The metabolic route by which oleate is converted into cholesterol in rat hepatocytes

Geoffrey F. GIBBONS, Christopher P. ATTWELL THOMAS and Clive R. PULLINGER

Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, U.K.

The effect of (–)-hydroxycitrate on the conversion of [1-¹⁴C]oleate into cholesterol was dependent on the time of day at which the cells were prepared and on the extracellular oleate concentration. In hepatocytes prepared during the light phase of the diurnal cycle (L₆-hepatocytes), (–)-hydroxycitrate inhibited the conversion of L-[U-¹⁴C]lactate (2 mM) and of 0.13 mM-[1-¹⁴C]oleate into cholesterol. However, when [1-¹⁴C]oleate was present at 1.3 mM, most of the sterol carbon was derived from this source, and under these conditions (–)-hydroxycitrate had no inhibitory effect on [¹⁴C]cholesterol formation. In these cells, non-radioactive acetoacetate blocked the conversion of 1.3 mM-[1-¹⁴C]oleate, but not of 0.13 mM-[1-¹⁴C]oleate, into cholesterol. In cells prepared during the dark phase of the diurnal cycle (D₆-hepatocytes), irrespective of the concentration of [1-¹⁴C]oleate, (–)-hydroxycitrate decreased its conversion into cholesterol. In both types of cell preparation, the inhibitory effect of (–)-hydroxycitrate on the conversion of L-[U-¹⁴C]lactate into cholesterol was greater than that on the overall rate of cholesterol production from all endogenous sources. These results provide evidence for the following. (1) The major metabolic route by which oleate is converted into cholesterol is dependent on its extracellular concentration. (2) When oleate is the major source of hepatic sterol carbon, the flux of substrate through citrate into cholesterol is dependent on the nutritional state of the animal. (3) When endogenous substrates are the sole source of sterol carbon, a substantial proportion of the carbon enters the cholesterol pathway through routes not involving citrate cleavage.

INTRODUCTION

Some time ago it was proposed that ketone bodies may be an important source of carbon for cholesterol synthesis in the liver (Buckley & Williamson, 1975). However, the low maximal activity of the cytosolic acetoacetyl-CoA synthetase, the enzyme responsible for conversion of acetoacetate into acetoacetyl-CoA, appeared to preclude a significant role for this substance in the production of fatty acids. However, a more recent study has provided evidence that the maximal activity of the synthetase is indeed sufficiently high to permit acetoacetate to contribute substantially to lipogenic carbon flux (Bergstrom *et al.*, 1982). Other investigators have also provided evidence that extracellular acetoacetate is a significant source of carbon for lipogenesis and, to an even greater extent, for cholesterol synthesis, in the liver (Endemann *et al.*, 1982; Geelen *et al.*, 1983). Further evidence for the role of acetoacetate in cholesterologenesis was obtained by Bergstrom *et al.* (1984), who showed a close parallel between the activity of cytosolic acetoacetyl-CoA synthetase and the hepatic demand for cholesterol.

In the livers of rats fed on a high-carbohydrate diet, a large proportion of the carbon required for cholesterol synthesis is considered to arise from the oxidation of glycogen or lactate (Salmon *et al.*, 1974; Gibbons & Pullinger, 1979), by a process which involves the cleavage of cytosolic citrate to give acetyl-CoA (Lowenstein, 1971; Barth *et al.*, 1972; Brunengraber *et al.*, 1972; Triscari & Sullivan, 1977). However, hepatocytes from carbohydrate-fed rats rapidly develop the capacity to utilize exogenous oleate for cholesterologenesis, and, under these

