Evidence for the Operation of the Extrahepatic Lipoprotein Receptor System *in vivo* in Rats

EFFECT OF DIETARY CHOLESTEROL AND OROTIC ACID, ALONE OR IN COMBINATION, ON THE RATE OF SYNTHESIS OF CHOLESTEROL AND FATTY ACID IN VARIOUS TISSUES, MEASURED BY USING $^3\text{H}_2\text{O}$

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1. The biosynthesis of cholesterol *in vivo* was studied at a number of tissue sites in rats by using $^3\text{H}_2\text{O}$ as precursor. Overall, the mass of cholesterol synthesized was in good agreement with the rate of cholesterogenesis, as determined by kinetic analysis of cholesterol specific-radioactivity–time curves after administration of radiolabelled cholesterol. 2. Dietary cholesterol increased the circulating concentration of cholesterol and inhibited endogenous cholesterogenesis, with concomitant increases in the concentration of esterified cholesterol, in all tissues studied. Addition of orotic acid to the cholesterol-supplemented diet tended to reverse each of these changes in extrahepatic tissues. 3. The co-ordinated change in cholesterol biosynthesis and esterification, with no change in total cholesterol content, in extrahepatic tissues, is attributed to control by receptor-mediated lipoprotein uptake. 4. Further reduction of the concentration of the apoprotein B-containing lipoproteins by addition of orotic acid to a diet without supplementary cholesterol did not further enhance cholesterogenesis. We consider that the relatively high rates of extrahepatic cholesterogenesis in normolipidaemic rats are attributable to the low concentration of low-density lipoprotein.

The liver and small intestine are usually considered to be the principal sites for the production of cholesterol, and the regulation of cholesterogenesis in these tissues has been investigated extensively. Cholesterogenesis in peripheral tissues is more difficult to study because of the relatively low rates found and because conventional precursors, such as acetate, may be poorly utilized (Miettinen & Penttilä, 1971). The use of $^3\text{H}_2\text{O}$ for the quantitative measurement of sterol synthesis in the liver has been described (Fears & Morgan, 1976; Lakshmanan & Veech, 1977), and we have extended our analyses to extrahepatic tissues (Fears & Umpleby, 1978*a,b*). Our observation that dietary cholesterol represses cholesterogenesis in extrahepatic tissues disagrees with the results of Dietschy & Siperstein (1967) obtained by using acetate as substrate *in vitro*, although other workers have described an inhibition, by dietary cholesterol, of rat leucocyte β -hydroxy- β -methylglutaryl-CoA reductase, for example (Young & Rodwell, 1977).

Studies by Brown & Goldstein (1976) have suggested that cholesterol metabolism in extrahepatic tissues is subject to control by lipoprotein binding at a cell-surface receptor, with subsequent uptake, and some assessment has been made of the effects of individual lipoprotein classes in the rat *ex vivo* (Andersen & Dietschy, 1977*a,b*). It was the purpose of the present experiments to use $^3\text{H}_2\text{O}$ to quantify the relative contributions made by various tissues to the synthesis of sterol and fatty acid overall and to investigate in more detail the co-ordinate regulation of cholesterol biosynthesis and esterification in peripheral tissues. Additional information was obtained with $[\text{U-}^{14}\text{C}]$ leucine, a substrate that is known to be well utilized by extrahepatic tissues.

Materials and Methods

Animals and diets

Male CFY rats were obtained from Carworth (Europe) Ltd., Alconbury, Hunts., U.K. The rats were fed on a stock pelleted diet (Oxoid Breeding Diet obtained from Lillico and Sons, Wonham Mill, Betchworth, Surrey, U.K.) or on a semisynthetic diet with or without cholesterol plus bile acid (Fears,

Abbreviations used: VLD lipoprotein, very-low-density lipoprotein; LD lipoprotein, low-density lipoprotein; HD lipoprotein, high-density lipoprotein.

