Carbohydrate Metabolism of the Isolated Perfused Liver of Normal and Genetically Obese-Hyperglycaemic (*ob/ob*) Mice

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1. A technique for perfusion of the mouse liver has been developed, and aspects of carbohydrate metabolism have been investigated in the perfused liver of normal and genetically obese mice, homozygous for the recessive gene *ob*. 2. Rates of gluconeogenesis in perfused mouse liver were faster than those reported for slices of mouse liver, particularly from lactate and pyruvate. 3. The rate of glycogen breakdown to glucose, but not to lactate, was faster in liver from fed obese mice. 4. The capacity for glycogen synthesis from glucose was enhanced in liver from 20h-starved obese mice. 5. The capacity for gluconeogenesis from a number of substrates was not significantly altered in livers from fed or starved obese mice when compared with that of lean mice. 6. These results suggest that the liver contributes to the hyperglycaemia of the obese mice by increased glycogenolysis, and that liver glycogen in obese mice is maintained by synthesis from dietary glucose.

Genetically obese mice, homozygous for the recessive gene *ob*, have high blood concentrations of glucose and insulin when allowed free access to food (Christophe, Dagenais & Mayer, 1959; Westman, 1968; Stauffacher, Lambert, Vecchio & Renold, 1967; Genuth, 1969; for reviews see Hellman, 1965, 1967; Renold, 1968; Mayer 1960). However, when food is restricted, the high blood concentrations of glucose and insulin decline (Abraham, Dade, Elliott & Hems, 1971).

The combination of high blood concentrations of glucose and insulin is possibly a major cause of obesity in these mice, because insulin can stimulate the conversion of glucose to fat in adipose tissue for reviews (see Jeanrenaud & Hepp, 1970; Halperin & Robinson, 1971; Jeanrenaud, 1968). A technique for perfusion of the isolated mouse liver has been developed to investigate the role of hepatic glycogen metabolism and gluconeogenesis in the hyperglycaemia of obese mice. In the present paper the rates of these processes in the perfused liver of normal and obese mice are reported.

EXPERIMENTAL

Animals. Mice into which the Bar Harbor recessive gene for obesity *ob* was incorporated were bred in the Biochemistry Department at Imperial College (Abraham & Beloff-Chain, 1971). Obese mice, *ob/ob*, reached a weight of about 90 g at the age of 6 months, and were in good general health. If their dietary intake was restricted to 5 g/day (approximately that of normal mice) they reached a weight of 35-45 g; these mice are designated *ob/ob*-RD ('restricted diet'). Starved mice were deprived of food for 24 h, from about 10.00 h.

Chemicals. Enzymes and substrates for analytical procedures, and sodium pyruvate for perfusion were from C. F. Boehringer Corp. (London) Ltd., London W.5, U.K. L-Lactic acid was from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. D-Glucose and D-fructose were from Fisons Ltd., Loughborough, Leics., U.K. L-Alanine, L-serine and glycerol were from BDH Chemicals Ltd., Poole, Dorset, U.K. These and other chemicals were AnalaR grade. U-¹⁴C-labelled L-alanine (sodium salt) and L-lactate (sodium salt) were from The Radiochemical Centre, Amersham, Bucks., U.K.

Perfusion of isolated liver. The perfusion technique was essentially that described by Hems, Ross, Berry & Krebs (1966), which was based upon the method of Miller, Bly, Watson & Bale (1951). The standard medium consisted of 50 ml of bicarbonate-saline (Krebs & Henseleit, 1932) containing 2.5% (w/v) bovine serum albumin (Fraction V; Armour Pharmaceutical Company, Eastbourne, Sussex, U.K.), that had been dialysed against four changes of bicarbonate-saline for 2 days, and aged washed human erythrocytes. The haemoglobin concentration used was 3% (w/v) if livers were from starved mice, and 8% (w/v) in most perfusions of livers from fed animals, because of the possible relevance of haemoglobin concentration to glucose metabolism (Glinemann, Hern & Lynch, 1969). There was no haemoglobin in anaerobic perfusions.

