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Synthesis of Fatty Acids in the Perfused Mouse Liver

By D. MICHAEL W. SALMON, NEIL L. BOWEN and DOUGLAS A. HEMS
Department of Biochemistry, Imperial College, London S.W.7, U.K.

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1. Fatty acid synthesis *de novo* was measured in the perfused liver of fed mice. 2. The total rate, measured by the incorporation into fatty acid of ^3H from ^3H_2O (1–7 μmol of fatty acid/h per g of fresh liver), resembled the rate found in the liver of intact mice. 3. Perfusions with L-[U-^{14}C]lactic acid and [U-^{14}C]glucose showed that circulating glucose at concentrations less than about 17mM was not a major carbon source for newly synthesized fatty acid, whereas lactate (10mM) markedly stimulated fatty acid synthesis, and contributed extensive carbon to lipogenesis. 4. The identification of 50% of the carbon converted into newly synthesized fatty acid lends further credibility to the use of ^3H_2O to measure hepatic fatty acid synthesis. 5. The total rate of fatty acid synthesis, and the contribution of glucose carbon to lipogenesis, were directly proportional to the initial hepatic glycogen concentration. 6. The proportion of total newly synthesized lipid that was released into the perfusion medium was 12–16%. 7. The major products of lipogenesis were saturated fatty acids in triglyceride and phospholipid. 8. The rate of cholesterol synthesis, also measured with ^3H_2O, expressed as acetyl residues consumed, was about one-fourth of the basal rate of fatty acid synthesis. 9. These results are discussed in terms of the carbon sources of hepatic newly synthesized fatty acids, and the effect of glucose, glycogen and lactate in stimulating lipogenesis, independently of their role as precursors.

Long-chain fatty acids are synthesized in the liver from the acetyl component of tissue acetyl-CoA. The nature of the circulating (or tissue) precursors of this active C_2 residue is not clear. The purpose of the present paper is to report experiments with the perfused liver of the mouse, in which the total rate of fatty acid synthesis was measured by the incorporation of ^3H_2O into fatty acids (Windmueller & Spaeth, 1966). Perfusions with ^{14}C-labelled glucose or lactate are described which suggest that blood glucose does not provide significant carbon for fatty acid synthesis, whereas precursors which are converted rapidly into pyruvate, such as glycogen and lactate, can more easily fulfil this role. The results also show that glucose, glycogen and lactate can stimulate the total rate of fatty acid synthesis independently of their function as carbon sources.

Materials and Methods

Perfusion of mouse liver

Female mice (aged about 3 months) from a mixed strain bred in the Department of Biochemistry at Imperial College (London S.W.7, U.K.) were fed on a standard mixed supplemented (Thompson's) diet (Pilsburys Ltd., Birmingham, U.K.). Livers (average weight about 1.4g) were perfused (usually between 11.00 and 14.00h) as described by Elliott *et al.* (1971), with the following minor modifications: (i) during the operative procedure, after portal vein cannulation, warm gassed (O_2+CO_2, 95:5) bicarbonate-buffered saline (Krebs & Henseleit, 1932), containing no albumin or erythrocytes, was circulated through the liver; (ii) a larger input cannula was used (internal diam. 0.5mm, outer diam. 0.75mm) which was cut to a bevelled point and a length of 1cm, from a Portex cannula with a Luer fitting (Portland Plastics Ltd., Hythe, Kent, U.K.); (iii) heparin was not administered (before perfusion). The perfusion medium, which consisted of bicarbonate-buffered saline (60ml) containing bovine serum albumin (3.5%, w/v) and 'aged' human erythrocytes (haemoglobin about 8%, w/v), was gassed with O_2+CO_2 (95:5).

The total rate of synthesis of fatty acids or cholesterol was followed by measuring the incorporation into lipids of ^3H from ^3H_2O (2–4mCi in each perfusion). Quantitative aspects of this method have been evaluated by Jungas (1968) in adipose tissue, and Windmueller & Spaeth (1966) in liver. The perfusion medium also contained about 2 μCi of U-^{14}C-labelled D-glucose (3–43mM) or L-lactate (initially 9–12mM). All labelled precursors were present from the start of perfusion.

Sequential samples of liver and perfusion medium were taken for the determination of ^3H and ^{14}C radioactivity in lipids. Liver samples were dropped into liquid N_2 and weighed. The usual order of sampling of the liver (see Hems *et al.*, 1972) was to take one-half of the median (bifurcated) lobe initially, followed by the other half, and finally, the left lateral (largest) lobe; these were removed after

1, 2 and 3h of perfusion respectively. Perfusion medium, removed at 30min intervals, was then centrifuged briefly to obtain plasma for lipid extraction (see below) or extraction with $HClO_4$ for measurement of glucose and lactate (see Elliott *et al.*, 1971).

Chemicals and analytical methods

Chemicals were of the highest grade commercially available (for sources see Elliott *et al.*, 1971; Salmon & Hems, 1973).