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1978). Unless otherwise indicated all rats received food and water *ad libitum* and were killed between 10:00 and 12:00h.

Methods

Lipogenesis *in vivo*. The synthesis of digitonin-precipitable sterols and fatty acid from $^3\text{H}_2\text{O}$ in liver and small intestine was measured by the method of Fears & Morgan (1976) with the modification that the pooled light petroleum (b.p. 40–60°C) extract was washed with 0.1M-KOH (sterols) or 0.05M- H_2SO_4 (fatty acid). Preliminary experiments in which lipid extracted from samples of liver was fractionated by t.l.c. showed that cholesterol formed more than 85% of the total sterol fraction.

Lipogenesis in other tissues and lipogenesis from [^{14}C]leucine was measured by similar procedures. Substrate pool sizes cannot be calculated satisfactorily for precursors supplying carbon atoms for lipogenesis *in vivo*, so the results for leucine are expressed as d.p.m. rather than as substrate fluxes. Adipose tissue was always taken from the epididymis, and skeletal muscle was a mixture of gastrocnemius and soleus muscles (and may include a small proportion of undissected adipose tissue).

Measurement of serum lipids and lipoproteins. Total cholesterol in serum was measured by using the Technicon Auto Analyser method no. SE4-001 6FH4 (Technicon Instruments Corp., 1974). Total triacylglycerol in serum was measured by using the Technicon Auto Analyser method no. SE4-0023 FE5 (Technicon Instruments Corp., 1975).

Lipoproteins from fresh serum samples were fractionated by agarose-gel electrophoresis (Papadopoulos & Kintzios, 1969) by using samples pre-stained with acetylated Sudan Black (Ribeiro & McDonald, 1963), and the concentrations of the separated lipoprotein classes were determined *in situ* by densitometry (Joyce-Loebl Chromoscan 200 with 530nm filter). Apoprotein B-containing lipoproteins were precipitated from serum by the addition of MnCl_2 solution (to give final concentration 0.096M) and sodium heparin (to give final concentration 1.6mg/ml) (Burstein & Scholnick, 1973). After being left overnight at 4°C, the precipitate and supernatant were separated by centrifugation, and total cholesterol was measured in the chloroform phase of the total lipid extract. Preliminary experiments where the precipitate was reconstituted in 4% NaCl and fractionated by agarose-gel electrophoresis confirmed that all of the HD lipoprotein remained in the supernatant.

Measurement of tissue lipids. Tissue lipids were extracted by the method of Folch *et al.* (1957) as modified by Bligh & Dyer (1959). Total cholesterol and triacylglycerol were measured as for serum. Free and esterified fractions of cholesterol were separated by t.l.c. performed on layers of silica gel HF₂₅₄+366

(Merck) in the solvent system light petroleum (b.p. 40–60°C)/diethyl ether/acetic acid (70:30:1, by vol.).

Measurement of cholesterol-production rate in 'rapidly turning-over pool'. Eight rats received an intravenous dose of 5 μCi of [^{14}C]cholesterol in 0.2 ml of ethanol/sterile NaCl (3:47, by vol.). Blood samples were taken by cardiac puncture at various time intervals for 7 weeks. Total serum cholesterol was constant over this period; the specific-radioactivity-time curve for serum cholesterol was analysed by the multi-exponential equation:

$$y = \sum_{i=1}^{nC} A_i e^{-\alpha_i t}$$

where y is concentration of tracer, A_i is zero-time intercept, α_i is rate constant and nC is the number of compartments.

In fact, the curve was satisfactorily described by the double-exponential function (Goodman & Noble, 1968):

$$\text{Specific radioactivity} = Ae^{-\alpha t} + Be^{-\beta t}$$

where A , B , α and β are constants.

Production rate (PR) in pool A ('rapidly turning-over pool') is:

$$\text{PR}_A = \frac{R_A \alpha \beta}{\alpha B + \beta A}$$

where R_A is amount of isotope injected into pool A.

