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INTERRELATION OF AEROBIC GLYCOLYSIS AND LIPOGENESIS IN ISOLATED PERFUSED LIVER OF WELL-FED RATS

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Summary

The rates of glycolysis and lipogenesis in isolated perfused liver of well-fed rats were studied. When liver was allowed to synthesize [^{14}C]glycogen prior to perfusion, no more than 9% of the degraded [^{14}C]glycogen was recovered in lactate and 6% in lipid. Addition of glucose, fructose and sorbitol enhanced concomitantly the formation of lactate and pyruvate and the rate of release of triglyceride and free fatty acid. Glucose was less efficient than fructose or sorbitol. The incorporation of ^{14}C from these ^{14}C-labelled substrates into lactate, pyruvate and lipids confirmed their role as carbon sources. Incorporation of ^{14}C into the glycerol moiety of neutral lipid exceeded that found in the fatty acids, suggesting that these substrates contributed largely to the esterification of fatty acids. The total rate of de novo fatty acid synthesis was correlated with the formation of lactate and pyruvate. It is concluded that increased rates of aerobic glycolysis are related to increased rates of lipogenesis.

Introduction

The metabolism of carbohydrate and lipid are closely related in the liver. Glycolysis furnishes precursors for several biosynthetic pathways including lipogenesis. It is well known that the liver is not only an important source of plasma triglyceride released in very low density lipoprotein, but also a site of de novo synthesis of fatty acids and triacylglycerols [1]. Low rates of glycolysis and fatty acid synthesis are associated with diabetes, starvation or the feeding of carbohydrate-free high fat diets [1]. From experiments showing that liver has a potential for quite high rates of glycolysis, it was suggested that the anaerobic glycolytic capacity of the isolated perfused liver from well-fed rats is equivalent to its capacity for fatty acid synthesis [2]. Experiments with isolated hepatocytes indicate that the rate of de novo fatty acid synthesis is

dependent on the accumulation of pyruvate and lactate in the incubation medium [3]. The glycolytic activity of the liver, as based on the formation of lactate, has been assessed in earlier studies using tissue slices incubated under anaerobic conditions [4,5] and more recently in studies on the perfused liver [2,6—8] and isolated hepatocytes [9] under a variety of experimental conditions. However, there is little information available on the extent of glycolysis from endogenous glycogen, the main carbohydrate reserve of the liver.

The present work was undertaken to investigate the rates of aerobic glycolysis and lipogenesis from endogenous ^{14}C-labelled glycogen in the perfused liver of normal fed rats and, by employing ^{14}C-labelled six-carbon substrates, to determine the extent of aerobic glycolysis and lipogenesis from exogenous substrates by measuring the incorporation of ^{14}C into lactate, pyruvate and lipid. The paper also reports on the rate of lipogenesis in the presence and absence of exogenous six-carbon substrates, as estimated from the release of free fatty acids and triglyceride by the liver into the perfusion medium and the incorporation of tritium from tritiated water into fatty acids of all lipid classes. The results show that the rates of aerobic glycolysis and lipogenesis in the perfused liver of well-fed rats are closely linked.

Materials and Methods

Male albino Wistar strain rats weighing 200—280 g were supplied by Thomae, Biberach, Germany and fed ad libitum on a standard diet obtained from Altromin, Lage/Lappe, Germany. They were always taken at the same time (09:00—10:00 h) for perfusion.

All enzymes and coenzymes were obtained from Boehringer G.m.b.H., Mannheim, Germany and lipid standards from Fluka A.-G., Buchs, Switzerland and Applied Science Laboratories, Pa., U.S.A. The ion-exchange resins Dowex 50-WX2, H^+-form, and AG 1-X8, chloride form, both 200—400 mesh, were from Serva, Heidelberg, G.F.R. and Bio-Rad Laboratories, Munich, respectively. Silicic acid (—325 mesh) for separation of phospholipids from neutral lipids was from Serva, Heidelberg, and silica gel H for thin-layer chromatography of lipids was from Merck, Darmstadt, G.F.R. [U-^{14}C]Glucose, [U-^{14}C]-fructose, [U-^{14}C]sorbitol, tritiated water and N.C.S. tissue solubilizer were obtained from Amersham-Buchler G.m.b.H., Braunschweig, G.F.R. Bovine serum albumin (fraction V, fatty acid poor) for the perfusion medium was obtained from Miles Biochemicals, Kankakee, Ill., U.S.A. Cab-O-Sil, scintillation grade, was obtained from Eastman Kodak Co., Rochester, N.Y., U.S.A. All other chemicals were analytical reagent grade and obtained from Merck, Darmstadt.

The perfusion technique is described elsewhere [10,11] except that glucose, lactate and pyruvate were omitted from the medium. Perfusions were started with 120 ml medium. The first 20 ml was allowed to pass through the liver and then collected and discarded, because of its high concentration of glucose and lactate, which are produced in the liver during the anoxic period of liver operation. Then recirculating perfusion was commenced. The livers were pre-perfused without the addition of exogenous substrate for 1.5 h, prior to the addition of freshly prepared solutions of substrates in 0.9% NaCl solution or tritiated water to the collection reservoir. When ^{14}C-labelled substrates were used,

CO_2 was trapped in 1 M NaOH. Perfusions lasted 2.5 h, that is 1 h after the addition of substrate.

In one series of perfusions the glycogen in the liver was pre-labelled with ^{14}C. Rats which had been starved for 24 h were given intragastrically 25 μCi [U-^{14}C]fructose (3 Ci/mol) with 3 mmol unlabelled fructose in 0.5 ml 0.9% NaCl. Two such doses were administered with a time interval of 1.5 h between doses, so that the total amount of radioactivity given was 50 μCi. After a further 1.5 h the rats were operated for perfusion. By this technique about 20% of administered fructose is recovered in glycogen. These perfusions were carried out for 2 h.

Glucose, fructose, sorbitol, pyruvate and lactate in the perfusion medium were analysed by standard enzymatic methods [12]. Glycogen was extracted from liver by hydrolysis in 30% KOH and then isolated and hydrolysed according to ref. 13, the resulting glucose being determined enzymatically [12].