Because of the smaller size of mouse blood vessels, modifications of the published procedure were required. The portal venous cannula was polythene (Portex, internal diameter 0.02 in; Portland Plastics Ltd., Hythe, Kent, U.K.), cut to a bevelled point; its length was about 2 cm and at flow rates of 2 ml of medium/min the pressure drop across it was about 10 cm of medium. This cannula was connected to the oxygenator by silicone-tubing filled with oxygenated medium. Immediately after the insertion of the cannula, the inferior vena cava was cut below the renal vein, and a slow flow of medium was allowed, which

emerged from the cut vein. The gall-bladder was then cut open, unless bile was to be collected. The inferior vena cava was cannulated through the right atrium with a curved, firm cannula about 8 cm long (Portex Ltd., internal diameter 0.04 in). The platform carrying the mouse was then placed beside the perfusion reservoir in the cabinet, and the inferior vena cava was tied above the right renal vein. The effluent medium was discarded for 3–4 min and then the cannula was placed in a side-arm of the reservoir, so that its end was about 7 cm below the liver. Thus, allowing for input cannula resistance, and output negative pressure, the input pressure was equivalent to about 17 cm of medium. Flow rates were 1.5–2.5 ml/min per g of wet liver in aerobic perfusions of lean or *ob/ob* mouse liver if the concentration of haemoglobin was 3% (w/v) and about 0.8–1.6 ml/min per g if it was 8%. The temperature of the cabinet was 37°C. Care was taken to prevent drying of the liver, by the use of saline-soaked tissue supported on a gauze arch over the organ. During perfusion, the surface of the liver remained moist and evenly brown-coloured. After perfusion the liver could be extensively cleared of medium, as judged by the even olive colour of the liver that appeared immediately after saline was substituted for the perfusion medium.

The perfusion medium, containing 25 mM-NaHCO₃, was gassed with O₂+CO₂ (95:5) in aerobic experiments, and N₂+CO₂ (95:5) in anaerobic experiments. At the point of entry to the liver, *p*O₂ was 320–370 mmHg in aerobic experiments. This is less than in the gas mixture at atmospheric pressure, because of inefficiency of the gas-exchanger, humidification, and leakage of gas from the tubing carrying medium to the liver. There was no major loss of CO₂, because the pH of the medium on entry to the liver was 7.4–7.5 (measured with a glass electrode). The rate of bile flow during aerobic perfusion was 0.74 µl/min (average from two perfusions of livers from starved mice with medium containing 10 mM-lactate).

Substrates were usually added to the medium at the beginning of perfusions. Samples of medium, 0.5 or 1.0 ml, were taken at 10–20 min intervals. When liver samples were taken during the perfusion, the base of the lobe was tied firmly with thread, and the liver was cut away, to be immediately frozen (see below). During the measurement of glycogen synthesis, the larger liver lobes were sampled in random order, in different perfusions.

Preparation of extracts. Samples of mouse liver were obtained *in vivo* within 12 s of cervical dislocation (Hems & Brosnan, 1970). These, or perfused liver samples, were clamped in metal tongs at the temperature of liquid N₂ (Wollenberger, Ristau & Schoffa, 1960). Frozen liver samples were ground, then weighed and homogenized in 4 vol. of 6% (w/v) perchloric acid or boiled in 10 vol. of 30% (w/v) KOH for isolation of glycogen. Samples of perfusion medium were mixed with an equal volume of 6% (w/v) perchloric acid. Perchloric acid extracts were centrifuged and neutralized with 30% (w/v) KOH, and potassium perchlorate was removed by centrifugation.

Analytical methods. Glucose, lactate, pyruvate, adenine nucleotides and haemoglobin were determined as described by Hems *et al.* (1966). Glycogen was isolated by ethanol precipitation after alkali treatment (Good, Kramer & Somogyi, 1933), and then determined as glucose after hydrolysis at pH 4.8 with amyloglucosidase from *Aspergillus niger* (kindly provided by Pro-

fessor W. J. Whelan), as described by Lee & Whelan (1966).

The O₂ content of the perfusion medium was calculated from the *p*O₂, which was measured with an O₂ electrode, in a Beckman gas analyser. The amount of O₂ bound to haemoglobin was calculated by assuming that dilution of the human erythrocytes did not alter the O₂ dissociation curve for aged cells at pH 7.4 given by Valtis & Kennedy (1954). The amount of O₂ in solution was calculated from a solubility coefficient at 37°C of 0.024 ml/ml of medium at 1 atmosphere pressure.

Dry weight and fat content of the liver were determined by weighing; fat was extracted with ethanol-diethyl ether (2:1, v/v). The N content was determined by a Kjeldahl method.