The radioactivity in total lipid was measured in washed extracts (Folch *et al.*, 1957) of liver or perfusion medium, and that in liver fatty acids after saponification (in 5M-NaOH in 30%, v/v, ethanol for 3h at 80°C) and extraction (Brunengraber *et al.*, 1973). The ^{14}C or ^{3}H radioactivity in fatty acids separated according to saturation [as methyl esters (Dunn & Robson, 1965)] or in major lipid classes, was determined after t.l.c. (Salmon & Hems, 1973). The recovery of free cholesterol after t.l.c. was about 90%; the amount of ^{3}H-labelled cholesterol, after t.l.c. bands were scraped into vials and counted, was about 70% of that in the total non-saponifiable petroleum-soluble fraction. Amounts of ^{3}H and ^{14}C radioactivity in samples were determined in a Packard Tri-Carb liquid-scintillation spectrometer, in a fluid containing 6g of 5-(4-biphenylyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazol (CIBA Ltd., Basle, Switzerland)/l of toluene. Each vial contained 10ml of this fluid, plus 2ml of 2-methyoxyethanol when counting the radioactivity of small aqueous samples. The d.p.m. were computed from the c.p.m. by channels-ratio methods for the determination of ^{3}H or ^{14}C alone (Baillie, 1960) or simultaneously (Hendler, 1964). The specific radioactivity of the perfused $^{3}H_2O$ (d.p.m./mol of total water) was calculated by measuring ^{3}H in cell-free perfusion medium, and by presuming water concentration to be 53M (Windmueller & Spaeth, 1966). The initial specific radioactivity of [^{14}C]lactate or [^{14}C]glucose was determined by measuring lactate (9–12mM) or glucose (4–32mM) within 5min of the start of perfusion, and presuming that all ^{14}C in the perfusate at that time was in lactate, or glucose respectively.

Results are expressed as μmol of fatty acid or cholesterol synthesized. The total rates of synthesis were calculated from the quotient: (^{3}H in lipid in d.p.m.)/(sp. radioactivity of $^{3}H_2O$), dividing by 13.3 to obtain μmol of fatty acid, and by 19 to obtain μmol of cholesterol, as described by Windmueller & Spaeth (1966, 1967). Rates of synthesis from ^{14}C-labelled precursors were determined from the μg-atom of C in lipid (calculated from the initial specific radioactivity of precursors) by presuming that 16μg-atoms of C were incorporated/μmol of newly synthesized fatty acid [since C_{16}-fatty acid is the main product of synthesis *de novo* in the liver (Foster & Bloom, 1963)], and 27μg-atom/μmol of cholesterol.

Glycogen, glucose and lactate were determined as described by Elliott *et al.* (1971).

Results

Time-course of hepatic fatty acid synthesis

During perfusion of the liver in the presence of added glucose (4–32mM) the time-course of incorporation of ^{3}H from $^{3}H_2O$ into liver fatty acid was approximately linear for 3h, although tending to rise slightly during the second and third hour of perfusion under some conditions (Table 1). There was no evidence that the incorporation of ^{3}H varied significantly between liver lobes in experiments where lobes were sampled simultaneously, or in an order different from the standard sequence. Thus the total rate of fatty acid synthesis in perfusion could be calculated from the change in the ^{3}H content of fatty acids in sequential liver samples (Table 1, Fig. 1). The time-course of incorporation of ^{14}C from [^{14}C]-glucose (4–32mM) or [^{14}C]lactate (10mM) into hepatic fatty acids was also approximately linear for 3h (results not shown in detail; such linearity is implicit in the results in Table 1). This observation means that rates of fatty acid synthesis from ^{14}C-labelled precursors may reasonably be calculated from the initial specific radioactivity of the precursor. The linear time-courses probably imply a relatively constant specific radioactivity of precursor as would be expected during perfusion of the small mass of the mouse liver with 60ml of medium.

Dependence of hepatic fatty acid synthesis on glucose

The dependence of fatty acid synthesis on glucose was investigated. This aim was facilitated by the fact that during perfusion for 3h at various glucose concentrations (4–32mM; no other substrates added) there were no marked changes in glucose concentrations (see also Elliott *et al.*, 1971), presumably due to the small mass of the mouse liver. Over the range 3–15mM-glucose there was little variation in the 'basal' rate of fatty acid synthesis, whereas at higher concentration (15–30mM), synthesis was enhanced (Fig. 1).

The contribution of circulating glucose carbon to the synthesis *de novo* of fatty acids was investigated with [U-^{14}C]glucose (present in the perfusion medium in addition to $^{3}H_2O$). The percentage of carbon in fatty acid provided by glucose was 15% or less, unless the glucose concentration was greater than about 17mM (Table 1). The proportion of ^{3}H from $^{3}H_2O$, or [^{14}C]glucose in the fatty acid portion of hepatic lipids was 55–80% of the radioactivity in the total (washed but not saponified) lipid extract, depending on the glucose concentration in the perfusate (Fig. 2). Most of the non-fatty acid radioactivity was in

Table 1. *Synthesis of fatty acids from glucose and lactate in the perfused liver*

Livers were perfused with medium containing ^3H_2O and precursors (including ^{14}C) at the initial concentrations indicated. The ^3H and ^{14}C contents of hepatic fatty acids were determined in sequential samples. The total amount of newly synthesized fatty acid was calculated from the ^3H values, and that formed from glucose or lactate from the ^{14}C results; the latter quantity is expressed as a percentage of the former. The rate of synthesis of total new fatty acid was calculated for the third hour of perfusion. Other details are in the text. Results are means ± S.E.M. of the number of observations in parentheses.

