Biochemical Pharmacology, Vol. 35, No. 20, pp. 3489–3494, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

CYCLOHEXANOL AND METHYLCYCLOHEXANOLS

A FAMILY OF INHIBITORS OF HEPATIC HMGCoA REDUCTASE *IN VIVO*

ANN MICIAK, DAVID A. WHITE and BRUCE MIDDLETON*
Department of Biochemistry, The Medical School, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH, U.K.

(*Received* 17 *March* 1986; *accepted* 29 *April* 1986)

Abstract—Oral dosing of rats with cyclohexanol and methylcyclohexanols resulted in the inhibition of hepatic HMGCoA reductase. Neither cyclohexane or cyclohexane diols exerted any effects. Inhibition was not due to alcohol dehydrogenase mediated changes in redox state since 3,3′,5-trimethylcyclohexanol (TMC), a non substrate for alcohol dehydrogenase, was a potent inhibitor of HMGCoA reductase. Following a single dose of TMC there was no alteration in total hepatic HMGCoA reductase activity for more than 6 hr after which the enzyme activity was depressed in a dose-dependent manner. The normal diurnal rhythm of HMGCoA reductase was reduced in amplitude following TMC administration but the phase was unaltered and the $t_{\frac{1}{2}}$ for activity decay following the peak of activity was unaffected. Prior to the inhibitory effect of a TMC dose becoming apparent in total HMGCoA reductase activity we found that the expressed activity of the enzyme (after isolation in F^- medium to suppress endogenous protein phosphatase) was depressed by 43%. The inhibitory effect of TMC on total HMGCoA reductase activity seen 8 hr or more after dosing was reflected by inhibition of sterol synthesis in liver measured *in vivo* after [^{3}H]-H_2O administration.

The vasoactive compound cyclandelate (Cyclospasmol, Gist Brocades N.V. Delft, The Netherlands) is the mandelic acid ester of 3,3′,5-trimethylcyclohexanol (TMC). Administration of a single oral dose of the compound to rats caused a 50% inhibition of hepatic HMGCoA reductase when measured 17 hr after dosing. A similar effect was seen with a single equimolar dose of TMC and appeared to be specific since other parameters of microsomal function, arylesterase activity and the amounts of cytochromes b_5 and P_{450} were unchanged [1]. Using the tritiated water technique for the measurement of *in vivo* rates of lipogenesis it was shown that cyclandelate administration caused a significant inhibition of both sterol and fatty acid synthesis [2]. These inhibitions were independent of the 3.2-fold diurnal variation in the rates of hepatic sterol and fatty acid synthesis. These actions are similar to the effects of cyclic monoterpenes on hepatic lipogenesis [3, 4] and may reflect structural similarities between the cyclic monoterpenes and TMC. The present paper describes a study of the structure–activity relationship with respect to the inhibition of HMGCoA reductase by simpler compounds derived from cyclohexanol and uses TMC to gain further insight into the mechanism of this inhibition.

MATERIALS AND METHODS

^3H_2O, [3-^{14}C]HMGCoA, [4-^{14}C]cholesterol and [1-^{14}C]oleic acid were purchased from Amersham International Ltd. (Little Chalfont, Bucks., U.K.). [5-^{3}H]Mevalonic acid (DBED salt) was purchased from New England Nuclear (Boston, MA). Glucose-6-phosphate, glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and $NADP^+$ were obtained from Boehringer (Mannheim, F.R.G.). Aluminium-backed silica gel precoated sheets for tlc were from Merck (Darmstadt, F.R.G.) and Fisofluor scintillation cocktail was from Fisons Ltd. (Loughborough, U.K.). 3,3′,5-Trimethylcyclohexanol and some of its metabolites were kindly donated by Dr. W. E. van den Hoven, Gist Brocades N.V., Delft, The Netherlands.

