REVIEW ARTICLE

The contribution of the cholesterol biosynthetic pathway to intermediary metabolism and cell function

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Introduction

The biosynthesis of cholesterol *de novo* was first demonstrated by Schoenheimer & Breusch (1933) and most vertebrate cells possess the capacity for cholesterogenesis (Siperstein, 1970). The long-term control of HMG-CoA reductase, usually considered to be the rate-limiting step in the cholesterol biosynthetic pathway, has received particular attention (Rodwell *et al.*, 1976). The acute regulation of activity by phosphorylation (Beg *et al.*, 1980) was reviewed recently by Geelen *et al.* (1980) within the context of the overall control of hepatic lipogenesis. However, it should be noted that the decrease in activity when homogenous HMG-CoA reductase is incubated with ATP and Mg^{2+} was suggested not to involve phosphorylation (Ness *et al.*, 1980) and the physiological relevance of this control has not been established in view of the observation that the level of enzyme activity apparently expressed *in situ* is insufficient to account for the mass of cholesterol synthesized (Brown *et al.*, 1979) unless microsomes are treated with alkaline phosphatase prior to assay. The activity of HMG-CoA reductase may not always correlate well with the rate of sterol biosynthesis as measured by other methods so that, for example, assay of the isolated enzyme activity overestimates the response of the pathway to food intake (Fears & Morgan, 1976; Fears, 1978).

It is the purpose of the present review to consider those aspects of the pathway which have received relatively less attention previously. First, to discuss the role of HMG-CoA reductase in providing non-sterol intermediates required for cell function. Secondly, to describe the inter-relationship between cholesterol biosynthesis and other pathways of intermediary metabolism at the level of substrate supply. Thirdly, to compare the contribution made by endogenous synthesis relative to exogenous supply of tissue sterol requirements.

Abbreviations used: HMG-CoA reductase, β -hydroxy- β -methylglutaryl-CoA reductase (EC 1.1.1.34); VLD lipoprotein, very-low-density lipoprotein; LD lipoprotein, low-density lipoprotein; HD lipoprotein, high-density lipoprotein.

HMG-CoA reductase and the synthesis of non-sterol intermediates

When the supply of exogenous cholesterol is limited, a relatively high rate of synthesis is required in the liver for bile acid and lipoprotein secretion, in the steroid-hormone producing organs and in rapidly-dividing tissues for membrane production. The rate of endogenous cholesterogenesis is repressed by cholesterol and by certain oxysterols, for example, 5 α -lanost-8-ene-3 β ,32-diol and 3 β -hydroxy-5 α -lanost-8-en-32-al, which may be natural precursors of cholesterol (Gibbons *et al.*, 1980). However, other important metabolic intermediates are synthesized from isopentenyl pyrophosphate (Fig. 1) and their supply presumably must be maintained when sterol synthesis is decreased. Studies on the control of the branched pathway of mevalonate metabolism (Brown & Goldstein, 1980) have been advanced by the use of compactin (ML 236B) (Fig. 2), a competitive inhibitor of HMG-CoA reductase isolated independently from the culture broth of *Penicillium brevicompactum* by Brown *et al.* (1976) and of *Penicillium citrinum* by Endo *et al.* (1976). We find 50% inhibition of HMG-CoA reductase in rat liver *in vitro* at 8.5×10^{-8} M-compactin (Fears *et al.*, 1980). Potent inhibition is also obtained in liver, small intestine, spleen and kidney *in vivo* 2-3 h after dosing (20-50 mg/kg body wt.) although there is little net effect on the mass of cholesterol synthesized during a period of 24 h because of subsequent enzyme induction.

Isopentenyladenine

When cultured fibroblasts are grown in the presence of 10^{-6} M-compactin HMG-CoA reductase activity is initially inhibited and then subsequently induced so that increased enzyme activity is expressed if compactin is removed by dilution (Brown *et al.*, 1978). If a saturating level of LD lipoprotein is added to cells incubated with compactin then the induced HMG-CoA reductase activity is suppressed by up to 85% and the residual activity can be almost completely abolished by 5×10^{-5} M-mevalonate (Brown & Goldstein, 1980).

