Effect of drugs, peptide hormones and lipogenic precursors on the relative incorporation of $[^3H]H_2O$ and carbon into hepatic cholesterol

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Measurement of the weight of desmosterol produced during its biosynthesis in the presence of tritiated water and triparanol has permitted a direct determination of the relative flux of carbon and tritium (the *H/C* ratio) into sterol in hepatocytes. The *H/C* ratio increased with time of incubation irrespective of the nutritional state of the donor animals. This increase was more marked in hepatocytes from starved animals. Pyruvate and lactate increased, and glucagon decreased, the sterol *H/C* ratio. Addition of pyruvate to incubations containing glucagon resulted in a 32–67% increase in the *H/C* ratio depending upon nutritional status. Insulin had no effect whilst (–)-hydroxycitrate decreased the ratio by 25%.

Insulin *Glucagon* *Pyruvate* *Lactate* *NADPH* *Cholesterol synthesis*

1. INTRODUCTION

The accuracy of the ^3H_2O incorporation method to measure the absolute rate of sterol synthesis relies upon the assumption that the relative incorporation of tritium and carbon (the sterol *H/C* ratio) is constant under different experimental conditions. However, it has been calculated that, dependent upon the source of NADPH for sterol synthesis, the *H/C* ratio may vary between the extremes of 0.26 and 0.81 [1,2]. In addition, the source of cytosolic acetyl-CoA for sterol synthesis may also affect the *H/C* ratio [3]. A non-isotopic technique has recently been developed which enables the flux of carbon into cholesterol to be measured directly in isolated hepatocytes in the presence of ^3H_2O and this has facilitated a direct measurement of the sterol *H/C* ratio [3]. Tritiated water has previously been used to assess the effects of pancreatic hormones [4], lipogenic substrates [5] and metabolic inhibitors [6] on hepatic cholesterol synthesis. The object of the present work was to determine whether these substances affected the sterol *H/C* ratio in hepatocytes derived from rats in different nutritional states.

2. MATERIALS AND METHODS

The sources of materials used in this work have been described previously [3,7]. In addition, α-cyano-3-hydroxycinnamic acid and α-cyano-4-hydroxycinnamic acid were obtained from Sigma. Rats were maintained on a strict lighting and feeding schedule as described [8]. Hepatocytes were prepared at 09.00 h (the start of the 8 h feeding period), 12.00, 15.00, 17.00 h (the end of the feeding period) and at 22.00 and 03.00 h (the interprandial period). Hepatocytes were also prepared at 12.00, 15.00 and 17.00 h from animals which had not had access to food on the day of the experiment (starved). The procedures for prepara-

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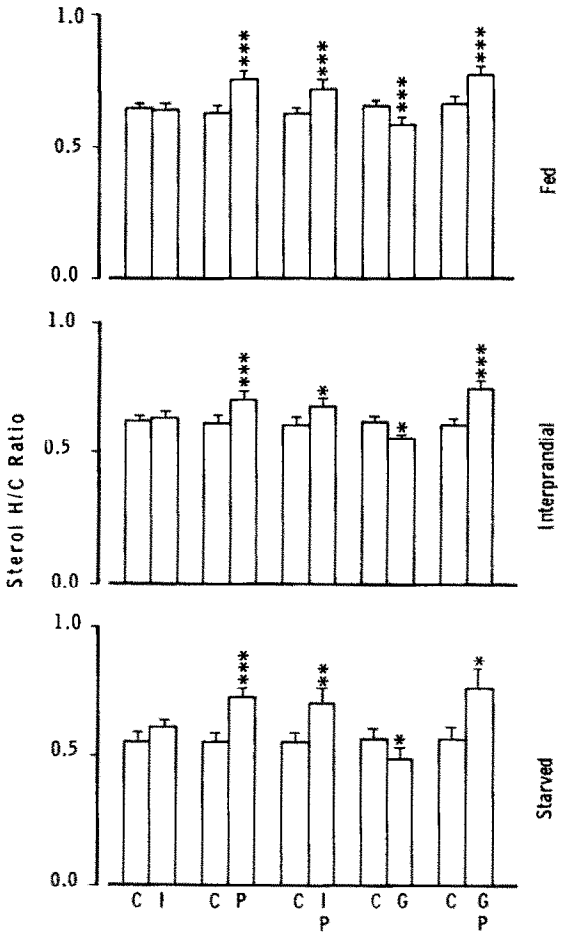
tion and incubation of hepatocytes have been described [3,7] and, where appropriate, solutions of insulin, glucagon, pyruvate and lactate were added [8]. The absolute rate of sterol synthesis was determined by measuring the weight of desmosterol that accumulated in cells during sterol biosynthesis in the presence of triparanol. The incorporation of ^3H into the newly synthesized desmosterol was determined and the *H/C* ratio was calculated as in [3].



