mM		5 mM		10 mM		20 mM		40 mM	
Time-period (min)	Glucose	Lactate	Glucose	Lactate	Glucose	Lactate	Glucose	Lactate	Glucose	Lactate
0–30	2.16 ± 0.22	0.73 ± 0.07	1.50 ± 0.18	0.52 ± 0.05	1.14 ± 0.11	0.77 ± 0.20	−0.65 ± 0.44	2.32 ± 0.39	−1.85 ± 0.35	1.64 ± 0.34
30–60	0.88 ± 0.15	0.37 ± 0.04	0.12 ± 0.05	0.28 ± 0.10	0.41 ± 0.20	0.32 ± 0.14	−1.07 ± 0.12	2.31 ± 0.22	−1.82 ± 0.25	1.57 ± 0.42
60–90	0.26 ± 0.13	0.02 ± 0.08	0.41 ± 0.03	−0.10 ± 0.01	0.08 ± 0.23	0.12 ± 0.15	−0.89 ± 0.06	1.56 ± 0.36	−3.31 ± 0.85	0.45 ± 0.03
90–120	0.58 ± 0.28	0.18 ± 0.09	−0.01 ± 0.20	0.13 ± 0.07	−0.08 ± 0.21	0.29 ± 0.07	−0.97 ± 0.15	1.38 ± 0.21	−0.58 ± 0.79	0.74 ± 0.35

Table 4. *Effect of added glucose on the final concentrations of glucose and lactate in the perfusion medium*

The data refer to the experiments described in Table 3. The results are means of three or four experiments. Pyruvate was produced with the lactate, the [lactate]/[pyruvate] ratio being maintained between 8 and 15.

Concn. of glucose (mM)		Concn. of lactate (mM)	
Initial	Final	Initial	Final
0.27	6.00	0.26	2.11
5.11	8.07	0.24	1.67
9.80	11.8	0.13	2.13
19.6	13.9	0.37	13.2
41.2	28.7	0.10	7.89

than at 20mM-glucose, so that the total amount of lactate formed accounted for no more than about 30% of the glucose removed. Most of the glucose was presumably converted either into glycogen or into fat. The [lactate]/[pyruvate] ratio (not recorded in Table 4) was not much affected by the glucose concentration.

Livers from 48h-starved animals formed much less lactate than did those from well-fed rats. The steady-state concentration of lactate in the medium was 0.61mM (mean of two observations) for livers from rats starved for 24h and 0.32mM (mean of two observations) when the rats were starved for 48h. The latter value is very similar to that in the blood of rats starved for 48h, namely 0.35mM (Williamson, Veloso, Ellington, & Krebs, 1969).

Addition of 10mM- or 20mM-glucose to the medium did not increase the lactate production.

Liver damage and lactate production. When the perfusion technique was inadequate as indicated by patchy colouring of the liver, swelling and the formation of an exudate on the surface (an infrequent event except with anaerobic perfusions of starved livers), lactate production was much increased and continued throughout the perfusion. The [lactate]/[pyruvate] ratio rose above the physiological limits. Swelling alone, produced by clamping the outflow tubing and thus raising the back pressure to the liver, did not cause extra lactate formation.

'Anaerobic' production of lactate and glucose by the liver from well-fed rats. Replacement of O_2 in the gas mixture by N_2 caused a relatively high rate of production of both lactate (1.80μmol/min per g wet wt., S.E.M. $\pm$0.10, six observations) and glucose (2.43μmol/min per g wet wt., S.E.M. $\pm$0.12, six observations). When erythrocytes were present the addition of 1mM-cyanide increased lactate production significantly [2.68μmol/min per g wet wt., S.E.M. $\pm$0.19, four observations ($P<0.0025$)] though the production of glucose was not affected. It is difficult to exclude O_2 entirely from the perfusion system, especially in the presence of erythrocytes (see the Materials and Methods section), and the fact that the rate of glycolysis was not at its maximum in the absence of cyanide can be ascribed to incomplete anaerobiosis. Omission of erythrocytes, which is likely to decrease contamination of the system by O_2, gave the same high rates of glycolysis as the cyanide-treated erythrocyte-containing medium. In the absence of erythrocytes cyanide had no effect on the production of lactate. Passing the gas mixture through the oxygen-absorbing vanadous sulphate–amalgamated zinc reagent of Meites & Meites (1948) did not alter the rate of glycolysis. In the absence of erythrocytes sodium azide was also without effect. The addition of 10mM-glucose made no difference to the rates of lactate production by the liver.