For the assay of lipids perfusion medium was centrifuged at 2500 $\times g$ for 10 min at 4°C and the resulting cell-free extracts were stored at −40°C until analysis. Liver samples were rapidly frozen in liquid nitrogen and kept frozen at −40°C until analysis. Weighed quantities of powdered frozen liver were extracted with 20 volumes of chloroform/methanol (2 : 1, v/v) containing 0.002% 2,6-di-*tert*-butyl-4-methyl phenol as anti-oxidant with a glass Potter-Elvehjem homogeniser and left to stand overnight at 4°C. The extracts were then filtered and washed [14] and the chloroform fraction was evaporated to dryness under nitrogen. A similar procedure was employed to extract lipids from the cell-free extracts of perfusate. Extracts were shaken with 20 vols. of chloroform/methanol (2 : 1, v/v) and then filtered and washed, as for liver. For determination of the concentration of neutral triglycerides and free fatty acids the dried residues of the total lipid extracts were redissolved in a small volume of chloroform. Phospholipids were removed by shaking an aliquot of the chloroform solution with silicic acid [15]. Aliquots were then taken for assay of total glycerides, calculated as triglycerides, by enzymatic measurement of the free glycerol [12] released after saponification [12] and for the estimation of free fatty acids by a colorimetric method [16].

The separation of ^{14}C-labelled metabolites was performed by cation-exchange chromatography on Dowex 50-WX2 (H^+-form) and by anion-exchange chromatography on AG1-X8 (formate) and AG1-X8 (borate). The formate and borate forms of the resins were prepared from the AG1-X8, chloride form, by first converting it to the hydroxide form with 1 M NaOH and then to the formate form with 1 M formic acid or to the borate form with saturated boric acid. Neutralized samples of perchloric extracts of perfusate were passed through small columns (2 $\times$ 1 cm) of Dowex-50 H^+ resin and the eluate was collected. The columns were washed with water (15 ml) and the combined eluates containing the neutral and acidic fractions were kept. The adsorbed amino acids were eluted from the H^+ column with 2 M NH_4OH (10 ml). The combined neutral and acidic fraction was then applied to a column (3 $\times$ 1 cm) of formate resin, which was washed with water to remove the neutral fraction. Lactate was then eluted with 0.1 M formic acid and pyruvate with 3 M formic acid. When the substrate was fructose or sorbitol, the neutral fraction was lyophilised and the dried extract redissolved in 0.12 M phosphate

buffer, pH 5.6. Glucose was oxidised enzymatically to gluconic acid [12]. A solution of 0.1 M sodium tetraborate was then added, so that the final concentration was 10 mM. The incubation mixture was transferred to a column (6 × 1 cm) of borate resin. Fructose was eluted with 40 mM sodium tetraborate, sorbitol with 60 mM sodium tetraborate and finally gluconate with 0.5 M acetic acid.

For the measurement of the incorporation of ^{14}C and tritium into lipids, an aliquot of the dried extracts of total lipids, redissolved in chloroform, was taken for direct counting of the total radioactivity in a toluene scintillation fluid. Another aliquot was dried under nitrogen and saponified for 3 h at 80°C in 2 ml 5 M NaOH. The fatty acids were extracted three times from the acidified solution with four volumes of light petroleum (b.p. 40—60°C). After evaporation of the solvent under nitrogen, the total radioactivity of the free fatty acids was measured in a toluene scintillation fluid. The radioactivity of the remaining aqueous solution containing the glycerides was measured in a dioxane-based scintillation fluid. Separation of the various lipid classes in the total lipid extracts was achieved by thin-layer chromatography on silica gel H in the solvent system light petroleum (b.p. 40—60°C)/diethyl ether/acetic acid/methanol (81 : 18 : 3 : 2, v/v). The lipids were detected with a 0.5% solution of Rhodamine B in ethanol. Each of the lipid components, corresponding to standard spots, was scraped from the plate directly into toluene scintillation fluid and Cab-O-Sil was added in a concentration of 4%. Recovery of applied radioactivity from the chromatogram was approx. 90%.

The total rate of de novo fatty acid synthesis in liver was determined from the incorporation of tritium from tritiated water into lipids between 1.5 and 2.5 h of perfusion [17—20]. The tritium content of lipids was determined in liver removed at 2.5 h after extraction of total lipids, saponification and extraction of fatty acids, as already described. Results are expressed as μmol of newly synthesised fatty acid [18,19].

The activity of ^{14}C and ^{3}H was measured in a liquid scintillation spectrometer (Nuclear Chicago) with counting efficiency of approx. 85% for ^{14}C and 30% for ^{3}H. Quenching was corrected by the use of an external standard. Aliquots in aqueous solution were counted in a dioxane-based scintillation fluid [21]. Non-aqueous samples were counted in a toluene scintillation fluid containing 6 g/l PPO and 0.7 g/l POPOP. The total radioactivity of the perfusion medium or the liver tissue was measured by incubating samples in N.C.S. solubilizer at 37°C. When fully dissolved aliquots were neutralized with acetic acid and counted in toluene scintillation fluid.

Results

Glycolysis and lipid formation from endogenous glycogen

When livers of well-fed rats are perfused with medium and no exogenous substrate, there is a rapid initial rise in the levels of glucose, lactate and pyruvate in the medium. Once the circulating glucose levels in the medium reach a concentration of 5—7 mM, the rate of glycogenolysis is greatly diminished [2,22]. In the first hour of perfusion the rate of glycogen breakdown is approx. 30 μmol/g per h and thereafter approx. 9 μmol/g per h.

Since in liver, where the glycolytic pathway can operate in the direction of both glycolysis and gluconeogenesis, pyruvate can arise from sources other than glycogen. Furthermore pyruvate arising from glycogen via the glycolytic pathway may be removed by transamination reactions or the pyruvate dehydrogenase reaction. This means that the measurement of pyruvate and lactate production alone is a doubtful index of the glycolytic rate. In order to overcome these difficulties, experiments employing radioactivity in the form of either labelled glycogen itself in the liver or labelled six-carbon substrates were undertaken. Glycogen has been implicated as a source of fatty acids in studies on rat liver slices [23], perfused mouse liver [24] and isolated hepatocytes [25].