Additions to medium (mM)	Time of liver sample (min) ...	Total newly synthesized fatty acid (μmol/g of fresh liver)			Calculated percentage of synthesized fatty acid derived from ^{14}C-labelled precursors			Calculated total rate of fatty acid synthesis (μmol/h per g)
		60	120	180	60	120	180	
[^{14}C]Glucose (4–6)		1.4±0.2 (3)	2.8±1.0 (3)	4.8±0.5 (3)	6±1 (3)	9±1 (3)	11±1 (3)	2.0
[^{14}C]Glucose (11–17)		1.2±0.2 (3)	2.1±0.5 (3)	4.0±0.7 (4)	15±3 (3)	14±2 (3)	14±2 (3)	1.9
[^{14}C]Glucose (21–23)		3.2±0.1 (3)	6.4±0.5 (3)	9.6±1.6 (3)	20±1 (2)	23±2 (3)	22±1 (3)	3.2
[^{14}C]Glucose (24–32]		2.3±0.3 (6)	5.4±0.6 (7)	10.3±0.9 (7)	36±3 (3)	38±4 (3)	40±5 (3)	4.9
[^{14}C]Lactate (10) +glucose (5–10)		2.3±0.4 (6)	4.5±0.7 (7)	7.0±1.0 (7)	34±7 (5)	32±4 (6)	31±7 (6)	2.5
[^{14}C]Lactate (10) +glucose (12–17)		2.4±0.4 (4)	6.9±0.6 (6)	12.8±0.5 (4)	49±13 (4)	36±4 (6)	34±3 (4)	5.9
[^{14}C]Lactate (10) +glucose (20–25)		2.1±0.2 (5)	5.1±0.7 (5)	8.0±1.1 (5)	30±4 (5)	29±4 (5)	26±4 (5)	2.9

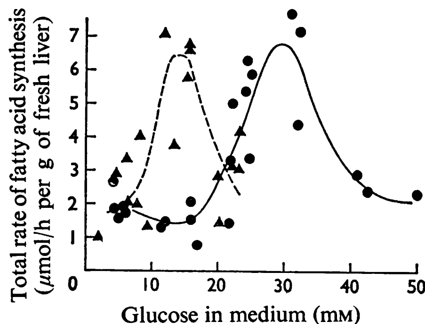

Fig. 1. *Dependence of total fatty acid synthesis in the perfused mouse liver on glucose concentration*

Livers were perfused with ^3H_2O and glucose at various initial concentrations (measured after 5 min of perfusion) as described in Table 1. Rates of synthesis were calculated from the increase in liver ^3H-labelled fatty acids between 2 and 3 h of perfusion. Other details are in the text. Results are from single perfusions, including those of Table 1; ●, no added lactate; ▲, initial lactate 9–12 mM.

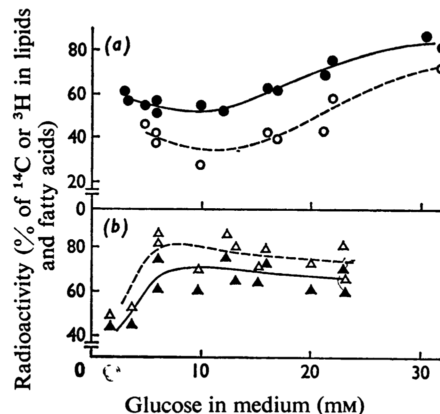

Fig. 2. *Distribution of ^3H or ^{14}C in lipids in the perfused mouse liver*

Livers were perfused as described in Table 1 or Fig. 1. After 3 h ^3H and ^{14}C were determined in the total lipid fraction and in fatty acids after saponification. Other details are in the text. Results are from single perfusions, including those of Table 1 and Fig. 1: (*a*) no added lactate; (*b*) initial lactate 9–12 mM. ●, ▲, ^3H in lipid; ○, △, ^{14}C in lipid.

lipid glycerol (as shown by determining water-soluble ^3H and ^{14}C after saponification; results not given). The proportion of ^3H in lipids, or ^{14}C (from ^{14}C-labelled glucose or lactate) in fatty acid increased with the glucose concentration in the perfusate, in the same manner as the corresponding rate of fatty acid synthesis (cf. Figs. 1 and 2). The time-course of incorporation of ^{14}C from ^{14}C-labelled glucose or lactate into total (un-saponified) lipid was linear for 3 h (results not shown). The proportion of ^{14}C-labelled lipid in fatty acid was consistently less than that for ^3H in perfusions with [U-^{14}C]glucose, whereas the converse was true for perfusions with [U-^{14}C]lactate (Fig. 2).