conditions, oleate provides about 80% of the carbon required for cholesterol production (Pullinger & Gibbons, 1983a). The metabolic pathway by which oleate carbon is made available for cholesterol synthesis is not known. In view of the above evidence in support of a precursor role for ketone bodies, it seems reasonable to suggest that mitochondrial acetyl-CoA, formed by β -oxidation of extracellular oleate, may enter the cholesterol pathway via the intermediacy of acetoacetate (Scheme 1). Alternatively, oleate-derived acetyl-CoA, in a manner similar to that derived from carbohydrate, may enter into cholesterol biosynthesis via a pathway involving mitochondrial citrate synthesis and cytosolic citrate cleavage (Scheme 1). One of the objectives of the present work was to discriminate between these two alternative pathways by which oleate-derived mitochondrial acetyl-CoA could be utilized for cholesterol synthesis. This was achieved by comparing the effect of (–)-hydroxycitrate, an inhibitor of the citrate cleavage enzyme (ATP citrate lyase, EC 4.1.3.8) (Watson *et al.*, 1969), on the conversion of [1-¹⁴C]oleate into cholesterol with that on the conversion of L-[U-¹⁴C]lactate, a lipogenic substrate from which cytosolic acetyl-CoA is thought to be derived predominantly via citrate cleavage (Goodridge, 1973).

The rates of hepatic fatty acid (Hems *et al.*, 1975; Munday & Williamson, 1983; Gibbons *et al.*, 1984) and cholesterol (Rodwell *et al.*, 1976) synthesis fluctuate with a diurnal periodicity, and these variations reflect the changing nutritional status of the liver over the 24 h cycle. A further aim of the present study was to investigate whether there are any changes in the relative contribution

Scheme 1. Alternative pathways of oleate conversion into cholesterol
Abbreviation used: HMG, 3-hydroxy-3-methylglutaryl.

of citrate cleavage in sustaining the observed rates of cholesterol synthesis over the diurnal cycle.

EXPERIMENTAL

Maintenance of animals, preparation of hepatocytes and incubation conditions

Male Wistar rats were housed in a windowless room lit artificially between 08:00 h and 20:00 h (Schedule A) or between 16:00 h and 04:00 h (Schedule B). The animals were fed on a commercially available pelleted diet high in carbohydrate (Diet PRM; E. Dixon & Sons, Ware, Herts., U.K.). This contained (w/w) 47.5% carbohydrate (mainly starch), 2.7% fat and 18.7% protein. Food and water were available *ad libitum*. Animals were acclimatized under these conditions for 2–4 weeks before use, at which time they weighed between 230 and 300 g. Hepatocytes were prepared at 10:00 h from animals on both lighting schedules. For animals on Schedule A, this point was 2 h into the light phase of the cycle (L_2 -hepatocytes); for those on Schedule B, this represented a time 6 h into the dark phase of the cycle (D_6 -hepatocytes). Procedures for the preparation and incubation of hepatocytes have been described previously (Seglen, 1976; Pullinger & Gibbons, 1983b, 1985). In the present experiments, triparanol was not added to the incubation medium. Acetoacetate, (–)-hydroxycitrate and L-[U- 14 C]lactate were added to the hepatocyte suspensions as solutions in incubation medium. Solutions of [1- 14 C]oleate were added as albumin complexes prepared by the method of Van Harken *et al.* (1969).

Measurement of the incorporation of labelled substrates into cholesterol and fatty acids

In some incubations, the rates of cholesterol and fatty acid synthesis from all carbon sources were determined by addition of $^3\text{H}_2\text{O}$ to the incubation medium to give a final specific radioactivity of 80–90 d.p.m./nmol. In other incubations, either L-[U- 14 C]lactate (130–160 d.p.m./nmol) was added to give an initial concentration of 2.0 mM, or [1- 14 C]oleate (3000 and 300 d.p.m./nmol) was

