Independent regulation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase and chylomicron remnant receptor activities in rat liver

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1. Treatment of rats with pharmacological doses of oestrogen resulted in a 3-fold decrease in the activity of hepatic 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) and a 4-fold increase in saturable binding of ^{125}I -labelled chylomicron remnants to liver membranes *in vitro*. 2. Intragastric administration of mevalonolactone to rats did not affect the capacity of the liver membranes to bind to labelled chylomicron remnants even though there was a substantial decrease in the activity of HMG-CoA reductase. Similar results were obtained after cholesterol feeding. 3. Simultaneous treatment of rats with cholestyramine and compactin increased hepatic HMG-CoA reductase activity 6-fold. However, liver membranes derived from these animals showed no change in their capacity to bind to labelled chylomicron remnants *in vitro*. Administration of mevalonolactone to the cholestyramine/compactin-treated animals also failed to produce a change in remnant-binding capacity. 4. Although administration of mevalonolactone alone produced a significant 3-fold decrease in the activity of hepatic HMG-CoA reductase it was unable to suppress significantly the increase in enzyme activity caused by treatment with cholestyramine and compactin.

Lipoprotein and bile acid production and secretion by the liver create a large demand for cholesterol which can be satisfied by a combination of cholesterol uptake from external lipoprotein sources and by synthesis *de novo* from endogenous precursors. It is well known that the capacity for hepatic cholesterol production *de novo* in the rat is highly responsive to changes in the supply of, and demand for, liver cholesterol and that this is reflected by changes in the activity of HMG-CoA reductase (Rodwell *et al.*, 1976). For instance, increasing the rate of bile acid synthesis by feeding the drug cholestyramine increases the demand for liver cholesterol and HMG-CoA reductase activity increases (Goldfarb & Pitot, 1972; Higgins *et al.*, 1974). Conversely, increasing the supply of cholesterol either from external sources by dietary supplementation (Rodwell *et al.*, 1976) or from the biosynthetic source by administration of an easily utilizable cholesterol precursor such as mevalonolactone (Edwards *et al.*, 1977; Gould, 1977;

Mitropoulos *et al.*, 1978) results in a decrease in hepatic HMG-CoA reductase activity.

Since, in the rat, dietary cholesterol reaches the liver predominantly in the form of chylomicron remnants (Redgrave, 1970; Cooper, 1977; Sherrill & Dietschy, 1978) it is probably cholesterol or a related sterol delivered to the liver in these particles which is responsible for the decrease in the rate of hepatic cholesterol synthesis associated with cholesterol feeding (Rodwell *et al.*, 1976). Furthermore the capacity of rat liver to remove these particles is much greater than that for removal of other lipoprotein types (Cooper, 1977; Cooper & Yu, 1978; Sherrill & Dietschy, 1978; Windler *et al.*, 1980a) suggesting that, although it is difficult to calculate the contribution from other types of lipoprotein, rat liver preferentially acquires external cholesterol from the remnant source.

In several types of cultured cell the supply of cholesterol may be adjusted to meet demand, not only by changes in the capacity to synthesize sterol but also by variations in the capacity of the cells to obtain external sterol by regulating LDL receptor activity. The delivery of chylomicron-remnant

Abbreviations used: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; VLDL, LDL and HDL, very low, low and high density lipoproteins, respectively; SDS, sodium dodecyl sulphate.

cholesterol to the liver is mediated by plasma membrane receptor sites which recognize apoprotein E and/or apoprotein B of these particles (Carrella & Cooper, 1979; Cooper *et al.*, 1982). However it is not known whether, like non-hepatic cultured cells, liver cholesterol balance is also maintained by variations in the quantity of specific binding sites, as well as by changes in the capacity for cholesterol synthesis.

The present study was undertaken to ascertain the relative contribution of changes in the capacity for cholesterol uptake, as measured partly by variations in the capacity for chylomicron remnant binding, and changes in the capacity for cholesterol synthesis, as measured by HMG-CoA reductase activity, in determining the overall response of the liver to changes in the demand for, and availability of, hepatic cholesterol. These latter changes were effected by increasing the demand of the liver for cholesterol by feeding cholestyramine which induces an increase in bile acid production (Moutafis & Myant, 1969; Grundy *et al.*, 1971) and by varying the supply of cholesterol to the liver by treatment with mevalonolactone, or by feeding with cholesterol.