Radioactivity-counting procedures. These were carried out as described by Atkin *et al.* (1972).

Statistical analysis. Statistical analyses were done by using Student's t test.

Chemicals

L-[^{14}C]Leucine, [^{14}C]cholesterol and $^3\text{H}_2\text{O}$ were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Results

Comparison of rates of synthesis of sterols and fatty acid at various tissue sites

Rates of synthesis of sterol and fatty acid, measured with $^3\text{H}_2\text{O}$, in stock-fed rats (body wt. 230–250g) killed at 10:00h, were calculated by assuming a body composition of 10% adipose tissue and 45% skeletal muscle, and that the samples analysed were representative of the total tissue (Table 1).

It was confirmed that the liver is the major site of sterol synthesis with the small intestine next in importance. Skeletal muscle has a relatively low rate of synthesis per g of tissue, but does make a significant contribution to the total body cholesterol production. Rates per unit weight in the kidney, spleen and testis

Table 1. Comparison of sterol and fatty acid synthesis at various tissue sites

Each rat was given $^3\text{H}_2\text{O}$ (1.5mCi/100g body wt.) by intraperitoneal injection 1h before it was killed. Results are the means $\pm$ S.E.M. for six analyses; each analysis was of tissue from a single rat.

	Lipid synthesized ($\mu\text{g/h}$ per 100g body wt.)	
	Sterol	Fatty acid
Liver	240 $\pm$ 20	1000 $\pm$ 210
Small intestine	200 $\pm$ 15	550 $\pm$ 120
Adipose tissue	30 $\pm$ 6	4000 $\pm$ 600
Skeletal muscle	100 $\pm$ 8	250 $\pm$ 20
Kidney	15 $\pm$ 1	80 $\pm$ 10
Spleen	10 $\pm$ 2	40 $\pm$ 8
Testis	25 $\pm$ 2	90 $\pm$ 12

were on average, approx. 30% of the rate in the liver but the contribution to the total was, of course, much less. Adipose tissue was the major site for the synthesis of fatty acid with liver next in importance. Any undissected adipose tissue in the skeletal-muscle preparation will tend to contribute to an overestimation of the significance of fatty acid synthesis at that site. It is unlikely that any other sites make a significant contribution to the synthesis of cholesterol or fatty acid overall (Andersen & Dietschy, 1977a,b), although the contribution by endogenous synthesis to metabolism within a particular tissue may, of course, be important.

To determine the total daily production of cholesterol or fatty acid it is necessary to ascertain the influence of environmental variables, in particular, food intake. In Table 2 rates of lipogenesis were compared before and after a meal of stock diet in rats trained to meal-feeding. Fatty acid synthesis was increased after the meal in adipose tissue, liver, spleen and kidney, but the synthesis of sterol was unaffected by the meal. The results for the liver confirm a previous finding (Fears & Morgan, 1976) suggesting that cholesterogenesis is much less influenced by food intake than is fatty acid synthesis. Assuming a constant rate of cholesterogenesis, the total production of sterol at the sites described in Table 1 is calculated to be approx. 12mg/24h per 100g body wt. This rate can be compared with the cholesterol production rate, PR_A , in the 'rapidly turning-over pool', as determined by kinetic analysis of the specific-radioactivity-time curve obtained after administration of $[4\text{-}^{14}\text{C}]\text{cholesterol}$ in a separate experiment with larger rats (350–400g body wt.). PR_A , which equals the total input of cholesterol in animals not receiving dietary cholesterol, was calculated to be 9mg/24h per 100g body wt.

Table 2. Sterol and fatty acid synthesis before and after a meal of stock diet

Rats were maintained for 21 days on a reversed-light cycle, light period 16:00h–04:00h, with a single daily meal 10:00h–12:00h. Water was allowed *ad libitum*. Animals were killed at 10:00h and 14:00h. Each rat was given $^3\text{H}_2\text{O}$ as described in Table 1. Results are means $\pm$ S.E.M. for eight analyses; each analysis was of tissue from a single rat. Significant difference ($P < 0.05$) is indicated by *.