added to give concentrations of 0.13 mM and 1.3 mM respectively. When [1- 14 C]lactate was used, substrate depletion was minimized during incubation by adding similar quantities at the end of 1 h. After incubation for 2 h at 37 °C, the cell suspension was chilled on ice and the cell pellet was immediately sedimented by centrifugation for 1.5 min at 80 g. The resulting cell pellet was suspended in 0.1 M-potassium phosphate buffer (pH 7.5; 1.5 ml) containing Triton X-100 (0.125%), and the suspension was heated at 90 °C for 1 min. The mixture was saponified by addition of 3.0 ml of a (7.5%, w/v) solution of KOH in ethanol followed by heating at 60 °C for 0.25 h. After addition of water (10.0 ml), the solution was saturated with Na_2SO_4 and the non-saponifiable lipid was extracted with 2×10.0 ml of hexane. The combined hexane extracts were washed with 3×10.0 ml of distilled water and evaporated to dryness. The labelled cholesterol was isolated from the non-saponifiable mixture by t.l.c. on silica-gel G by using chloroform/methanol (100:1, v/v) as the mobile phase. The cholesterol-containing band was scraped from the plate and its radioactivity determined by scintillation counting. To account for losses of cholesterol during this procedure, internal standards of [4- 14 C]cholesterol (8200 d.p.m.) or [1,2- ^3H]cholesterol (8600 d.p.m.) were initially added to cell pellets obtained after incubation in the presence of $^3\text{H}_2\text{O}$ or of the ^{14}C -labelled substrates respectively.

The incorporation of ^3H or ^{14}C into the saponifiable fraction of the cell, containing the fatty acids, was determined as described previously (Pullinger & Gibbons, 1983b). To adjust for losses of biosynthetic fatty acid during the extraction procedure, [1- 14 C]oleate (6550 d.p.m.) was added to the cell pellet obtained after incubation in the presence of $^3\text{H}_2\text{O}$ and [^3H]oleate (48800 d.p.m.) was added to that obtained after incubation in the presence of ^{14}C -labelled substrates.

Calculation of total carbon flux into cholesterol and the contribution of [1- 14 C]oleate and L-[U- 14 C]lactate

The weight (nmol) of $^3\text{H}_2\text{O}$ incorporated into cholesterol was determined from its specific radioactivity. Since, under normal incubation conditions, 7.61 nmol of

$^3\text{H}_2\text{O}$ are incorporated per nmol of cholesterol synthesized (Pullinger & Gibbons, 1983b), this factor was used to determine the weight of cholesterol produced from all carbon sources. In the presence of (–)-hydroxycitrate, the corresponding factor was significantly decreased, to 5.71 (Björnsson *et al.*, 1985a). This factor has been used to calculate the weight of cholesterol synthesized under these conditions. It is clear, therefore, that ^3H incorporation gives an overestimate of the inhibitory effect of (–)-hydroxycitrate on carbon flux into cholesterol.

The weights of cholesterol (nmol) synthesized exclusively from L-[U- ^{14}C]lactate and [1- ^{14}C]oleate were calculated according to the known metabolic patterns of their incorporation into cholesterol (Gibbons & Pullinger, 1979; Pullinger & Gibbons, 1983a).

Materials

The sources of materials used in this work have been described previously (Gibbons & Pullinger, 1979). In addition, all radiochemicals were obtained from Amersham International, Little Chalfont, Bucks., U.K. Non-radioactive L(+)-lactic acid was obtained from Sigma (Poole, Dorset, U.K.) and was converted into the sodium salt before use. Acetoacetic acid (lithium salt) was also obtained from Sigma. (–)-Hydroxycitric acid (sodium salt) was given by Dr. P. A. Chalk and Dr. C. J. Coulson (Glaxo Research, Greenford, Middx., U.K.).