Experimental

Animals, treatment and diets

Male Wistar rats were obtained from a commercial source (Bantin & Kingman, Hull, U.K.) and were acclimatized under conditions of alternate 12 h periods of light and dark for at least 14 days before use. A diet containing cholestyramine was prepared by adding the drug to a powdered, commercially-available diet (Formula PRD, Lab-sure, Poole, Dorset, U.K.) to give a content of 5% (w/w). Water was added to give a thick slurry and pellets were prepared and dried at room temperature for 2 days. A diet containing cholesterol (2%) was prepared in the same way. Control rats received the re-pelleted chow only. Ethinyl oestradiol (5 mg/kg body wt.) was administered subcutaneously as a solution in propane-1,2-diol (1 mg/ml). Rats received two such doses at 24 h intervals and the animals were killed 24 h after the last dose. Control rats received identical quantities of propane-1,2-diol alone at the same time. An aqueous solution (5 mg/ml) of the sodium salt of compactin was prepared (Brown *et al.*, 1978) and administered to rats intragastrically in three doses, each of 17 mg/kg body wt., at 24 h intervals. The rats were killed 1 h after the last dose (i.e. at 10.00 h, 4 h into the dark period of the cycle). Mevalonolactone (0.67 g/kg body wt. per dose) was administered as a solution (200 mg/ml) in water in a similar fashion to the appropriate group of rats at identical times.

The animals were killed 1 h after the last dose. Control rats received the appropriate amounts of water only at the same times. The weight of each animal at the time of killing was 250–300 g.

Preparation of chylomicron remnants

Chylomicrons were prepared by thoracic duct cannulation of rats (250–400 g) which had received an intragastric load of olive oil (1.0 ml). A further 1.0 ml of olive oil was given 6 h later. A solution of glucose (4%) in saline (0.9% NaCl) was infused at a rate of 1 ml/h via a duodenal catheter during collection of the chyle. The lymph was adjusted with 20% sucrose solution to a final concentration of 2% sucrose, layered under an equal volume of solution of *d* 1.006 (NaCl/EDTA) and centrifuged in a Beckman L8-70 ultracentrifuge for 0.5 h at 32000 rev./min in a 60Ti rotor. The supernatant chylomicrons were separated by slicing, and washed by recentrifuging under the same conditions. The resultant chylomicron suspension was dialysed exhaustively against phosphate-buffered saline. Remnants were produced by injecting the chylomicrons into the femoral vein of functionally eviscerated rats (250–400 g) as described by Windler *et al.* (1980a). No more than 1.0 mg of chylomicron protein was injected per animal. One hour later blood was collected from the abdominal aorta and remnants were floated by centrifugation of the plasma for 16 h at 55000 rev./min in a Beckman 60Ti rotor at *d* 1.019 (Havel *et al.*, 1955). The remnant fraction was washed by recentrifugation for 16 h at 43000 rev./min in a Beckman 50.1 rotor at the same density. Freshly-prepared remnants were radiolabelled with ¹²⁵I by the iodine monochloride method (Bilheimer *et al.*, 1972) and the iodinated products were dialysed against buffer containing 100 mM-NaCl, 0.5 mM-CaCl₂ and 50 mM-Tris/HCl, pH 7.5 (buffer 1).

Extraction of an aliquot of the radioiodinated lipoprotein with chloroform/methanol (2:1, v/v) showed that an average of 5.3% of the total ¹²⁵I was associated with the lipid fraction.

Characterization of the lipoproteins

Differences in the apoprotein content of chylomicrons and their remnants were demonstrated by SDS/polyacrylamide-gel electrophoresis and the electrophoretic patterns are shown in Fig. 1. The major changes that occur when chylomicrons are converted into remnants are an increase in the amount of apoE and a decrease in the quantity of peptides which migrate with apoA-I and apoA-IV.