	Lipid synthesized ($\mu\text{g/h}$ per g of tissue)	
	Preprandial	Postprandial
Liver		
Sterol	167 $\pm$ 40	149 $\pm$ 20
Fatty acid	213 $\pm$ 62	540 $\pm$ 47*
Spleen		
Sterol	19 $\pm$ 4.2	22 $\pm$ 2.7
Fatty acid	53 $\pm$ 14	73 $\pm$ 16
Kidney		
Sterol	18 $\pm$ 6	25 $\pm$ 5
Fatty acid	44 $\pm$ 10	92 $\pm$ 15*
Adipose tissue		
Sterol	10 $\pm$ 2	10 $\pm$ 2
Fatty acid	30 $\pm$ 5	910 $\pm$ 280*

Effects of dietary cholesterol and orotic acid on lipogenesis

To investigate the influence of the circulating concentrations of lipoproteins on tissue lipogenesis, the quantitative relationship between HD and the lower-density lipoproteins was modulated by providing semisynthetic diets, with or without cholesterol plus bile acid and with or without orotic acid, to rats for 14 days (Table 3). Addition of cholesterol to the diet slightly reduced gain in body weight, but it is unlikely that any of the results described below can be attributed to this effect or to any change in the pattern of food intake.

Supplementation of the semi-synthetic diet with cholesterol led to an increased concentration of cholesterol and other lipids carried by the lower-density lipoproteins (LD, VLD and the newly appearing βVLD lipoproteins), whether measured by electrophoresis or by polyanion precipitation with MnCl_2 and heparin. Dietary cholesterol somewhat reduced the concentration of conventional HD lipoproteins; in our methods the newly appearing 'HDL_C' lipoprotein is precipitated by MnCl_2 and heparin, but is measured with conventional HD lipoprotein by electrophoresis. Addition of orotic acid to the diet, whether or not cholesterol was present, led to a reduction in serum total cholesterol, triacylglycerol and the concentration of the lower-density lipoproteins. HD+HDL_C lipoprotein total lipid (as measured by electrophoresis) was not

Table 3. *Effects of dietary cholesterol and orotic acid, alone or in combination, on serum lipoproteins and on lipogenesis in various tissues*

Each rat was given $^3\text{H}_2\text{O}$ as described in Table 1. Results are means $\pm$ S.E.M. for eight analyses, except for electrophoresis where only four samples were taken from each group; each analysis was of tissue from a single rat. Significant difference ($P < 0.05$) is indicated by * (compared with control diet) and by † (compared with cholesterol-supplemented diet). The values for food intake are means for two cages of four rats each.