Fig. 1. Mevalonate and isoprenoid metabolism

Fig. 2. Competitive inhibitors of HMG-CoA reductase

Thus, Brown & Goldstein (1980) have developed the concept of multivalent feedback regulation of HMG-CoA reductase involving both LD lipoprotein and a non-sterol metabolite of mevalonate. The inhibitory effect of mevalonate could not be reproduced by addition of squalene, ubiquinone or dolichol but isopentenyladenosine, a component of tRNA, caused a rapid decline in HMG-CoA reductase activity. A non-sterol metabolite of mevalonate was also required to support cell growth. Kaneko *et al.* (1978) found that growth of human fibroblasts and mouse L cells in the presence of LD lipoprotein was decreased by 10^{-5} M-compactin but restored by mevalonate. Further studies by Goldstein *et al.* (1979) demonstrated that 4×10^{-5} M-compactin inhibited growth of Chinese hamster ovary cells but that growth could again be restored by a combination of LD lipoprotein plus mevalonate. Similarly, Habenicht *et al.* (1980) found that mevalonate was required for DNA synthesis and cell survival in smooth muscle and Swiss 3T3 cells stimulated to divide by platelet-derived growth factor in the

presence of LD lipoprotein and 1.5×10^{-5} M-compactin. Other workers (Witte & Cornicelli, 1980) have shown that platelet-derived growth factor stimulates fibroblasts both to synthesize LD lipoprotein receptors and to increase cholesterol synthesis. Whilst extra cholesterol was required for new membranes it was suggested that the increased capacity to obtain exogenous cholesterol was important to conserve levels of the mevalonate-derived non-sterol intermediate essential for cell growth. Compactin (2.5×10^{-6} M) also inhibits HMG-CoA reductase by 95% and completely prevents the S-phase burst of DNA synthesis in synchronized cultures of BHK-21 cells (Quesney-Huneus *et al.*, 1979). The inhibition of DNA replication could be reversed by mevalonate and by isopentenyladenine and the hydroxylated analogue, zeatin, but not by dolichol, ubiquinone or isopentenyladenosine (Quesney-Huneus *et al.*, 1980).

Thus, in combination with cholesterol, isopentenyladenine, or a related substance, may be of critical importance both in the multivalent regulation of HMG-CoA reductase activity and in the control of cell growth although it should be noted that the presence of free isopentenyladenine in mammalian cells has yet to be demonstrated.

Ubiquinone

Compactin has also been used to examine the regulation of ubiquinone synthesis in cultured human fibroblasts (Faust *et al.*, 1979a). In the presence of LD lipoprotein plus 10^{-5} M-compactin the rate of incorporation of mevalonate into ubiquinone-10 was approx. 5-fold faster than into cholesterol, although sterol production was much increased in the absence of exogenous cholesterol.

The relative sparing of ubiquinone synthesis is consistent with inhibition by LD lipoprotein at an additional step distal to isopentenyl pyrophosphate as proposed by Gould & Swyrd (1966), for example, squalene synthetase (Faust *et al.*, 1979b) and with the observation (Rao & Olson, 1967) that dietary cholesterol inhibited mevalonate incorporation into sterols but not into ubiquinone in rat liver. Furthermore, feeding rats with ubiquinone-9 (1.5 mg/day) decreased hepatic cholesterogenesis between acetyl-CoA and mevalonate (Krishnaiah & Ramasarma, 1970) so that ubiquinone might also function in the multivalent regulation of HMG-CoA reductase although it was not found to be active in the studies of Brown & Goldstein (1980).