Because of these results a medium containing no erythrocytes was used in the subsequent anaerobic experiments. During the first 45min of perfusion the time-course of the anaerobic production of lactate by the liver from well-fed rats (Table 5) was almost linear whereas that of glucose slowly decreased. The rates then rapidly fell and after about 60min lactate and glucose production virtually ceased. The initial glycogen content of the liver was determined in a small lobe tied off before the beginning of the perfusion and a further specimen was collected at the end of the experiment. At this stage no glycogen was detectable in the liver. The amounts of lactate and glucose produced were identical, within the limits of error, with the loss of glycogen (Table 6). It follows that glycolysis stops when the glycogen has disappeared, and that the glucose present in the medium does not serve as a substrate for glycolysis. The proportions of glycogen that appeared as glucose and lactate were fairly constant, about two-thirds being glucose and the rest lactate. Similar results were reported by Gaja & Ferrero (1970) for experiments carried out with liver slices.

To test whether the accumulation of products affects the fate of glycogen, the experiments were repeated with 300ml of perfusion medium which is twice the normal volume. The rates of lactate and glucose production in these experiments were not significantly different from those measured under the standard conditions, nor were there differences in the time-course of lactate and glucose production. Thus the concentration of lactate in the medium did not affect the rate of glycolysis. Lowering of the perfusion rate to below 2ml/min per g wet wt. led to a marked decrease of the glycolytic rate, possibly because of local accumulation of lactate and a fall of pH. Male and female rats gave the same results wherever tested.

Table 5. *Time-course of lactate and glucose production during perfusion of rat liver under anaerobic conditions*

The liver from a fed rat was perfused in the absence of erythrocytes as described in the text. No substrate was added. The wet weight of the liver was 6.34g, the dry weight 1.41g. 1mM-HCN was present.

Time ...	0min	15min	30min	45min	60min	75min	90min	105min	120min	135min
Lactate found (μmol in total medium)	<0.01	232	441	643	766	788	781	809	830	806
Glucose found (μmol in total medium)	63	548	875	1130	1205	1180	1240	1225	1255	1235
Change in lactate (μmol/15min period)	—	+232	+209	+202	+123	+22	−7	+28	+21	−24
Change in glucose (μmol/15min period)	—	+485	+327	+255	+75	−27	+60	−15	+32	−20

Table 6. *Balance of glycogen, glucose and lactate changes on anaerobic perfusion*

Livers from well-fed rats were perfused with a medium containing no added substrate. The glycogen content of the livers was determined initially and after perfusion for 135min as described in the Materials and Methods section. The final glycogen content of the liver was below 0.2μmol/g wet wt. of liver.

Liver wet wt. (g)	Total initial glucose+glycogen in liver (as glucose) (μmol)	Total final glucose in medium (μmol)	Total final lactate in medium (μmol)	Total glucose+lactate formed (as glucose) (μmol)
7.02	1860	1346	1202	1947
7.40	1687	1099	1130	1654

Physiological effects of anaerobic conditions on livers of well-fed rats. As the perfusions progressed the wet wt./dry wt. ratio increased from the value *in vivo* of 3.45 (S.E.M. ±0.02; 11 observations) to 3.82 (S.E.M. ±0.07; seven observations) at 60min and then to 4.65 (S.E.M. ±0.05; 30 observations) after 135min. The changes were caused by uptake of fluid from the medium; there was a small contribution because of loss of glycogen. The major increase in the wet wt./dry wt. ratio occurred after the first hour of perfusion, when liver glycogen had become depleted. This suggests that it was the cessation of ATP production by glycolysis that led to an increased fluid uptake from the medium.

During anaerobic perfusion there was a small production of bile during the first hour: 21 (S.E.M. ± 3; four observations) mg/h per g wet wt.; compared with the normal rate of 101mg/h per g wet wt. Much of this probably represents the bile present in the intrahepatic biliary collecting system when the liver was placed on perfusion, because no bile was formed after 1h.

