The role of substrate supply in the regulation of cholesterol biosynthesis in rat hepatocytes

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1. Compactin, (–)-hydroxycitrate and dexamethasone gave rise to a decrease in the rate of cholesterol production in hepatocytes from fed rats by interfering with the flow of substrate into the sterol biosynthetic pathway. The cells responded to the deficit of biosynthetic sterol by increasing the activity of hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase). 2. Compactin and (–)-hydroxycitrate gave similar results in hepatocytes from rats starved for 24 h but in this case dexamethasone had no significant effect. 3. Exogenous oleate interferes with the production of carbohydrate-derived acetyl-CoA and also gives rise initially to opposing effects on the rate of sterol synthesis and HMG-CoA reductase activity. Over a longer period, however, oleate itself was capable of replacing carbohydrate as the major source of carbon for sterol synthesis. 4. The increase in HMG-CoA reductase activity observed when liver cells were incubated in the presence of compactin, (–)-hydroxycitrate or oleate could be partially reversed by the simultaneous presence of glucagon. 5. Under some physiological conditions, a deficiency of biosynthetic cholesterol or of a related precursor may lead to an increase in the activity of HMG-CoA reductase.

The activity of HMG-CoA reductase (EC 1.1.1.34) is generally considered to determine, and reflect, the overall rate of cholesterol biosynthesis in mammalian cells (Rodwell *et al.*, 1976). There are, however, some conditions under which this relationship does not hold. For instance, the fungal metabolite compactin is a competitive inhibitor of HMG-CoA reductase and results in a decrease in the rate of cholesterol synthesis in intact cells (Endo *et al.*, 1976*a,b*). However, in cells or animals pretreated with the drug, the levels of HMG-CoA reductase are substantially elevated (Brown *et al.*, 1978; Endo *et al.*, 1979; Brown & Goldstein, 1980; Fears, 1981). This effect has been ascribed to an induction of synthesis of HMG-CoA reductase protein in response to a deprivation of terpenoid or steroid products of mevalonic acid metabolism, which results from enzyme inhibition in the intact cells. Upon disruption of the cells the inhibitor is diluted out and this latent activity becomes apparent. If this explanation is correct, then it may be expected that other means of interruption of the normal flow of precursors into cholesterol would result in similar effects on HMG-CoA reductase. To investigate this

possibility, rat hepatocytes were incubated in the presence of (–)-hydroxycitrate, which prevents the formation of cytosolic acetyl-CoA by inhibiting ATP citrate lyase (EC 4.1.3.8) (Watson *et al.*, 1969). Similarly, the effects of dexamethasone, an inhibitor of HMG-CoA synthase (EC 4.1.3.5) in HeLa cells (Ramachandran *et al.*, 1978, 1980) on cholesterol synthesis and HMG-CoA reductase activity in freshly isolated liver cells were investigated.

In a preliminary report (Pullinger & Gibbons, 1981) we have shown that short-term exposure of hepatocytes to oleic acid also gives rise to a decrease in the rate of sterol synthesis and to an increase in HMG-CoA reductase activity. The present work extends these studies and has examined the ability of oleate to provide acetyl-CoA for sterol synthesis. Finally, the role of glucagon in the regulation of HMG-CoA reductase activity has been investigated during conditions under which the rate of cholesterol synthesis is restricted by substrate availability.

Experimental

Methods

Animals, preparation of hepatocytes and incubation procedures. The housing and treatment of

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Abbreviation used: HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase.

animals, preparation of isolated hepatocyte suspensions and incubation procedures have been described previously (Gibbons & Pullinger, 1977, 1979). All animals were given food and water *ad libitum*, except for experiments on liver cells from starved rats, in which case food was withdrawn for 24 h before hepatocyte preparation. In the present studies cell yield was considerably improved by including EGTA (0.5 mM) in the liver perfusate during the initial period in the absence of collagenase (Seglen, 1976). The final cell preparation was suspended in a solution containing glucose (11.1 mM), amino acids (East *et al.*, 1973) and bovine serum albumin (1.1%) in Krebs–Henseleit calcium and bicarbonate buffer (Krebs & Henseleit, 1932). This was the standard incubation medium. Solutions of albumin-bound sodium oleate were prepared by the method of Van Harken *et al.* (1969) and added to incubation flasks such that the initial oleate concentration was 2.0 mM. A solution of bovine serum albumin only was added to control flasks. The final albumin concentration in the oleate-containing and control incubations was 1.6%.