The appearance of high-molecular-weight apo-B in the chylomicron remnant fraction is of interest and may possibly be attributed to the presence of a small quantity of endogenous VLDL remnants in the plasma of the functionally hepatectomized

Fig. 1. SDS/polyacrylamide-gel electrophoresis of chylomicrons and chylomicron remnants

animals. Available data suggests that the metabolism of these particles by the liver is identical with that of chylomicron remnants (Windler *et al.*, 1980a). The quantity of apoB in lipoproteins was determined by precipitation with tetramethylurea (Faergeman *et al.*, 1975). The contents of cholesterol and cholesteryl ester were determined by g.l.c. after fractionation of the extracted total lipid material (Folch *et al.*, 1957) by t.l.c. on silica gel H with light petroleum/diethyl ether/acetic acid (84:15:1, by vol.). Trace quantities of radioactive cholesterol and cholesteryl ester were added to the lipoprotein fractions before analysis to account for manipulative losses. 5 α -Cholestan-3 β -ol was added as internal standard for g.l.c. The triacylglycerol content of the lipoproteins was measured by using a commercially available kit (Triglycerides Reagent Set, Dow Chemical Co., Indianapolis, IN,

U.S.A.). Total phospholipid was measured by the method of Marinetti (1962). The lipid compositions of chylomicrons and their remnants are given in Table 1, which shows a large increase in the relative content of cholesterol and a relative decrease in the triacylglycerol content of the remnants compared with the native chylomicrons.

Preparation of liver membranes and assay of ¹²⁵I-labelled chylomicron remnant binding

Animals were killed by a blow on the head and the livers were removed and perfused lightly with ice-cold 0.25M-sucrose solution to remove blood. All subsequent procedures were carried out between 0 and 4°C. The liver was passed through a stainless steel tissue mincer and homogenized in 10 vol. of a buffer containing 150mM-NaCl, 1mM-CaCl₂ and 10mM-Tris/HCl, pH 7.5, using a hand-driven glass/Teflon homogenizer. A microsomal fraction containing plasma membranes was prepared from this homogenate by the method of Kovanen *et al.* (1979). The pellet was stored under liquid N₂ and used within 2 weeks. On the day of the experiment, the membrane pellet was suspended in a buffer containing 50mM-NaCl, 1mM-CaCl₂ and 20mM-Tris/HCl, pH 7.5, to give a protein concentration of approx. 10mg/ml. The suspension was sonicated for 20s (6 μ m amplitude) in an MSE sonicator (MSE Scientific Instruments) and portions (5 μ l) were incubated for 40 min at 37°C in buffer 1 containing 20mg of bovine serum albumin/ml and various concentrations of labelled chylomicron remnants. The final concentration of membrane protein was approx. 1.0mg/ml and the total incubation volume was 50 μ l. At the end of the incubation period a portion (40 μ l) of the incubation mixture was removed and the extent of remnant binding was determined as described for LDL binding by Harders-Spengel *et al.* (1982). Saturable binding was expressed as μ g of remnant protein bound per mg of membrane protein and was determined by the difference between the amounts of labelled remnants bound in the presence and absence of a large excess of unlabelled remnants. At a labelled remnant concentration of 15 μ g/ml, in the presence of a 50-fold excess of unlabelled material, non-saturable binding accounted for less than 30% of the total.

Assay of HMG-CoA reductase activity

The microsomal fraction prepared as described above was suspended in a buffer containing 5mM-dithiothreitol, 1mM-EDTA and 50mM-potassium phosphate buffer, pH 7.4, such that the protein concentration was approx. 2.5mg/ml. The suspension was preincubated at 37°C for 20 min. Portions (25 μ l) were removed and assayed for HMG-CoA reductase activity by a previously described

Table 1. *Composition of chylomicrons and chylomicron remnants*

Each constituent of the lipoproteins was estimated as described in the Experimental section. All values represent the mean $\pm$ S.E.M. for three independent determinations except where otherwise specified. Abbreviation: TMU, tetramethylurea.