Fatty acid synthesis from lactic acid

Since glucose was the only substrate added to the perfusion medium in the experiments described above, the remaining (unidentified) carbon for fatty acid synthesis must have been derived either from endogenous precursors or from circulating material such as lactic acid. Lactate, which is unavoidably present from an early stage during perfusions of livers from fed animals (being formed from liver glycogen; Glinsmann *et al.*, 1969; Woods & Krebs, 1971), can markedly stimulate total fatty acid synthesis in chick hepatocytes (Goodridge, 1973). To test the role of lactate, livers were perfused with [U-^{14}C]lactate (initially 9–12mM) at various glucose concentrations. Lactate made a major contribution to the carbon skeleton of newly synthesized fatty acids, (26–49%) especially at glucose concentrations in the intermediate range (11–17mM, Table 1), when maximum rates of fatty acid synthesis in the presence of lactate were observed (Fig. 1).

During the perfusions with combinations of glucose and lactate, net changes in circulating substrates were followed. When glucose (10 or 25mM), but no added lactate, was present initially, lactate was gradually released to reach a steady concentration of 1–3 or 5–6mM respectively. When lactate was initially about 10mM, uptake of lactate did not occur when glucose was 5 or 25mM, presumably because lactate was being formed respectively from glycogen or glucose (Woods & Krebs, 1971). However, at intermediate glucose concentrations (about 15mM) uptake of lactate (initally 9–12mM) was discernible, mainly during the second hour of perfusion, at a rate of about 1 μmol/min per g of fresh liver. During perfusion with added lactate, at all glucose concentrations, glucose was released into the perfusion medium, at about the above rate.

Fatty acid synthesis in intact mice

For comparison with rates in the perfused mouse liver, fatty acid synthesis was measured *in vivo* with $^{3}H_2O$, in mice similar to those used in the perfusion experiments. The ^{3}H content of the total hepatic fatty acid fraction was determined after 60min. In conscious female mice (aged about 3 months studied between 10.00 and 14.00h, the rate of total fatty acid synthesis (mean value±S.E.M. with number of animals in parentheses) was 1.8±0.3 (8) μmol of fatty acid/h per g of fresh liver. Other experiments have shown that during the daily 24h cycle, the rate varies between 1 and 4μmol/h per g (D. Hems, E. Rath & T. Verrinder, unpublished results). Thus the rates of hepatic fatty acid synthesis in intact mice closely resemble those in the perfused mouse liver (1–7μmol/h per g).

Role of glycogen in hepatic fatty acid synthesis

It is likely that a significant part of the carbon for fatty acid synthesis in the perfused liver was obtained from liver glycogen. The relationship between glycogen and fatty acid synthesis was investigated as follows; two mild procedures were selected which altered (*in vitro*) the initial glycogen content of the perfused liver. These were (i) to perfuse briefly (30–60min) in the absence of added glucose or erythrocytes, which depleted glycogen, and (ii) to add glucose (20mM) to the medium perfusing the liver during the operative procedure, which caused retention of rather more glycogen than in the standard procedure. These livers, and 'standard' livers, were perfused for 1h with 5mM-[^{14}C]glucose. The rate of fatty acid synthesis, and the percentage contribution of glucose to fatty acid synthesis, were positively correlated with the initial glycogen content of the liver (Fig. 3).

If the glucose concentration in the medium perfusing livers from fed animals is increased, glycogen breakdown is gradually suppressed, and eventually ceases at about 20mM-glucose (Glinsmann *et al.*, 1969). This was indirectly confirmed in the present experiments, as shown by the presence of glycogen

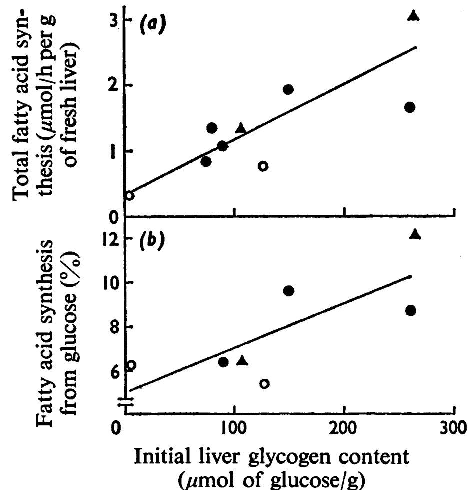

Fig. 3. *Influence of glycogen concentration on fatty acid synthesis in the perfused mouse liver*

Livers were perfused with medium containing $^{3}H_2O$ and 5mM-[U-^{14}C]glucose for 1h. Samples were taken after 5min for the determination of glycogen (in the median bifurcated lobe) and after 1h for the measurement of ^{3}H and ^{14}C in fatty acid (in the left lateral lobe). The procedures for varying the initial glycogen content and other details are given in Table 1 and the text. ▲, Pre-perfusion with 20mM-glucose; ●, 'standard' perfusions; ○, pre-perfusion without glucose. Results are from individual perfusions and lines have been fitted by regression analysis: (*a*) $r = 0.83$, $P<0.01$; (*b*) $r = 0.77$, $P<0.05$.

contents similar to that *in vivo* after 3h perfusion at high glucose concentrations (Fig. 4).