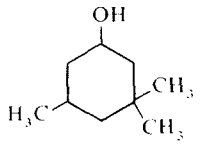
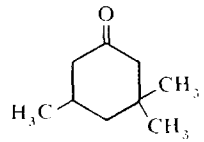
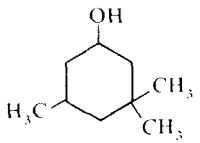
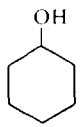
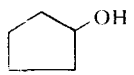
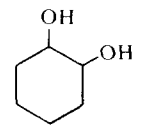
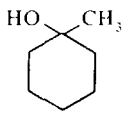
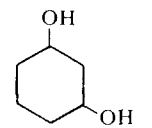
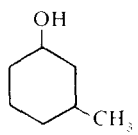
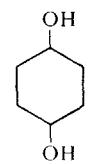
Male Wistar rats (approximately 230 g) from the Joint Animal Breeding Unit, Nottingham University School of Agriculture, Sutton Bonington, Leics., U.K. were subjected to normal (lights on 08.00–20.00 hr) and reversed (lights on 15.00–03.00 hr or as described) lighting schedules and fed 41B pellet diet *ad libitum*. Animals were allowed at least ten days to adapt to a changed lighting schedule before experimentation.

Solutions of pure cyclohexanol derivatives from commercial sources were prepared freshly in olive oil and administered in 0.5 ml at a dosage of 3 mmol per kg body weight. Animals were dosed by stomach tube, controls receiving 0.5 ml olive oil alone. Times are quoted in relation to hours of darkness (D) or light (L).

Hepatic microsomes were prepared from freshly excised livers as described previously [3] and resuspended in 100 mM-phosphate buffer (pH 7.5) containing 10 mM-EDTA and 5 mM-DTT to a final concentration of about 20 mg protein/ml. Total and expressed HMGCoA reductase activity was assayed as described in [5] except that animals were anaesthetized with pentobarbitol (60 mg/kg body wt.)

* Author to whom all correspondence should be addressed.

Table 1. The inhibition of HMGCoA reductase by some cyclohexanol derivatives

Compound	Structure	HMGCoA reductase activity (nmol/min/mg)	% Inhibition	Compound	Structure	HMGCoA reductase activity (nmol/min/mg)	% Inhibition
3,3,5 Trimethylcyclo hexanol (*Cis* TMC)		C: 0.18 ± 0.043 (4) T: 0.07 ± 0.016 (4)*	61.1	3,3,5 Trimethylcyclo-hexanone		C: 0.148 ± 0.06 (4) T: 0.111 ± 0.04 (4)	25.0
Trans TMC		C: 0.137 ± 0.022 (4) T: 0.056 ± 0.010 (4)†	59.1	Cyclohexane		C: 0.257 ± 0.052 (6) T: 0.244 ± 0.072 (6)	5.0
Cyclohexanol		C: 0.228 ± 0.094 (4) T: 0.095 ± 0.043 (4)‡	58.3	Tetrahydropyran		C: 0.159 ± 0.05 (4) T: 0.144 ± 0.09 (4)	9.4
Cyclopentanol		C: 0.114 ± 0.05 (4) T: 0.072 ± 0.08 (4)	36.8	Cyclohexane 1,2diol		C: 0.121 ± 0.067 (4) T: 0.070 ± 0037 (4)	21.5
1,Methylcyclohexanol		C: 0.144 ± 0.044 (4) T: 0.065 ± 0.034 (4)‡	54.9	Cyclohexane 1,3diol		C: 0.082 ± 0.009 (4) T: 0.070 ± 0.037 (4)	14.6
3,Methylcyclohexanol		C: 0.251 ± 0.073 (7) T: 0.124 ± 0.057 (7)	50.6	Cyclohexane 1,4diol		C: 0.138 ± 0.04 (4) T: 0.118 ± 0.036 (4)	14.5

Rats were dosed intragastrically with solutions of cyclohexanol derivatives (3 mmol/kg body weight) in 0.5 ml olive oil at L8.5 as described in Materials and Methods. They were killed 17 hr later at L1.5 and total HMGCoA reductase assayed in the liver microsomes. The results are expressed as nmol mevalonate formed/min mg microsomal protein ± SD. The numbers in parentheses give the number of animals/group. C = controls, T = test compound. ‡ $P < 0.05$, † $P < 0.01$, * $P < 0.001$ with respect to appropriate controls (Students *t*-test).

before removal of liver. *In vivo* rates of lipid synthesis were determined by measurement of ^3H incorporation into tissue lipids following injection with ^3H_2O [4]. Approximately 17 hr after dosing rats with TMC in olive oil, ^3H_2O (15 mCi/rat) in 0.9% w/v saline (0.3 ml) was injected intraperitoneally. One hour later the rats were decapitated and tissues processed as described previously [4] for measurement of incorporation of radioactivity into sterol and fatty acid fractions. Acetyl CoA carboxylase [6] and alcohol dehydrogenase activity [7] were assayed using methods described in the literature.