Measurement of the rate of cholesterol synthesis

The absolute rate of sterol synthesis in the cells was determined by measuring the weight of desmosterol (cholesta-5,24-dien-3 β -ol) that accumulates over a given period of time in the presence of triparanol (Gibbons & Pullinger, 1977, 1979). Briefly, this involved centrifugation of the incubated cell suspension, removal of the incubation medium and treatment of the cell pellet with a mixture of

argentation chromatography. Secondly, manipulative losses incurred during the extraction and purification of desmosterol (isolated as desmostenone) were accounted for by the addition of [26,27- 14 C]desmosterol (10000 d.p.m.) as internal standard. The relatively low mass (10–15 ng) associated with this added label was subtracted from the total mass of the desmosterol derivative as measured by g.l.c. The presence of triparanol in the medium of the cells had no effect on the rate of [14 C]acetate conversion into sterols (Gibbons & Pullinger, 1977). 3 H incorporation from 3 H $_2$ O into sterols and fatty acids was also unaffected and there was no change in the activity of HMG-CoA reductase (C. R. Pullinger & G. F. Gibbons, unpublished work).

Calculation of the contribution of labelled oleate to sterol synthesis

The contribution of [1- 14 C]oleate was determined from its specific radioactivity, its known pattern of incorporation into sterol and the radioactivity of the derived stenone fraction obtained after argentation chromatography. Thus 1 mol of [1- 14 C]oleate gives rise to 9 mol of [1- 14 C]acetyl-CoA, whose molar specific radioactivity is one-ninth that of the original oleate; 18 mol of acetyl-CoA are utilized/mol of sterol formed. However, during this process 6 of the 18 labelled carbon atoms of [1- 14 C]acetyl-CoA are lost as CO $_2$. Thus the contribution of [1- 14 C]oleate to sterol synthesis can be calculated from the formula:

$$\text{Sterol synthesis (nmol)} = \frac{\text{stenone radioactivity (d.p.m.)}}{12 \times [\text{oleate specific radioactivity (d.p.m./nmol)/9]}$$

cholesterol esterase and cholesterol oxidase in buffer containing Triton X-100. This resulted in the conversion of free and esterified desmosterol into desmostenone (cholesta-4,24-dien-3-one), which, after saponification of the enzyme-treated mixture, was separated from the much larger quantity of cholestenone (formed from cellular non-esterified and esterified cholesterol) by argentation chromatography. The pure desmostenone was converted into its heptafluorobutyryl enol ester by treatment with heptafluorobutyric anhydride and the mass of the derivative was determined by g.l.c. with electron-capture detection. The procedure employed in the present work differed from that previously reported (Gibbons & Pullinger, 1977, 1979) in the following respects. First, after saponification of the enzyme-treated cells, the alkaline mixture was saturated with sodium sulphate and the non-saponifiable lipid was extracted with hexane. The lipid fraction was subjected directly to thin-layer

Measurement of the rate of fatty acid synthesis

Cells were incubated in the standard medium containing 3 H $_2$ O (sp. radioactivity 39.8 mCi/mol). After extraction of the non-saponifiable lipid the aqueous phase was acidified to pH 1.0 by the addition of 5 M-HCl and extracted with hexane (10 ml) after addition of [1- 14 C]oleate (17500 d.p.m.) as internal standard. The organic phase was washed four times with water (10 ml) and a portion was assayed for 3 H and 14 C radioactivity.

Measurement of HMG-CoA reductase activity

At the end of each incubation period a portion of the cell suspension (0.3–0.5 ml) was transferred to an ice-cold 'Nunc' cryotube (Gibco Europe) and centrifuged at 80 g for 1.5 min. The supernatant was discarded. The pellet was resuspended in ice-cold Krebs–Henseleit (1932) buffer (0.5 ml) and re-centrifuged at 80 g for 1.5 min. The supernatant was discarded and the cell pellet was stored under liquid

N₂ until used (2–3 days). Immediately before assay the cell pellet was thawed and HMG-CoA reductase activity was measured on the whole-cell homogenate by the method of Kandutsch and his associates (Saucier & Kandutsch, 1979; Cavenee *et al.*, 1981).