3. RESULTS

3.1. *Effect of nutritional state and incubation time on the sterol* H/C *ratio*

Hepatocytes were prepared at various times during the feeding period, during the interprandial period and from starved animals. Each hepatocyte preparation was incubated for periods of 1, 2 and 3 h and after each time the sterol *H/C* ratio was determined (table 1). Irrespective of the nutritional state of the animal, the sterol *H/C* ratio increased with increasing time of incubation. This effect was particularly pronounced in hepatocytes from starved animals. There were no significant dif-

Fig.1. Effects of pyruvate, insulin and glucagon on the sterol *H/C* ratio. Hepatocytes were prepared from donor rats in 3 different nutritional states. Each hepatocyte preparation was incubated for 2 h in the presence or absence of pyruvate (25 mM), glucagon (10^{-7} M) or insulin (10^3 μU/ml), or in the presence of mixtures of pyruvate + glucagon or pyruvate + insulin. At the end of this period the sterol *H/C* ratio was determined in each case. Columns marked with a single, double or triple asterisk indicate that the values obtained were significantly different ($P < 0.05$, $P < 0.01$, $P < 0.001$, respectively) from the corresponding controls. C, control; I, insulin; G, glucagon; IP, insulin + pyruvate; GP, glucagon + pyruvate.

Table 1

Effects of nutritional state and incubation time on the sterol *H/C* ratio

Incubation time (h)	*H/C* ratio		
	Fed	Interprandial	Starved
1	0.46 ± 0.05 (22)[b]	0.51 ± 0.03 (19)[a]	0.42 ± 0.07 (13)[a]
2	0.64 ± 0.02 (30)	0.62 ± 0.02 (20)	0.55 ± 0.04 (13)
3	0.67 ± 0.04 (27)[a]	0.64 ± 0.02 (20)	0.74 ± 0.08 (13)[a]

[a] Significantly different from the respective 2 h value ($P < 0.05$)
[b] Significantly different from the respective 2 h value ($P < 0.01$)

ferences in the sterol *H/C* ratio after 2 h incubations of hepatocytes prepared at each of the 6 different times of the day from normal rats (see section 2) subjected to the controlled lighting and feeding schedule (not shown).

3.2. *Effects of lipogenic precursors and pancreatic hormones*

Insulin had no significant effect on the sterol *H/C* ratio in any of the 3 types of hepatocyte preparation (fed, interprandial, starved) (fig.1). However, incubation with glucagon suppressed the *H/C* ratio of sterol synthesized in all 3 groups (average 89.3 ± 0.2% of control values). Pyruvate increased the sterol *H/C* ratio in each of the 3 groups compared to their respective controls. These increases amounted to 22.9 ± 5.2% (n = 21), 15.1 ± 3.5% (n = 14) and 36.1 ± 12.4% (n = 13) in hepatocytes prepared from the fed, interprandial and starved animals, respectively. The addition of insulin had no further effect on the pyruvate-induced change in the sterol *H/C* ratio. This effect of pyruvate on the sterol *H/C* ratio was most pronounced when it was added to incubations containing glucagon. Thus, in cells from starved animals, a combination of pyruvate and glucagon produced an average increase in the sterol *H/C* ratio of 66.9 ± 17.8% (n = 10) compared to that observed with glucagon alone. In cells from rats in the fed and interprandial states, these increases amounted to 35.7 ± 3.4% (n = 14) and 32.4 ± 4.7% (n = 16), respectively (fig.1). In the presence of glucagon, lactate also gave rise to a highly significant ($P < 0.001$) 17.0 ± 2.4% (n = 12) increase in the sterol *H/C* ratio.

3.3. *Drug-induced variations in the sterol* H/C *ratio*

Several drugs inhibit cholesterol biosynthesis either by specific inhibition of a particular enzyme in the cholesterol pathway or by interfering with the production of common precursors which are normally utilized by pathways in addition to that leading to cholesterol. The effects of some of these drugs on the sterol *H/C* ratio are shown in table 2. Of these only (−)-hydroxycitrate produced a significant change. In this case the *H/C* ratio decreased to 75.6 ± 4.5% (n = 10; $P < 0.001$) of that observed in the control incubations.