Experiments with U-¹⁴C-labelled lactate and alanine. The [¹⁴C]glucose produced from [U-¹⁴C]lactate or alanine was separated from precursor material on a column (6 mm × 80 mm) of Bio-Rad AG (X-14, 100–200 mesh). To remove lactate from glucose, the resin was initially in the Cl⁻ form; the neutralized extract of medium (about 1 ml) was loaded on to the column, which was then eluted with water and the first 6 ml of eluate was collected. A sample (0.5 ml) was counted for radioactivity. The recovery of glucose was 92%, and all radioactivity in the commercial lactate was removed except for a non-anionic impurity, which comprised 2% of the total radioactivity of the original lactate. To remove alanine from glucose, the resin was initially in the OH⁻ form. The column was then equilibrated with 0.5 M-glucose. The sample was loaded on to the column, which was then eluted with 0.5 M-glucose, and again the first 6 ml was collected and the radioactivity of a 0.5 ml sample was determined.

Determination of radioactivity in glucose and glycogen. Glycogen was isolated from the liver (see above), washed twice in 70% (w/v) ethanol and hydrolysed in 3 M-HCl at 95°C for 3 h. Aqueous samples of partially purified [¹⁴C]glucose from perfusion medium, and from glycogen were counted in a Packard Tri-Carb liquid-scintillation counter. The scintillation fluid contained 6 g of 5-(4-biphenyl)-2-(4-*tert.*-butylphenyl)-1-oxa-3,4-diazole (Ciba Ltd., Basle, Switzerland) and 50 g of naphthalene/l of toluene; each vial contained 10 ml of this fluid, plus 10 ml of 2-methoxyethanol. The d.p.m. were computed from the c.p.m. by a channels-ratio method, as described by Baillie (1960); the efficiency of counting was about 70%. The extent of incorporation of ¹⁴C from ¹⁴C-labelled precursors into glucose or glycogen was calculated from the ratio: d.p.m. in product per g of tissue/d.p.m. per µmol of precursor at the beginning of the perfusion. The results are expressed as µmol of precursor converted into glucose/g of liver.

RESULTS

General characteristics of the perfused mouse liver. Preliminary experiments were done to ascertain the conditions required to maintain the aerobic condition of the liver. Two criteria were used to assess the aerobic state of the perfused liver. First, the concentrations of adenine nucleotides after perfusion (Schimassek, 1963; Hems *et al.* 1966) were compared with the values obtained *in vivo*, because

the concentration of the adenine nucleotides is known to alter rapidly in the hypoxic liver (Brosnan, Krebs & Williamson, 1970). Secondly, the [lactate]/[pyruvate] ratio in the tissue, which is an index of the redox state of the cytoplasmic NAD-couple (Williamson, Lund & Krebs, 1967; Brosnan *et al.* 1970), was measured.

In perfused livers, the concentrations of ATP compared favourably with those *in vivo* (Table 1). The [lactate]/[pyruvate] ratio was greater in perfused livers, suggesting the presence of some hypoxia. However, this ratio was not diminished by increasing the haemoglobin content of the medium, and the O₂ supply could not be increased by increasing the pressure-head of the medium, as this caused the livers to swell.

In the presence of 3% (w/v) haemoglobin, the pO₂ of effluent blood from starved lean mouse liver was 15–25 mmHg; the O₂ uptake was 1.5–2.5 $\mu\text{mol/min per g}$ of wet liver in the absence of substrates, and 2–4 $\mu\text{mol/min per g}$ in the presence of 10 mm-lactate or pyruvate. These rates are similar to those in the isolated rat liver perfused in similar conditions (Hems *et al.* 1966).

In anaerobic perfusions, there was a great increase in the ratio [lactate]/[pyruvate], and a decrease in the amount of ATP (Table 1). The flow rate of the medium was 3 ml/min per g of wet liver in lean mice, and only 1.3 ml/min per g in *ob/ob* mouse livers. The latter livers tended to swell and become patchy in anaerobic conditions.

Composition of perfused mouse liver. The compositions of the livers that were used in the present experiments are shown in Table 2. The livers from *ob/ob* mice were larger than those of lean mice, although not particularly fatty (Table 2). The composition of these perfused livers is similar to that of the livers of these mice *in vivo* (E. Dade & D. A. Hems, unpublished work).