Materials

The sources of materials used in the present work have been described previously (Gibbons & Pullinger, 1977, 1979; Pullinger & Gibbons, 1981). In addition, glucagon was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., and was dissolved in 0.001 M-HCl immediately before use. Sodium [1-¹⁴C]oleate was obtained from Amersham International, Amersham, Bucks., U.K. Compactin was obtained from Dr. R. Fears, Beecham Pharmaceuticals Research Division, Great Burgh, Epsom, Surrey, U.K., and was converted into the sodium salt by the method of Brown *et al.* (1978). (–)-Hydroxycitrate was obtained from Dr. A. C. Sullivan, Roche Research Center, Hoffmann–LaRoche Inc., Nutley, NJ, U.S.A.

Results and discussion

Effect of inhibitors of cholesterol synthesis on the activity of HMG-CoA reductase

Hepatocytes from fed animals were incubated in the standard medium either alone or in the presence

of (–)-hydroxycitrate (initial concentration, 2 mM). At hourly intervals, portions of the cell suspensions were removed for assay of HMG-CoA reductase activity and the rates of sterol and fatty acid synthesis were also measured. The results are presented in Fig. 1 and Table 1. In the presence of (–)-hydroxycitrate there was a substantial increase in the activity of HMG-CoA reductase even though the rate of sterol synthesis was suppressed (Fig. 1). The drug also gave rise to a decrease in the rate of fatty acid synthesis (Table 1). One of the effects of

Table 1. *Effect of (–)-hydroxycitrate, compactin and dexamethasone on fatty acid synthesis in hepatocytes of fed rats*

Suspensions of hepatocytes were incubated in the standard medium in the presence of ³H₂O (sp. radioactivity 39.8 mCi/mol) either alone or with dexamethasone (10 μM), (–)-hydroxycitrate (2.0 mM) or compactin (2 μM). After 3 h the radioactivity of each fatty acid fraction was determined. Results are means ± S.E.M. of at least four different hepatocyte preparations.

Addition	Fatty acid synthesis (nmol of ³ H ₂ O incorporated/mg of protein per 3 h)
—	113.0 ± 15.3
Dexamethasone	117.4 ± 21.8
(–)-Hydroxycitrate	48.1 ± 4.3
Compactin	106.9 ± 19.3

Fig. 1. *Effect of (–)-hydroxycitrate on the rate of sterol biosynthesis (a) and HMG-CoA reductase activity (b) in rat hepatocytes*

Suspensions of hepatocytes were incubated for various periods of time in the standard medium containing triparanol (2 μM) either in the absence or in the presence of (–)-hydroxycitrate (2 mM). After each hour of incubation the weight of sterol synthesized and the activity of HMG-CoA reductase were determined. Each point represents the average of separate determinations carried out on at least three independent preparations of hepatocytes. The vertical bars represent the S.E.M. Symbols: ●, (–)-hydroxycitrate absent; ▲, (–)-hydroxycitrate present. Statistical comparisons using Student's *t* test for paired data showed that after the third and fourth hours of incubation (–)-hydroxycitrate resulted in significant ($P < 0.02$) decreases in the rate of sterol synthesis and significant ($P < 0.02$) increases in the activity of HMG-CoA reductase.