The contribution of endogenous glycogen to glycolysis and lipid formation was estimated by pre-labelling the glycogen with ^{14}C from [U-^{14}C]fructose prior to perfusion (see Materials and Methods). About 20—30% of ^{14}C was

TABLE I

BALANCE SHEET OF METABOLITES OF [^{14}C]GLYCOGEN IN LIVER AND PERFUSION MEDIUM

Livers pre-labelled with [^{14}C]glycogen (for experimental details see Materials and Methods) were perfused with no added substrate for 2 h. The percentage distribution of glycogen as μmol and ^{14}C is calculated as a percentage of the change in glycogen content and radioactivity between 0—1 and 1—2 h of perfusion. The mean weight of the livers was 7.19 g. Results are expressed as means ± S.E. and are the means of five perfusions.

	Content and radioactivity of glycogen in liver and rise of metabolites in medium		
Perfusion time (h):	0	1	2
Total glycogen content (μmol)	986 ± 167	522 ± 114	374 ± 41
Total glucose content (μmol)	30 ± 6	497 ± 60	621 ± 102
Total lactate + pyruvate content (μmol)	80 ± 10	82 ± 12	111 ± 10
Total ^{14}C in glycogen (μCi)	8.4 ± 0.3	3.6 ± 0.2	2.5 ± 0.3

	Change in glycogen (%)	
Perfusion time (h):	0—1	1—2
Content (μmol)	47	28
Radioactivity (μCi)	57	23

	Distribution (%) of μmol glycogen and radioactivity from glycogen in metabolites	
Perfusion time (h):	0—1	1—2
Metabolite		
Glucose (μmol)	98	83
Lactate + pyruvate (μmol)	2	10
Glucose (μCi)	66.2	75.0
Lactate (μCi)	6.1	8.6
Pyruvate (μCi)	0.9	1.4
CO_2 (μCi)	7.2	7.6
Lipid (μCi)	5.0	5.9
Recovery of ^{14}C from glycogen	85.4	98.5

incorporated into the liver and about 80—95% of the total radioactivity of the liver was accounted as glycogen. This meant that when 50 μCi of [U-^{14}C]fructose was administered to a rat, the amount of [^{14}C]glycogen in the liver at the start of the perfusion was of the order of 10 μCi. The content of glycogen in the liver at the start of the perfusion was 137 ± 36 μmol/g (n = 5). The rate of glycogen breakdown was rapid and calculated on a percentage basis about 40—50% of glycogen was broken down in the first hour (Table I). Nearly all of this glycogen degraded could be accounted as glucose released into the medium. From the measurements of radioactivity about 66% of ^{14}C appeared in glucose, 6% in lactate and about 1% in pyruvate and in total lipid in the medium.

However, in the second hour of perfusion, the rate of glycogenolysis and the rate of formation of glucose were considerably lower. The production of pyruvate and lactate was 6.89 ± 0.70 μmol/g per h. Only 28% of the glycogen present at 1 h was broken down between 1 and 2 h and 83% was accounted in glucose, 9% in lactate and 1% in pyruvate. As calculated on the basis of the radioactivity present in glycogen at 1 h, 23% of [^{14}C]glycogen disappeared between 1 and 2 h and 75% of this ^{14}C appeared in glucose, 9% in lactate, about 1% in pyruvate and 6% in total lipid in the medium. The total CO_2 production during the entire 2-h period was about 7%. The total amount of ^{14}C recovered at 1 or 2 h was in the range 85—98%.

In agreement with the previous data [26], it is concluded that the contribution of endogenous degraded glycogen to lactate production does not exceed 9%. The increase in radioactivity of the total lipid fraction of the medium and liver suggests a significant role for glycogen as a carbon source of liver lipids.

Effects of substrates on production of lactate and lipid

Since isolated perfused livers release metabolites such as glucose, pyruvate and lactate into the perfusion medium until circulating levels approximating those found in vivo are reached [2,10], it was decided to study the effects of exogenous substrates on lactate and lipid production after allowing an equilibration period of 1.5 h. By this time the release of the above-mentioned metabolites from endogenous substrates such as glycogen is minimal. Table II summarizes the results of the addition of various concentrations of glucose, fructose and sorbitol on the release of lactate, triglyceride and free fatty acid and on the deposition of glycogen in the liver. The latter measurement was essential in order to fully account for the utilization of substrate.

Glucose

When the concentration of glucose in the medium is raised between 16 and 55 mM, a rise in the levels of pyruvate and lactate is noted. A continuous increase in the glycogen content in the liver is also observed, reaching a maximum rate of synthesis of 63.6 μmol/g per h at 54 mM glucose. In fact with all the glucose concentrations tested most of the glucose taken up by the liver is converted to glycogen. Even though over a 6-fold rise in lactate production in the presence of 40—50 mM glucose was observed, it represented only about 30 μmol/g per h, which was about 25% of the amount of substrate as compared to that accounted in glycogen. Increasing the glucose concentration above 20 mM brought about a 1.5—2.5-fold rise in the release of both triglycer-

TABLE II

BALANCE SHEET OF METABOLITES AFTER ADDITION OF GLUCOSE, FRUCTOSE AND SORBITOL TO THE PERFUSION MEDIUM

Livers from well-fed rats were perfused with medium for an equilibration period of 1.5 h. At 1.5 h 10, 15, 20, 30, 40 or 50 mM glucose; 2.5, 5, 7, 10, 15 or 20 mM fructose and 7, 10 or 15 mM sorbitol were added or continuous infusion of fructose or sorbitol at the rate of 600 or 1000 μmol/h into 100 ml perfusion medium was commenced. The concentrations of glucose given are the effective concentrations in the medium, as glucose was already present at a concentration of 4—5 mM at 1.5 h. Perfusions were continued unitl 2.5 h. The rates were calculated for the perfusion period 1.5—2.5 h. Negative rates denote uptake by the liver or glycogenolysis. Results are expressed as μmol/g per h and are means ± S.E. except for concentrations of 2.5 and 5 mM fructose, when results are expressed as μmol/g per 0.5 h and μmol/g per 0.75 h, respectively, that is during the period of uptake of the added fructose. Number of observations is given in parentheses.