	Control diet	Control diet + orotic acid (1%)	Control diet + cholesterol (1%) + cholic acid (0.2%)	Control diet + cholesterol (1%) + cholic acid (0.2%) + orotic acid (1%)
Gain in body wt. (g)	65.0 $\pm$ 2.0	60.5 $\pm$ 1.8	54.0 $\pm$ 1.8*	53.0 $\pm$ 2.7*
Food intake (g/day)	17.0	18.3	17.1	15.1
Serum cholesterol (mg/100ml)	77 $\pm$ 3.2	66 $\pm$ 4.9*	225 $\pm$ 20*	101 $\pm$ 21†
Serum triacylglycerol (mg/100ml)	113 $\pm$ 39	46 $\pm$ 10*	145 $\pm$ 15	84 $\pm$ 10†
Polyanion-precipitated cholesterol (mg/100ml)	35 $\pm$ 3	18 $\pm$ 2*	186 $\pm$ 25*	84 $\pm$ 16*†
Agarose-gel electrophoresis (densitometry units):				
HD lipoprotein	71 $\pm$ 25	87 $\pm$ 14	64 $\pm$ 15	77 $\pm$ 18
LD lipoprotein	52 $\pm$ 15	49 $\pm$ 11	71 $\pm$ 16	54 $\pm$ 17
VLD lipoprotein	261 $\pm$ 50	223 $\pm$ 14	336 $\pm$ 20	212 $\pm$ 30†
Sterol synthesis ($\mu\text{g/h}$ per g of tissue):				
Liver	134 $\pm$ 21	149 $\pm$ 33	28 $\pm$ 5*	15 $\pm$ 2*†
Spleen	26 $\pm$ 4.7	22 $\pm$ 2.8	7 $\pm$ 1.1*	9 $\pm$ 0.7*
Kidney	42 $\pm$ 10	58 $\pm$ 7.9	14 $\pm$ 5.2*	75 $\pm$ 22†
Skeletal muscle	6 $\pm$ 0.5	6 $\pm$ 0.4	4 $\pm$ 0.1*	5 $\pm$ 0.5
Fatty acid synthesis ($\mu\text{g/h}$ per g of tissue):				
Liver	189 $\pm$ 43	367 $\pm$ 73	701 $\pm$ 63*	1427 $\pm$ 195*†
Spleen	165 $\pm$ 22	127 $\pm$ 26	184 $\pm$ 21	168 $\pm$ 9.0
Kidney	95 $\pm$ 18	103 $\pm$ 14	78 $\pm$ 8.3	78 $\pm$ 4.2
Cholesterol (mg/g of tissue):				
Liver, free	1.9 $\pm$ 0.2	—	0.9 $\pm$ 0.2*	0.4 $\pm$ 0.1*†
Liver, esterified	0.4 $\pm$ 0.1	—	9.0 $\pm$ 1.0*	11 $\pm$ 1.3*
Kidney, free	1.1 $\pm$ 0.05	—	1.1 $\pm$ 0.10	1.3 $\pm$ 0.08
Kidney, esterified	0.07 $\pm$ 0.004	—	0.22 $\pm$ 0.04*	0.07 $\pm$ 0.03†
Spleen, free	1.7 $\pm$ 0.1	—	1.5 $\pm$ 0.3	1.8 $\pm$ 0.1
Spleen, esterified	0.018 $\pm$ 0.003	—	0.200 $\pm$ 0.032*	0.065 $\pm$ 0.090*†

decreased by orotic acid, but the content of cholesterol in HD lipoprotein (calculated by the difference between serum total cholesterol and polyanion-precipitated cholesterol) was moderately reduced. Dietary cholesterol significantly inhibited cholesterologenesis in liver, spleen, kidney and skeletal muscle, and these changes were associated with a large increase in the concentration of esterified cholesterol at each site. Tissue total cholesterol was not significantly increased at the extrahepatic sites in contrast with the liver. Fatty acid synthesis was markedly increased in the liver but little affected elsewhere.

Addition of orotic acid to the cholesterol-supplemented diet led to an even greater inhibition of cholesterologenesis and accumulation of cholesteryl esters in the liver. In contrast, cholesterologenesis in spleen, kidney and skeletal muscle tended to increase back towards the rates found on the unsupplemented control diet and the concentrations of cholesteryl esters decreased. The orotic acid-induced diminution

in lipoprotein output from the liver (Roheim *et al.*, 1965) leads to a comparative overestimation of the rate of hepatic fatty acid synthesis, and caution must be exercised when interpreting data on the measurement of lipogenesis at the central sites *in vivo*.