(-)-hydroxycitrate is inhibition of ATP citrate lyase (EC 4.1.3.8), resulting in a decrease in the availability of cytosolic acetyl-CoA for cholesterol synthesis and lipogenesis (Watson *et al.*, 1969). The observed decrease in the rate of steroidogenesis is thus consistent with the known effects of (-)-hydroxycitrate and probably results from a decreased availability of cytosolic acetyl-CoA. The drug compactin has similar opposing effects of HMG-CoA reductase activity and the rate of sterol synthesis during longer term incubations of cultured human fibroblasts (Brown *et al.*, 1978). However, the minimum time required for the effects on compactin on HMG-CoA reductase activity to become apparent is not known. To determine whether compactin acts as rapidly as (-)-hydroxycitrate in provoking an increase in HMG-CoA reductase activity in liver cells, the activity of the enzyme was measured at hourly intervals after addition of the drug to cell suspensions. Fig. 2 shows that a significant increase in enzyme activity occurred after as little as 1 h of incubation. The rate of sterol synthesis in the intact cells decreased during the first hour of incubation in the presence of compactin and this inhibition persisted for up to 4 h (Fig. 2). The rate of fatty acid synthesis was unaffected by compactin (Table 1).

Glucocorticoids inhibit cholesterol synthesis in several types of non-hepatic cells in culture (Cavenee & Melnykovich, 1977; Johnston *et al.*, 1979; Henze *et al.*, 1981) and in some, but not all, cell lines this is

accompanied by an increase in the activity of HMG-CoA reductase (Johnston *et al.*, 1979). However, there is no evidence for a similar glucocorticoid-induced uncoupling of the rate of sterol synthesis from HMG-CoA reductase activity in freshly isolated hepatocytes, which, unlike the previously studied cells, are derived from an organ that is responsible for the synthesis of a major part of the total body cholesterol. To investigate this possibility, hepatocytes were incubated for various periods of time in the presence or absence of dexamethasone. Changes in the rate of sterol synthesis and HMG-CoA reductase activity were determined and the results are shown in Fig. 3. It appears that, like compactin and (-)-hydroxycitrate, dexamethasone very rapidly gives rise to opposing changes in these activities, whereas the rate of fatty acid synthesis was unchanged (Table 1). In contrast, in HeLa cells, there is a lag period of 12–16 h before the effect of dexamethasone on HMG-CoA reductase activity becomes apparent (Cavenee & Melnykovich, 1977; Ramachandran *et al.*, 1982).

Both the rate of hepatic sterol synthesis and the activity of HMG-CoA reductase are decreased after depriving rats of food for 24 h (Rodwell *et al.*, 1976). To determine whether the above drugs are capable of further depressing this low rate of sterol synthesis and, if so, the effects of this on HMG-CoA reductase activity, freshly prepared liver cells derived from 24 h-starved rats were treated with compactin,

Fig. 2. Effect of compactin on the rate of sterol biosynthesis (a) and HMG-CoA reductase activity (b) in rat hepatocytes. Suspensions of hepatocytes were incubated in the standard medium containing triparanol ($2\mu\text{M}$) either in the absence (●) or presence (▲) of compactin ($2\mu\text{M}$). Statistical comparisons were made by Student's *t* test for paired data and show that at all time periods compactin led to a significant ($P < 0.05$) increase in HMG-CoA reductase activity. Compactin also resulted in a significant decrease ($73.3 \pm 3.5\%$; $n = 5$) in the rate of sterol synthesis between the second and third hour of incubation.

Fig. 3. Effect of dexamethasone on the rate of sterol biosynthesis and HMG-CoA reductase activity in rat hepatocytes

Suspensions of hepatocytes were incubated for up to 4 h in the standard medium containing triparanol (2 μ M) either in the absence or presence of dexamethasone (10 μ M). After each hour of incubation, the weight of sterol synthesized and the activity of HMG-CoA reductase were determined. The results are expressed as rates of sterol synthesis (nmol/mg of protein per h; $\blacktriangle$) or enzymic activity (pmol of mevalonate/min per mg of protein; $\bullet$) observed in the presence of the drug as a percentage of values observed in the control incubations at corresponding times. Values for HMG-CoA reductase activity and the rate of sterol synthesis after 3 h of incubation are the means of determinations carried out on five independent hepatocyte preparations. At all other times the values are the means of between two and four independent determinations. Vertical bars represent the means $\pm$ s.e.m. Statistical comparisons were made by Student's *t* test for paired data. Between the second and the third hour of incubation, dexamethasone resulted in a significant ($P < 0.005$) decrease in the rate of sterol synthesis. After 3 h, the drug gave rise to a significant ($P < 0.05$) increase in the activity of HMG-CoA reductase.