Adriamycin (Fig. 2) is an anthracycline antibiotic from *Streptomyces peucetius*, effective against a variety of neoplasms, which is also a competitive inhibitor of HMG-CoA reductase in rat liver *in vitro* (5×10^{-4} M) but, like compactin, subsequently induces new enzyme synthesis *in vivo* (Satish Menon & Ramasarma, 1980). Ubiquinone synthesis is also induced 24 h after dosing although inhibition is obtained at high levels of adriamycin (3×10^{-4} M) in beef heart *in vitro* (Folkers *et al.*, 1977). It is of interest that supplementary ubiquinone protects against adriamycin-induced cardiomyopathy (Kishi *et al.*, 1976), perhaps by compensating for diminished synthesis, although there may also be interference with ubiquinone function (Folkers *et al.*, 1977) because of the structural relationship between the benzoquinone ring of ubiquinone and the tautomeric quinone and quinol rings of adriamycin.

Dolichol

Lucas *et al.* (1980) showed that dietary cholesterol increased dolichyl monophosphate concentration in rat adipose tissue and this response was associated with an increased transfer of mannose into mannosyl lipids. Tavares *et al.* (1981) found that 1% (w/w) dietary cholesterol also increased the dolichol content of rat liver and stimulated the incorporation of mevalonate into dolichol and dolichyl diphosphate oligosaccharide with an associated enhancement of protein *N*-glycosylation. Similarly, feeding rabbits on a diet containing 2% (w/w) cholesterol (White *et al.*, 1981) decreased HMG-CoA reductase activity and cholesterol synthesis but stimulated mevalonate incorporation into dolichol and the activity of dolichyl phosphate-dependent mannosyltransferase. In contrast, James & Kandutsch (1980b), using rats given 5% (w/w) dietary cholesterol, observed decreased dolichol biosynthesis although mannose incorporation into liver and plasma glycoproteins was unchanged. As the dolichol pathway is saturated at a lower concentration of isoprenoid intermediates than is cholesterogenesis, then the synthesis of dolichol, like

that of ubiquinone, may be maintained when HMG-CoA reductase is incompletely repressed, or even may be increased if inhibition of squalene synthetase leads to accumulation of farnesyl pyrophosphate. A change in hepatic dolichol synthesis during severe hypercholesterolaemia, if accompanied by changes in protein glycosylation and glycoprotein secretion, might contribute to the appearance of lipoproteins of altered composition, for example, β -VLD lipoprotein. Dolichyl phosphate is also required for the assembly of membrane-bound glycoproteins and it would be interesting to establish whether the decreased dolichol concentration in spleen, lung, testis, heart and skeletal muscle found after dietary cholesterol (Tavares *et al.*, 1981), in contrast with the liver, is associated with the suppression of LD lipoprotein-receptor activity found at those sites (Fears & Uempleby, 1979).

Dolichol was recently discovered to be of key importance for cell function during embryonic development (Carson & Lennarz, 1981). Compactin induced abnormal gastrulation in the sea urchin with concomitant reduction in glycolipid and glycoprotein synthesis. Supplementation with dolichol maintained glycoprotein synthesis and normal gastrulation whereas ubiquinone and cholesterol were ineffective. Elevated rates of dolichol synthesis were also found to be important in the mouse during development of the brain (James & Kandutsch, 1980c) and spermatogenesis (James & Kandutsch, 1980a).

Supply of substrate

Acetyl-CoA

In addition to providing essential metabolites for cell function the cholesterol biosynthetic pathway also interacts with other areas of intermediary metabolism at the level of substrate supply. In normolipidaemic rats the rate of hepatic cholesterogenesis is approx. 30% of the rate of fatty acid synthesis, and the latter is of the same order as the rate of degradation of C_2 units in the tricarboxylic acid cycle (Salmon *et al.*, 1974). However, using rats adapted to meal-feeding on a diet rich in fat (Fears, 1978) the postprandial hepatic synthesis of cholesterol and fatty acid is similar ($90 \pm 10 \mu\text{g/h}$ per g and $85 \pm 15 \mu\text{g/h}$ per g respectively; $n = 8$) so that the mass of cholesterol synthesized per day accounts for a significant proportion of acetyl-CoA turnover. Although many workers have used acetate as a precursor for cholesterogenesis it is unlikely to be a substrate of physiological relevance in nonruminants and acetate incorporation may not reflect the throughput of other substrates if activation by acetyl-CoA synthetase is the rate-limiting step. In agreement with comparable studies on fatty acid