RESULTS AND DISCUSSION

Inhibition of hepatic HMGCoA reductase by in vivo *administration of cyclohexanol derivatives*

The effect of cyclohexanol derivatives administered *in vivo* on hepatic HMGCoA reductase activity measured 17 hr after dosing is shown in Table 1. Of the derivatives tested only those containing a single hydroxy substituent were inhibitory. *Cis* and *trans* TMC, 1-methylcyclohexanol, 3-methyl cyclohexanol and cyclohexanol itself caused a significant inhibition (50–60%, $P < 0.05$) of total HMGCoA reductase activity. Neither cyclohexane nor tetrahydropyran were inhibitory. This is similar to results obtained on dosing mono- and bicyclic monoterpenes, compounds which bear some structural similarity to the cyclohexanol derivatives [3]. These compounds inhibited HMGCoA reductase only if they contained a hydroxyl group or were metabolized rapidly to a hydroxy derivative. Thus menthol which is structurally similar to TMC inhibited hepatic total HMGCoA reductase activity by 70% when administered *in vivo* 17 hr prior to enzyme assay. Cyclopentanol inhibited the enzyme by 37% but this was not statistically significant suggesting a certain degree of ring size specificity for inhibition by compounds of this nature. It was of interest to test cyclohexane diols for inhibitory effect since it is known that cyclohexanol is rapidly converted to these compounds. However, the diols tested (Table 1) were inactive suggesting that the active agent is not this metabolite of cyclohexanol.

The effect of cyclohexanol on HMGCoA reductase activity in starved rats

Since cyclohexanol and the methyl cyclohexanols are substrates for alcohol dehydrogenase and might give rise to an increased NADH/NAD ratio in the liver on metabolism it is possible that the inhibition of HMGCoA reductase after dosing might be due to an altered redox state. An increase in NADH/NAD ratio in the liver can also be generated by starving animals for 24 hr, during which time liver glycogen is virtually depleted [4] as the animals rely on degradative pathways for energy. Hepatic HMGCoA reductase was reduced by 76% ($P < 0.05$) in starved rats compared with normal fed rats as observed earlier [8]. Dosing starved animals with cyclohexanol gave rise to a 64% ($P < 0.05$) inhibition of HMGCoA reductase compared with starved controls (Table 2) which compares well with the 58% inhibition seen in fed rats. Thus when the NADH/NAD ratio is increased by starvation, the effect of cyclohexanol on reductase activity is still found. These results imply that the inhibition seen is independent of the redox state and is also not mediated via an effect on those hormones (e.g. insulin, glucagon, glucocorticoids) whose plasma concentrations are known to change during starvation. Further evidence against any involvement of redox state on HMGCoA reductase activity was obtained when we found that 3,3,5-trimethylcyclohexanol (TMC) (which inhibits HMGCoA reductase as potently as cyclohexanol) was not a substrate for NAD^+- or $NADP^+$-linked alcohol dehydrogenases of liver cytosol under conditions where cyclohexanol was.

The effect of increasing doses of TMC on the inhibition of hepatic HMGCoA reductase

The inhibition of HMGCoA reductase increased as the administered dose of TMC increased and a maximum inhibition of 70% ($P < 0.05$) was observed at a dose of 3 mmol/kg body weight (Fig. 1). Doses greater than this had no further effect on enzyme activity. A similar dose dependent inhibition of the enzyme was observed with menthol [5] where maximal inhibition was achieved at 1.5 mmol/mg body

Table 2. The effect of cyclohexanol on hepatic HMGCoA reductase in starved and fed rats

Treatment	HMGCoA reductase activity (pmol/min/mg)	% Inhibition
Starved rats:		
Control (3)	55 ± 15	
Cyclohexanol (3)	20 ± 5	63.6*
Fed rats:		
Control (4)	228 ± 94	
Cyclohexanol (4)	95 ± 43	58.3*

Rats were starved for 24 hr prior to death. Cyclohexanol (3 mmol/g of body weight) in 0.5 ml of olive oil was administered to rats at 16.30 hr as described in Materials and Methods. The rats were killed 17 hr later at 09.30 hr and hepatic total HMGCoA reductase was assayed in microsomes as described in the Materials and Methods. HMGCoA reductase activity is expressed as pmol mevalonate formed/min/mg of microsomal protein ± SD. * $P < 0.05$ with respect to controls (Students *t*-test).