Table 2. Effect of drugs on the rate of sterol synthesis and HMG-CoA reductase activity in hepatocytes from 24 h-starved rats

Suspensions of hepatocytes were incubated in the standard medium either alone or in the presence of the compounds shown below. After 2 h triparanol was added to give a concentration of 2 μ M and the weight of sterol synthesized during the next hour was measured. The activity of HMG-CoA reductase was also determined at the end of this period. The results are expressed as rate of sterol synthesis (nmol/h per mg of protein) or HMG-CoA reductase activity (pmol/min per mg of protein) observed in the presence of each drug as a percentage of that in the control incubations. The control activities were: sterol synthesis, 0.126 ± 0.028 nmol/h per mg of protein; HMG-CoA reductase, 35.4 ± 2.8 pmol/min per mg of protein. The above values are means $\pm$ s.e.m. of determinations in six animals. Statistical comparisons using Student's *t* test for paired data showed that compactin and (–)-hydroxycitrate led to significant ($P < 0.02$) decreases in the rate of sterol synthesis and significant increases ($P < 0.02$) in HMG-CoA reductase activity. In the presence of dexamethasone there was no significant change in either the rate of sterol synthesis or in HMG-CoA reductase activity.

Drug	Rate of sterol synthesis (% of control)	HMG-CoA reductase activity (% of control)
Dexamethasone (10 μ M)	106.7 $\pm$ 3.1	115.4 $\pm$ 9.7
Compactin (2 μ M)	67.7 $\pm$ 4.2	130.1 $\pm$ 11.8
(–)-Hydroxycitrate (2 mM)	46.9 $\pm$ 2.9	124.7 $\pm$ 8.1

the end of this period (Table 2). Compactin and (–)-hydroxycitrate both gave rise to a decrease in the rate of sterol biosynthesis during this period. This effect of (–)-hydroxycitrate appears to contrast with the results of a previous investigation in which it was reported that the drug had no effect on the rate of $^3\text{H}_2\text{O}$ conversion into cholesterol in the liver of starved rats *in vivo* (Triscari & Sullivan, 1977). In the present work dexamethasone had no effect on the rate of sterol synthesis in the liver cells of starved animals (Table 2). In non-hepatic cells, the inhibitory effect of this drug on cholesterol synthesis has been attributed to its effect on HMG-CoA synthase (Ramachandran *et al.*, 1978, 1980). If this is the case in liver, then during a period of starvation, the enzyme somehow acquires a resistance to the inhibitory effects of the glucocorticoid. It is of interest that dexamethasone, which failed to inhibit cholesterol synthesis under these circumstances, was also the only one of the three compounds tested that failed to provoke a significant increase in HMG-CoA reductase activity (Table 2).

(–)-hydroxycitrate and dexamethasone. The rates of sterol synthesis were determined between the second and third hours in the presence of the drugs and the activity of HMG-CoA reductase was measured at

Effect of exogenous oleate on the supply of substrate for cholesterol biosynthesis

An increase in the activity of HMG-CoA reductase similar to that observed above also occurs when sterol synthesis is inhibited during short-term incubations of hepatocytes with oleate (Pullinger & Gibbons, 1981). Exogenous fatty acids inhibit pyruvate dehydrogenase (EC 1.2.4.1) (Wieland *et al.*, 1972; Patzelt *et al.*, 1973) and since under normal circumstances carbohydrate oxidation is the major process by which acetyl-CoA is produced for sterol synthesis in liver cells (Gibbons & Pullinger, 1979; Fears, 1981), the observed decrease in the rate of sterol biosynthesis may be attributed to a

Fig. 4. Contribution of $[1-^{14}\text{C}]$ oleate to sterol biosynthesis in rat hepatocytes