Constituent	Relative content	
	(mg/mg of total protein)	(mg/mg of TMU-insoluble protein)
Chylomicrons		
Triacylglycerol	23.9 $\pm$ 8.3	201 $\pm$ 143.5
Unesterified cholesterol ($n = 2$)	0.11 $\pm$ 0.06	0.44 $\pm$ 0.12
Esterified cholesterol	0.13 $\pm$ 0.02	0.92 $\pm$ 0.38
Phospholipid ($n = 2$)	2.54 $\pm$ 0.11	8.70 $\pm$ 4.30
TMU-soluble protein	0.74 $\pm$ 0.15	5.80 $\pm$ 3.03
TMU-insoluble protein	0.26 $\pm$ 0.15	—
Total protein	—	6.83 $\pm$ 3.04
Chylomicron remnants		
Triacylglycerol	15.3 $\pm$ 1.95	42.3 $\pm$ 16.0
Unesterified cholesterol ($n = 2$)	1.32 $\pm$ 0.18	2.55 $\pm$ 0.60
Esterified cholesterol ($n = 2$)	0.46 $\pm$ 0.39	0.67 $\pm$ 0.46
TMU-soluble protein	0.54 $\pm$ 0.15	1.61 $\pm$ 0.70
TMU-insoluble protein	0.46 $\pm$ 0.15	—
Total protein	—	2.62 $\pm$ 0.70

method (Saucier & Kandutsch, 1979; Cavenee *et al.*, 1981).

In most cases, protein was determined by the method of Lowry *et al.* (1951) except in the assay of HMG-CoA reductase during which the method of Gieger & Bessman (1972) was used.

Materials

All radiochemicals were obtained from Amersham International, Amersham, Bucks., U.K. NADP, glucose 6-phosphate, glucose-6-phosphate dehydrogenase, dithiothreitol and ethinyl oestradiol were obtained from Sigma. Cholestyramine was obtained from Merck, Sharp and Dohme Inc., West Point, PA, U.S.A. Mevalonolactone was obtained from Koch-Light, Colnbrook, Berks., U.K. Compactin was a gift from Dr. R. Fears, Beecham Pharmaceuticals, Epsom, Surrey, U.K.

Results

Effect of oestrogen treatment on 125 I-labelled chylomicron remnant binding to liver membranes and on HMG-CoA reductase activity

To test the feasibility of using isolated liver membranes in studies of the regulation of chylomicron remnant metabolism it was required to show that remnant binding to the hepatic membranes is responsive to treatments which are known to increase liver receptor activity for lipoproteins containing apoprotein B and/or apoprotein E. Such an increase has been described after treatment of animals with pharmacological quantities

of oestrogen derivatives *in vivo* (Chao *et al.*, 1979; Kovanen *et al.*, 1979; Windler *et al.*, 1980b). Although this treatment has not yet been shown to increase chylomicron remnant binding to liver membranes *in vitro*, such a change would be expected in view of the apoB and apoE content of the remnant particles. Fig. 2 shows that oestrogen treatment for 2 days led to a substantial increase in the capacity of hepatic membranes to bind in a saturable manner to 125 I-labelled chylomicron remnants. The concentration required for half-maximal binding of the remnants to membranes from control animals ($7.15 \pm 1.93 \mu\text{g}$ of remnant protein/ml) was similar to that observed after oestrogen treatment ($9.54 \pm 4.11 \mu\text{g}$ of remnant protein/ml), suggesting that the nature of the binding site remained unchanged. However, the maximal amount of 125 I remnant bound increased from $1.03 \pm 0.29 \mu\text{g}$ of remnant protein/mg of membrane protein per 40 min (control) to $3.72 \pm 2.19 \mu\text{g}$ /mg per 40 min (oestrogen-treated). The activity of HMG-CoA reductase in the microsomal fraction of the oestrogen-treated animals decreased by over 65% compared with that observed in the controls (Table 2).

Effects of changes in the supply of hepatic cholesterol on chylomicron remnant uptake and HMG-CoA reductase activity in the liver

The capacity of the liver to synthesize cholesterol, as measured by the activity of HMG-CoA reductase, is decreased when the supply of exogenous cholesterol to this organ is increased by

Fig. 2. Effect of oestrogen treatment on the saturable binding of ¹²⁵I-labelled chylomicron remnants to hepatic membranes

Liver membranes were incubated with different concentrations of labelled chylomicron remnants to determine total binding. Non-saturable binding was determined by the amount of ¹²⁵I bound in the presence of a 50-fold excess of unlabelled remnants and these values have been subtracted from the total. Each point represents the mean (±S.E.M.) of determinations on seven animals in the control group and three in the oestrogen-treated group. ▲, Control; ●, oestrogen-treated.