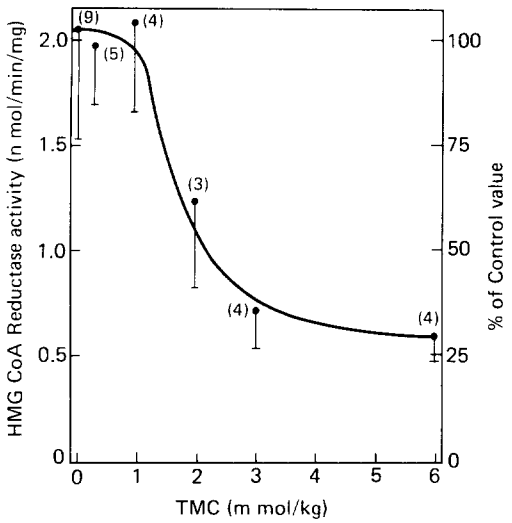


Fig. 1. Dose response curve for TMC. Solutions of TMC (up to 6 mmol/kg body weight) dissolved in 0.5 ml olive oil were given intragastrically to rats at L8.5. The rats were sacrificed 17 hr later and total hepatic HMGCoA reductase was assayed (see Materials and Methods). Results are expressed as nmol mevalonate formed/min/mg microsomal protein ± SD. The number of animals/group are shown in parentheses.

weight. The failure to increase further the inhibition with higher doses contrasts with inhibition seen after dosing with mevalonolactone [9] (97% 4 hr after dose), 25-hydroxycholesterol [9] (78% after 1 hr) and cholesterol [10] (88% 12 hr after inclusion of 1% cholesterol in the diet).

The effect of TMC and some metabolites on HMGCoA reductase in vitro

It was possible that TMC or one of its metabolites might be acting directly as inhibitors of HMGCoA reductase. The inclusion of TMC, 3′ and 5′ carboxylic derivatives at final concentrations of 0.1 or 0.5 mM had no significant inhibitory effect in the direct assay of HMGCoA reductase in hepatic microsomes prepared from control rats (data not shown), contrasting with the inhibition seen when the parent compound was given *in vivo* (Fig. 1). This observation is similar to that shown by Clegg *et al.* [5] for the monoterpene menthol and it rules out any direct effect of TMC or its metabolites on HMGCoA reductase activity.

Time course for the inhibition of hepatic HMGCoA reductase after dosing with TMC

Rats which had been dosed with TMC (3 mmol/kg body weight) in olive oil were killed at various times up to 50 hr after dosing and livers were assayed for HMGCoA reductase. Control animals received olive oil alone. Hepatic total HMGCoA reductase activity as a function of time after dosing is shown in Fig. 2. As has been noted by others [11–14] a diurnal variation in HMGCoA reductase activity of approximately 10-fold was seen. Maximal activity occurred at about hour 4 of the dark period while minimum activity was about hour 4 of the light period. HMGCoA reductase activity rose and fell sharply in contrast to the recent data of Easom and Zammit [14] who found that enzyme activity increased rapidly at the onset of darkness reaching a maximum at hour 2 of darkness and remaining high for a further 6 hr before declining rapidly to basal