Rates of formation of glucose and lactate by perfused liver from fed mice. To investigate glycogen breakdown, mice received glucose before perfusion of their livers (see Table 3 for details) to ensure a sufficient initial amount of glycogen. The results given in Table 3 show that in livers from *ob/ob* and lean mice with similar initial glycogen amounts, the rate of glucose output in the first 30 min of perfusion under aerobic conditions was more than 4 times as great in livers from *ob/ob* mice than in livers from lean animals. The time-course of glucose release from the liver is indicated in Fig. 1. This shows that a high rate of glycogenolysis in the liver of the *ob/ob* animal is maintained for a period of perfusion of at least 100 min. At this time the glucose concentrations in perfusates of livers from *ob/ob* mice were 9–12 mM and 2–3 mM in the livers from lean mice, and the liver glycogen contents still exceeded 60 μmol of glucose/g.

Table 1. *Content of lactate, pyruvate and adenine nucleotides in mouse liver*

Mice	Substrate (10 mM)	Content of intermediates ($\mu\text{mol/g}$ of wet liver)				Total adenine nucleotides ($\mu\text{mol/g}$)		[ATP] [AMP]	
		Lactate	Pyruvate	ATP	ADP	AMP	[Lactate]	[Pyruvate]	[ADP] ²
Fed lean	—	2.50 $\pm$ 0.55 (4)	0.10 $\pm$ 0.01 (4)	2.75 $\pm$ 0.35 (4)	1.02 $\pm$ 0.11 (4)	0.18 $\pm$ 0.02 (4)	—	25	0.48
Fed <i>ob/ob</i>	—	1.65 $\pm$ 0.01 (4)	0.06 $\pm$ 0.01 (4)	2.90 $\pm$ 0.18 (4)	1.24 $\pm$ 0.14 (4)	0.23 $\pm$ 0.03 (4)	—	28	0.43
Fed lean, anaerobic	—	8.88 $\pm$ 1.96 (4)	<0.02	0.41 $\pm$ 0.12 (4)	0.74 $\pm$ 0.04 (4)	0.75 $\pm$ 0.07 (4)	—	>450	0.56
Starved lean	Alanine	1.59 $\pm$ 0.21 (3)	0.07 $\pm$ 0.01 (3)	1.20 $\pm$ 0.10 (3)	1.28 $\pm$ 0.26 (3)	0.33 $\pm$ 0.01 (3)	23	23	0.24
Starved <i>ob/ob</i>	Alanine	1.63 $\pm$ 0.08 (3)	0.08 $\pm$ 0.02 (3)	1.64 $\pm$ 0.20 (3)	1.20 $\pm$ 0.11 (3)	0.36 $\pm$ 0.14 (3)	20	20	0.41
Starved lean	Pyruvate	—	—	1.50 $\pm$ 0.08 (3)	1.33 $\pm$ 0.12 (3)	0.16 $\pm$ 0.02 (3)	—	—	0.14
Fed lean <i>in vivo</i>	—	1.37 $\pm$ 0.18 (3)	0.08 $\pm$ 0.01 (4)	2.84 $\pm$ 0.32 (4)	1.38 $\pm$ 0.08 (3)	0.19 $\pm$ 0.07 (3)	17	17	0.28
Fed <i>ob/ob in vivo</i>	—	1.54 $\pm$ 0.37 (3)	0.12 $\pm$ 0.02 (4)	2.95 $\pm$ 0.15 (4)	1.74 $\pm$ 0.08 (3)	0.24 $\pm$ 0.05 (3)	13	13	0.23
Starved lean <i>in vivo</i>	—	0.42 $\pm$ 0.06 (3)	0.03 $\pm$ 0.01 (4)	2.02 $\pm$ 0.11 (4)	1.43 $\pm$ 0.18 (4)	0.29 $\pm$ 0.07 (4)	14	14	0.29
Starved <i>ob/ob in vivo</i>	—	0.45 $\pm$ 0.06 (3)	0.03 $\pm$ 0.01 (3)	2.91 $\pm$ 0.08 (4)	1.50 $\pm$ 0.07 (4)	0.27 $\pm$ 0.05 (4)	15	15	0.35

Livers of male mice 3–4 months old were either perfused with standard medium and sampled after 100 min or were sampled *in vivo* within 12 s of cervical dislocation. Further details are in the text. Results are means $\pm$ s.e.m.; the numbers of measurements are in parentheses.

Table 2. *Composition of perfused mouse livers*

Livers of male mice 2–4 months old were removed after 120 min of perfusion with standard medium and analysed as described in the text. Results are the means $\pm$ s.e.m.; the numbers of observations are in parentheses.