RESULTS

Effect of (–)-hydroxycitrate on the flux of total carbon, L-[U- ^{14}C]lactate and [1- ^{14}C]oleate into cholesterol

To determine the extent to which [1- ^{14}C]oleate was converted into cholesterol via the intermediacy of citrate, measurements were made of the extent to which (–)-hydroxycitrate (2 mM) was able to inhibit [^{14}C]cholesterol formation from this substrate. The varying nutritional status of the liver over the 24 h cycle is dependent on the animals' feeding habits (Scott & Potter, 1970) and is reflected in widely different rates of fatty acid synthesis (Gibbons *et al.*, 1984) and cholesterologenesis (Edwards,

1975; Björnsson *et al.*, 1985b) in hepatocytes prepared at different times of the day. In view of this, the effects of (–)-hydroxycitrate were compared in hepatocytes prepared either at the mid-point of the dark phase of the cycle (D_6 -hepatocytes) or at a point 2 h into the light phase of the cycle (L_2 -hepatocytes). Visual examination of the stomach size and the intestinal contents confirmed that, at the latter time, the animals were in the post-absorptive state. These results are shown in Table 1, in which labelled substrates were incubated in the presence or absence of (–)-hydroxycitrate for 2 h. Previous studies have shown that the rates of cholesterol synthesis, with or without (–)-hydroxycitrate, do not decline during this period (Pullinger & Gibbons, 1983a). In L_2 -cells, when [1- ^{14}C]oleate was present at a concentration of 0.13 mM, (–)-hydroxycitrate inhibited [^{14}C]cholesterol formation by 37%. However, this effect was largely abolished when [1- ^{14}C]oleate was present at a higher extracellular concentration (1.3 mM; Table 1). There was no difference in the response of cholesterol synthesis to (–)-hydroxycitrate in the L_2 - and D_6 -cells at the low concentration of oleate (Table 1). However, in the D_6 -cells, at the higher (1.3 mM) concentration of oleate, there was a significant inhibition of [^{14}C]cholesterol formation by (–)-hydroxycitrate, in contrast with the apparent resistance of cholesterol synthesis to inhibition from [1- ^{14}C]oleate in the L_2 -cells under similar conditions.

In order to estimate the proportion of oleate carbon that was converted into cholesterol via the citrate pathway, parallel experiments were carried out with L-[U- ^{14}C]lactate as the [^{14}C]cholesterol precursor. Carbon flux from this substrate is considered to pass predominantly through citrate (Goodridge, 1973), and it was thus of interest to compare the effects of (–)-hydroxycitrate on cholesterol synthesis from each of the two labelled precursors ([1- ^{14}C]oleate and L-[U- ^{14}C]lactate). In addition, the effect of the inhibitor on the production of cholesterol from all endogenous hepatocyte carbon sources was determined by measuring the incorporation of $^3\text{H}_2\text{O}$ into cholesterol and converting this into carbon flux by application of the ratios determined previously (Pullinger & Gibbons, 1983b; Björnsson *et al.*, 1985a). These results are shown in Table 2. When [U- ^{14}C]lactate

Table 1. Effect of (–)-hydroxycitrate on the formation of cholesterol from [1- ^{14}C]oleate by hepatocytes prepared at different times of the day

Hepatocytes were prepared from animals either at the mid-point of the dark phase of the 24 h cycle (D_6 -cells) or 2 h into the light phase of the cycle (L_2 -cells). Each hepatocyte preparation was incubated for 2 h in the absence or presence of (–)-hydroxycitrate (2 mM). In each case [1- ^{14}C]oleate was present at an extracellular concentration of 0.13 mM or 1.3 mM. The values below represent the weight of cholesterol formed exclusively from the added oleate (see the Experimental section). The relative effects of (–)-hydroxycitrate were calculated as a percentage of the rate observed when hepatocytes from the same animal were incubated in the absence of the drug. Each value represents the mean $\pm$ S.E.M. of measurements made on seven hepatocyte preparations at L_2 and six preparations at D_6 . Values marked *, ** or *** are significantly different at $P < 0.05$, $P < 0.01$ or $P < 0.001$ respectively.