Secretion of newly synthesized fatty acid

The liver is able to release fatty acid into blood as glycerolipid (mainly triglyceride). In the present experiments, the proportion of newly synthesized lipid which was released into the perfusion medium increased from 12% of the ^{3}H-labelled lipid in liver plus perfusate in the first hour, to 16% in the second and third hours of perfusion. These values were obtained by determination of ^{3}H in unsaponified lipid (both of liver and perfusate), but also apply to the fatty acid moiety, since the proportion of ^{3}H-labelled lipid in fatty acid in the perfusate was similar to that found in the liver (i.e. 55–80%; Fig. 2). The proportion of secreted ^{3}H-labelled lipid is comparable with values obtained in the rat liver perfused with a medium containing erythrocytes (Windmueller & Spaeth, 1966, 1967). Among the variables which were investigated in the present experiments (e.g. dependence on glucose, lactate and glycogen) none altered the proportion of ^{3}H-labelled lipid that was secreted.

Nature of newly synthesized hepatic lipids

The fatty acids of the liver are contained within a variety of constituent lipids. The predominant lipid classes are the triglycerides and phospholipids. The distribution of ^{14}C in these classes was measured in unsaponified fractions; this approach was valid since

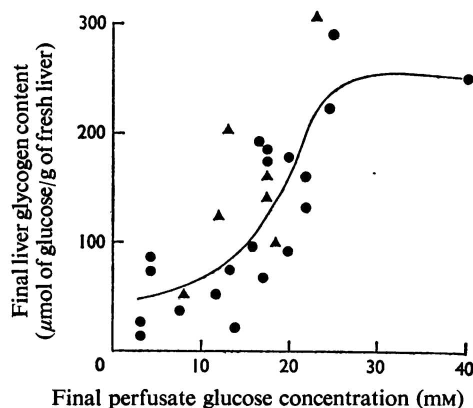

Fig. 4. *Glycogen concentration in the perfused mouse liver*

Livers were perfused as described in Table 1 and Fig. 1. The glycogen content was measured in the left lateral lobe after 3h. For comparison the hepatic glycogen concentration in similar mice (μmol of glucose/g fresh wt.) was 255±12 in untreated animals and 157±14 at the beginning of perfusion (means±S.E.M. of three measurements). Other details are in the text. Results are from single perfusions. ●, No added lactate; ▲, initial lactate 9–12mM.

Table 2. *Distribution of ^{14}C from [U-^{14}C]glucose in lipid classes during perfusion of mouse liver*

Livers were perfused with medium containing [U-^{14}C]-glucose. The major lipid classes in liver were separated by t.l.c. and ^{14}C was determined (without saponification). Other details are in the text. Results, expressed as a percentage of total d.p.m. recovered (in all bands) after t.l.c., are from single perfusions; where two values are given, all first or all second values are from the same perfusion.

Initial glucose concn. (mM)	Time of sample (h)	Total ^{14}C-labelled lipid (μg-atom/g of fresh liver)	^{14}C-labelled lipid (%)	
			Phospholipid	Triglyceride
5	2	9	54	28
5	3	13, 15	54, 67	32, 26
10	2	11	60	27
10	3	14	70	24
15	2	9, 7	66, 70	21, 14
15	3	21, 14	60, 78	27, 13
25	2	65	54	38
25	3	89	59	31

Table 3. *Degree of saturation of fatty acids synthesized in the perfused mouse liver*

Livers were perfused with medium containing ^3H_2O and [^{14}C]glucose at the concentrations indicated. After 3h of perfusion, fatty acids in the liver were separated (as methyl esters) according to their degree of saturation by t.l.c., and ^{3}H and ^{14}C were determined in each band. Other details are given in the text. Each set of results is from a single perfusion.

Initial glucose concn. (mM)	Radioactivity in total fatty acids (%)					
	Saturated		Monoenoic		Polyenoic	
	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H
None	78	87	14	8	8	5
3	91	93	7	5	3	2
22	86	88	12	10	2	3
22	87	90	9	7	4	3

^{14}C-labelled fatty acid tended to parallel ^{14}C-labelled total lipid in the liver (Fig. 2). As would be expected, phospholipid and triglyceride were the main fates of fatty acid synthesized *de novo* in the perfused liver (Table 2); of the ^{14}C radioactivity in other lipid classes, 3–6% was located in diglyceride and less than 2% in free fatty acid or monoglyceride. During fatty acid synthesis in the perfused liver, more than 85% of the ^{3}H (from ^3H_2O) or ^{14}C (from [^{14}C]glucose) was incorporated into saturated fatty acids (Table 3), confirming results in liver slices (Foster & Bloom, 1963).

Table 4. *Cholesterol synthesis in the perfused mouse liver*

Livers were perfused with medium containing ^3H_2O and [U-^{14}C]glucose. Liver samples were removed after 3h and ^3H and ^{14}C were determined in free cholesterol after separation by t.l.c. The methods for the calculation of the rates of cholesterol synthesis, and other details, are given in the text. Values are from the same single perfusions as in Table 3.