Mice	Mouse wt. (g)	Total liver wet wt. (g)	Composition of perfused liver (% wet wt.)		
			Dry wt.	N	Fat
Fed lean	37 $\pm$ 1 (10)	1.53 $\pm$ 0.1 (10)	28 $\pm$ 1 (9)	3.2 $\pm$ 0.2 (3)	5.5 $\pm$ 1.0 (3)
Fed <i>ob/ob</i>	68 $\pm$ 3 (22)	2.34 $\pm$ 0.3 (11)	30 $\pm$ 1 (8)	3.1 $\pm$ 0.4 (4)	6.1 $\pm$ 0.5 (3)
Starved lean	31 $\pm$ 1 (22)	1.43 $\pm$ 0.1 (11)	33 $\pm$ 1 (40)	3.0 $\pm$ 0.1 (11)	11.3 $\pm$ 1.1 (15)
Starved <i>ob/ob</i>	64 $\pm$ 2 (14)	1.77 $\pm$ 0.1 (17)	34 $\pm$ 1 (17)	3.1 $\pm$ 0.3 (7)	12.4 $\pm$ 0.9 (11)

Table 3. *Initial rates of release of glucose and lactate by perfused livers of fed mice*

Livers of male mice 3–4 months old were perfused with standard medium. The haemoglobin concentration was 8% (w/v) in aerobic perfusions. To ensure comparable initial amounts of liver glycogen, mice were given 1 ml of 2 M-glucose intragastrically 90 min before perfusion; lean mice received in addition 0.5 ml of 2 M-glucose intraperitoneally 45 min before perfusion. Results are the means $\pm$ s.e.m.; the numbers of observations are in parentheses. The initial glycogen amount was measured after 3 min of perfusion. Initial metabolic rates were derived from time-courses over the first 30 min of perfusion.

Mice	Gas phase (95:5)	Rate of flow of medium (ml/min per g of wet liver)	Initial content of glycogen (μ mol of glucose/g of wet liver)	Initial metabolic rates (μ mol/min per g of wet liver)	
				Glucose release	Lactate release
Lean	O ₂ + CO ₂	1.1 $\pm$ 0.3 (6)	254 $\pm$ 17 (5)	0.97 $\pm$ 0.14 (6)	0.26 $\pm$ 0.10 (6)
<i>ob/ob</i>	O ₂ + CO ₂	0.9 $\pm$ 0.1 (7)	234 $\pm$ 28 (5)	4.19 $\pm$ 0.69 (7)	0.23 $\pm$ 0.12 (7)
Lean	N ₂ + CO ₂	2.9 $\pm$ 0.6 (4)	269 $\pm$ 60 (4)	3.52 $\pm$ 0.31 (5)	2.82 $\pm$ 0.42 (5)
<i>ob/ob</i>	N ₂ + CO ₂	1.3 $\pm$ 0.4 (4)	221 $\pm$ 44 (4)	4.98 $\pm$ 0.97 (4)	2.65 $\pm$ 0.66 (4)

Fig. 1. Time-course of release of glucose into the medium during perfusion of livers from fed mice. Livers were perfused as described in Table 3. Other details are in the text. The bars indicate the s.e.m. ■, Lean (six perfusions); ○, *ob/ob* (seven perfusions); △, *ob/ob*-RD (three perfusions).

This very high rate of glycogenolysis was not present in the perfused liver of mice that were partially food-deprived, i.e. *ob/ob*-RD mice (Fig. 1).

In aerobic perfusions, the initial lactate concentration of 1–3 mM was presumably derived from the erythrocytes. The rate of lactate accumulation was slow (Table 3) and tended to decrease after 1–2 h, reaching a concentration of 2–4 mM during perfusion of either lean or *ob/ob* mouse liver. The rate of lactate release was the same in lean and *ob/ob* mice under aerobic conditions (Table 3).

In aerobic perfusions of liver from fed rats, autoregulation of glucose concentration (i.e. a tendency to constant concentration of glucose in the medium) can be demonstrated, and is influenced by the haemoglobin concentration (Glinsmann *et al.* 1969). Because *ob/ob* mice are hyperglycaemic, we decided to investigate this aspect of hepatic metabolism. The results are shown in Fig. 2. The glucose concentration in the medium did not decrease from the high initial values in perfusions of livers from fed lean or *ob/ob* mice. Thus autoregulation of glucose concentration was not detected. In these perfusions, it was observed that

the concentration of lactate in the medium did not increase above that in aerobic perfusions where no initial glucose was present.