Suspensions of hepatocytes were incubated in the standard medium containing triparanol ($2\mu\text{M}$) either alone or in the presence of $[1-^{14}\text{C}]$ oleate (2mM ; 0.31 Ci/mol). The volume of each incubation was 15.0 ml , containing 6.88 mg of protein/ ml . At hourly intervals, portions of cells were removed from each type of incubation and both the weight and radioactivity of the newly-synthesized sterol were determined. The contribution of $[1-^{14}\text{C}]$ oleate to sterol synthesis was calculated by use of the formula shown in the Experimental section. In this experiment, recovery of desmosterol was determined by measuring the mass of cholesterol (cholestenone) recovered after argentation chromatography (Gibbons & Pullinger, 1979). This was related to protein content on the basis of $100\mu\text{g}$ of cholesterol = 6.38 mg of protein (Campbell & Kosterlitz, 1947; Cook, 1958). Although this method is less convenient, the biosynthetic labelling of desmosterol with ^{14}C from $[1-^{14}\text{C}]$ oleate precluded the use of $[26,27-^{14}\text{C}]$ desmosterol as an internal standard to account for losses during sterol isolation. Sterol synthesis: ■, oleate absent; ●, oleate present; ▲, sterol synthesized exclusively from oleate.

decreased activity of this enzyme. During longer periods in the presence of oleate, there is a marked increase in the rate of sterol synthesis compared with that observed in control cells (Goh & Heimberg, 1973, 1979; Pullinger & Gibbons, 1981). Under these conditions, it was of interest to determine the extent to which exogenous oleate is capable of providing acetyl-CoA for sterol synthesis. Hepatocytes were therefore incubated in the presence of $[1-^{14}\text{C}]$ oleate and the mass and radioactivity of the newly-synthesized sterol were determined after intervals of 1, 2, 3 and 4 h. For comparison, the rates of sterol synthesis were also determined at the same times in cells incubated in the absence of oleate. The results of a typical experiment are shown in Fig. 4. The previously observed biphasic effect of exogenous oleate on the rate of sterol synthesis is again apparent, the rate being much lower at shorter time periods and higher at longer time periods than in the control cells. The contribution of $[1-^{14}\text{C}]$ oleate to sterol synthesis was calculated from its specific radioactivity and the incorporation of radioactivity into newly-synthesized sterol using the formula shown in the Experimental section. It appears that acetyl-CoA produced by the β -oxidation of oleate is capable of providing a large proportion of the sterol carbon over the first 3 h of the incubation (Fig. 4). In three experiments of this type oleate contributed $85.9 \pm 5.0\%$ (mean $\pm$ s.e.m.) and $79.7 \pm 6.5\%$ over the first 2 and 3 h of incubation respectively.

Partial reversal of the drug- and oleate-induced increase in HMG-CoA reductase activity by glucagon

Glucagon inhibits the normal levels of HMG-CoA reductase in untreated liver cells (Ma *et al.*, 1978; Edwards *et al.*, 1979; Ingebritsen *et al.*, 1979), but it is not known whether the hormone is capable of suppressing the compactin-mediated increase in enzyme activity. Fig. 5 shows that glucagon partially prevented the rise in HMG-CoA reductase activity when simultaneously present during incubations of hepatocytes with compactin and had a similar effect in the presence of oleate and $(-)$ -hydroxycitrate. If the effect of these compounds on HMG-CoA reductase activity is mediated by an interference with substrate supply, these observations suggest that glucagon is capable of overriding the stimulation of enzyme activity caused by the resulting deficiency of sterol and/or sterol precursors.

Mechanism of the drug-mediated increase in HMG-CoA reductase activity

There is a body of evidence that suggests that pre-existing HMG-CoA reductase protein is susceptible to inactivation and activation by a reversible mechanism involving phosphorylation and dephos-

Fig. 5. Effect of glucagon on the compactin-, (–)-hydroxycitrate- and oleate-induced increase in HMG-CoA reductase activity in rat hepatocytes

Suspensions of hepatocytes were incubated for up to 4 h in the standard medium in the presence of oleate (2 mM) (a), compactin (2 μM) (b), (–)-hydroxycitrate (2 mM) (d) or with none of these substances (c). In each case glucagon (initial concentration 2.9 μM) was either present (▲) or absent (●). Each point represents the mean determined on hepatocytes derived independently from at least three different animals. Statistical analysis using Student's *t* test for paired data showed that in all cases glucagon resulted in significant decreases ($P < 0.05$) in HMG-CoA reductase activity after the 2 and 3 h of incubation.