In two experiments where neutralized sodium cyanide (instead of potassium cyanide) was added as the inhibitor of respiration potassium efflux into the medium continued throughout the perfusions: 27% of the initial K^+ content of the livers, i.e. 99 (S.E.M. ±5; three observations) mequiv./kg wet wt.) passed into the medium in 135min.

Effect of the glucose concentration on the anaerobic production of lactate and glucose. The initial rates of anaerobic production of lactate were not altered by adding 5mM- or 10mM-glucose to the medium, but 20mM-glucose increased the rate significantly, as shown in Table 7 ($P<0.025$). With 20mM-glucose, lactate production continued for 120min at a gradually declining rate (Table 8), whereas without added glucose (Table 5) lactate formation almost stopped after 60min. The initial anaerobic rate of glucose production was significantly decreased by 10mM-glucose ($P<0.0025$). Thus the addition of glucose to the medium had a protective effect on the liver glycogen. However, this was essentially caused by a delay of glycogen breakdown: the total amount of glucose produced after 135min was not significantly changed by the addition of 10mM-glucose. The amount of glucose produced from glycogen was 170μmol/g wet wt., S.E.M. ±14, seven observations; with 10mM-glucose the corresponding figures were 143μmol/g wet wt., S.E.M. ±16, seven observations, $P>0.15$.

Anaerobic fate of fructose. Rat liver slices are known to convert added fructose readily into lactate when the conditions are anaerobic, provided that the rat is well-fed (Rosenthal, 1930*a*,*b*, 1931; Dickens & Greville, 1932). On perfusion of livers from well-fed rats in the presence of fructose the anaerobic changes with regard to carbohydrate

metabolism are the sum of the metabolism of endogenous glycogen and the added fructose. As shown in Table 9 both glucose and lactate production from endogenous sources become almost negligible after 60min perfusion. On addition of fructose the production of glucose during the first hour was not significantly changed, but during the second hour fructose, especially at the higher concentrations, increased the glucose production. Lactate production during the first hour was increased by 10mM- and 20mM-fructose and during the second hour also by 5mM-fructose.

When the initial fructose concentrations were 5 or 10mM, all the fructose removed could be accounted for as lactate (on the assumption that glycogen contributed little to the lactate formation; Table 10). But with 20mM-fructose the rate of lactate formation was far too low to account for the fructose removal, and therefore some of the fructose must have been converted into glucose. This conclusion is borne out by the fact that the total glucose production in the presence of 20mM-fructose was much greater than can be accounted for by the glycogen store of the liver.

The initial rates of fructose removal rose with increasing fructose concentrations (Table 10). They remained steady for up to 60min when the initial concentrations were 5mM or 10mM. With an initial concentration of 5mM the fructose uptake virtually ceased after 60min, when the concentration of fructose in the medium had fallen to about 1.3mM. With 10mM-fructose the rate fell after 60min and averaged 0.53 (S.E.M. ±0.03; four observations) μmol/min per g wet wt. between 60 and 135min.

Table 7. *Effect of added glucose on the initial rates of anaerobic lactate and glucose production by the perfused liver*

Livers from fed rats were perfused under anaerobic conditions in the presence of 1mM-HCN. Values are means±S.E.M. with the numbers of observations in parentheses. The initial rates refer to the first 40min of perfusion.

Initial concn. of glucose (mM)	Lactate production (μmol/min per g wet wt.)	Glucose production (μmol/min per g wet wt.)
0	2.47±0.07 (7)	3.20±0.13 (7)
5	2.39±0.11 (4)	2.94±0.04 (4)
10	2.48±0.10 (7)	2.53±0.11 (7)
20	2.85±0.15 (5)	—

Table 8. *Time-course of lactate production during anaerobic perfusion of rat liver with a medium containing 20mM-glucose*

The liver from a fed rat was perfused with a medium containing an initial concentration of 20mM-glucose in the presence of 1mM-HCN. The wet weight of the liver was 7.49g, the dry weight 1.77g.

Time ...	0min	15min	30min	45min	60min	75min	90min	105min	120min	135min
Lactate found (μmol in total perfusion medium)	58	570	788	1100	1320	1435	1615	1750	1920	1920
Change in lactate (μmol/15min interval)	—	+512	+218	+314	+215	+118	+180	+135	+170	—

Table 9. *Glucose and lactate production by livers from fed rats perfused with D-fructose*

The livers were perfused under anaerobic conditions in the presence of 1mM-HCN with a medium containing D-fructose. Glucose and lactate production were calculated from the experiments in Table 10. The results are expressed as μmol of C_6 units/g wet wt. and are means±S.E.M. with the numbers of observations in parentheses.