In anaerobic perfusions, the rates of release of glucose and lactate from glycogen were faster than in aerobic conditions, and the release of glucose, but not lactate, from the liver of *ob/ob* mice was slightly faster than in the liver of lean mice (Table 3).

Rates of glycogen synthesis in perfused mouse liver. Measurement of glycogen synthesis in liver is complicated by glycogen that is already present. Hence the liver glycogen must be depleted before net synthesis can be satisfactorily measured. Mice were deprived of food from 17.00h, and the livers were perfused with 30mM-glucose about 20h later. The results (Table 4) show that synthesis of glycogen from glucose was faster in livers from *ob/ob* mice.

Rates of gluconeogenesis in perfused mouse liver. In one group of experiments, gluconeogenesis was measured in liver from fed mice. Glucose formation

from U-¹⁴C-labelled precursors was measured, to obviate the complication in the interpretation of the results that arises from the effects of glycogenolysis (Krebs, 1964). The results are given in Table 5. The amount of glucose formed from lactate or alanine was not significantly different in the *ob/ob* and lean mice (Table 5). In these experiments, the rate of gluconeogenesis was measured by sampling the medium every 10 min. After addition of ¹⁴C-labelled precursor the eventual rate of glucose formation was linear with time, but tended to show an apparent lag phase, mainly caused by the delay in mixing of the labelled precursor throughout the perfusion medium. This accounts for the fact that rates of gluconeogenesis in Table 5, especially from alanine, are greater than is implied by the amount of ¹⁴C in glucose after 50 min. The amount of glycogen in the perfused liver from *ob/ob* mice was about 7 times that in the livers from lean mice, but the amount of [U-¹⁴C]lactate converted into glycogen was no greater in the *ob/ob* animals (Table 5).

Fig. 2. Concentration of glucose in the medium during perfusion of livers from fed mice with high initial concentrations of glucose. Livers were perfused as described in Table 3, except that glucose was added to the medium. Each symbol denotes a single perfusion. (a) Lean; (b) *ob/ob*.

Table 4. *Synthesis of glycogen from glucose by perfused liver*

Livers from 20 h-starved male mice 3–4 months old were perfused with standard medium containing 30 mM-glucose and 8% (w/v) haemoglobin. Experimental details are in the text. There were six perfusions in each group; results are expressed as means $\pm$ S.E.M.

Mice	Time of sampling ...	Liver glycogen content (μ mol of glucose/g of wet liver)			Calculated rate of glycogen synthesis (μ mol of glucose/min per g)	
		15 min	45 min	75 min	15–45 min	45–75 min
Lean		3 $\pm$ 1	11 $\pm$ 3	32 $\pm$ 9	0.27	0.70
<i>ob/ob</i>		6 $\pm$ 2	17 $\pm$ 3	50 $\pm$ 6	0.37	1.10

In perfused liver from starved mice, net gluconeogenesis was measured directly. The rate of glucose formation was linear for 1–2 h. The rates of glucose formation from various precursors by isolated perfused liver of starved *ob/ob* mice were similar to the rates of formation by liver from normal mice (Table 6). No glycogen was deposited in the liver during the perfusion; the initial glycogen amount in the liver from starved (lean or *ob/ob*) mice was 1–10 μmol of glucose/g of wet liver and, after 100 min perfusion, 1–15 μmol of glucose/g. The rate of gluconeogenesis was not increased by raising the concentration of haemoglobin above 3% (w/v). Krebs, Notton & Hems (1966) reported that gluconeogenesis was faster in liver slices from female than those from male mice. This was not observed in the present experiments (Table 6).

DISCUSSION

Characteristics of isolated perfused mouse liver. The metabolic integrity of the mouse livers perfused by the present technique is demonstrated by the content of adenine nucleotides, and the estimated redox state of the cytoplasmic NAD-couple, the values of which compare satisfactorily with those *in vivo*. Further, in the isolated perfused mouse liver the rates of gluconeogenesis were 1–3 times as fast as those obtained with liver slices from male mice (Krebs *et al.* 1966); in particular, the rates were faster with lactate and pyruvate as substrates. Hence the perfused mouse liver seems to be a better preparation than tissue slices for the measurement of rates of hepatic metabolic processes, as is true for the rat liver perfused in similar conditions (Ross, Hems & Krebs, 1967).