Oleate concn. (mM)	(–)-Hydroxycitrate concn. (mM)	Rate of sterol synthesis (nmol of cholesterol/2 h per mg of protein)		Relative effect of (–)-hydroxycitrate (% of control values)	
		L_2 -cells	D_6 -cells	L_2 -cells	D_6 -cells
0.13	—	0.19 ± 0.04	0.29 ± 0.04		
0.13	2.0	0.12 ± 0.03	0.17 ± 0.02	$63.0 \pm 5.0^{***}$	$62.7 \pm 9.1^{**}$
1.30	—	1.98 ± 0.43	2.80 ± 0.53		
1.30	2.0	1.79 ± 0.31	2.21 ± 0.68	94.6 ± 5.2	$71.9 \pm 10.2^*$

Table 2. Effect of (–)-hydroxycitrate on the absolute rate and the contribution of [U-¹⁴C]lactate to cholesterol synthesis

Hepatocytes were prepared from animals either at the mid-point of the dark phase of the 24 h cycle (D₆-cells) or 2 h into the light phase of the cycle (L₂-cells). Each hepatocyte preparation was incubated in the absence or presence of (–)-hydroxycitrate (2 mM). For measurement of lactate conversion into cholesterol, L-[U-¹⁴C]lactate was present at 2.0 mM. The weight of cholesterol formed exclusively from this precursor was calculated (see the Experimental section). Measurement of the total weight of sterol synthesized was determined by addition of ³H₂O (to give a specific radioactivity of 80–90 d.p.m./nmol) to the hepatocyte suspension. The total weight of sterol synthesized was calculated from the incorporation of ³H (see the Experimental section). Values in parentheses are the numbers of independent hepatocyte preparations used. Values marked ** and *** represent a significant decrease at *P* < 0.01 and *P* < 0.001 levels respectively.

Type of hepatocytes	(–)-Hydroxycitrate	Weight of sterol formed (nmol/2 h per mg of protein)		Relative effect of (–)-hydroxycitrate (% of control)	
		Total	From lactate	Total	From lactate
L ₂	–	2.50 ± 0.60 (8)	0.65 ± 0.15 (7)		
	+	1.82 ± 0.33 (8)	0.45 ± 0.12 (7)	78.3 ± 5.1 (8)**	68.9 ± 9.2 (7)**
D ₆	–	3.74 ± 0.34 (11)	0.75 ± 0.15 (6)		
	+	3.10 ± 0.47 (11)	0.38 ± 0.12 (6)	85.2 ± 8.5 (11)	45.6 ± 7.5 (6)***

Fig. 1. Effect of non-radioactive acetoacetate on the rate of conversion of [1-¹⁴C]oleate into cholesterol

L₂- and D₆-hepatocytes were incubated with [1-¹⁴C]oleate at concentrations of 0.13 mM (▲) or 1.3 mM (■). In each case acetoacetate was either absent or present at concentrations of 2, 5 and 10 mM. The effects of acetoacetate are expressed in terms of the decrease in the incorporation of [¹⁴C]oleate into cholesterol as a % of the control values (i.e. no acetoacetate). The control values are given in Table 1. Each point is the mean ± S.E.M. of independent determinations on hepatocytes from three to six rats (except when acetoacetate was present at 10 mM with 0.13 mM oleate in D₆-hepatocytes; in this case two rats only were used).

was present at 2 mM, (–)-hydroxycitrate gave rise to a 31% and 55% inhibition of labelled cholesterol formation in the L₂- and D₆-cells respectively. (–)-Hydroxycitrate had quantitatively similar effects when lactate was present at 1 mM (results not shown). However, total carbon flux into cholesterol from all exogenous sources was not as sensitive to inhibition by (–)-hydroxycitrate (Table 2).