synthesis, glucose, when present in physiological concentrations (5–15 mM), does not provide more than 6–12% of substrate for mammalian hepatic cholesterogenesis (Salmon *et al.*, 1974; Gibbons & Pullinger, 1979). However, glycogen may be an important source of acetyl units for lipogenesis and this supply constitutes a major role of hepatic glycolysis (Woods & Krebs, 1971). Using avian hepatocytes, Reisenfeld *et al.* (1981) have recently found the incorporation of glucose into lipids to be much stimulated in the presence of glutamine, alanine and, to some extent, asparagine, serine and glycine. Lactate and pyruvate (3 mM) and their circulating precursors, for example alanine and serine, are considered to be the major substrates for hepatic cholesterogenesis (Salmon *et al.*, 1974; Gibbons & Pullinger, 1979). The contribution from other amino acids has not been studied systematically but in an experiment using DL-[benzene- ^{14}C]tryptophan (Fears & Murrell, 1980) we found less than 1% converted to total lipids. Davis (1978) suggested that propionate could be used by rat liver for cholesterogenesis and that this precursor was not in equilibrium with the acetyl-CoA substrate pool but was metabolized via malonyl-CoA. The participation of malonyl-CoA in the synthesis of isoprenoids had been proposed in earlier studies (Rudney *et al.*, 1966) but it may also be relevant to note that propionyl-CoA can be converted to hydroxyethylglutaryl-CoA and thence to homo-mevalonate in rat liver (Baker & Schooley, 1981).

As regards the provision of substrate for extrahepatic tissues, Edmond (1974) and Ramsey (1976), using 1–12-day-old rats, showed that 3-hydroxybutyrate was the preferred substrate for sterol synthesis in brain, spinal cord and skin, i.e. the three organs of ectodermal origin. Thus, not only is the developing central nervous system dependent on ketone bodies for energy requirements but also for lipid for myelination. 3-Hydroxybutyrate was also used for cholesterogenesis by foetal tissues, in particular brain, liver and placenta (Secombe *et al.*, 1977) although the quantitative contribution was difficult to assess. In addition, acetoacetate may act to transport acetyl-CoA out of mitochondria, an important function for species where ATP citrate lyase activity is low (Robinson & Williamson, 1980). Under certain conditions, leucine compares favourably with mevalonate as a precursor of tissue cholesterol (Miettinen & Penttilä, 1971) and we observe that leucine gives results qualitatively comparable to those obtained using $^3\text{H}_2\text{O}$ (Fears & Umpleby, 1979). When compared with the rate of protein synthesis the incorporation of label from [U- ^{14}C]leucine into cholesterol is small, ranging from 5% of the total amino acid metabolized in epididymal adipose tissue to 0.7% in liver (R. Fears, unpublished work). The contribution made by

leucine to the total mass of cholesterol synthesized is high in adipose tissue (20%) but much lower elsewhere, for example, skeletal muscle (6%) and liver (3%) (R. Fears, unpublished work).