feeding cholesterol in the diet (Rodwell *et al.*, 1976). A similar decrease in hepatic HMG-CoA reductase also occurs when the supply of biosynthetic cholesterol is increased after administration of an easily-utilizable cholesterol precursor such as mevalonolactone (Gould, 1977; Edwards *et al.*, 1977; Mitropoulos *et al.*, 1978). It is not known whether these treatments result in similar changes in the capacity of the liver to acquire cholesterol from external sources as determined by the ability of the liver membranes to bind chylomicron remnants. To test this, liver membranes were prepared from groups of rats which had either been fed cholesterol for 6 days or had received intragastric doses of mevalonolactone over a period of 48 h. The chylomicron remnant binding characteristics of the liver membranes prepared from animals in each group were compared with those derived from control groups fed a standard chow diet. The results are presented in Fig. 3, which shows that neither of the above treatments had any significant effect. The concentrations required for half-maximal binding were (in µg of protein/ml) 7.15 ± 1.93 (control) 6.35 ± 0.30 (cholesterol-fed) and 5.74 ± 1.40 (mevalonolactone-treated). The corresponding values for the maximum quantities of lipoprotein bound were (in µg of remnant protein/mg of membrane protein per 40 min) 1.03 ± 0.29 , 1.41 ± 0.08 and 1.11 ± 0.12 . Despite this, the various treatments produced substantial changes in the activity of HMG-CoA reductase (Table 2).

Effects of increased hepatic cholesterol demand on chylomicron remnant uptake by the liver and on the activity of hepatic HMG-CoA reductase

Cholestyramine increases the rate of hepatic cholesterol conversion into bile salts by interrupt-

Table 2. Response of hepatic HMG-CoA reductase to drugs, cholesterol and mevalonolactone administered to rats *in vivo*
Rats were treated with the compounds below as described in the Experimental section. After the prescribed period the animals were killed and the activities of HMG-CoA reductase in the liver microsomal fraction were determined. The values in parentheses represent the number of animals in each group. Enzyme activities are expressed as the mean ± S.E.M. Statistical analysis was carried out using Student's *t*-test and the *P* values represent the degree of significance compared with the control. There was no significant difference between rats treated with cholestyramine plus compactin and those treated with cholestyramine plus compactin plus mevalonolactone.

Treatment	Hepatic HMG-CoA reductase activity (pmol of mevonolate/min per mg of microsomal protein)
Control (7)	238 ± 11.2
Oestrogen (4)	82.6 ± 23.2 (<i>P</i> < 0.005)
Cholesterol (3)	22.3 ± 4.9 (<i>P</i> < 0.001)
Mevalonolactone (4)	74.7 ± 29.5 (<i>P</i> < 0.001)
Compactin + cholestyramine (5)	1656 ± 199 (<i>P</i> < 0.001)
Compactin + cholestyramine + mevalonolactone (5)	1206 ± 257 (<i>P</i> < 0.005)

Fig. 3. Effects of drug treatment on ^{125}I -labelled chylomicron remnant binding to isolated liver membranes

Rats were treated as described in the Experimental section and their liver membranes were incubated with different concentrations of ^{125}I -labelled chylomicron remnants to determine total binding. Non-saturable binding was determined by the amount of ^{125}I bound in the presence of a large excess of unlabelled remnants and these values have been subtracted from the total. Each point represents the mean ($\pm$ S.E.M.) of determinations on several animals in each group, the actual numbers used appearing in the appropriate section of the diagram. (a) Compactin/cholestyramine-treated; (b) compactin/cholestyramine/mevalonolactone-treated; (c) control; (d) cholesterol-treated; (e) mevalonolactone-treated.