Substrate addition (mM)	Substrate infusion (μmol/h)	Rates of metabolic change						
		Substrate uptake	Glucose production	Lactate production	Pyruvate production	Glycogen synthesis	Triglyceride production	Fatty acid production
Control (8)		—	7.1 ± 1.4	5.6 ± 0.4	0.7 ± 0.1	—9.1 ± 1.9	0.45 ± 0.07	0.44 ± 0.07
Glucose								
16.1 (4)		—24.8 ± 3.5	—	7.2 ± 0.7	0.9 ± 0.3	21.2 ± 2.0	0.42 ± 0.09	0.40 ± 0.09
20.4 (3)		—32.2 ± 1.2	—	10.0 ± 0.3	1.2 ± 0.3	25.2 ± 2.5	0.42 ± 0.02	0.37 ± 0.08
23.7 (8)		—57.7 ± 5.4	—	21.9 ± 0.7	2.4 ± 0.2	37.3 ± 6.2	0.66 ± 0.15	0.67 ± 0.15
35.2 (6)		—76.4 ± 3.3	—	23.6 ± 0.6	2.7 ± 0.4	50.8 ± 3.8	0.64 ± 0.16	0.76 ± 0.16
44.7 (6)		—90.8 ± 3.7	—	30.3 ± 2.7	3.2 ± 0.5	61.1 ± 7.2	1.10 ± 0.10	1.01 ± 0.11
54.9 (3)		—118.4 ± 3.1	—	32.6 ± 2.0	3.1 ± 0.4	63.6 ± 2.3	1.09 ± 0.16	1.16 ± 0.11
Fructose								
2.5 (6)		—29.8 ± 2.0	22.5 ± 3.0	5.7 ± 0.1	1.4 ± 0.1	—5.2 ± 1.4	0.45 ± 0.05	0.38 ± 0.03
5 (7)		—41.9 ± 3.0	28.0 ± 2.0	15.5 ± 0.3	2.1 ± 0.2	8.5 ± 1.2	0.65 ± 0.10	0.67 ± 0.13
7 (3)		—55.4 ± 4.5	33.4 ± 3.5	21.8 ± 0.5	1.9 ± 0.1	5.1 ± 0.5	0.93 ± 0.09	0.89 ± 0.10
10 (6)		—86.1 ± 7.1	57.6 ± 7.7	25.7 ± 2.5	2.3 ± 0.3	12.0 ± 3.1	1.20 ± 0.12	1.38 ± 0.09
15 (4)		—107.4 ± 2.9	47.5 ± 6.3	32.2 ± 0.3	2.7 ± 0,2	20.8 ± 2.8	1.23 ± 0.20	1.53 ± 0.15
20 (6)		—123.1 ± 5.6	64.2 ± 5.8	36.3 ± 3.0	3.1 ± 0.4	33.2 ± 5.8	1.32 ± 0.04	1.61 ± 0.12
	Fructose							
	600 (7)	—51.0 ± 2.2	13.1 ± 3.1	24.1 ± 1.5	2.1 ± 0.2	14.7 ± 3.1	1.09 ± 0.06	1.01 ± 0.08
	1000 (5)	—70.1 ± 3.5	25.6 ± 2.5	28.2 ± 2.5	3.4 ± 0.5	20.3 ± 2.5	1.19 ± 0.10	1.49 ± 0.09
Sorbitol								
7 (3)		—65.5 ± 2.8	30.9 ± 3.3	31.9 ± 1.3	—0.4 ± 0.1	13.9 ± 1.6	0.59 ± 0.13	0.67 ± 0.12
10 (4)		—82.0 ± 6.5	39.0 ± 1.4	36.4 ± 2.9	—1.2 ± 0.2	20.4 ± 2.4	0.74 ± 0.18	0.74 ± 0.12
15 (3)		—99.9 ± 3.8	52.0 ± 2.5	35.2 ± 3.0	—1.2 ± 0.1	19.0 ± 1.9	0.77 ± 0.18	0.94 ± 0.09
	Sorbitol							
	600 (3)	—39.2 ± 1.0	27.5 ± 2.4	31.4 ± 1.1	—0.2 ± 0.02	10.1 ± 1.7	0.86 ± 0.13	1.16 ± 0.16
	1000 (3)	—61.9 ± 4.3	31.3 ± 2.0	30.2 ± 1.6	—0.3 ± 0.04	17.6 ± 2.0	0.91 ± 0.10	1.48 ± 0.13

TABLE III

DISTRIBUTION OF ^{14}C DERIVED FROM [U-^{14}C]GLUCOSE INTO METABOLITES IN PERFUSION MEDIUM AND LIVER

Livers were perfused with 10—20 μCi [U-^{14}C]glucose added together with 10, 15 or 20 mM unlabelled glucose at 1.5 h. Medium and liver were sampled at 2.5 h and the ^{14}C-labelled metabolites separated as described in Materials and Methods. Total activity of the liver means the amount of radioactivity measured in a sample of liver tissue dissolved in N.C.S. solubilizer (see Materials and Methods). Results are expressed as the percentage of the added radioactivity incorporated and as the change in metabolites calculated as a percentage of the amount of added unlabelled substrate in μmol. The number of perfusions is given in parentheses.

Metabolite	Substrate addition (mM):	10		15		30	
	Effective substrate concentration (mM):	16.3 (n = 3)		20.2 (n = 3)		24.1 (n = 3)	
		^{14}C recovered (%)	Substrate accounted as μmol	^{14}C recovered (%)	Substrate accounted as μmol	^{14}C recovered (%)	Substrate accounted as μmol
Medium							
Glucose		54.3	67.1	67.0	70.5	60.4	66.4
Lactate		7.1	9.0	8.1	9.2	7.0	7.1
Pyruvate		1.2	1.7	1.4	1.1	1.1	0.7
Amino acids		0.8	—	0.9	—	0.7	—
Lipid		1.0	—	0.9	—	0.9	—
Liver							
Total		15.4	—	18.1	—	19.5	—
Glycogen		4.1	6.2	10.0	14.0	12.0	15.0
Lipid		1.9	—	1.6	—	2.0	—
CO_2		4.4	—	4.1	—	4.1	—
Total recovered		84.2	84.0	100.5	94.8	93.7	89.2

ide and fatty acid. At a circulating glucose concentration of 44.7 mM a saturation of the release of lipid was reached.