In contrast with the results obtained with a cholesterol-supplemented diet, when orotic acid was added to the diet without cholesterol (Table 3) no effect was observed on the rate of synthesis of cholesterol or fatty acid at any site. This finding was confirmed in a subsequent experiment where orotic acid was again added to a semisynthetic diet, without supplementary cholesterol or bile acid, given to rats for 14 days (Table 4). As observed previously orotic acid significantly decreased serum cholesterol, triacylglycerol and VLD- plus LD-lipoprotein cholesterol, as measured by polyanion precipitation. HD-lipoprotein cholesterol was again slightly decreased. Orotic acid significantly increased the concentration of liver total cholesterol and triacylglycerol, but again did

Table 4. *Effect of orotic acid on lipid metabolism*

Each rat was given $^3\text{H}_2\text{O}$ as described in Table 1. Results are means $\pm$ S.E.M. for eight analyses; each analysis was of tissue from a single rat. Significant difference ($P < 0.05$) is indicated by *.

	Control diet	Control diet + orotic acid (1%)
Gain in body wt. (g)	45.4 $\pm$ 1.1	38.1 $\pm$ 2.9
Liver wt. (g)	7.6 $\pm$ 0.2	8.6 $\pm$ 0.2*
Serum cholesterol (mg/100ml)	65 $\pm$ 3.1	42 $\pm$ 3.7*
Serum triacylglycerol (mg/100ml)	73 $\pm$ 5.1	24 $\pm$ 2.8*
Polyanion-precipitated cholesterol (mg/100ml)	29 $\pm$ 1.5	14 $\pm$ 0.7*
Supernatant cholesterol (mg/100ml)	36 $\pm$ 2.3	28 $\pm$ 3.4
Liver cholesterol (mg/g)	4.2 $\pm$ 0.1	6.1 $\pm$ 0.2*
Liver triacylglycerol (mg/g)	11.7 $\pm$ 0.7	61.7 $\pm$ 4.7*
Sterol synthesized ($\mu\text{g/h}$ per g of tissue):		
Kidney	30 $\pm$ 2.0	29 $\pm$ 0.8
Spleen	30 $\pm$ 1.8	29 $\pm$ 1.6
Skeletal muscle	4.5 $\pm$ 0.9	5.5 $\pm$ 1.9
Testis	21 $\pm$ 4.4	16 $\pm$ 1.2
Fatty acid synthesized ($\mu\text{g/h}$ per g of tissue):		
Kidney	72 $\pm$ 9.0	92 $\pm$ 7.0
Spleen	64 $\pm$ 7.8	72 $\pm$ 6.8
Skeletal muscle	22 $\pm$ 1.5	19 $\pm$ 1.2
Testis	65 $\pm$ 3.3	62 $\pm$ 2.7

Table 5. *Effect of dietary cholesterol on synthesis of sterol from leucine*

Each rat was given L-[U- ^{14}C]leucine (324 mCi/mmol, 15 $\mu\text{Ci}/100\text{g}$ body wt.) in 0.9% NaCl by intraperitoneal injection 1 h before it was killed. Results are the means of two analyses; each analysis was of tissue from a single rat.

	Control diet	Control diet + cholesterol (1%) + cholic acid (0.2%) for 7 days
Sterol (d.p.m./h per g):		
Liver	360	60
Kidney	100	65
Skeletal muscle	40	20
Adipose tissue	70	170
Serum	80	10

cholesterol, in all the extrahepatic tissues examined (results not shown). The reduction in radioactivity as serum sterols, in the rats receiving cholesterol, presumably reflects the marked inhibition of cholesterol synthesis in the liver. The disparity in d.p.m. in the serum between the groups was very much greater than any of the changes observed in the extrahepatic tissues and supports our initial assumption that extrahepatic tissue lipid radioactivity after 1 h is not substantially derived from the serum.

Discussion

The comparison of cholesterol synthesis at various sites *in vivo* is subject to a number of difficulties. It is important to ascertain that the number of ^3H atoms incorporated per molecule of cholesterol synthesized is similar under different metabolic conditions. It is claimed (Kekki *et al.*, 1977) that the distribution of $^3\text{H}_2\text{O}$ in body water shows no disparity between the sites of cholesterol synthesis in the body, but in any series of experiments it is desirable to establish that equilibrium has been reached between $^3\text{H}_2\text{O}$ in the serum and in the tissues (Fears & Morgan, 1976; Hopkirk & Bloxham, 1977). Furthermore, the number of ^3H atoms incorporated may vary if the contributions from various substrates were to change appreciably in proportion to one another (Gibbons & Pullinger, 1977) or if the contribution of reducing equivalents from NADPH generated by the pentose cycle was to change. It is possible to calculate the number of ^3H atoms incorporated into cholesterol

not affect the synthesis of cholesterol or fatty acid in kidney, spleen and skeletal muscle, nor in testis.

