the first hour after an anesthetic dosage of pentobarbital. 5-HT was significantly elevated in only two areas of the rat brain. Therefore, it is not surprising that Efron and Gessa [7] found no significant effect of a single dosage of pentobarbital on 5-HT content in the whole rat brain. These data provide a strong argument for the study of the effects of drugs on brain biogenic amines in discrete brain areas rather than in the whole brain.

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Transformation of Δ^1-tetrahydrocannabinol (THC) by rabbit liver microsomes

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The major transformation products of Δ^1-tetrahydrocannabinol (Δ^1-THC) by rabbit liver have been reported by Nilsson *et al.* [1] and Wall [2]. They found that subcellular fractions of rabbit liver readily metabolized Δ^1-THC to 7-hydroxy-Δ^1-THC [1, 2], 6β-hydroxy-Δ^1-THC [2] and 6α,7-dihydroxy-Δ^1-THC [2]. In order to provide a more complete picture of the biotransformations in the rabbit, we have reinvestigated this process with a view toward identifying some of the less abundant metabolites. This report describes the isolation and identification of two pharmacologically active metabolites.

Female albino rabbits approximately 6 months old were used as the tissue source. The livers were homogenized and the microsomal fraction was obtained as previously described by Burstein and Kupfer for the rat [3]. ^{14}C-Δ^1-THC was obtained from the National Institute on Drug

Table 1. Metabolism of Δ^1-THC by rabbit liver microsomes

T.l.c. zone	*Rf*	Assignment*	Retention time (min)	Principal ions† (M/e)
1	0·67	Δ^1-THC acetate		
2	0·40	1,2α-Epoxyhexahydrocannabinol acetate	5·7	372(25), 357(25), 330(50), 315(75), 312(45) 298(100), 288(55), 274(75), 231(8·8)
		6α-Hydroxy-Δ^1-THC diacetate	7·0	372(3·4), 354(100), 339(21), 312(82), 297(65) 295(18)
3	0·30	7-Hydroxy-Δ^1-THC diacetate	7·5	372(3·8), 354(43), 312(100), 297(28), 259(31)
4	0·13	6α,7-Dihydroxy-Δ^1-THC triacetate	11·3	412(34), 397(5), 370(9), 355(12), 337(47) 310(46), 295(100)

* All materials were acetylated prior to t.l.c. with a mixture of acetic anhydride and pyridine. T.l.c. system: Silica gel G, hexane–ether (7:3).

† The spectra were obtained on a Finnegan 1015 at 70 eV. The column conditions were: 2 ft, 2% OV-1; 180–240° (8°/min); carrier gas, He; injector temp., 255°. Numbers in parentheses refer to relative intensities.

Abuse and was purified to 99 per cent by thin-layer chromatography (t.l.c.) using a hexane–acetone (4:1) system. The incubation conditions were based on those previously reported [3], and the products were extracted and isolated in the same manner as those from a similar study in the mouse [4]. Briefly, this involved exhaustive solvent extraction with ethyl acetate of the incubation media, separation into radioactive zones by t.l.c. and isolation and identification of the metabolites by gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.). The results are summarized in Table 1.

More than 70 per cent of the recovered radioactivity consisted of unchanged Δ^1-THC. This is in marked contrast to the extensive conversions previously observed by us in the rat and mouse [4]. The known metabolites, 7-hydroxy-Δ^1-THC and 6α,7-hydroxy-Δ^1-THC, were found in t.l.c. zones 3 and 4 respectively; their chromatographic mobilities and GLC–MS properties were identical to those of authentic standards.*

Zone 2 was further separated by g.l.c. into two monooxygenated metabolites of Δ^1-THC. Comparison with standards showed that the more mobile peak was 1,2α-epoxyhexahydrocannabinol [5, 6] and the other was 6α-hydroxy-Δ^1-THC.† The epoxide has been previously reported in only one species, the squirrel monkey [5], while the other product has been found in the rat and mouse [4] and in human plasma [7]. Both of these metabolites showed activity in the rhesus monkey [6, 8] and, in addition, 6α-hydroxy-Δ^1-THC showed some activity in the mouse [7].

Our finding that the epoxide is not a unique metabolite of the squirrel monkey suggests that this metabolic route may also exist in man. It seems unlikely that a reactive species such as an epoxide would be a final product, raising the question of further transformation products being formed *in vivo*. Since epoxides are believed to react with macromolecules such as proteins [9], one can speculate on the occurrence of such a process here. This could give rise to immunologically reactive substances, as has been postulated by Nahas *et al.* [10], and may also account for the unextractable products which have been observed in various experiments *in vitro*.‡ In fact, Willinsky *et al.* [11] have also reported non-extractable binding of metabolites to tissue constituents possibly involving epoxides.

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* Obtained from the National Institute on Drug Abuse.

† A sample of the epoxide was prepared using the procedure in reference 6; authentic 6α-hydroxy-Δ^1-THC was obtained from the National Institute on Drug Abuse.

‡ Unpublished results from this laboratory.

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Effect of penicillamine on some metals and metalloproteins in the rat

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In an attempt to gain a better understanding of the mechanism of copper exchange between plasma proteins and cellular compartments of rat tissues, we have altered the mobilization and the excretion of this metal by administration of penicillamine, the chelating agent commonly used in Wilson's disease. In humans, D-penicillamine (D-PAM) is well tolerated even in cases of prolonged treatment. The substance is not cytotoxic although, some side effects, ascribed to allergic, idiosyncratic or immunological mechanisms, have been reported [1].

In this study we have determined the extent to which copper, zinc and iron are removed by treatment with D-PAM. In addition, we have checked if the activity of cytoplasmic superoxide dismutase (SOD) and serum ceruloplasmin are affected by depletion of copper and zinc.

Male Wistar rats weighing about 300 g were kept in metabolic cages made of plexiglas and fed dried pellets of standard composition. Each rat was injected intramuscularly with 15 mg/100 g body wt of D-PAM at intervals of 12 hr for 20 days. Control animals were kept under the same environmental conditions. Twenty animals were used in each group.

Copper, zinc and iron in urine and in homogenized tissues were estimated by atomic absorption (Perkin Elmer model 300) after oxidation with nitric and perchloric acids.

SOD activity in the supernatant obtained from homogenates after centrifugation at 105,000 *g* for 4 hr was determined according to Beauchamp and Fridovich [2]. The oxidase activity of ceruloplasmin was estimated using *p*-phenylendiamine according to Ravin [3], and D-PAM in the blood was estimated by the method of Pal [4]. Water was deionized on Amberlite resins and checked