Table III shows the results obtained with [U-^{14}C]glucose in the presence of varying concentrations of unlabelled substrate. Over 83% of the total radioactivity was recovered, of which 64—70% was found in the medium. Between 54 and 67% of this total radioactivity remained in glucose, 7—8% in lactate and less than 2% in pyruvate, amino acids and lipid, respectively. Between 15 and 19% was found in the liver. About 26—63% of the total radioactivity in the liver was accounted in glycogen, depending upon the glucose concentration, while 8—12% was accounted in lipid. The CO_2 production amounted to about 4% of the added radioactivity. These experiments suggest that the rate of aerobic glycolysis from 10 to 20 mM glucose is fairly low.

Fructose

Since glucose uptake in liver is regulated by the activity of glucokinase [27], high concentrations of glucose are needed in the perfusion medium before isolated perfused liver manifests a net glucose uptake. As is evident from Table II, glucose increases both lactate output as well as triglyceride and fatty acid production at concentrations above 20 mM. Fructose is readily taken up by liver because of the much lower K_m value for fructokinase as compared to glucokinase [28]. Thus at an initial concentration of 10 mM, fructose uptake of 2.58 μmol/g per min for perfused liver [29] and about 4 μmol/g per min for hepatocytes [9] has been reported. In view of these considerations fructose was employed in this study. Since the addition of fructose in a concentration of 10 mM causes a pronounced diminution in the hepatic content of adenine nucleotides in vivo [30] as well as in perfused liver [29] and changes the activities of the glycogen-metabolising enzymes [31], this substrate was also infused at a rate of either 600 or 1000 μmol/h into 100 ml perfusion medium. These rates of infusion do not alter the levels of adenine nucleotides in the perfused liver (Walli, R.A., unpublished). Fructose enters the glycolytic pathway at the triose phosphate level and increases the glycolytic flux between triose phosphates and pyruvate. There is evidence of a link between fructose feeding of rats and a rise in the plasma level [32] and liver content [33] of triglyceride. Low concentrations of fructose stimulate fatty acid synthesis in isolated hepatocytes [3,25] and fructose increased the secretion of triglyceride in very low density lipoprotein in the perfused rat liver [34].

Because of the rapid removal of fructose by the liver, rates of substrate uptake and metabolite formation at low initial concentrations (2.5 and 5 mM) of substrate are calculated for the initial period of linear uptake. As seen in Table II, with increasing concentration of added fructose both the rates of formation of pyruvate and lactate and the release of triglyceride and fatty acid increase. At fructose concentrations of 10—20 mM the production of lactate was 26—36 μmol/g per h, which represents a 5—6-fold increase compared with control values. Simultaneously a 3—4-fold increase in the release of lipid was noted. On an equimolar basis lactate production was 3-fold higher and lipid release 2—4-fold greater with fructose as substrate than with glucose. The lower rates of lactate production from fructose, as well as glucose, as compared to previous reports [2,6—8,29] could be accounted for, as the substrate was added

TABLE IV

DISTRIBUTION OF ^{14}C DERIVED FROM [U-^{14}C]FRUCTOSE INTO METABOLITES IN PERFUSION MEDIUM AND LIVER

Livers were perfused with 10—25 μCi [U-^{14}C]fructose added together with 7, 10 or 20 mM fructose at 1.5 h. For other details see Table III. Values are means and the number of perfusions is given in parentheses.

Metabolite	Substrate addition (mM):	7 (n = 4)		10 (n = 3)		20 (n = 3)	
		^{14}C recovered (%)	Substrate accounted as μmol	^{14}C recovered (%)	Substrate accounted as μmol	^{14}C recovered (%)	Substrate accounted as μmol
Medium							
Fructose		20.5	23.1	17.0	18.2	19.0	24.4
Glucose		30.1	39.4	37.2	46.1	29.4	31.1
Lactate		7.4	9.3	8.0	12.0	10.3	13.2
Pyruvate		1.9	1.4	2.1	0.8	2.2	1.4
Amino acids		1.1	—	1.0	—	1.3	—
Lipid		0.8	—	1.3	—	1.2	—
Liver							
Total		21.1	—	20.3	—	22.3	—
Glycogen		4.0	5.1	8.0	12.2	7.1	10.0
Lipid		2.2	—	3.3	—	5.4	—
CO_2		6.5	—	5.3	—	5.1	—
Total recovered		89.4	78.3	92.2	89.2	90.8	80.1

when the concentration of lactate in the medium was already about 2 mM and the liver was more poised for glycogen synthesis, because of glycogenolysis occurring until 1.5 h [22]. As can be seen from Table II, a greater percentage of the substrates glucose and fructose was directed towards glycogen synthesis than towards lactate formation.

Table IV summarizes the results of experiments with [U-^{14}C]fructose and various fructose concentrations. About 80% of the added fructose was converted into ^{14}C-labelled products, in contrast to 20—30% of [U-^{14}C]glucose. This may be explained by the difference in the rates of uptake of glucose and fructose by the liver. Of the fructose removed from the medium, only 7—10% of ^{14}C could be accounted as lactate in the medium and 5—6% in CO_2, confirming the relatively low rate of lactate production. About 1% of the radioactivity appeared in lipid in the medium and about 2—5% in liver lipid. With the exception of 15 and 20 mM fructose, less than 20% of the substrate was accounted in glycogen synthesized in the liver. Even with 15 and 20 mM fructose, although higher rates of glycogen synthesis were noted, the amount synthesized accounted for only 10% of the added substrate. In agreement with previous studies [7,29], the glucose concentration in the medium increased continuously, parallel with the disappearance of fructose from the medium and 30—40% of ^{14}C from [U-^{14}C]fructose was recovered in glucose in the perfusion medium. This represented 40—50% of the total ^{14}C recovered in the metabolic products. In the liver tissue itself only 19—40% of ^{14}C from [U-^{14}C]-fructose was accounted in glycogen, the rest of the activity being found in $HClO_4$-soluble intermediates, which may represent the accumulated phosphorylated intermediates as well as free glucose.