Initial concn. of D-fructose (mM)	Glucose production		Lactate production		Fructose removed	
	0–60min	0–135min	0–60min	0–135min	0–60min	0–135min
0	163±11 (7)	170±14 (7)	68±4 (7)	81± 7 (7)	—	—
5	189±14 (4)	250±10 (4)	86±6 (4)	132±12 (4)	58±2 (4)	69± 4 (4)
10	154±18 (4)	268±29 (4)	100±5 (4)	169±14 (4)	86±4 (4)	119± 5 (4)
20	158±19 (4)	308±32 (4)	126±5 (4)	197± 6 (4)	168±9 (4)	253±16 (4)

Table 10. *Effect of fructose concentrations on the initial rates of metabolic changes in the anaerobic perfused liver*

Livers from well-fed rats were perfused in the presence of 1 mM-HCN with a medium containing 5 mM-, 10 mM- and 20 mM-D-fructose. The conditions were as described in Table 9. Results are means ± S.E.M. with the numbers of observations in parentheses. The initial rates refer to the first 40 min of perfusion.

Initial concn. of fructose ...	Initial rate (μmol/min per g wet wt.) 5 mM	10 mM	20 mM
Metabolic changes			
Fructose removal	1.02 ± 0.01 (4)	1.80 ± 0.06 (4)	3.14 ± 0.29 (4)
Glucose formation	2.96 ± 0.30 (4)	2.64 ± 0.30 (6)	2.36 ± 0.12 (8)
Lactate formation	3.11 ± 0.15 (4)	3.34 ± 0.12 (6)	4.07 ± 0.12 (8)
Pyruvate formation	0.03 ± 0.006 (4)	0.05 ± 0.009 (4)	0.09 ± 0.01 (4)
D-Glyceraldehyde formation	—	0.10 (2)	0.04 (2)

With 20 mM-fructose the uptake was linear throughout the 135 min of perfusion. Lactate production was linear while fructose removal was linear, except with 20 mM-fructose, when the lactate production decreased after about 100 min. The initial rates of lactate production were higher with fructose than with glucose (3.1 μmol/min per g wet wt. with 5 mM-fructose and 4.7 μmol/min per g wet wt. with 20 mM-fructose). After 60 min of perfusion the lactate concentrations in the medium had risen to above 10 mM.

Some pyruvate was formed during the first 45 min of perfusion; afterwards the pyruvate concentration remained fairly constant, so that the [lactate]/[pyruvate] ratio in the medium rose to very high values.

Small amounts of D-glyceraldehyde were formed anaerobically from fructose (Table 10). A major accumulation of D-glyceraldehyde was not to be expected, because anaerobic liver is known to convert it into lactate (Rosenthal, 1930*b*).

Anaerobic perfusion of livers from starved rats. Livers from 48 h-starved rats, unlike those from well-fed rats, began to deteriorate after about 60 min when perfused anaerobically in the presence of 1 mM-cyanide. The tissue became oedematous and there was copious leakage of fluid from the surface of the organ. For this reason experiments on the 48 h-starved rats were limited to 60 min; samples of the medium were analysed every 10 min. After 60 min the wet wt./dry wt. ratio was 4.77 (S.E.M. ± 0.14; 18 observations). Livers from rats starved for 24 h did not leak fluid from their surface, but the wet wt./dry wt. ratio rose to 5.43 (S.E.M. ± 0.22; 12 observations) after 135 min of perfusion. Livers from 48 h-starved rats did not produce measurable amounts of lactate when no substrate was added (Table 11). Addition of 10 mM- or 20 mM-glucose resulted in measurable rates of lactate production that were 10–15% of that observed with liver from well-fed rats. Slightly higher rates were observed with 10 mM- and 20 mM-fructose. In livers from 24 h-starved rats there was a low, just measurable, lactate production in the absence of added substrate, indicating that the glycogen stores had not been completely exhausted. Addition of glucose or fructose was no more effective than with liver from 48 h-starved rats. These results are in broad agreement with earlier observations on the low glycolytic capacity of the livers of starved animals (Rosenthal, 1930*a*).

