Specificity of the Effect of Dietary Cholesterol on Rat Liver Microsomal 3-Hydroxy-3-methylglutaryl-Coenzyme A Reductase Activity

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Dietary cholesterol lowers the activity of rat liver microsomal 3-hydroxy-3-methylglutaryl-CoA reductase without affecting various other liver microsomal enzymes. This is consistent with a specific regulatory mechanism and distinguishes the action of cholesterol on 3-hydroxy-3-methylglutaryl-CoA reductase from that of at least one other stimulus known to affect this enzyme.

Dietary cholesterol is known to lower the rate of liver cholesterol biosynthesis, probably through its inhibitory action on microsomal 3-hydroxy-3-methylglutaryl-CoA reductase (Linn, 1967; Shapiro & Rodwell, 1971; Edwards & Gould, 1974). Although the mechanism whereby cholesterol acts on this enzyme is not fully understood, it has been generally accepted as a negative-feedback system, controlling the rate of cholesterol biosynthesis in response to a changing cholesterol intake (Siperstein, 1970).

There seemed, however, to be no evidence on the specificity of action of dietary cholesterol on liver microsomal enzymes. In view of the profound effects which various drugs, hormones and nutritional stimuli are now known to exert on both structure and function of the liver endoplasmic reticulum, it seemed essential to know whether dietary cholesterol affects many liver microsomal enzymes, or whether it specifically influences 3-hydroxy-3-methylglutaryl-CoA reductase. To distinguish between these two possibilities, the activities of several microsomal enzymes were measured in the livers of cholesterol-fed rats, and the results are reported in this paper.

Experimental

Animals. Female Wistar rats, weighing 90–100 g on arrival, were purchased from The Manston Research Centre, Margate, Kent, U.K.. They were kept in a room artificially illuminated between 06:00h and 18:00h, and in darkness between 18:00h and 06:00h, and allowed continuous access to food and water. The rats were divided into two groups and individually weighed daily for 4 days. In the afternoon of the fourth day, the period of experimental feeding was begun, and the test rats were given a 2% cholesterol diet prepared by crushing ordinary rat cubes, mixing in 2 g of cholesterol to each 98 g of crushed cube, and re-forming pellets after moistening the powdered food with a little water. Control rats

received similar pellets lacking added cholesterol. These diets were continued until the morning of the seventh day, when the rats were killed between 09:00h and 10:00h and the microsomal fraction was prepared from their livers as described by Tata (1969).

Enzymes. Enzyme assays were carried out on resuspended microsomal fraction. 3-Hydroxy-3-methylglutaryl-CoA reductase was measured as previously described (Gregory *et al.*, 1972); 1 unit of activity forms 1 nmol of mevalonate in 1 min. Glucose 6-phosphatase was assayed by the method of Swanson (1955), the P_i released being measured by the procedure of King (1932); 1 unit of activity yields 1 μ mol of P_i in 1 min. The cytochrome *c* reductases, diaphorases and neotetrazolium reductases were assayed by the methods of Dallner *et al.* (1966). For the two cytochrome *c* reductases 1 unit of activity reduces 1 μ mol of cytochrome *c* in 1 min, and for the diaphorases 1 unit of activity oxidizes 1 μ mol of NADH or NADPH in 1 min. For the neotetrazolium reductases 1 unit of activity oxidizes 1 nmol of NADH or NADPH in 1 min. All enzyme activities are expressed as specific activity, which is units/mg of microsomal protein. Protein was measured as described by Lowry *et al.* (1951).

Chemicals. Cholesterol was obtained from Organon Laboratories, Newhouse, Lanarkshire, U.K., and was purified by recrystallization as described by Leffler & McDougald (1963). Cytochrome *c* (horse heart), NADPH (tetrasodium salt, grade II) and NADH (disodium salt, grade II) were purchased from Boehringer Corp., London W.5, U.K. Glucose 6-phosphate and neotetrazolium were purchased from Sigma (London) Chemical Co., London S.W.6, U.K.

Results and discussion

The results summarized in Table 1 show that the rats' daily increase in weight was not affected by the 2% (w/w) cholesterol diet for 3 days; weight gains by

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the test rats were not significantly different from those of the controls during the same period. The test rats were therefore not in a state of starvation on the day of the experiments, and any difference from the control animals must have been due to the cholesterol in the diet. This point is important because starvation is known to lower the activity of liver microsomal 3-hydroxy-3-methylglutaryl-CoA reductase (Linn, 1967; Hamprecht *et al.*, 1969).