Sorbitol

Since the effects of fructose were more marked than those of glucose, sorbitol was chosen as a substrate. Sorbitol is rapidly converted into fructose in the liver [35], stoichiometric amounts of fructose and NADH being produced. Because of this additional H^+ it is of interest to examine the effects of this substrate on glycolysis and lipogenesis. Addition of sorbitol to the perfusion medium brought about an immediate rise in the concentration of lactate, a decline in that of pyruvate and an increase in the concentration of triglyceride and fatty acid in the medium. Even though sorbitol is associated with a more reduced cytoplasmic redox state as compared with glucose or fructose, yet the rates of lactate production, 32—36 μmol/g per h, did not exceed those observed with high concentrations of these two substrates, while the rates of lipid release were slightly less, being 1.5—2-fold higher than control values (Table II). It is also noteworthy that the lactate production was not greatly enhanced with increasing concentrations of added sorbitol and that sorbitol at corresponding equimolar concentrations was less effective than fructose in increasing the rate of lipid release. Although similar rates of lactate production were noted with continuous infusion of sorbitol, the rates of production of triglyceride and fatty acid were higher as compared with the addition of single doses of the substrate.

Only 5—10% of ^{14}C from [U-^{14}C]sorbitol was accounted in lactate, 5—6% in CO_2, less than 1% in lipid in the medium and 1—3% in liver lipid (Table V). As

TABLE V

DISTRIBUTION OF ^{14}C DERIVED FROM [U-^{14}C]SORBITOL INTO METABOLITES IN PERFUSION MEDIUM AND LIVER

Livers were perfused with 20—25 μCi [U-^{14}C]sorbitol added together with unlabelled 7, 10 or 15 mM sorbitol at 1.5 h. For other details see Table III. Values are means of three perfusions.

Metabolite	Substrate addition (mM):	7		10		15	
		^{14}C recovered (%)	Substrate accounted as μmol	^{14}C recovered (%)	Substrate accounted as μmol	^{14}C recovered (%)	Substrate accounted as μmol
Medium							
Sorbitol		23.3	28.6	25.0	28.2	30.3	34.2
Glucose		38.0	41.1	30.9	32.0	21.1	25.7
Lactate		5.5	4.0	10.0	11.4	9.4	10.1
Pyruvate		—	—	—		—	—
Amino acids		0.7	—	1.1	—	0.8	—
Lipid		0.6	—	0.9	—	0.8	—
Liver							
Total		16.1	—	18.3	—	24.1	—
Glycogen		5.4	10.2	7.7	13.3	8.4	12.4
Lipid		1.1	—	3.1	—	2.1	—
CO_2		5.4	—	5.0	—	6.4	—
Total recovered		89.6	83.9	90.2	84.9	92.9	82.4

with fructose, the glucose production in the medium increased with increasing concentrations of sorbitol and 20—40% of the radioactivity was recovered in glucose.

Formation of lipid from ^{14}C-labelled precursors

The total lipid fractions obtained from liver sampled from the perfusions performed with ^{14}C-labelled substrates (Tables III—V) were separated into their main constituents by thin-layer chromatography. The phospholipid fraction was predominantly labelled (Fig. 1) and more of the radioactivity was found in this fraction with glucose than with fructose or sorbitol as precursor. Slightly more ^{14}C was recovered in the triglyceride and diglyceride fractions with fructose and sorbitol than with glucose as the source of ^{14}C. Moreover the sum of the radioactivity found in the three separated glycerides was about 10% greater in perfusions with fructose or sorbitol as compared with glucose. Smaller amounts of ^{14}C were found in cholesterol esters and free fatty acids.

Upon saponification of the total lipid extracts of perfusate and liver, the radioactivity recovered in the glycerol fraction exceeded that in the fatty acid fraction by 2—4-fold (Fig. 2). This suggests that the contribution of carbon from these six-carbon precursors to the [^{14}C]glycerol moiety for esterification with existing free fatty acids exceeded that for the synthesis of new fatty acid molecules.

Rate of de novo fatty acid synthesis

In some of the perfusions described in Table II the rate of de novo fatty acid synthesis was estimated. Over 85% of the tritium-labelled fatty acids in the liver were detected in the form of neutral glycerides and the rest in phospholipid and cholesterol esters. Only 13.5 ± 1.3% (n = 11) of the newly synthesized fatty acids were found in the perfusate. Incorporation of tritium into fatty

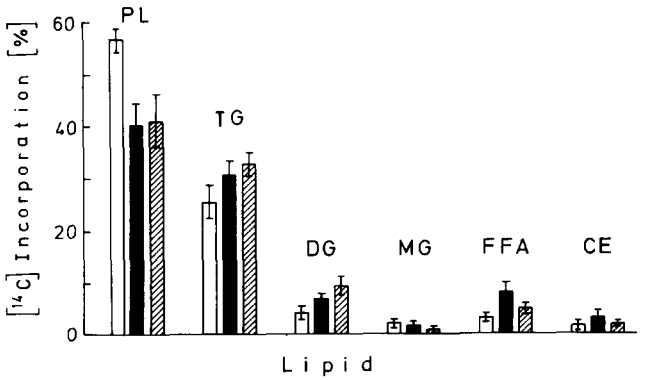

Fig. 1. Distribution of ^{14}C from ^{14}C-labelled substrates in liver lipid classes. The total lipid fractions obtained from livers perfused from 1.5 to 2.5 h with 10—25 μCi [U-^{14}C]glucose (open bars), [U-^{14}C]-fructose (filled bars) or [U-^{14}C]sorbitol (cross-hatched bars) in the presence of unlabelled substrate of concentration 10—40 mM glucose, 7—20 mM fructose and 7—15 mM sorbitol (Tables II—V) were resolved into lipid classes by chromatography (see Materials and Methods). Results are expressed as percent of radioactivity applied to the chromatogram and are the means of eight perfusions with glucose and six perfusions with fructose and sorbitol, respectively. Vertical lines indicate S.E. PL, phospholipid; TG, triglyceride; DG, diglyceride; MG, monoglyceride; FFA, free fatty acid and CE, cholesterol ester.