Effect of non-radioactive acetoacetate on the rate of cholesterol synthesis from [1-¹⁴C]oleate

The lack of any effect of (–)-hydroxycitrate on cholesterol formation from 1.3 mM-oleate in the L₂-cells suggested that very little, if any, carbon from this source passed through the citrate pathway. A possible alternative

pathway is that involving acetoacetate (Scheme 1). If this pathway is active, it would be reasonable to expect non-radioactive acetoacetate to block the conversion of label from [1-¹⁴C]oleate into cholesterol as a result of isotope dilution. In Fig. 1, L₂- and D₆-hepatocytes were incubated with oleate (0.13 mM and 1.3 mM). In each case the effect of increasing the extracellular concentration of acetoacetate on the accumulation of labelled cholesterol was determined. In the L₂-hepatocytes, with 1.3 mM-oleate, [¹⁴C]cholesterol formation was inhibited in a concentration-dependent manner. However, there were no similar inhibitory effects at the lower oleate concentration. In the D₆-hepatocytes, the concentration-dependent inhibition of [¹⁴C]cholesterol formation was similar in extent, irrespective of the concentration of [1-¹⁴C]oleate.

DISCUSSION

The pathway of substrate flux from oleate into cholesterol

Under certain conditions, extracellular oleate is capable of providing about 80% of the carbon required for cholesterol synthesis by isolated hepatocytes (Pullinger & Gibbons, 1983a). The present work has shown that, in similar circumstances, in L_2 -hepatocytes, when $[1-^{14}\text{C}]$ oleate was present at 1.3 mM, (–)-hydroxycitrate had no significant effect on its conversion into cholesterol (Table 1). Under the same conditions, the conversion of $[\text{U-}^{14}\text{C}]$ lactate (2 mM) into cholesterol was significantly inhibited by over 30% (Table 2). This suggests that, in hepatocytes prepared during the light phase of the diurnal cycle, when extracellular fatty acids are available as the predominant substrate for sterol synthesis, little, if any, sterol carbon is derived via the cleavage of citrate to give cytosolic acetyl-CoA. There is considerable evidence implicating ketone bodies as important cholesterol precursors (Endemann *et al.*, 1982; Geelen *et al.*, 1983; Bergstrom *et al.*, 1984), and carbon flux from oleate into cholesterol via acetoacetate instead of citrate (Scheme 1) would provide a reasonable explanation for the resistance of this process to inhibition by (–)-hydroxycitrate. This hypothesis is supported by the observation that non-radioactive acetoacetate showed an isotope-dilution effect in blocking the formation of labelled cholesterol from 1.3 mM- $[1-^{14}\text{C}]$ oleate (Fig. 1). However, in these experiments, the effects of acetoacetate were not as great as might be expected, and became pronounced only at concentrations greater than 5 mM. This could be explained if the labelled acetoacetate formed from $[1-^{14}\text{C}]$ oleate did not completely equilibrate with the exogenous, non-radioactive, material.

The above effects were observed in L_2 -hepatocytes when $[1-^{14}\text{C}]$ oleate was present at 1.3 mM. In contrast, at a lower (0.13 mM) concentration, $[1-^{14}\text{C}]$ oleate conversion into cholesterol was as sensitive to inhibition by (–)-hydroxycitrate as was that of $[\text{U-}^{14}\text{C}]$ lactate (Tables 1 and 2). In addition, non-radioactive acetoacetate had no effect on the formation of $[^{14}\text{C}]$ cholesterol in the presence of a low concentration of $[1-^{14}\text{C}]$ oleate. Under these conditions, oleate contributed little carbon for cholesterol synthesis (0.19 nmol) (Table 1) compared with a total of 2.5 nmol (Table 2). Of this small proportion, it would appear that most passed through citrate rather than through acetoacetate.

Thus in L_2 -hepatocytes the proportion of oleate-derived sterol carbon that passes through the citrate pathway is dependent on the extracellular oleate concentration. In this respect, the favoured pathway is obviously determined by the fate of the acetyl-CoA produced by β -oxidation of the exogenous oleate (Scheme 1). This is influenced to a large extent by the concentration of mitochondrial oxaloacetate, a high concentration favouring citrate synthesis, and a low concentration the formation of acetoacetate (Lane & Mooney, 1981). In the presence of a high concentration of $[1-^{14}\text{C}]$ oleate, mitochondrial oxaloacetate concentration is depressed, a situation which channels acetyl-CoA into ketone-body formation (Siess *et al.*, 1982, 1984). Citrate is thus by-passed, and $[^{14}\text{C}]$ cholesterol synthesis is insensitive to inhibition by (–)-hydroxycitrate. Conversely, when exogenous $[1-^{14}\text{C}]$ oleate concentration is low, the continuing high mitochondrial oxaloacetate concentration would direct acetyl-CoA into citrate synthesis (Siess *et al.*, 1982, 1984),

and $[^{14}\text{C}]$ cholesterol synthesis now becomes susceptible to inhibition by (–)-hydroxycitrate.