Table 11. *Anaerobic lactate production by perfused livers from starved rats*

The medium contained 1 mM-HCN and the substrates indicated. The results are initial rates of lactate production expressed as μmol/min per g wet wt. and are means ± S.E.M. with the numbers of observations in parentheses.

Substrate added	Initial rate of lactate production (μmol/min per g wet wt.) 24 h-starved	48 h-starved
None	0.19 ± 0.02 (5)	0.02 (4)
10 mM-Glucose	—	0.21 ± 0.02 (4)
20 mM-Glucose	0.22 ± 0.02 (3)	0.27 ± 0.03 (4)
10 mM-D-Fructose	0.30 ± 0.01 (3)	0.40 ± 0.10 (3)
20 mM-D-Fructose	0.43 ± 0.04 (3)	0.44 ± 0.06 (5)

DISCUSSION

Control of lactate production of the perfused rat liver. The experimental conditions differ from those in previous work on the perfused rat liver in this laboratory in that the initial lactate concentration in the perfusion medium was kept as low as possible by repeated washing of the erythrocytes in the medium and by using aged erythrocytes, which no longer form lactate. There was a rapid output of lactate by the liver from well-fed rats during the first 30 min until the lactate concentration in the medium approached that found in blood of well-fed

rats, i.e. 2mM. Broadly speaking, these results are similar to those obtained by Schimassek (1962) and by Glinsmann, Hern & Lynch (1969), who also found a rapid initial output of lactate. The increased aerobic formation of lactate that occurred on addition of 20mM- or 40mM-glucose to the medium coincided with the conditions where glucokinase (EC 2.7.1.2) is significantly active in raising the rate of supply of glucose 6-phosphate. Unlike the case of supply of glucose 6-phosphate catalysed by hexokinase, the reaction catalysed by glucokinase is not controlled by feedback (see Sols, 1968). Thus though an increased rate of formation of glucose 6-phosphate is expected at high glucose concentrations it is not evident why all the glucose 6-phosphate formed at 40mM-glucose is not either converted into glycogen or degraded to acetyl-CoA to be used for fatty acid synthesis. The formation of lactate at high glucose concentrations may be due to an overloading of the pathways responsible for the conversion of glucose 6-phosphate into either glycogen or fatty acids.

Liver from starved rats formed very little lactate. When the rats had been starved for 24h lactate production stopped when its concentration in the medium had reached 0.6mM, and when the rats had been starved for 48h it stopped when the concentration in the medium had reached 0.3mM. These final concentrations are very similar to those in the blood of starved rats. That in liver from starved rats there was no major increase in lactate production on addition of 20mM-glucose to the medium is to be expected in view of the decreased activity of glucokinase in the liver of starved rats. Evidently hexokinase activity *in situ* is too low to initiate a significant rate of glycolysis. It follows from these experiments that higher rates of glycolysis depend either on the presence of a store of glycogen or on conditions favouring the glucokinase reaction.

Glycolytic capacity of the liver and the function of liver glycolysis. The maximum glycolytic capacity of the liver, like that of other organs, manifests itself under anaerobic conditions and the end product is then mainly lactate (with trace amounts of pyruvate). The rates of anaerobic lactate formation in the present experiments (up to 2.5μmol/min per g wet wt.) are relatively high and comparable with the rates observed in those tissues that exhibit a high glycolytic capacity (brain, embryonic tissue, testicle, leucocytes, tumours). There is, however, a major difference between the glycolytic capacity of the liver and that of other tissues. Tissues other than liver glycolyse glucose at near-maximum rates at physiological glucose concentrations (5–10mM), whereas liver forms lactate only from the glycogen stores or from glucose at exceptionally high concentrations (20mM and above). This difference between liver and other tissues is presumably related to the special function of glycolysis in the liver. Whereas in other tissues (except adipose tissues) glycolysis is the means of generating ATP, liver glycolysis is mainly a pathway for the conversion of carbohydrate into fat. An additional function, quantitatively of minor importance, is the provision of precursors for the synthesis of cell constituents such as alanine, aspartate, glutamate, serine, glycine, glucosamine and cholesterol.