phorylation respectively (Gibson *et al.*, 1982). However, in hepatocytes, the presence of compactin is believed to result predominantly in an increased quantity of enzyme protein rather than in an activation of existing enzyme (Edwards *et al.*, 1980). In the present work, in most cases, enzyme preparations were obtained using buffers that did not contain fluoride, an inhibitor of dephosphorylation and therefore activation of HMG-CoA reductase. Under these conditions most of the enzyme should be present in the activated, dephosphorylated form. The fact that increases in enzyme activity persisted under these conditions tentatively suggests that at least some of the increase was due to an increase in the number of enzyme molecules. Nevertheless, in those experiments in which fluoride (50 mM) was

present in the buffer before assay of reductase, the increase in the activity of HMG-CoA reductase in cells incubated in the presence of the drugs was somewhat lower than that observed when fluoride was absent (C. R. Pullinger & G. F. Gibbons, unpublished work).

Relationship between the activity of HMG-CoA reductase and the availability of substrate for cholesterol synthesis

A common feature of all the substances used in the present work is their ability to decrease the quantity of biosynthetic cholesterol produced by the hepatocyte by restricting the availability of cholesterol precursors either at or before the HMG-CoA reductase step. It seems likely therefore

that the decreased flux of precursors into cholesterol, into an oxysterol intermediate (Gibbons *et al.*, 1980) or into some other post-mevalonate metabolite is directly responsible for the elevation of HMG-CoA reductase activity. This explanation is also supported by the decrease in HMG-CoA reductase activity observed when the flux of precursors through the cholesterol pathway is increased in hepatocytes incubated in the presence of millimolar concentrations of mevalonolactone (Edwards *et al.*, 1977; Gould, 1977), a situation that is the exact reverse of that described in the present report. It would appear therefore that under some circumstances HMG-CoA reductase responds to a deficiency of biosynthetic cholesterol or cholesterol precursor in a manner similar to that by which the low-density lipoprotein receptor responds to a deficiency of extracellular, lipoprotein cholesterol (Brown *et al.*, 1981). Whether certain physiological circumstances during which substrate supply for cholesterol synthesis is restricted also give rise to elevated levels of HMG-CoA reductase is presently a matter for conjecture. However, the effects of exogenous oleate described in the present report and previously (Pullinger & Gibbons, 1981) suggest that this may occur as the liver cell adapts to a metabolism based on fatty acid oxidation rather than one in which the major source of acetyl-CoA is carbohydrate. As glycogen normally provides a large quantity of the sterol carbon in liver (Gibbons & Pullinger, 1979; Fears, 1981), it is possible that the marked diurnal change in glycogen metabolism (Walker, 1977) may produce cyclical variations in substrate availability for sterol synthesis and may thus contribute to the well-known diurnal variation in the activity of HMG-CoA reductase (Rodwell *et al.*, 1976).