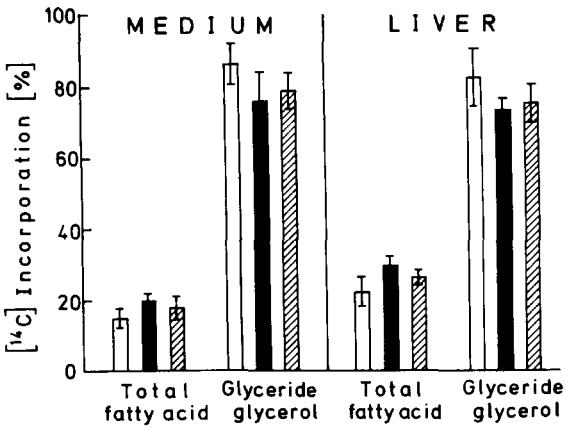

Fig. 2. Distribution of ^{14}C in total lipid between fatty acid and glycerol. Total lipid extracts of perfusate and liver sampled at 2.5 h from perfusions performed with substrate additions at 1.5 h of [U-^{14}C]glucose (open bars), [U-^{14}C]fructose (closed bars) and [U-^{14}C]sorbitol (cross-hatched bars) in the presence of 7—20 mM unlabelled substrate (Tables II—V) were saponified and free fatty acids extracted (see Materials and Methods). Results are expressed as percent of total ^{14}C in the lipid extract before saponification and are the means of six perfusions. Vertical lines indicate S.E.

acids was approximately linear with time and in control perfusions proceeded at a rate of 2.34 ± 0.27 μmol/g per h, $n = 4$, which resembles the rates observed in vivo in the intact liver of rats fed ad libitum [17,36], in isolated perfused liver from fed rats [18—20,37] and mice [24] and in isolated hepatocytes prepared from rats fed ad libitum [25,38,39]. The rate of fatty acid synthesis was dependent on the initial concentration of substrate. When glucose was added to the medium in a concentration of 10—20 mM, the rate of fatty acid synthesis remained slightly below the basal level, being within the range 1.84—2.22 μmol/g per h. However, at higher concentrations of 30 and 40 mM glucose the

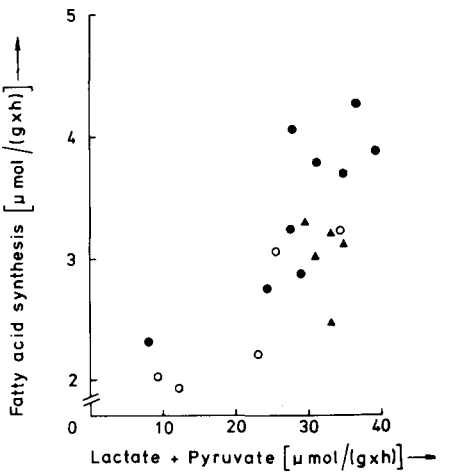

Fig. 3. Relation of lactate and pyruvate production to total fatty acid synthesis. Livers were perfused with medium and at 1.5 h, 1—2 mCi ^3H_2O and various concentrations of glucose (○), fructose (●) or sorbitol (▲) were added. The data for the rates of lactate and pyruvate production are from Table II. Rates of de novo synthesis of fatty acid in liver and rates of lactate and pyruvate production are expressed as μmol/g per h. Results are from single perfusions. Correlation coefficient: 0.86 ($P < 0.001$).

rate of synthesis was enhanced by about 30%. With the exception of 2.5 mM fructose all concentrations of fructose tested, as well as infusions of 600 or 1000 μmol/h into 100 ml perfusion medium, resulted in a small rise in the rate of fatty acid synthesis of the order of 20% for 7 and 10 mM fructose, 60% for 15 and 20 mM fructose and 60—80% for infusion of fructose. When concentrations of 10 or 15 mM sorbitol were presented to the medium or when sorbitol was infused at the rate of 600 or 1000 μmol/h, there was a small enhancement of 30—55% in the rate of fatty acid synthesis. Highest rates were observed in the presence of 20 mM fructose and after infusion of fructose, being 3.90 and 3.12—3.74 μmol/g per h, respectively. It is interesting to compare the rates of glycolysis with the rates of de novo fatty acid synthesis. As seen in Fig. 3 a good correlation exists between the rate of lactate and pyruvate production and the rate of fatty acid synthesis. This is in agreement with the conclusion of ref. 3 that fatty acid synthesis is dependent upon the concentration of pyruvate.

Discussion

The results of this study suggest that there is a parallelism between the aerobic glycolytic rate and the synthesis of lipids in the liver and their rate of release into the perfusion medium. Increased de novo synthesis of fatty acids and esterification of fatty acids in the liver tissue occur concurrently with increased production of pyruvate and lactate. Incorporation of ^{14}C into the various lipid classes confirms that glycogen and the six-carbon substrates employed in the present experiments contributed carbon atoms for the formation of lipid, as well as the glycolytic products pyruvate and lactate.

Aerobic glycolysis is considerably less than the maximum glycolytic capacity of the liver. Thus in isolated perfused livers from fed rats lactate is produced at a rate of approx. 150 μmol/g per h under conditions of anaerobiosis [2]. This high rate is essential in order to meet the energy requirements of the tissue during anaerobiosis. Furthermore as long as liver produces lactate under anaerobic conditions, no cellular damage such as enzyme release from the tissue occurs [6]. The relation observed between glycolysis and lipogenesis, illustrated by the experiments reported here, lends support to the view that glycolysis in the liver may be regarded as a pathway functioning mainly for the purpose of converting carbohydrate to fat [2]. Studies with meal-fed rats or rats fed high carbohydrate diets have established that liver can synthesize fatty acids quite rapidly in amounts which exceed the basal synthesis in rats fed ad libitum by 2—3-fold [7,25]. It therefore appears that under normal conditions the pathways of glycolysis and lipogenesis in the liver operate at low rates and that higher rates represent a response of the organ to a sudden excess amount of carbohydrate.