The comparison of the rates of hepatic triglyceride synthesis and glycolysis support the view that the potential rate of anaerobic formation of lactate corresponds essentially to the aerobic pathway of fatty acid synthesis. The rate of hepatic triglyceride synthesis has been estimated by Otway & Robinson (1967), who measured the entry of triglycerides into the circulation when the uptake of plasma triglycerides by extrahepatic tissues was inhibited by Triton W.R. 1339 (Robinson, 1963). If it is assumed that the rate of triglyceride release after Triton treatment is equivalent to triglyceride synthesis and that the liver represents 4% of the body weight, the rate of triglyceride synthesis in the experiments of Otway & Robinson (1967) was equivalent to 3.02μmol of C_3 units/min per g wet wt. for female rats and 1.76μmol of C_3 units/min per g wet wt. for male rats in the fed state. The corresponding mean values for glycolysis are of the same order of magnitude, namely 2.47μmol for female and 2.31μmol for male rats.

The synthesis of fatty acids requires, apart from the acetyl-CoA generated via glycolysis, reducing equivalents in the form of NADPH generated via the pentose phosphate cycle. This pathway of glucose degradation, however, would not be maintained anaerobically because of the exhaustion of the supply of $NADP^+$.

Comparison of glycolysis in the perfused liver and in slices. The rates of anaerobic glycolysis in the perfused organ are not only much higher than in slices (Table 12) but are also maintained for a much longer period. In liver slices the rate of glycolysis is usually very low, being about one-fifth of the rate found in the perfused organ. As Rosenthal & Lasnitzki (1928) have shown (see also Negelein & Noll, 1963), the rate is increased by a brief aerobic incubation of slices or by the addition of pyruvate and NAD^+ (Burk *et al.* 1967; Gaja, Ragnotti, Cajone & Bernelli-Zazzera, 1968), but even after aerobic preincubation the rates are no more than two-thirds of those observed in the perfused organ and they tend to decrease very much more rapidly with time. The factors responsible for the loss of glycolytic capacity in the liver slices have not been fully established.

Fructolysis. Lactate formation from fructose in the perfused liver differed from that from added

Table 12. *Comparison of rates of anaerobic formation of lactate in rat liver under various experimental conditions*

Nutritional state	Substrate added	Rate of lactate formation (μmol/min per g wet wt.)	Reference
Slices			
Well-fed	None	0.7	Bernelli-Zazzera *et al.* (1966)
Well-fed*	None	1.4	Bernelli-Zazzera *et al.* (1966)
Well-fed*	30mM-Glucose	1.9	Gaja *et al.* (1967)
Well-fed	22mM-Fructose	0.6	Rosenthal (1931)
Well-fed	44mM-Fructose	0.9	Rosenthal (1931)
Starved (48h)	None	0.17	Rosenthal (1930*a*)
Starved (48h)	11mM-Glucose	0.19	Rosenthal (1930*a*)
Starved (48h)	11mM-Fructose	0.24	Rosenthal (1930*a*)
Perfused organ			
Well-fed	None	2.47	Present work
Well-fed	20mM-Glucose	2.85	Present work
Well-fed	5mM-Fructose	3.11	Present work
Well-fed	10mM-Fructose	3.34	Present work
Well-fed	20mM-Fructose	4.07	Present work
Starved (48h)	None	<0.02	Present work
Starved (48h)	20mM-Glucose	0.27	Present work
Starved (48h)	20mM-Fructose	0.44	Present work

* The slices were preincubated in O_2.

glucose in several ways, as already established by earlier workers who used slices. Lactate formation from fructose was somewhat more rapid than that from endogenous glycogen (Table 12). The fact that added fructose is readily converted into lactate whereas added glucose is not is only to a minor extent due to the different capacities of fructokinase and hexokinase. Under identical conditions (25°C, pH 7.4, crude homogenate, liver from fed or starved rats) the activities determined *in vitro* under optimum conditions are 3.4 μmol/min per g wet wt. for fructokinase and 0.31 μmol/min per g wet wt. for hexokinase (unpublished values of L. V. Eggleston; see also Heinz, Lamprecht & Kirsch, 1968). An important factor responsible for the differences between glucose and fructose metabolism is the feedback regulation of hexokinase by glucose 6-phosphate (derived in the liver of fed rats mainly from glycogen), whereas fructose 1-phosphate and the pathway of fructose degradation, as far as is known, are not subject to feedback controls.

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