chromatography. The oven temperature was programmed from 150° to 280°C at 10°/min and the run isothermally for 5 min.

Results. Figure A shows the gas chromatogram of a sample of bile which was permethylated and indicates the N-glucuronide metabolite of carbamazepine. It is the largest peak in the chromatogram and appeared at the end of the programmed run with the instrument maintained at 275° for 5 min. Figure B is the structure and mass spectrum of the permethylated N-glucuronide of carbamazepine. The molecular ion is at *m/e* 482 and there are ions at *m/e* 451, 425, 394, 306, 278, 249, 233, 220, 201, 192, 173, 169, 157, 141 and 101. The ions at *m/e* 233, 201, 169, 141 and 101 are caused by fragmentation of the permethylated glucuronic acid moiety[8]. The ion at *m/e* 425 is caused by loss of CH_3–N–C=0 (57 atomic mass units) and a molecular rearrangement.

Discussion. Previous studies have shown that intact glucuronide metabolites of drugs can be permethylated and identified by gas chromatography and mass spectrometric analysis[8]. Glucuronide metabolites of menadione[9], 5,5-diphenylhydantoin[10], phenyramidol[11], methocarbamol[12] and other drugs have been identified by this technique. The present observations establish that carbamazepine is metabolized to an N-glucuronide by the liver of the rat. This metabolite is excreted in the bile and is likely to be hydrolyzed by glucuronidases in the gastrointestinal tract. This would release the parent drug which could therefore be reabsorbed and undergo extensive enterohepatic recirculation. Carbamazepine occasionally produces serious and sometimes fatal side effects in the blood including agranulocytosis and aplastic anemia. These abnormalities may be caused by the parent compound or a toxic metabolite produced in the liver. Intestinal bacteria may also modify the drug during enterohepatic recirculation[13] and lead to the formation of toxic biotransformation products. It is therefore necessary to study the metabolic profile of carbamazepine in normal subjects and those who develop adverse reactions in order that the latter may be predicted or avoided.

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3-Hydroxy-3-Methylglutaric Acid and Experimental Atherosclerosis in Rats

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Summary. In rats 3-hydroxy-3-methylglutaric acid effectively counteracts the lipemic and atherosclerotic response of massive doses of vitamin D_2. It regressed the formation of atheromatous arterial lesions. Furthermore the significant decrease in serum β-lipoprotein levels on HMG treatment could be due to decrease in VLDL triglyceride and cholesterol levels.

Hypolipidemic properties of 3-hydroxy-3-methylglutaric acid (HMG) have been shown in rats[3,4], rabbits[5,6], and man[7,8]. The experiments in rabbits indicated that HMG decreased lipid levels in serum, liver and aorta and also prevented the atheromatous plaque formation, when they were fed atherogenic diet[9]. More recently, atherosclerosis has been produced in rats supposed to be highly resistant to atherosclerosis by intubating a mixture containing olive oil, vitamin D_2 and cholesterol[10,11]. In view of these findings, and also of the fact that no definite information is available about the hypolipidemic compounds and severity of atherosclerosis in rats, we report now that HMG prevents the severity of atherosclerosis in rats also.

Materials and methods. 15 male albino rats (stock colony of Indian Veterinary Research Institute, India) weighing about 160 g were divided into 3 equal groups. They were maintained on Hind Lever basal diet (Hindustan Lever Co., India) and received by gastric intubation 1.5 ml olive oil mixture containing per ml: 8 mg vitamin D_2 320,000 I.U. (E. Merck, Germany) and 40 mg cholesterol/kg body weight for 5 consecutive days as described by Altman[10]. The 1st group receiving 1 ml saline i.p. served as control group. Each animal of the 2nd and 3rd groups received i.p. HMG at the concentration of 25 and 50 mg/kg body weight respectively in 1 ml saline. After 5 days treatment, the animals were fasted overnight, ether anaesthetized, blood was withdrawn by cardiac puncture and serum obtained by centrifugation. The methods of extraction and tissue lipid analysis were as described in a previous publication[6]. Serum β-lipoproteins were estimated by the method of Voelker[12]. Atheromatous arterial lesions were visually graded on 0–4 scale.