There seems to be an apparent discrepancy between the rates of aerobic lactate formation from glucose and fructose observed in the present study and values reported in other work on the perfused liver. Thus comparing with ref. 2 the rate of lactate production with 20 mM glucose was about 6-fold higher and with 40 mM glucose about 3-fold higher. However, substrate addition was made at the start of perfusion, when the initial lactate concentration was less than

0.3 mM. Moreover the rate of lactate production declined appreciably in the second hour. A similar high lactate production in the initial stages of the perfusion, followed by a subsequent marked reduction, was observed by ref. 7. The aerobic lactate formation from 10 mM fructose reported by ref. 29 in the perfused liver of fed rats was about 4-fold higher then noted in the present study. Again fructose was added at the start of perfusion. In the present experiments it was also found that the uptake of fructose was extremely rapid in the first 15—30 min and coincided with an initial rapid output of lactate, which thereafter declined to lower rates. However, the rates shown in Table II were calculated for 1 h of perfusion and hence a direct comparison with ref. 29 is not valid. Similar high initial rates of lactate production were reported by ref. 8 in a study of livers from starved rats, perfused for 10 min with medium containing fructose in a non-recirculating system.

The circulating level of lactate in the perfusion medium warrants consideration. In the present experiments lactate had already attained a mean concentration of 1.93 ± 0.07 mM (n = 50) at 1.5 h, the time at which exogenous substrates were introduced. On the other hand in the perfusions of [2,29] the initial lactate concentration was only 0.26 mM. As already suggested by ref. 2, a feedback control mechanism with respect to lactate may be operating and end-product inhibition of glycolysis by lactate has been claimed for isolated hepatocytes [9]. Thus the differences in the extent of lactate formation may be explained in terms of a restriction being imposed on the glycolytic flux by high levels of circulating lactate.

Addition of glucose in the concentrations used in this study leads to inactivation of the active form of glycogen phosphorylase and stimulation of the active form of glycogen synthase [40,41] and this favours glycogen deposition. Thus in the present study a considerable amount of the substrate was directed towards glycogen formation and this is further substantiated by a simultaneous decline in the hepatic content of UDP-Glc of 40—66% and a rise in that of glucose 6-phosphate of 88—225% (Walli, R.A., unpublished). Similarly addition of fructose in high concentrations (10 mM and above) also causes drastic alteration in the activities of the enzymes involved in glycogen metabolism [31]. Thus in perfusion studies 0.5 h after the addition of 10 mM fructose the activity of the active form of glycogen synthase is increased several-fold [31] and the hepatic content of UDP-Glc declines, while that of glucose 6-phosphate rises (Walli, R.A., unpublished). This sequence of events favours glycogen synthesis.

When fructose was infused from the start of perfusion, at the rate of 600 μmol/h, the uptake of fructose was about 61 μmol/g per h and lactate formation was about 50 μmol/g per h (Walli, R.A., unpublished). Calculating on the basis of substrate removed and lactate output, about 37% of the substrate uptake was accounted in lactate. Comparing on the basis of substrate uptake, the percentage of substrate appearing in lactate agrees favourably with the lactate production reported earlier [29]. It must, however, be pointed out that no changes in the activity of glycogen-metabolising enzymes or in the intracellular content of hepatic metabolites or glycogen were noted.

Even though isolated hepatocytes are being used extensively in metabolic studies, care should be taken in directly comparing the results with those from

experiments on isolated perfused liver. When hepatocytes isolated from fed rats are incubated, they manifest high rates of glycogenolysis [9,42,43]. The activity of the key glycogenolytic enzyme phosphorylase is high and returns to lower levels only after prolonged incubation or in the presence of high concentrations of glucose or K^+ in the medium. Moreover glycogen degradation is not always correlated with the activity of the active form of phosphorylase [42]. Therefore the high rates of lactate formation from 10 mM fructose in hepatocytes [9,43,44] cannot be directly compared with the present study, since hepatocytes synthesize negligible amounts of glycogen at this concentration of substrate.

Physiological concentrations of glucose are associated with very low rates of glycolysis and lipogenesis. This is in agreement with the finding of ref. 24 in the perfused mouse liver. Moreover in an in vivo study of the mouse liver, plasma glucose did not provide extensive carbon for fatty acid synthesis [45]. It therefore appears that blood glucose at the physiological levels encountered in vivo, is only of minor importance in fatty acid synthesis. Nevertheless from the results of ^{14}C labelling of the lipid classes, glucose may be important in the synthesis of the glycerol moiety of triacylglycerols.

The nature of the circulating precursor for the synthesis of long chain fatty acids in liver remains to be clarified. Glycogen, lactate and high concentrations of glucose, fructose and sorbitol, as well as glycerol and dihydroxyacetone [3, 25] can all provide acetyl residues necessary for this synthesis. It has already been shown in vivo that fructose brings about rapid interconversion of the inactive to the active form of pyruvate dehydrogenase [46].

Fructose and sorbitol support higher rates of glycolysis than corresponding equimolar concentrations of glucose. Likewise the rates of release of triglyceride and fatty acid are 2—3-fold higher and the total rate of fatty acid synthesis is 1.5-fold greater in the presence of fructose and sorbitol, as compared with glucose. In a recent study on the perfused rat liver [47] infusion of fructose, but not glucose, increased the esterification of free fatty acids and the secretion of very low density lipoprotein triglycerides. Sorbitol is a less efficient substrate for lipogenesis than fructose. This may be related to the rapid diminution of pyruvate levels with increasing concentrations of sorbitol. In isolated hepatocytes [3] it has been shown that pyruvate accumulation in the medium is essential for fatty acid synthesis and decreased rates are associated with substrates such as xylitol and glycerol, which decrease the pyruvate concentration [3,48,49].

Most studies on hepatic lipogenesis have been concerned with the synthesis of fatty acids and there is less information available on their esterification. Since only a small proportion (13.5%) of newly synthesized fatty acids were released mainly in an esterified form into the perfusion medium, most of the newly synthesized triglyceride released presumably arose from esterification of existing fatty acid molecules. This suggests that esterification is a more rapid process than the synthesis of fatty acids under the conditions of the present experiments. This enhancement by six-carbon substrates of the esterification of fatty acids may not be a direct effect. Instead it may be explained as an inhibition of the oxidation sequence of long chain fatty acids, as shown recently for a rat liver mitochondrial and 140 000 $\times g$ supernatant system [50]. Previous

studies have demonstrated the reciprocal effects of fructose and sorbitol on the stimulation of esterification and the simultaneous inhibition of the oxidation pathway of fatty acid in rat liver slices [51], in hepatocytes isolated from starved rats [52] and in perfused rat liver [34,53].

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