Diurnal variations in the pathway of oleate metabolism

The above observations were made with hepatocytes prepared from rats at a point in the diurnal cycle when little or no food was being consumed. Quantitatively different results were obtained in hepatocytes prepared from rats at a time close to that of maximum food consumption (i.e. D_6 -hepatocytes). In this case, when $[1-^{14}\text{C}]$ oleate was present at a concentration (1.3 mM) sufficient to make a major contribution to cholesterol production, (–)-hydroxycitrate significantly inhibited its conversion into cholesterol. This suggests that D_6 -hepatocytes, in contrast with those prepared at L_2 , have the capacity to convert a significant quantity of oleate into cholesterol via a pathway involving citrate. Nevertheless, the inhibition by (–)-hydroxycitrate of $[\text{U-}^{14}\text{C}]$ lactate conversion into cholesterol was significantly greater than that of the conversion of 1.3 mM- $[1-^{14}\text{C}]$ oleate $[45.6 \pm 7.5\%$ of control (Table 2) and $71.9 \pm 10.2\%$ of control (Table 1) respectively; $P < 0.05$]. This indicates that, in the D_6 -cells, some of the oleate is converted into cholesterol via a non-citrate pathway, although this proportion is less than in the L_2 -hepatocytes. Consistent with this hypothesis was the observation that non-radioactive acetoacetate remained able to block the conversion of label from $[1-^{14}\text{C}]$ oleate (1.3 mM) into cholesterol, presumably by isotopic dilution of the intermediate ^{14}C -labelled acetoacetate.

When a lower concentration of $[1-^{14}\text{C}]$ oleate (0.13 mM) was used as the cholesterol precursor, the effect of (–)-hydroxycitrate in the D_6 -cells was quantitatively similar to that observed in the L_2 -hepatocytes (Table 1). This observation suggested that similar relative proportions of oleate carbon passed through the citrate and non-citrate pathways in both cell types. If the non-citrate pathway involves acetoacetate in both types of hepatocyte preparations, then it is difficult to explain why non-radioactive acetoacetate exhibits an isotope-dilution effect in the D_6 -cells, but not in the L_2 -cells.

Relative importance of citrate cleavage in providing carbon for cholesterol synthesis from endogenous hepatocyte sources

Isolated hepatocytes are able to maintain a substantial rate of cholesterol synthesis which is sustained entirely from endogenous sources and which remains undiminished for at least 2 h (Gibbons & Pullinger, 1979). Unlike fatty acid synthesis, this 'endogenous' rate cannot be increased by presenting the cells with an increased concentration of lipogenic precursors such as lactate. The present results show that the overall flux of carbon from the sum of these endogenous sources into cholesterol was inhibited by (–)-hydroxycitrate (Table 2). However, the extent of this inhibition was not as great as that observed when carbon flux from lactate alone was measured in the presence or absence of the drug. Similar results have been obtained previously in liver *in vivo* (Sullivan *et al.*, 1974) and in isolated hepatocytes *in vitro* (Hamilton *et al.*, 1977) when $[^{14}\text{C}]$ alanine was the C_3 precursor. One possible explanation for this discrepancy is that lactate, or lactate precursors such as glycogen or alanine, do not constitute the sole source of endogenous sterol carbon, and that other precursors, the metabolism of which is less sensitive to inhibition by (–)-hydroxycitrate, make a significant contribution.

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Received 29 August 1985/28 October 1985; accepted 12 November 1985