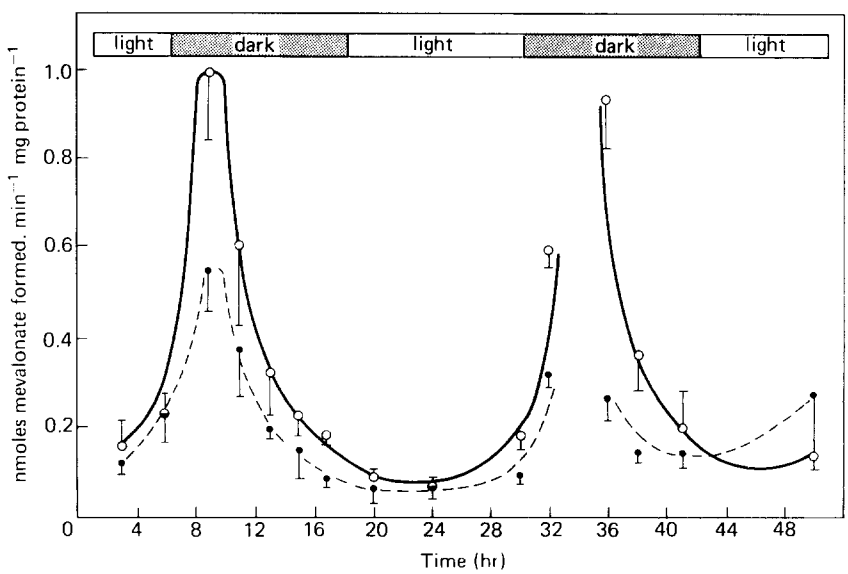


Fig. 2. Time course for the inhibition of HMGCoA reductase by TMC. Rats were dosed intragastrically with TMC (3 mmol/kg of body weight) in olive oil (0.5 ml) at L6.5. Control (○) and TMC treated (●) rats (3/group) were killed at time intervals during the following 50 hr. Total HMGCoA reductase was assayed in the liver microsomes as described in Materials and Methods and is expressed as nmol mevalonate formed/min/mg of microsomal protein ± SD.

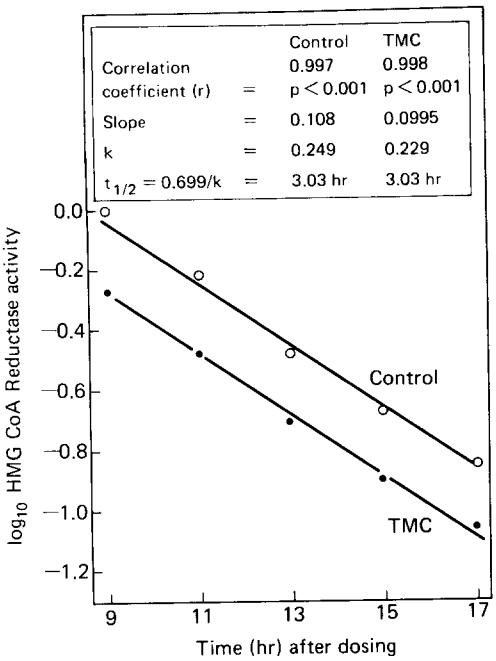


Fig. 3. Degradation of HMGCoA reductase. Plot of $\log_{10}$ HMGCoA reductase activity vs time after dosing. The degradation rate of HMGCoA reductase can be calculated from the downward slope of the diurnal variation (Fig. 2), when enzyme synthesis is switched off, as shown in the insert.

values. In the present experiments HMGCoA reductase activity declined to basal levels by hour 8 of the dark period in both control and TMC treated animals. Inhibition of the enzyme by TMC was not measurable until 6–8 hr after dosing. The phase of the diurnal rhythm was unaltered, only its amplitude being decreased and this inhibition was maintained for at least 38 hr after dosing. The cause of the delay in inhibition by TMC after dosing is unexplained and contrasts with the rapid inhibitions (1–2 hr) seen after oral dosing of mevalonolactone or cholesterol [15]. It is possible that it takes about 6 hr for TMC to reach some threshold concentration in the liver or to be metabolized to an inhibitory species or initiate some other event which gives rise indirectly to the observed inhibition. The half life of HMGCoA reductase in both control and treated rats can be calculated from the data in Fig. 2 for the decline in enzyme activity with time. As seen in Fig. 3 dosing with TMC did not alter the half life of the enzyme and the value of 3 hr for both control and treated animals agrees well with published data [13, 16]. This lack of change in degradation rate suggested that the inhibition observed might be due to decreased rate of synthesis of the enzyme. To test this hypothesis rats were dosed with TMC at the diurnal high of HMGCoA reductase activity and enzyme activity measured 8 hr after dosing, i.e. after a period of time when enzyme synthesis was switched off [17, 18]. No inhibition of enzyme activity was seen at this time: control group and TMC group activities (N = 4) were respectively 0.18 ± 0.05 and 0.17 ± 0.04 nmol/min/mg (means ± SD). This contrasts with the inhibition seen where dosing of TMC preceded the diurnal rise in enzyme activity due to enzyme synthesis ([1] and Table 1).