To determine if the results obtained with $^3\text{H}_2\text{O}$ could to some extent be confirmed by using a substrate that provided carbon atoms for cholesterol biosynthesis, the effects of dietary cholesterol on the incorporation of radioactivity from L-[U- ^{14}C]leucine *in vivo* was examined (Table 5). In agreement with the results obtained with $^3\text{H}_2\text{O}$ the incorporation of radioactivity was inhibited in liver, kidney and skeletal muscle, although the conversion of leucine appeared to be increased in adipose tissue. Fatty acid synthesis was increased, after feeding of dietary

by calculating the $^3\text{H}/^{14}\text{C}$ ratio from the simultaneous incorporation of ^3H from $^3\text{H}_2\text{O}$ and ^{14}C from a suitable carbon precursor. With studies, *in vivo*, the dilution of the ^{14}C -labelled substrate can be corrected by comparing the experimentally derived $^3\text{H}/^{14}\text{C}$ ratio for fatty acid synthesis with that quoted in the literature (Brunengraber *et al.*, 1972). In such studies (R. Fears, unpublished work), comparing $^3\text{H}_2\text{O}$ and sodium $[1-^{14}\text{C}]$ octanoate, the number of ^3H atoms incorporated per molecule of cholesterol synthesized in liver or ileum was not substantially affected by food intake or by a 20% fat diet in comparison with a 4% fat diet. The incorporation of ^3H atoms into cholesterol in adipose tissue may be more variable.

When measuring lipogenesis *in vivo* it is also necessary to establish that lipid transported from the central sites does not contribute to radiolabelling in peripheral tissues during the experimental period. Such transport would underestimate the rates of lipogenesis in liver and small intestine but would greatly overestimate the relatively lower rates elsewhere. That this transport is not significant is suggested by three pieces of evidence. First, data calculated from the rate of appearance in serum of lipoproteins newly synthesized *in vivo* from $[1-^{14}\text{C}]$ -acetate and their subsequent uptake into extrahepatic tissues suggests that after 1 h less than 10% of the radioactivity as sterols in epididymal adipose tissue and gastrocnemius muscle could be attributed to uptake rather than endogenous synthesis (Fears & Umpleby, 1978a). Secondly, the detergent Triton WR1339 impairs the clearance of chylomicrons and VLD lipoproteins by lipoprotein lipase and reduces the subsequent uptake of LD lipoprotein, yet has no significant effect on radiolabelling as sterol or fatty acid in adipose tissue, kidney, skeletal muscle or spleen (Fears & Umpleby, 1978a). Thirdly, there is a lack of correlation between changes in rates of cholesterologenesis at central sites and in peripheral tissues, for example, after the addition of orotic acid to the cholesterol-supplemented diet (Table 3).

The experiments with $^3\text{H}_2\text{O}$ described in the present paper confirm that liver and small intestine are the principal sites of cholesterologenesis, although the peripheral tissues are more active than is usually considered. The relatively large contribution made by skeletal muscle overall may well be related to the active membrane turnover in these growing rats. Cholesterologenesis at the sites examined is sufficient to account for the total production rate measured by the kinetic analysis of the specific-radioactivity-time curve obtained after the administration of radiolabelled cholesterol, and it is unlikely that other tissue sites make a major contribution. The slight discrepancy between the results from the two methods of measurement *in vivo* can be attributed to the fact that cholesterologenesis decreases with age (Bortz, 1973) and that the kinetic analysis was determined

with growing animals, which are not under true steady-state conditions.