tion of their enterohepatic circulation (Moutafis & Myant, 1969; Grundy *et al.*, 1971). To determine whether this increased demand for hepatic cholesterol could be satisfied by an increased capacity for chylomicron remnant uptake, rats were fed a diet containing cholestyramine for 6 days. To minimize the possibility of a compensatory increase in the rate of cholesterol synthesis, the rats were also given compactin, a specific inhibitor of this

process (Endo *et al.*, 1976a,b; Brown *et al.*, 1978). Treatments such as these, involving a combination of bile acid sequestrants and specific inhibitors of cholesterol biosynthesis, have previously been employed to increase hepatic demand for cholesterol *in vivo* (Kovanen *et al.*, 1981a; Brown *et al.*, 1981). Under these conditions there was no increase in the capacity of hepatic membranes to bind to ^{125}I -labelled chylomicron remnants *in vitro*

(Fig. 3). The maximum quantity of remnants bound was $1.41 \pm 0.51 \mu\text{g}$ of remnant protein/mg of membrane protein per 40 min compared with $1.03 \pm 0.29 \mu\text{g}/\text{mg}$ per 40 min in the controls. The concentration of remnant protein required for half-maximal binding was $7.60 \pm 2.38 \mu\text{g}/\text{ml}$, which was not significantly different from that observed with membranes derived from untreated rats. Despite an unchanged capacity for remnant uptake, the potential capacity for cholesterol synthesis increased 6–7-fold during this treatment, as reflected by the activity of HMG-CoA reductase (Table 2). Simultaneous administration of mevalonolactone also failed to produce any change in the capacity of liver membranes from the cholestyramine/compactin-treated rats to bind to labelled remnants (Fig. 3). Although administration of mevalonolactone to cholestyramine/compactin-treated rats apparently resulted in a decline in HMG-CoA reductase activity, the extent of the decrease was less than expected and on a percentage basis did not achieve statistical significance. This was in contrast to the large percentage decline in HMG-CoA reductase activity observed after administration of mevalonolactone alone.

Discussion

In the rat, external cholesterol of dietary origin appears in the plasma initially as chylomicrons which are metabolized by extrahepatic tissues to chylomicron remnants. These particles are removed more rapidly by the intact liver than are other types of lipoprotein (Cooper, 1977; Cooper & Yu, 1978; Sherrill & Dietschy, 1978; Windler *et al.*, 1980a), suggesting that dietary cholesterol reaches the liver predominantly in this form. This high rate of removal is also reflected *in vitro* by the high affinity with which isolated hepatic membranes bind to labelled chylomicron remnants and their large capacity for this process (Carrella & Cooper, 1979; Mahley *et al.*, 1981; Cooper *et al.*, 1982). In the present work, the concentration of chylomicron remnant protein required for half-maximal binding to hepatic membranes from normal rats was $7.15 \pm 1.93 \mu\text{g}/\text{ml}$, a value in reasonable agreement with that of $14.3 \mu\text{g}/\text{ml}$ reported previously (Cooper *et al.*, 1982). It has been proposed that the liver of the immature dog, pig and man contains at least two different types of lipoprotein receptor sites, one of which is responsible for the metabolism of lipoproteins containing apoE and apoB (the B,E receptor) and the other which recognizes lipoproteins containing apoE only (the E receptor) (Mahley *et al.*, 1981). It has also been suggested that, particularly in adult animals, the apoE receptor is primarily responsible for the metabolism of lipoproteins such as chylomicron remnants