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References

- Brown, M. S. & Goldstein, J. L. (1980) *J. Lipid Res.* **21**, 505–517
- Brown, M. S., Faust, J. R., Goldstein, J. L., Kaneko, I. & Endo, A. (1978) *J. Biol. Chem.* **253**, 1121–1128
- Brown, M. S., Kovanen, P. T. & Goldstein, J. L. (1981) *Science* **212**, 628–635
- Campbell, R. M. & Kosterlitz, H. W. (1947) *J. Physiol. (London)* **106**, 12P
- Cavenee, W. K. & Melnykovich, G. (1977) *J. Biol. Chem.* **252**, 3272–3276
- Cavenee, W. K., Chen, H. W. & Kandutsch, A. A. (1981) *J. Biol. Chem.* **256**, 2675–2681
- Cook, R. P. (1958) in *Cholesterol* (Cook, R. P., ed.), p. 145, Academic Press, New York
- East, A. G., Louis, L. N. & Hoffenberg, R. (1973) *Exp. Cell Res.* **76**, 41–46
- Edwards, P. A., Popják, G., Fogelman, A. M. & Edmond, J. (1977) *J. Biol. Chem.* **252**, 1057–1063
- Edwards, P. A., Lemongello, D. & Fogelman, A. M. (1979) *J. Lipid Res.* **20**, 2–7
- Edwards, P. A., Lemongello, D., Kane, J., Shechter, I. & Fogelman, A. M. (1980) *J. Biol. Chem.* **255**, 3715–3725
- Endo, A., Kuroda, M. & Tanzawa, K. (1976a) *FEBS Lett.* **72**, 323–326
- Endo, A., Kuroda, M. & Tsujita, Y. (1976b) *J. Antibiot.* **29**, 1346–1348
- Endo, A., Tsujita, Y., Kuroda, M. & Tanzawa, K. (1979) *Biochim. Biophys. Acta* **575**, 266–276
- Fears, R. (1981) *Biochem. J.* **199**, 1–7
- Gibbons, G. F. & Pullinger, C. R. (1977) *Biochem. J.* **161**, 321–330
- Gibbons, G. F. & Pullinger, C. R. (1979) *Biochem. J.* **177**, 255–263
- Gibbons, G. F., Pullinger, C. R., Chen, H. W., Cavenee, W. K. & Kandutsch, A. A. (1980) *J. Biol. Chem.* **255**, 395–400
- Gibson, D. M., Parker, R. A., Stewart, C. S. & Evenson, K. J. (1982) *Adv. Enzyme Regul.* **20**, 263–283
- Goh, E. H. & Heimberg, M. (1973) *Biochem. Biophys. Res. Commun.* **55**, 382–388
- Goh, E. H. & Heimberg, M. (1979) *Biochem. J.* **184**, 1–6
- Gould, R. G. (1977) in *Cholesterol Metabolism and Lipolytic Enzymes* (Polonovski, J., ed.), pp. 13–38, Masson Publishing (U.S.A.) Inc., New York
- Henze, K., Kudchodkar, B. J., Chait, A., Albers, J. J. & Bierman, E. L. (1981) *Biochim. Biophys. Acta* **666**, 199–204
- Ingebritsen, T. S., Geelen, M. J. H., Parker, R. A., Evenson, K. J. & Gibson, D. M. (1979) *J. Biol. Chem.* **254**, 9986–9989
- Johnston, D., Cavenee, W. K., Ramachandran, C. K. & Melnykovich, G. (1979) *Biochim. Biophys. Acta* **572**, 188–192
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
- Ma, G. Y., Gove, C. D. & Hems, D. A. (1978) *Biochem. J.* **174**, 761–768
- Patzelt, C., Löffler, G. & Wieland, O. H. (1973) *Eur. J. Biochem.* **33**, 117–122
- Pullinger, C. R. & Gibbons, G. F. (1981) *Biochem. Biophys. Res. Commun.* **99**, 37–43
- Ramachandran, C. K., Gray, S. L. & Melnykovich, G. (1978) *Arch. Biochem. Biophys.* **189**, 205–211
- Ramachandran, C. K., Gray, S. L. & Melnykovich, G. (1980) *Biochim. Biophys. Acta* **618**, 439–448
- Ramachandran, C. K., Gray, S. L. & Melnykovich, G. (1982) *Biochem. J.* **208**, 47–52
- Rodwell, V. W., Nordstrom, J. L. & Mitschelen, J. J. (1976) *Adv. Lipid Res.* **14**, 1–74
- Saucier, S. E. & Kandutsch, A. A. (1979) *Biochim. Biophys. Acta* **572**, 541–556
- Seglen, P. O. (1976) *Methods Cell Biol.* **13**, 29–83
- Triscari, J. & Sullivan, A. C. (1977) *Lipids* **12**, 357–363
- Van Harken, D. R., Dixon, C. W. & Heimberg, M. (1969) *J. Biol. Chem.* **244**, 2278–2285
- Walker, P. R. (1977) *Biochim. Biophys. Acta* **496**, 255–263
- Watson, J. A., Fang, M. & Lowenstein, J. M. (1969) *Arch. Biochem. Biophys.* **135**, 209–217
- Wieland, O. H., Patzelt, C. & Löffler, G. (1972) *Eur. J. Biochem.* **99**, 37–43