Whether or not the supply of acetyl-CoA can limit the rate of cholesterogenesis has received relatively little attention. Bortz (1973) suggested that the increased fatty acid flux to the liver that is generated by a fat-rich diet increases the supply of acetyl-CoA and thus enhances the rates of cholesterogenesis and ketogenesis. Gibbons & Pullinger (1979), using isolated hepatocytes, demonstrated that the provision of acetyl-CoA was not rate-limiting in short-term studies and contrasted the behaviour of the key enzyme(s) in cholesterol synthesis with pyruvate dehydrogenase and acetyl-CoA carboxylase, both of which can be activated by substrate. Acetyl-CoA generated intramitochondrially must be transferred to the cytosol for cholesterogenesis and this transfer is accomplished, in part, by conversion to citrate which is then cleaved extramitochondrially by ATP citrate lyase. The competitive inhibitor (–)hydroxycitrate inhibits cholesterogenesis (Sullivan *et al.*, 1977) but this action cannot necessarily be attributed to depletion of substrate because fluoroacetate also increases tissue citrate but does not influence lipogenesis (Brownsey *et al.*, 1977).

Mevalonate

Whilst in general the extrahepatic tissues may be dependent, in part, on substrate obtained from the liver, the kidney appears to have a specific role in the clearance of blood mevalonate. Circulating mevalonate is mainly of hepatic origin and is metabolized principally by the kidney (Edmond *et al.*, 1976; Feingold *et al.*, 1980), the renal extraction rate being in the range 114–720 pmol/min for two kidneys (Kopito & Brunengraber, 1980). In addition to rapid metabolism to sterols, mevalonate is converted to CO_2 and fatty acid via 3,3-dimethylallylpyrophosphate and *trans*-3-methylglutaconyl-CoA (Fogelman *et al.*, 1975). Nephrectomy decreases the clearance of blood mevalonate through the *trans*-methylglutaconate shunt (Edmond *et al.*, 1976) and increases hepatic uptake for cholesterol synthesis and secretion.

We have recently studied the response to acute renal damage induced by a single intravenous dose of uranyl nitrate, 5 mg/kg body wt. (Flamenbaum *et al.*, 1974). After 7 days, serum cholesterol was increased to 85 ± 5.3 mg/100 ml compared with the control level of 59 ± 4.6 mg/100 ml ($n = 8$, $P < 0.05$) with a specific elevation in the VLD lipoprotein fraction as measured by agarose electrophoresis. Serum urea N increased from a control level of 19.0 ± 0.4 to 123 ± 47 mg/100 ml ($P < 0.01$). [5- ^{14}C]Mevalonate was given as an intraperitoneal dose

(2 μ Ci/100g body wt.) 3h before killing and the proportion of $^{14}\text{CO}_2$ recovered from the shunt pathway was decreased from 27% to 23% in the uranyl nitrate-treated rats. The impairment of the *trans*-methylgluconate shunt was accompanied by significantly decreased renal utilization of mevalonate for the synthesis of sterols (170 000 $\pm$ 52 000 d.p.m./h per g compared with the control incorporation of 386 000 $\pm$ 35 000 d.p.m./h per g, $P < 0.01$) and other nonsaponifiable lipids (22 000 $\pm$ 5500 d.p.m./h per g compared with 34 000 $\pm$ 3400, $P < 0.05$) accounting, in total, for 6% of the administered dose compared with 13% in control rats. Hepatic cholesterologenesis was significantly increased (44 000 $\pm$ 5200 d.p.m./h per g compared with the control incorporation of 32 000 $\pm$ 1500 d.p.m./h per g, $P < 0.01$), accounting for 3% of the administered dose compared with 2% in the control rats, and was associated with increased appearance of radiolabelled sterols in the serum (14 000 $\pm$ 4700 d.p.m./ml compared with 9000 $\pm$ 1400 d.p.m./ml). Thus, we confirm the observation that impaired kidney function leads to increased hepatic use of mevalonate for sterol production but the physiological relevance of the association between decreased renal clearance of mevalonate and hypercholesterolaemia in kidney disease and during pregnancy (Feingold *et al.*, 1980) has yet to be established.