*Glycogen metabolism in *ob/ob* mice.* The formation of an excess of glucose from glycogen by isolated liver from *ob/ob* mice presumably contributes to the maintenance of their hyperglycaemia, especially as the liver is enlarged in these mice. The increased rate of glycogenolysis was not caused by tissue hypoxia, as shown by adenine nucleotide levels and the [lactate]/[pyruvate] ratio. The high capacity for glycogen breakdown is probably attributable to the high activity of liver glycogen phosphorylase in *ob/ob* mice (Shull, Ashmore & Mayer, 1956). The origin of this high activity has not been established. It is secondary to the obesity and the associated events, since it was not present in *ob/ob*-RD mice, and it may possibly be associated with the increased secretion of adrenal cortical steroids in *ob/ob* mice (see Hellman, 1965), as the adrenal cortex has been suggested to control the activity of liver phosphorylase (Shull, Cahill, Gadsden & Mayer, 1956; Schaeffer, Chenoweth & Dunn, 1969).

Table 5. Incorporation of ^{14}C from $[\text{U}-^{14}\text{C}]\text{lactate}$ or $[\text{U}-^{14}\text{C}]\text{alanine}$ into glucose and glycogen by perfused liver of fed mice

Mice	Average liver wet wt. (g)	Substrate (initially 10 mM)	Number of perfusions	Glucose in medium (mM)	Metabolic changes after 50 min of perfusion				Rate of gluconeogenesis (μmol of precursor/g per min)	Calculated quantity of precursor converted into glucose in 50 min (μmol /g of wet liver)	
					Specific radioactivity of ^{14}C glucose in medium (d.p.m./ μmol)	Liver glycogen content of glucose/g of wet liver	Specific radioactivity of total ^{14}C glucose in liver glycogen (d.p.m./ μmol)	Calculated concentration of glucose in medium derived from precursor (mM)		Glucose in 50 min	Glycogen in 50 min
Lean	1.2	$[\text{U}-^{14}\text{C}]\text{lactate}$	3	2.9 ± 0.6	7890 ± 990	10 ± 4	4800 ± 2600	0.5 ± 0.1	0.61 ± 0.06	28 ± 3	1.5 ± 0.3
<i>ob/ob</i>	2.5	$[\text{U}-^{14}\text{C}]\text{lactate}$	3	12.5 ± 2.0	2110 ± 465	69 ± 24	313 ± 234	0.7 ± 0.2	0.49 ± 0.06	26 ± 6	0.7 ± 0.4
Lean	1.4	$[\text{U}-^{14}\text{C}]\text{alanine}$	6	2.2 ± 0.4	1647 ± 245	—	—	0.08 ± 0.02	0.14 ± 0.03	5.1 ± 0.1	—
<i>ob/ob</i>	2.3	$[\text{U}-^{14}\text{C}]\text{alanine}$	5	7.2 ± 1.8	898 ± 244	—	—	0.15 ± 0.04	0.15 ± 0.02	6.1 ± 0.8	—

Livers of male mice 3–4 months old were perfused with 35–50 ml of standard medium containing 8% (w/v) haemoglobin, plus substrate (initially 10 mM). After 30 min a trace dose of $[\text{U}-^{14}\text{C}]\text{alanine}$ or $[\text{U}-^{14}\text{C}]\text{lactate}$ was added: the initial specific radioactivity of precursors was $1.8\text{--}2.2 \times 10^4$ d.p.m./ μmol . Glycogen in the liver was analysed 50 min after adding ^{14}C -labelled precursor. Further details are in the text. Results in each group are means $\pm$ S.E.M. for the numbers of perfusions indicated.

Table 6. *Rates of gluconeogenesis by perfused liver of starved mice*

The livers of mice 3–4 months old were perfused with the standard medium; the haemoglobin concentration was 3% (w/v). Substrates were added at the beginning of the perfusion. Further details are in the text. Results are the means $\pm$ S.E.M.; the numbers of observations are in parentheses.