and β -VLDL (Hui *et al.*, 1981; Kovanen *et al.*, 1981b), at least in dogs and rabbits. In the rat, there is as yet no evidence for different lipoprotein receptor sites in the liver. Nevertheless, in this species, irrespective of the nature of the binding site responsible, the present work has shown that treatment with a combination of cholestyramine and compactin which increases hepatic demand for cholesterol fails to increase the capacity of liver membranes to bind lipoproteins containing apoE and apoB, such as chylomicron remnants, and thus has no effect on the capacity of the liver to obtain cholesterol from this source. In contrast the capacity of the liver to synthesize cholesterol, as measured by HMG-CoA reductase activity, increases substantially under these conditions. It is important to recognize that in these experiments an increase in HMG-CoA reductase activity measured *in vitro* reflects only the capacity of the liver to synthesize cholesterol and probably bears little relation to the actual rate of cholesterol production *in vivo*. This situation results from the partial dependence of the latter process upon the availability of cholesterol precursors, the production of which is diminished in the presence of compactin. However, it should be noted that there have been no reported measurements of changes in the rates of cholesterol synthesis over the period of treatment with cholestyramine/compactin and the possibility of an initial decrease followed by a longer term increase cannot be completely discounted. Because of this we cannot wholly eliminate the possibility that during very short-term treatments remnant receptor activity temporarily increased. Nevertheless, the longer term resistance of rat hepatic chylomicron remnant receptor activity to treatment with cholestyramine is in contrast with the effect of this treatment on hepatic binding to apoE- and apoB-containing lipoproteins in other species. For instance, treatment of adult dogs with cholestyramine results in an increase in the hepatic binding capacity for HDL_c (Hui *et al.*, 1981) which, like chylomicron remnants, contains apoE and whose transport kinetics resemble those of chylomicron remnants in perfused rat liver (Sherrill *et al.*, 1980). Neither does cholesterol feeding give rise in the rat to a decreased capacity of the isolated liver membranes to bind to labelled chylomicron remnants, an observation which supports a previous finding that cholesterol feeding does not decrease the rate of uptake of chylomicron remnants in perfused rat liver (Cooper & Yu, 1978). This again contrasts with the effects of cholesterol feeding on the ability of rabbit liver to take up apoE-containing lipoproteins (Kovanen *et al.*, 1981b). A large increase in the supply of hepatic cholesterol derived from the biosynthetic source after the administration of

mevalonolactone to either normal or cholestyramine/compactin-treated animals was also ineffective in modifying the capacity of liver membranes to bind labelled chylomicron remnants (Fig. 3).

We conclude therefore that, in the rat, oestrogen induces an increased capacity for chylomicron remnant binding to liver membranes. Nevertheless, this process is unresponsive to manipulations *in vivo* which produce large variations in hepatic cholesterol flux and which are accompanied by substantial changes in the capacity of the liver to synthesize cholesterol as reflected by the activity of HMG-CoA reductase (Table 2). Variations in the cholesterol content of the diet are accompanied, in the rat, not only by changes in HMG-CoA reductase activity, but by large changes in the rate of bile acid production (Myant & Mitropoulos, 1977). The present results suggest, therefore, that in this species hepatic cholesterol homeostasis is regulated exclusively by changes in the rate of cholesterol synthesis and output rather than by changes in uptake, the capacity for which remains large and constant. In any species, the relative importance of the means by which liver cholesterol homeostasis is regulated may be a key factor in the susceptibility of the species to dietary cholesterol-induced hypercholesterolaemia. For instance, feeding rabbits a high cholesterol diet does not lead to such a marked increase in bile acid output as that which occurs in the rat (Dietschy & Wilson, 1970). In this case, hepatic cholesterol balance appears to be maintained, at least in part, by a down-regulation of hepatic receptors which recognize the apoE of the cholesterol-containing lipoproteins, the rate of clearance of which is diminished and a marked hypercholesterolaemia results (Kovanen *et al.*, 1981b). In contrast, in the rat, regulation occurs predominantly by changes in cholesterol synthesis and output, rather than by changes in the capacity for hepatic cholesterol uptake, which remains high. This ensures a speedy removal of cholesterol-containing lipoproteins and thus prevents their accumulation in the plasma. Finally, a somewhat unexpected finding is that the relative decrease in the activity of HMG-CoA reductase observed after administration of mevalonolactone to animals treated with cholestyramine/compactin was much less than that which occurred in untreated animals (Table 2). It is possible that the quantity of mevalonolactone used was insufficient to significantly suppress the very high induced reductase activity under these conditions. However, mevalonolactone in relatively small quantities was effective in suppressing the compactin-mediated increase in HMG-CoA reductase activity in human fibroblasts (Brown & Goldstein, 1980) and in bovine vascular endothelial cells (Cohen *et al.*, 1982). There is evidence

which suggests that the increased enzyme activity which results from cholesterol removal from cells *in vivo*, induced by cholestyramine treatment (Tanaka *et al.*, 1982), or *in vitro*, in the presence of HDL (Cohen *et al.*, 1982) is of a different type from that induced by compactin. The first type is more difficult to suppress with mevalonolactone (Cohen *et al.*, 1982). In the present work it is possible that the cholestyramine-mediated contribution to the increase in enzyme activity is of a type which is more resistant to suppression by mevalonolactone.

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