In vivo *expressed activity of HMGCoA reductase after dosing with TMC*

HMGCoA reductase can be regulated acutely *in vivo* by covalent modification involving phosphorylation and dephosphorylation [19]. Phosphorylation of the enzyme causing inhibition of activity is an early regulatory response after intragastric administration of cholesterol and mevalonolactone [9, 15, 20, 21] and it precedes the irreversible inhibition seen later after dosing with these two compounds suggesting an initial inhibition due to phosphorylation followed by a decreased enzyme amount. Since an apparent change in enzyme amount was seen 8 hr after dosing with TMC in the present experiments it was possible that an initial inhibition by phosphorylation might occur at earlier times after dosing. Hepatic microsomes were thus prepared in the presence and absence of sodium fluoride from dosed animals 4 hr after administration at the diurnal high of activity and HMGCoA reductase activity assayed (Table 3). In agreement with the data in Fig. 2 total HMGCoA reductase activity was unchanged by TMC treatment after 4 hr. However, expressed

Table 3. Total and expressed HMGCoA reductase activity 4 hr after TMC treatment

Treatment	HMGCoA reductase activity after isolation in medium −NaF (Total) (nmol/min/mg)	+NaF (Expressed) (nmol/min/mg)	Activity + NaF / Activity − NaF %
Control (4)	0.366 ± 0.032	0.189 ± 0.054	54.8 ± 16
TMC (4)	0.374 ± 0.101	0.107 ± 0.103*	29.8 ± 13†

Rats were kept in light cupboards with the lights switched on between 03.00 and 15.00 hr for 10 days prior to the experiment. They were dosed intragastrically with TMC (3 mmol/kg of body weight) in 0.5 ml of olive oil or with olive oil alone at 09.00 hr. The uppermost liver lobe was removed under anaesthesia 4 hr later and portions were immediately homogenized in ice cold isolation buffer ± 50 mM NaF. Total and expressed HMGCoA reductase was assayed in the microsomes as described in the Materials and Methods. Activities are expressed as nmol mevalonate formed/min/mg microsomal protein ± SD. The number of animals/group is shown in parenthesis. * $P < 0.05$ (Student's *t*-test), † $P < 0.05$ (Mann Whitney U test).

Table 4. Rate of incorporation of H from $[^3H]H_2O$ into digitonin precipitable sterol and fatty acids in TMC treated rats

Treatment	Digitonin precipitable sterol (μg atoms H/hr/g tissue)	Fatty acids
Liver:		
Control (8)	6.39 ± 2.7	36.6 ± 4.8
TMC (8)	3.38 ± 2.6*	16.6 ± 3.7†
Serum:		
Control (4)	1.2 ± 0.28	3.35 ± 0.92
TMC (4)	0.95 ± 0.16	2.48 ± 0.6

Rats were dosed with TMC (3 mmol/kg of body weight) in olive oil at 16.30 hr, control rats received olive oil alone as described in Materials and Methods. They were injected with $[^3H]H_2O$ (15 mCi/rat) intraperitoneally as described in Materials and Methods 1 hr prior to death 17 hr after dosing. Rates of incorporation of H into digitonin precipitable sterol and fatty acids were determined for liver and serum as described in Materials and Methods. Results are expressed as μg atoms H incorporated/hr/g of tissue or μg atoms H/hr/ml of serum ± SD. The number of animals are given in parentheses. * $P < 0.01$, † $P < 0.001$ (Students *t*-test).

enzyme activity, i.e. activity in the presence of fluoride, in the treated animals was 57% ($P < 0.05$) of the expressed control values. The percentage of total activity expressed in control rats was 54.8 ± 16% while in TMC treated rats it was 29.8 ± 13% suggesting that like cholesterol and mevalonolactone the first stage of inhibition of HMGCoA reductase by TMC might involve increased phosphorylation of the enzyme.

Effect of TMC on in vivo *rates of sterol and fatty acid synthesis*

The rates of *in vivo* synthesis of sterol and fatty acid were measured by the rate of incorporation of ^3H from intraperitoneally injected ^3H_2O into digitonin precipitable sterol (DPS) and total saponifiable lipid. The effect of TMC on the incorporation of H into hepatic DPS and saponifiable lipid is shown in Table 4. The rate of incorporation of ^3H into DPS was inhibited by 47% ($P < 0.01$) 17 hr after dosing with TMC agreeing well with the observed inhibition of hepatic HMGCoA reductase at this time (Table 1) and suggesting that the inhibition measured *in vitro* is responsible for the decreased rate of sterol synthesis *in vivo*. Hepatic fatty acid synthesis was also inhibited by 55% ($P < 0.001$) 17 hr after administration of TMC although the activity of hepatic acetyl CoA carboxylase in dosed animals was unchanged (B. Middleton, unpublished results). The possible relationship between the inhibitions of synthesis of both sterol and fatty acid is unexplained. Both sterol and fatty acid derived molecules are required for assembly of very low density lipoproteins which are then exported to blood. However, evidence is divided as to whether the rates of synthesis of sterol and fatty acid are functionally linked in liver [22, 23] or are independent [24, 25]. Therefore it is not clear whether inhibition of hepatic sterol synthesis would necessarily result in decreased fatty acid synthesis. However, the accumulation of newly synthesized serum sterol and fatty acid was not affected by TMC treatment (Table 4) implying either that lipoprotein export from liver was unaffected or that compensatory changes in uptake by peripheral tissues occurred.