Substrate (initially 10 mM)	Mice ...	Rates of gluconeogenesis ($\mu\text{mol/min per g of wet liver}$)		
		Lean male	<i>ob/ob</i> male	Lean female
None		0.18 $\pm$ 0.05 (6)	0.25 $\pm$ 0.01 (4)	0.22 $\pm$ 0.05 (4)
Lactate		0.89 $\pm$ 0.08 (7)	0.95 $\pm$ 0.13 (5)	0.81 $\pm$ 0.08 (3)
Pyruvate		0.84 $\pm$ 0.05 (3)	—	—
Alanine		0.38 $\pm$ 0.05 (6)	0.37 $\pm$ 0.04 (6)	0.26 $\pm$ 0.05 (3)
Serine		0.29 $\pm$ 0.05 (3)	—	—
Fructose		1.27 $\pm$ 0.06 (3)	1.29 $\pm$ 0.06 (3)	1.34 $\pm$ 0.14 (3)
Glycerol		0.51 $\pm$ 0.03 (5)	0.65 $\pm$ 0.03 (5)	—

The main precursors of the excess of liver glycogen and blood glucose in *ob/ob* mice are presumably the glucose-containing constituents of ingested food, since the rate of formation of glucose or glycogen by gluconeogenesis is not increased in *ob/ob* mice. Thus it is not surprising that the hyperglycaemia and hyperinsulinaemia of *ob/ob* mice disappear on starvation (Abraham *et al.* 1971).

As the *ob/ob* mice have consistently higher amounts of liver glycogen (Abraham *et al.* 1971) it is likely that glycogen is more rapidly synthesized *in vivo*. This has been shown in *ob/ob* mice with [^{14}C]glucose (Shull & Mayer, 1956) and by re-feeding glucose to the starved animal (Abraham *et al.* 1971). The present results show that the capacity for glycogen synthesis from glucose by perfused liver is greater in *ob/ob* mice. These observations are consistent with the known increase in hepatic glucokinase activity in *ob/ob* mice (Creutzfeldt, Mende, Willms & Soling, 1970; Hellerstrom *et al.* 1970).

Lactate formation in perfused mouse liver. Because of the increased rates of synthesis of liver lipid from glucose in obese-hyperglycaemic mice (Shigeta & Shreeve, 1964; Winand, Furnelle, Georis & Christophe, 1968; Jansen, Zanetti & Hutchison, 1967; Shreeve, Lamdin, Oji & Slavinski, 1967; Zomzely & Mayer, 1959; Lamdin, Shreeve, Slavinski & Oji, 1969), we assessed the capacity of the glycolytic pathway in liver. This capacity may be reflected in the rate of lactate release, perhaps especially in anaerobic conditions. The formation of lactate from glycogen or glucose was not enhanced in *ob/ob* mice, in aerobic or anaerobic conditions.

Gluconeogenesis in *ob/ob* mice. The lack of a detectable enhancement of gluconeogenesis in the liver of *ob/ob* mice, which in general confirms the results obtained by perfusion of New Zealand obese mouse liver with [^{14}C]alanine (Rudorff, Huchzermeyer,

Windeck & Staib, 1970), makes it unlikely that gluconeogenesis contributes significantly to the excess of circulating glucose of these animals. Thus the increased conversion of the carbon of precursors such as lactate and glycerol into fat in the liver of *ob/ob* mice (Shreeve *et al.* 1967), is probably not associated with an increase in their conversion into glucose. Interestingly, there was some enhancement in gluconeogenesis from endogenous substrates in the *ob/ob* mouse liver; this enhancement is similar to that observed by Krebs *et al.* (1966) in liver slices from alloxan-diabetic animals.

Several investigators have measured the activity of potentially regulatory gluconeogenic enzymes in the liver of *ob/ob* mice. Enhancement of glucose 6-phosphatase activity has been reported (Seidmann, Horland & Teebor, 1967, 1970), which is compatible with the increased rate of glycogenolysis reported in the present paper. Pyruvate carboxylase and phosphoenolpyruvate carboxykinase activities are not increased in *ob/ob* mouse liver (Herberg *et al.* 1970). Seidmann *et al.* (1967) reported a small increase in fructose diphosphatase activity, but there is also an increase in phosphofructokinase activity in *ob/ob* mouse liver. No increase in fructose diphosphatase activity was observed by Creutzfeldt *et al.* (1970) or Willms, Ben-Ami & Soling (1970) in the liver of *ob/ob* mice. The above observations are thus consistent with the present finding that the capacity for hepatic gluconeogenesis is not increased in *ob/ob* mice.

Herberg *et al.* (1970) reported that [^{14}C] from [^{14}C]CO₂ is incorporated more rapidly into acid-soluble metabolites in liver slices from *ob/ob* animals. However, several interpretations of this finding are possible, and it cannot be regarded as evidence for an increased capacity for gluconeogenesis in isolated liver, especially because of the present results.

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