Table 2

Effects of various drugs on the rate of sterol synthesis and on the sterol *H/C* ratio

Addition to hepatocytes		Rate of sterol synthesis (% of control)	Sterol *H/C* ratio
−, control	(26)	100	0.670 ± 0.024
(−)-Hydroxycitrate	(10)	57.3 ± 6.8[a]	0.510 ± 0.060[a]
Dexamethasone	(10)	67.6 ± 2.7[a]	0.681 ± 0.036
Compactin	(10)	20.3 ± 2.6[a]	0.637 ± 0.068
Cyanohydroxycinnamate	(14)	104.8 ± 8.8	0.723 ± 0.026

[a] Significantly different ($P < 0.001$) from the controls

Hepatocytes were incubated for 3 h in the presence or absence of (−)-hydroxycitrate (2 mM), dexamethasone (10 μM), compactin (2 μM), α-cyano-3-hydroxycinnamate (2 mM) or α-cyano-4-hydroxycinnamate (2 mM). The weight of sterol synthesized and the incorporation of ^3H_2O during this period was determined in each case and the *H/C* ratio was calculated. There was no significant difference in the effects of the two cyanohydroxycinnamate derivatives and these two sets of data have been combined

4. DISCUSSION

Here, determination of the relative incorporation of tritium and carbon into sterol was facilitated by measurement of the weight, and ^3H content, of desmosterol which accumulated in the presence of triparanol. The validity of this method has been established previously [3,8] and it was shown that triparanol had no effect on the flux of tritium from ^3H_2O into total sterols, and fatty acids, nor did it affect the activity of 3-hydroxy-3-methylglutaryl CoA reductase [3].

The present results show that, in common with hepatocytes taken from rats fed ad libitum [3], the sterol *H/C* ratio also increased with time of incubation when hepatocytes taken from rats in the interprandial and starved states were incubated in a chemically defined medium. In the latter case, these differences were greater than those observed in hepatocytes prepared from normal rats during the controlled diurnal cycle (table 1).

As regards the relative incorporation of ^3H, the importance of the source of NADPH for sterol synthesis has been emphasized [1,2]. In particular, any increase in the proportion of NADPH arising from a specific increase in malic enzyme activity may be expected to increase the relative incorporation of ^3H and vice versa [2]. In this respect, glucagon, by inhibiting fatty acid synthesis, may interrupt the generation of malic enzyme-derived NADPH by decreasing the flux of substrate through the anaplerotic sequence required to maintain a normal rate of lipogenesis [9,10]. This could produce the observed decrease in the sterol *H/C* ratio. The extent of the glucagon-mediated decrease was dependent upon the presence or absence of a lipogenic substrate. The present results may help to explain the conflicting reports of the effect of glucagon on sterol biosynthesis in isolated liver preparations [4,11–14]. When carbon flux into sterol was measured directly using the technique described above, glucagon had no significant effect [8].

The increase in the relative incorporation of tritium into sterol synthesized in the presence of pyruvate could have resulted partly from ^3H labelling of the pyruvate methyl group. This could occur as a result of an increased 'futile' cycling of pyruvate through oxaloacetate and phospho*enol*-pyruvate [15] or by interconversion of the enzyme-bound keto and enol forms of pyruvate [16].

Of the metabolic inhibitors studied, all but the cyanohydroxycinnamate derivatives inhibited the absolute rate of sterol synthesis. However, only (−)-hydroxycitrate led to a significant change in the sterol *H/C* ratio. The reason for this change is not clear but may be related to a decreased flux of oxaloacetate through the malic enzyme pathway resulting in a decreased production of labelled NADPH.

In general, in vitro manipulations which caused changes in the rate of fatty acid synthesis also gave rise to corresponding changes in the sterol *H/C* ratio. Thus lactate and pyruvate which increase fatty acid synthesis in hepatocytes, also increase the sterol *H/C* ratio. In contrast, glucagon and (−)-hydroxycitrate, which inhibit hepatocyte lipogenesis resulted in a decrease in the sterol *H/C* ratio. Finally, the present results suggest that it would be unwise to conclude that a change in the incorporation of tritium always reflects a corresponding change in the flux of carbon into sterol. The extent of any change in the *H/C* ratio would need to be known before any such conclusion could be drawn with certainty.

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