Initial glucose concn. (mM)	No. of experiments	Cholesterol synthesis (nmol/g of fresh liver)		Calculated average percentage of synthesized cholesterol formed from glucose
		Total	From [^{14}C]glucose	
5	2	220, 390	17, 35	8
10	1	160	14	9
15	2	145 150	16, 19	12
25	1	295	92	31

Cholesterol synthesis in the perfused mouse liver

Apart from fatty acids, a product of the conversion of carbohydrate into lipid in the liver is cholesterol. For comparison, rates of cholesterol synthesis were measured in the perfused mouse liver (Table 4). As in the case of newly synthesized fatty acids, a minor portion of ^3H-labelled cholesterol was released into the perfusion medium (less than 10% during 3h of perfusion). The glucose-dependence of cholesterol synthesis was evaluated by following ^3H incorporation into hepatic free cholesterol (separated by t.l.c.); this fraction contained more than three times as much ^{14}C (per g of liver) as the esterified cholesterol. Cholesterol synthesis was not increased by increasing the glucose concentration in the perfusate (Table 4). At low concentrations glucose carbon did not contribute significantly to cholesterol synthesis, whereas at higher glucose concentrations, the proportional contribution of glucose to cholesterol synthesis increased (Table 4), as in the case of fatty acid synthesis.

The rate of cholesterol synthesis, expressed in terms of acetyl residues consumed, may be calculated from the rates in Table 4; the value is about 3μmol of acetate/h per g of fresh liver, i.e. about one-fourth of the basal rate of fatty acid synthesis (also calculated as acetate consumed).

Discussion

Rates of lipid synthesis in the perfused mouse liver

The rates of fatty acid synthesis in the mouse liver perfused in the present conditions resemble those *in vivo* in the liver of mice of the same age, in the intact rat liver (Lowenstein, 1971) and in the perfused rat liver (Windmueller & Spaeth, 1966, 1967; Brunengraber *et al.*, 1973; Mayes & Topping, 1974). They are faster than those which have generally been reported in other liver preparations from rodents fed *ad lib* (e.g. slices, isolated cells), presumably as a result of the greater functional viability of the perfused preparation.

In the reports cited above, fatty acid synthesis was measured, as in the present work, by the incorporation of ^3H from ^3H_2O into lipid. The rates obtained are higher than in most experiments with ^{14}C-labelled substrates. The question arises of the validity of measurements made with ^3H_2O. This has been discussed by the authors cited above, and by Jungas (1968) and Foster & Bloom (1963). The credibility of the method is attested by the absence of significant ^3H incorporation into polyunsaturated fatty acids (Foster & Bloom, 1963; present work), and by the expected alterations in response to, e.g., variation in the glucose concentration, or starvation (Brunengraber *et al.*, 1973; Windmueller & Spaeth, 1966). In particular, the validity of the use of ^3H_2O is supported by the facts that in liver as in adipose tissue (Jungas, 1968), a substantial portion of the carbon of newly synthesized fatty acid can be identified from experiments with ^{14}C-labelled substrates (up to 50% in the present work if lactate and glucose contributions are taken together; see also Clark *et al.*, 1974) and that $^{14}C/^3H$ ratios in cholesterol resemble those in fatty acid (present work; Brunengraber *et al.*, 1972).

The fastest rates of fatty acid synthesis in the present experiments, expressed as μmol of acetate consumed, were about 1.0/min per g of fresh liver. This is of the same order as the rate of degradation of acetate in the tricarboxylic acid cycle (see also Brunengraber *et al.*, 1973; McGarry & Foster, 1971). Hence the process of fatty acid synthesis can represent a major fate of acetyl-CoA in the liver, at least in rodents ingesting a carbohydrate-based low-fat diet. In contrast, hepatic cholesterol synthesis is not fast enough to be significant in the control of rapid metabolic events; this was reflected in its lack of dependence on circulating glucose concentration.

The perfused liver releases a proportion of newly synthesized lipid to blood, as triglyceride, phospholipid or cholesterol (Windmueller & Spaeth, 1967). This process, which must be considered in assessing the functional state of a perfused liver preparation, is extensive in the presence of erythrocytes (Windmueller & Spaeth, 1967). The proportion of secreted ^3H-labelled lipid in the present experiments resembled that in rat livers perfused with media

containing erythrocytes (Windmueller & Spaeth, 1967; Topping & Mayes, 1972, value calculated from their Tables 5 and 6). Although the present measurements of the proportional secretion of newly synthesized lipid cannot be compared with events *in vivo*, for which adequate information is not available, there is no reason to suppose that the values obtained imply impairment of function in the perfused liver.

The above considerations suggest that the characteristics of fatty acid synthesis observed in the liver perfused in the present conditions, may reasonably be presumed to obtain in the liver of the intact animal.

Nature of carbon sources of newly synthesized hepatic fatty acid

From the present measurements, a general picture can be obtained of the net carbon sources of fatty acid which is synthesized *de novo* at different glucose concentrations in the liver of the fed mouse.