The present results for the effects of orotic acid and dietary cholesterol confirm and extend the conclusions drawn from a previous experiment (Fears & Umpleby, 1978b). Dietary cholesterol was found to increase the concentration of cholesterol carried both by the apoprotein B-containing lipoproteins and by a newly appearing lipoprotein HDL_C (Mahley & Holcombe, 1977). Cholesterologenesis was inhibited and esterified cholesterol accumulated both in the liver and in the extrahepatic tissues. The inhibition of cholesterologenesis was confirmed in a separate experiment with $[\text{U}-^{14}\text{C}]$ -leucine to supply carbon atoms for biosynthesis. However, unlike the liver, there was no increase in the total mass of cholesterol in the extrahepatic tissues. Addition of orotic acid to the cholesterol-supplemented diet reduced the concentration of all classes of serum lipoproteins, with a concomitant de-repression of extrahepatic cholesterologenesis and diminished accumulation of esterified cholesterol. Our results, obtained *in vivo*, support the observations by Brown & Goldstein (1976), based on work with cultured human cells, on control of cellular events by receptor-mediated lipoprotein cholesterol uptake. Thus increased uptake of cholesterol from LD and other lipoproteins leads to suppression of β -hydroxy- β -methylglutaryl-CoA reductase, activation of acyl-CoA-cholesterol acyltransferase and suppression of LD-lipoprotein-receptor synthesis, thereby preventing overaccumulation of cholesterol by the cell. A similar regulation presumably does not apply to the liver. The finding that increasing the concentration of cholesterol in serum (and in interstitial fluid) allows the cell to satisfy a greater proportion of its sterol requirements by uptake rather than endogenous synthesis suggests that if the circulating concentration of lipoprotein cholesterol were reduced below the normolipidaemic value, the rates of cholesterologenesis in extrahepatic tissues might be increased above normal. This possibility was tested in rats receiving a semisynthetic diet without cholesterol where orotic acid decreased VLD plus LD lipoprotein, again in association with an increased concentration of liver lipids. The moderate effect on serum HD lipoprotein distinguishes this experimental state from the one investigated by Andersen & Dietschy (1976) using 4-aminopyrazolo-(3,4-d)-pyrimidine in which all lipoprotein classes were markedly decreased in concentration. No change was observed in the rate of cholesterologenesis in any peripheral tissue in comparison with the rates in the normolipidaemic animals. It is possible therefore that the rates are already maximal, at least within a reasonably 'physiological range' of serum lipid concentrations. This is different from the metabolic state in the human cells studied by Brown & Goldstein

(1976), where the relatively high circulating LD-lipoprotein concentration is sufficient to saturate the lipoprotein receptor and thus to permanently repress extrahepatic cholesterogenesis (Reichl *et al.*, 1978).

It seems likely that the maximal rates of extrahepatic cholesterogenesis observed in normal rats can be attributed to the low concentration of LD lipoprotein in the serum and interstitial fluid. It should be emphasized that we have not identified a single lipoprotein species that represses extrahepatic cholesterogenesis in cholesterol-fed rats. The appearance of the novel lipoproteins HDL_C and β -VLD may well result in a similar interaction at the peripheral-tissue lipoprotein receptor to that expected to result from the increases in the VLD and LD lipoproteins.

Although other workers have been able to increase the rate of cholesterogenesis in rat extrahepatic tissues assayed *ex vivo*, by a variety of experimental manipulations, it is not clear how these results relate to the present work because the maximum rate supported *ex vivo* is still much less than the control rate measured *in vivo*. We consider that the use of ³H₂O gives more meaningful values because the rates of cholesterogenesis reported in the present paper were measured in the presence of all the physiological controlling factors and because our results are in agreement with the cholesterol turnover rate as measured by the other best methods [analysis of specific-radioactivity-time curves and the method of Raicht *et al.* (1975)].

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