Lipoprotein uptake

Cellular cholesterol requirements can also be satisfied by lipoprotein binding and internalization (Goldstein & Brown, 1977) and rat tissues can be classified as follows, according to their capacity for cholesterol uptake (Andersen & Dietschy, 1977).

(i) Non-endocrine extrahepatic tissues possess high-affinity LD lipoprotein receptors to supply sterol for membrane synthesis. Increased uptake of lipoprotein results in co-ordinated regulation involving inhibition of HMG-CoA reductase, activation of acyl-CoA-cholesterol acyltransferase and suppression of receptor synthesis. Macrophages also exhibit high-affinity binding sites for modified LD lipoprotein (Brown *et al.*, 1980) and β -VLD lipoprotein (Mahley *et al.*, 1980) and this scavenger cell pathway may be important in the development of foam cells.

(ii) Endocrine tissues have an additional demand for sterol, for steroid hormone production, which can be satisfied by uptake of HD and LD lipoproteins. In both adrenal gland (Andersen & Dietschy, 1978) and ovary (McNamara *et al.*, 1980) steroid hormone biosynthesis is dependent on cholesterol uptake rather than endogenous synthesis.

(iii) The liver has a major role in clearance of

chylomicron remnants although LD lipoprotein receptor synthesis can also be induced, for example, by 17 α -ethinyl oestradiol (Kovanen *et al.*, 1979) and by cholestyramine in rabbits (Slater *et al.*, 1980).

In man it is likely that the high-affinity LD lipoprotein receptor system is saturated at the concentration of cholesterol found in interstitial fluid (Reichl *et al.*, 1978). LD lipoprotein levels are usually lower in laboratory animals and extra-hepatic cholesterologenesis may not be derepressed by further small decreases in the apoprotein B-containing lipoproteins (Fears & Umpleby, 1979). The physiological control of tissue metabolism in response to circulating lipoproteins is difficult to study because the concentration of cholesterol in the fluid surrounding cells is not usually known. The quantitative relationship between extracellular cholesterol levels and cell metabolism was studied using Ehrlich ascites tumour cells growing *in vivo* in the mouse peritoneum (Esmail & Fears, 1980; Fears & Esmail, 1980). Transfer of lipoproteins from serum to fluid surrounding the ascites cells is by diffusion through the double barrier of capillary wall and peritoneal surface, a process that may resemble the passage of lipoproteins from serum to the interstitial fluid surrounding other cells. Feeding mice on dietary cholesterol (Fig. 3) increased the concentration of cholesterol carried by apoprotein

Fig. 3. Sterol metabolism in Ehrlich ascites tumour cells. Results are taken, in part, from Fears & Esmail (1980). Groups of eight mice were studied 10 days following transplantation of 1×10^7 cells. $\square$, Control animals; ▨ , cholesterol-fed animals. Significance: * $P < 0.05$.

B-containing lipoproteins in serum and ascites fluid. Increased uptake of cholesterol by the ascites cells enhanced sterol esterification and repressed synthesis. Large amounts of cholesteryl esters accumulate in the ascites cells relative to the change in the surrounding fluid, which may be attributed to potent stimulation by β -VLD lipoprotein (Mahley *et al.*, 1980) which appears in the fluid and serum. Whilst it is apparent that ascites cells exhibit feedback repression, in contrast with other tumours (Siperstein, 1970), it is noteworthy that the rate of cholesterogenesis remains relatively high suggesting perhaps that the pathway is maintained to provide other key intermediates for new membrane synthesis and cell proliferation.

In conclusion, the role of the liver and small intestine in providing cholesterol for lipoprotein and bile acid secretion has long been recognised but it is now clear that the operation of the pathway is also important in other tissues for the supply both of sterols and nonsterol intermediates. Certain sites may have particular substrate requirements, for example, ketone bodies (developing central nervous system), leucine (adipose tissue), mevalonate (kidney) but it is likely that homeostatic regulation is normally exerted at each site for lack of control may contribute to the development of foam cells and tumours.

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