The present experiments with [^{14}C]glucose [the results of which resemble those obtained in the perfused rat liver (Brunengraber *et al.*, 1973)] suggest that glucose, in the concentration range usually present in blood (about 4–15mM), is not a major carbon source for fatty acids (or cholesterol) synthesized *de novo* in the liver. However, glucose at higher concentrations can contribute more extensively to fatty acid synthesis.

In contrast with glucose, lactate can serve as a major carbon source of fatty acids in the perfused liver, even in the presence of an equivalent (10mM) glucose concentration. This result is reminiscent of that obtained in a study of the precursors of glycogen in the perfused rat liver (Hems *et al.*, 1972). The highest rates of fatty acid synthesis *de novo* in the perfused mouse liver at intermediate glucose concentrations (about 7μmol/h per g) were obtained in the presence of 10mM-lactate. In this situation, lactate contributed up to 50% of the carbon for fat synthesis. The general implication of this observation is that circulating precursors that can rapidly form pyruvate, such as lactate and alanine, and perhaps serine (but not glucose) may provide significant carbon for fatty acid synthesis in the liver.

Although lactate (1–3mM) was present during perfusions in which glucose alone was added, the addition of lactate (9–12mM) produced a several-fold acceleration of fatty acid synthesis, which was not entirely due to provision of carbon. Lactate appears to control lipogenesis through substrate supply, in the range 1–10mM, as is true for gluconeogenesis (Exton, 1971) and anti-ketogenesis (McGarry *et al.*, 1973). Even though lactate may provide a significant portion of the total carbon for hepatic fatty acid synthesis in certain conditions, this represents a minor fate compared with the conversion of lactate into glucose (and glycogen) and CO_2 (Exton *et al.*, 1972; Elliott *et al.*, 1974; Clark *et al.*, 1974).

Role of glycogen in the synthesis de novo of fatty acids in the liver

The use of circulating ^{14}C-labelled precursors during perfusion identified the carbon sources of up to 50% of the fatty acid which was synthesized at low glucose concentrations (4–17mM). The remainder of the carbon was probably derived from an endogenous source, such as glycogen, protein or lipid. Of these sources, glycogen is the most likely, as it is known to exhibit rapid rates of breakdown during perfusion.

The possibility that glycogen contributes extensively to the carbon requirement for lipid synthesis is not open to direct testing, as glycogen cannot be exclusively pre-labelled with ^{14}C (e.g. before liver perfusion). However, circumstantial evidence supports the above suggestion that glycogen can provide significant carbon for hepatic fatty acid synthesis. Thus in the present experiment fatty acid synthesis was proportional to the initial glycogen content. Further, glycogen availability tends to precede or be associated with rapid rates of fatty acid synthesis, e.g. in well-fed or starved–re-fed, compared with starved or diabetic states [see Masoro (1962) for review]. Glycogen can provide substantial carbon to the glycolytic pathway in aerobic conditions, especially at low glucose concentrations (Glinsmann *et al.*, 1969; Woods & Krebs, 1971). Presumably glycogen is then a major source of CO_2 in the liver; thus glycogen breakdown may not be totally accounted for as lactate or glucose (Glinsmann *et al.*, 1969; Hems & Whitton, 1973). If glycogen can provide acetyl-CoA for oxidation, it is reasonable to suggest that it is also a potential source of acetyl-CoA for fatty acid synthesis [as discussed by Woods & Krebs (1971)]. The extent of glycogen breakdown in the present experiments, at glucose concentrations less than 15mM, was more than sufficient to account for fatty acid synthesis (see also Clark *et al.*, 1974).

In the intact animal, when the blood glucose concentration is in the normal range (4–15mM), glycogen could in certain conditions be a major carbon source of fatty acid synthesized *de novo* in the liver. This follows because a significant role for glucose appears to be precluded by the present results, and because other precursors in blood may not be present in sufficient amounts. Such a role for glycogen in providing acetyl residues for lipogenesis would permit simultaneous release of glucose and rapid fatty acid synthesis (i.e. zero contribution of glucose as a net carbon source of fat), especially in the presence of, e.g., rapid lactate uptake (as in the perfusions with glucose plus lactate). The conjunction of hepatic glucose release and fatty acid synthesis, which may be quite common *in vivo*, e.g. in post-

absorptive animals, appears to be exaggerated in genetically obese mice [see Elliott *et al.* (1971) and Salmon & Hems (1973) respectively].

Glycogen appears to exert a controlling influence on fatty acid synthesis, apart from being a likely carbon source. This follows because in perfusions with high initial glycogen concentrations, the extent of conversion of [^{14}C]glucose into fatty acid was increased, as well as the total rate of synthesis. A similar result was obtained in liver slices, with [^{14}C]acetate (Haugaard & Stadie, 1952). This effect of glycogen could have a variety of origins, and its mechanisms remain to be elucidated.