In conclusion we have shown that structurally simple cyclohexane derivatives (cyclohexanol and methyl substituted cyclohexanols) can cause specific inhibition of hepatic HMGCoA reductase. This effect occurs only *in vivo*, is dose and time dependent, but is not related to food intake. The inhibition of HMGCoA reductase *in vivo* appears to be due to decreased synthesis of HMGCoA reductase in liver following an initial acute effect in the degree of phosphorylation of the enzyme.

REFERENCES

1. B. Middleton, A. Middleton, A. Miciak, D. A. White and G. D. Bell, *Biochem. Pharmac.* **32**, 649 (1983).
2. A. Middleton, D. A. White, G. D. Bell and B. Middleton, *Biochem. Pharmac.* **32**, 3079 (1983).
3. R. J. Clegg, B. Middleton, G. D. Bell and D. A. White, *Biochem. Pharmac.* **29**, 2125 (1980).
4. B. Middleton and K-P. Hui, *Biochem. Pharmac.* **31**, 2897 (1982).
5. R. J. Clegg, B. Middleton, G. D. Bell and D. A. White, *J. biol. Chem.* **257**, 2294 (1982).
6. H. Inoue and J. M. Lowenstein, *Methods Enzymol.* **35**, 3 (1975).
7. R. Bonnichsen, in *Methods of Enzymatic Analysis* (Ed. H-U. Bergmeyer), pp. 285–289. Academic Press, London (1973).
8. J. M. Dietschy and M. S. Brown, *J. Lipid Res.* **15**, 508 (1974).
9. S. K. Erickson, M. A. Shrewsbury, R. G. Gould and A. D. Cooper, *Biochim. biophys. Acta* **620**, 70 (1980).
10. M. S. Brown, J. L. Goldstein and J. M. Dietschy, *J. biol. Chem.* **254**, 5144 (1979).
11. D. J. Shapiro and V. W. Rodwell, *Biochem. biophys. Res. Commun.* **37**, 867 (1969).
12. S. Shefer, S. Hansen, V. Lapar and E. H. Mosbach, *J. Lipid Res.* **13**, 571 (1972).
13. R. E. Dugan, L. L. Slakey, A. V. Breidis and J. W. Porter, *Archs Biochem. Biophys.* **152**, 21 (1972).
14. R. A. Easom and V. A. Zammit, *Biochem. J.* **220**, 739 (1984).
15. Z. H. Beg and H. B. Brewer, *Fedn Proc. Fedn Am. Soc. exp. Biol.* **41**, 2634 (1982).
16. M. E. Hickman, B. J. Horton and J. R. Sabine, *J. Lipid Res.* **13**, 17 (1972).
17. P. A. Edwards and R. G. Gould, *J. biol. Chem.* **247**, 1520 (1972).
18. P. A. Edwards and R. G. Gould, *J. biol. Chem.* **249**, 2891 (1974).
19. Z. H. Beg and H. B. Brewer, *Curr. Top. Cell. Regn.* **20**, 139 (1981).
20. R. E. Arebalo, J. E. Hardgrave, B. J. Noland and T. J. Scallen, *Proc. natn. Acad. Sci. U.S.A.* **39**, 6429 (1980).
21. R. E. Arebalo, J. E. Hardgrave, B. J. Noland and T. J. Scallen, *Fedn Proc. Fedn Am. Soc. exp. Biol.* **39**, 1776 (1980).
22. E. H. Goh and M. Heimberg, *Biochem. J.* **184**, 1 (1979).
23. N. Fukuda and J. A. Ontko, *J. Lipid Res.* **25**, 831 (1984).
24. W. Patsch, T. Tamai and G. Schonfeld, *J. clin. Invest.* **72**, 371 (1983).
25. C. R. Pullinger and G. F. Gibbons, *Biochim. biophys. Acta* **833**, 44 (1985).