Results. The data shown in the Table confirm the findings of Altman[10] that a mixture of vitamin D_2 and cholesterol dissolved in olive oil was not only able to

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Effect of HMG on tissue lipids of atherosclerotic rats (mean ± SE)

Lipids	Group I	HMG-treated groups			
		Group II		Group III	
Serum (mg/100 ml)					
Cholesterol	225 ± 9	215 ± 8 (4)[a]	—	140 ± 9 (38)	$p < 0.01$
Triglyceride	265 ± 12	202 ± 10 (24)	$p < 0.05$	159 ± 13 (40)	$p < 0.01$
Phospholipid	300 ± 14	240 ± 8 (20)	$p < 0.05$	225 ± 11 (25)	$p < 0.05$
Liver (mg/g)					
Cholesterol	3.5 ± 0.2	3.2 ± 0.1 (9)		2.5 ± 0.1 (30)	$p < 0.01$
Triglyceride	8.9 ± 0.8	8.1 ± 0.8 (8)		5.6 ± 0.7 (37)	$p < 0.01$
Phospholipid	15.8 ± 1.3	15.9 ± 1.0 —		13.2 ± 1.1 (17)	$p < 0.05$
Aorta (mg/g)					
Cholesterol	2.6 ± 0.2	2.5 ± 0.2 (4)		2.0 ± 0.1 (22)	$p < 0.02$
Triglyceride	6.3 ± 0.3	5.9 ± 0.6 (6)		4.7 ± 0.2 (26)	$p < 0.05$
Phospholipid	10.5 ± 1.2	10.1 ± 1.6 (4)		8.9 ± 2.5 (15)	—
Serum (mg/100 ml)					
β-lipoproteins	175 ± 8	135 ± 6 (23)	$p < 0.05$	110 ± 6 (37)	$p < 0.01$

[a] The values in parenthesis indicate percent change with respect to group I (control values).

produce hyperlipidemic condition in serum and aorta but also formed atheromatous plaque in rats. Except in the case of cholesterol, 25 mg HMG/kg significantly lowers serum triglyceride and phospholipid levels. There was little or no lipid lowering effect of HMG observed in liver and aorta. All serum, liver and aorta lipids except aortic phospholipids were significantly decreased by administration of 50 mg HMG/kg. Serum β-lipoprotein levels were also significantly decreased at both concentrations of HMG.

On gross examination, the animals of group I (control) had visible atheromatous lesions scattered throughout the aorta, but prominently at aortic arch (++++). The atheromatous arterial lesions regressed most effectively in group III rats treated with 50 mg HMG/kg (+). However, the animals of group II had visible lesions much closer to group I (++++). The animals remained active throughout the experimental period. The weights of treated animals were same as that of control animals.

Discussion. The results obtained show that HMG at 50 mg/kg concentration effectively counteracts the lipemic and atherosclerotic response of massive doses of vitamin D_2. Animals receiving 25 mg HMG/kg significantly decreased serum triglyceride and phospholipid levels to the extent of 24 and 20% respectively. Serum β-lipoproteins were also significantly decreased at both concentration of HMG. The level of lipid parameters did not return to normal value (unpublished observation) even with 50 mg HMG/kg. This could be due to short duration of treatment. The serum cholesterol, liver and aorta lipid did not record any significant change on 25 mg HMG/kg treatment. This could be attributed to lesser dose of HMG.

HMG inhibits biosynthesis of cholesterol[13,14]. The maximum decrease in triglyceride and cholesterol levels of HMG treatment could be due to decrease in VLDL triglyceride and cholesterol levels as a significant decrease was observed in serum β-lipoprotein levels.

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Antagonism of Prostaglandin-Induced Cyclic AMP Accumulation in the Rat Anterior Pituitary in vitro by Somatostatin Analogues

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Summary. The PGE_2-induced cyclic AMP accumulation in the rat anterior pituitary in vitro is inhibited by [desamino[1]]-, [desamino[1]] [descarboxy[14]]- and [D-Lys[4]]-somatostatin similarly to somatostatin, while the [descarboxy[14]]-somatostatin exhibits reduced activity; [D-Lys[9]]-somatostatin is ineffective at a higher concentration.

Prostaglandins cause release of growth hormone in the rat[2–5] and bovine[6,7] anterior pituitaries in vitro. In vivo the plasma growth hormone levels are increased by administration of prostaglandin in the rat[8], sheep[9] and man[10]. Cyclic AMP appears to be a mediator in the release as prostaglandins increase both the accumulation of cyclic AMP and the release of growth hormone in the rat[5] and bovine[7] anterior pituitaries in vitro. Somatostatin, a tetradecapeptide (H-Ala-Glys-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH) inhibits the release of immunoreactive growth hormone in vitro and in vivo[11,12].