A New N-Glucuronide Metabolite of Carbamazepine[1]

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Summary. An N-glucuronide metabolite of carbamazepine was identified in the bile of the isolated perfused rat liver by means of permethylation, gas chromatography and mass spectrometry.

Carbamazepine (5*H*-dibenz [*b*, *f*]azepine-5-carboxamide) is currently used in clinical medicine as an antiepileptic agent and for the treatment of trigeminal and glossopharyngeal neuralgia. The metabolic fate of the drug has been studied in man and the rat. The disposition of the entire dose of carbamazepine, however, remains to be established in both species. The biotransformation products of carbamazepine which have been identified in urine account for $< 50\%$ of the administered dose. Carbamazepine-10, 11-epoxide was identified as a urinary metabolite of the drug in 1972[3]. Subsequently it was reported that 10, 11-dihydro-10, 11-dihydroxy-5*H*-dibenz [*b*, *f*]-azepine-5-carboxamide was a urinary metabolite of carbamazepine in man and the rat[4]. The latter workers also provided evidence that 10, 11-dihydro-10-hydroxy-5*H*-dibenz [*b*, *f*] azepine-5-carboxamide may be a metabolite of the drug. They administered ^{14}C-carbamazepine to rats and reported that 27% of the radiolabel appeared in the urine in 48 h. Iminostilbene was also reported as a minor (2% of the dose) urinary metabolite of carbamazepine in the rat[5]. Recently, it was reported that when rats were given carbamazepine-10, 11-epoxide, i.p., iminostilbene-10, 11-epoxide and iminostilbene-10, 11-dihydrodiol were excreted in the urine[6]. The latter workers therefore inferred that these iminostilbene metabolites were also metabolites of carbamazepine.

Methods. The isolated perfused rat liver was prepared as previously described[7]. Control bile was collected for about 15 min and then 50 mg of carbamazepine was added to the perfusate. The perfusion was continued for about 2 h until approximately 1 ml of bile had been collected. A 50 µl aliquot of bile was evaporated to dryness under a stream of nitrogen and the residue permethylated with DMSO$^-$ (sodium salt) and methyl iodide as previously described[8]. The permethylated mixture was dissolved in 25 µl of chloroform for gas chromatography and mass spectrometry. Gas chromatography was carried out on a Varian 2700 instrument equipped with a $5' \times 1/8''$ stainless steel column packed with 3% OV 210 on Chromosorb W (HP), 80/100 mesh. The column oven temperature was programmed from 150° to 275° at 6°/min. The injector and detector temperatures were 240° and 270°C, respectively, and the nitrogen flow rate was 30 ml/min. At 275° the instrument was run isothermally for 5 min.

The mass spectra were obtained on a Dupont, Model 21-491 B, gas chromatograph/mass spectrometer under the following conditions: accelerating potential, 1,8 kV, ionizing potential, 70 eV, trap current = 300 µA, source temperature, 200°. A $6' \times 2$ mm (internal diameter) glass column packed as described above was used for the gas

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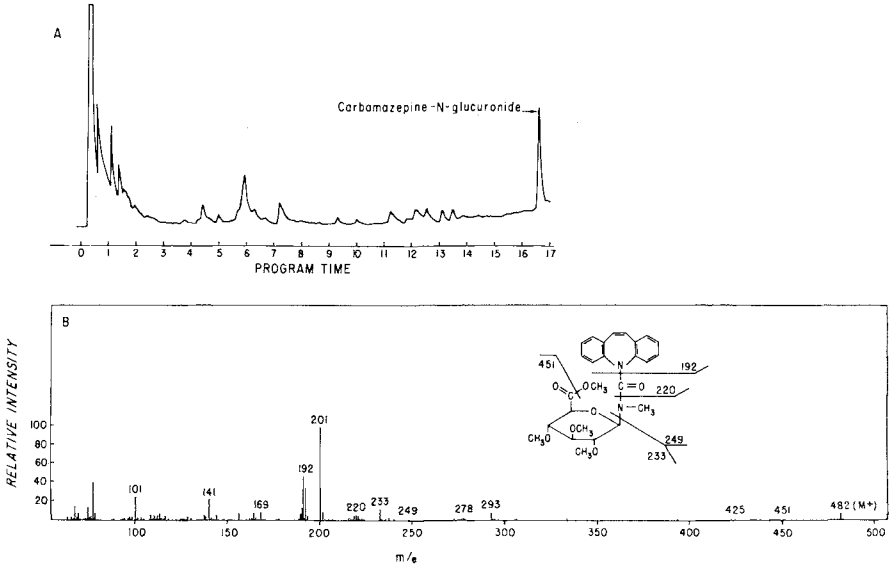

A) The chromatogram of an extract of bile which was permethylated shows the N-glucuronide of carbamazepine. B) The mass spectrum and fragmentation pattern of the N-glucuronide of carbamazepine after permethylation.

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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