Table 2 confirms the lowering effect of dietary cholesterol on liver microsomal 3-hydroxy-3-methylglutaryl-CoA reductase specific activity (Linn, 1967; Shapiro & Rodwell, 1971; Edwards & Gould, 1974), and shows that this lowering is not due to a change in the amount of microsomal protein; the yield of microsomal protein from a given weight of liver was not significantly different ($P>0.4$) between test and control rats. The results in this Table would therefore show the same features irrespective of whether enzyme activities were expressed as units/mg of microsomal protein or as units/g of liver. None of the enzymes tested, apart from 3-hydroxy-3-methylglutaryl-CoA reductase, was affected by the cholesterol-rich diet (Table 2). This observation provides the first reported evidence that dietary cholesterol does not grossly impair the enzymic functioning of liver endoplasmic reticulum, and therefore greatly strengthens the view that its action on microsomal 3-hydroxy-3-methylglutaryl-CoA reductase represents a specific control mechanism.

A distinction may now be drawn between the way in which dietary cholesterol and the ways in which other stimuli affect the activity of this enzyme. For example, thyroid hormone, when injected into experimentally hypothyroid animals, causes a marked increase in the activity of liver 3-hydroxy-3-methylglutaryl-CoA reductase (Guder *et al.*, 1968; Ness *et al.*, 1973). This hormone has been suggested to act by lowering the blood cholesterol, and con-

sequently the amount of cholesterol in the liver, thus relieving the inhibition due to cholesterol (Ness *et al.*, 1973), but available evidence does not support this view. First, although the amount of cholesterol does rise in the livers and circulation of rats fed on cholesterol-rich diets (Shapiro & Rodwell, 1971; Harry *et al.*, 1973; Edwards & Gould, 1974), there is no evidence that this rise must occur if the activity of liver 3-hydroxy-3-methylglutaryl-CoA reductase is to be lowered. It cannot therefore be taken for granted that a stimulus which lowers liver cholesterol will also increase the activity of this enzyme. Secondly, injected thyroid hormone is known to cause the proliferation of liver endoplasmic reticulum and the induction of many microsomal enzymes (Tata, 1968). This is therefore an effect not specific for 3-hydroxy-3-methylglutaryl-CoA reductase and, on the evidence presented here, is unlikely to work in the way suggested by Ness *et al.* (1973).

Possibly other stimuli affecting liver 3-hydroxy-3-methylglutaryl-CoA reductase, such as starvation (Linn, 1967; Hamprecht *et al.*, 1969), dietary bile acids (Hamprecht *et al.*, 1971) and other dietary components (Craig *et al.*, 1972), do so non-specifically. At any rate, a test, such as that described in the

Table 1. *Effect of a 2% (w/w) cholesterol diet on rat growth*

During days 1-4, all rats were fed on a normal diet; during days 5-7, the test rats received a 2% (w/w) cholesterol diet as described under 'Experimental'. Each value is the mean $\pm$ S.E.M. of separate weighings of the number of rats given in parentheses.

	Weight gain (g/day per rat)	
	Days 1-4	Days 5-7
Control rats	5.3 $\pm$ 0.8 (4)	4.9 $\pm$ 0.7 (4)
Test rats	5.5 $\pm$ 0.8 (4)	4.5 $\pm$ 0.7 (4)

Table 2. *Effect of a 2% (w/v) cholesterol diet on liver microsomal enzymes of the rat*

The rats were treated as described under 'Experimental', where details of enzyme assays are also given, and where units of enzyme activity are defined. Each value is the mean $\pm$ S.E.M. of separate measurements on the number of rats given in parentheses. Differences between means were evaluated by Student's *t* test; values of *P* are given, where relevant, under 'Results and discussion'.

	Control rats	Test rats
Liver weight (g/100g body wt)	4.1 $\pm$ 0.2 (9)	4.0 $\pm$ 0.3 (9)
Microsomal protein (mg/g wet wt. of liver)	18.7 $\pm$ 3.1 (9)	23.2 $\pm$ 4.8 (9)
NADPH-cytochrome <i>c</i> reductase	0.10 $\pm$ 0.02 (5)	0.11 $\pm$ 0.02 (5)
NADH-cytochrome <i>c</i> reductase	0.95 $\pm$ 0.11 (5)	0.89 $\pm$ 0.19 (5)
NADPH diaphorase	0.06 $\pm$ 0.01 (5)	0.06 $\pm$ 0.01 (5)
NADH diaphorase	1.22 $\pm$ 0.26 (5)	1.24 $\pm$ 0.29 (5)
Glucose 6-phosphatase	0.24 $\pm$ 0.03 (5)	0.23 $\pm$ 0.03 (5)
Neotetrazolium-NADH reductase	9.3 $\pm$ 1.0 (4)	9.0 $\pm$ 0.4 (4)
Neotetrazolium-NADPH reductase	1.7 $\pm$ 0.4 (4)	1.3 $\pm$ 0.2 (4)
3-Hydroxy-3-methylglutaryl-CoA reductase	0.051 $\pm$ 0.007 (5)	0.006 $\pm$ 0.001 (5)

present paper, would distinguish between stimuli acting specifically on this enzyme and those altering a whole spectrum of liver microsomal enzymes, and would therefore clarify the ways in which various factors affect this enzyme.

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