Role of glucose in fatty acid synthesis

The rate of total fatty acid synthesis depended on the supply of glucose (or lactate). Glucose at concentrations above about 15mM (although not the major carbon source), produced a marked acceleration of synthesis, as would be expected if glucokinase is implicated in glucose uptake, since the K_m of this enzyme (for glucose) is 10–20mM (Di Pietro *et al.*, 1962; Walker & Rao, 1964). This behaviour does not resemble that reported for the perfused rat liver (Brunengraber *et al.*, 1973), in which rates of fatty acid synthesis were relatively higher at glucose concentrations in the range 4–12mM. In the rat experiments, conditions differed from those of the present perfusions, particularly in the absence of erythrocytes. Suppression of glycogen breakdown by glucose is greater in the presence of erythrocytes (Glinsmann *et al.*, 1969). Thus if glycogen were a carbon source [as suggested by Brunengraber *et al.* (1973), and as implied by the present experiments] this effect could explain the high rates of lipogenesis in liver perfused in the absence of erythrocytes at intermediate glucose concentrations.

The total rate of fatty acid synthesis *de novo* in the perfused mouse liver was influenced by the glucose concentration in the perfusate, in the presence or absence of added lactate, to a greater extent than could be explained by its carbon contribution to fatty acid synthesis. Thus when glucose alone was added, fatty acid synthesis was markedly increased at glucose concentrations above 15mM, to a maximum rate of about 7μmol/h per g of fresh liver. In the perfusions with glucose plus lactate, net glucose output occurred in association with high rates of lipogenesis, and yet glucose was required to achieve these rates (as shown by the lower rates at lower glucose concentrations). This effect of glucose resembles that on glycogen synthesis in the perfused liver of starved rats, in which glucose was again not the net carbon source of the synthetic product (Hems *et al.*, 1972). Hence glucose appears to exert a regulatory or 'initiatory' role in the hepatic biosynthesis of glycogen (see Hers *et al.*, 1970) or fatty acid, the nature of which is not fully clear.

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References

Baillie, L. A. (1960) *Int. J. Appl. Radiat. Isotop.* **8**, 1–11
Brunengraber, H., Sabine, J. R., Boutry, M. & Lowenstein, J. M. (1972) *Arch. Biochem. Biophys.* **150**, 392–396
Brunengraber, H., Boutry, M. & Lowenstein, J. M. (1973) *J. Biol. Chem.* **248**, 2656–2669
Clark, D. G., Rognstad, R. & Katz, J. (1974) *J. Biol. Chem.* **249**, 2028–2036
Di Pietro, D. L., Sharma, C. & Weinhouse, E. (1962) *Biochemistry* **1**, 455–462
Dunn, E. & Robson, P. (1965) *J. Chromatogr.* **17**, 501–505
Elliott, J., Hems, D. A. & Beloff-Chain, A. (1971) *Biochem. J.* **125**, 773–780
Elliott, J., Dade, E., Salmon, D. M. W. & Hems, D. A. (1974) *Biochim. Biophys. Acta* **343**, 307–323
Exton, J. H. (1971) *Metab. Clin. Exp.* **21**, 945–990
Exton, J. H., Corbin, J. G. & Harper, S. C. (1972) *J. Biol. Chem.* **247**, 4996–5003
Folch, J., Lees, M. & Sloane-Stanley, G. N. (1957) *J. Biol. Chem.* **226**, 497–509
Foster, D. W. & Bloom, B. (1963) *J. Biol. Chem.* **238**, 888–892
Glinsmann, W. H., Hern, E. P. & Lynch, A. (1969) *Amer. J. Physiol.* **216**, 698–703
Goodridge, A. G. (1973) *J. Biol. Chem.* **248**, 1924–1931
Hauggard, E. S. & Stadie, W. C. (1952) *J. Biol. Chem.* **199**, 741–744
Hems, D. A. & Whitton, P. D. (1973) *Biochem. J.* **136**, 705–709
Hems, D. A., Whitton, P. D. & Taylor, E. A. (1972) *Biochem. J.* **129**, 529–538
Hendler, R. W. (1964) *Anal. Biochem.* **7**, 110–120
Hers, H.-G., deWulf, H., Stalmans, W. & Van den Berghe, G. (1970) *Advan. Enzyme Regul.* **8**, 171–190
Jungas, R. L. (1968) *Biochemistry.* **1**, 3708–3717
Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
Lowenstein, J. M. (1971) *J. Biol. Chem.* **246**, 629–632
Masoro, E. J. (1962) *J. Lipid Res.* **3**, 149–164
Mayes, P. A. & Topping, D. L. (1974) *Biochem. J.* **138**, 111–114
McGarry, J. D. & Foster, D. W. (1971) *J. Biol. Chem.* **246**, 1149–1159
McGarry, J. D., Meier, J. M. & Foster, D. W. (1973) *J. Biol. Chem.* **248**, 270–278
Salmon, D. M. W. & Hems, D. A. (1973) *Biochem. J.* **136**, 551–563
Topping, D. L. & Mayes, P. A. (1972) *Biochem. J.* **126**, 295–311
Walker, D. G. & Rao, S. (1964) *Biochem. J.* **90**, 360–368
Windmueller, H. G. & Spaeth, A. E. (1966) *J. Biol. Chem.* **241**, 2891–2899
Windmueller, H. G. & Spaeth, A. E. (1967) *Arch. Biochem. Biophys.* **122**, 362–369
Woods, H. F. & Krebs, H. A. (1971) *Biochem. J.* **125**, 129–139