TABLE 2. IMIPRAMINE INHIBITION OF LUNG, LIVER AND BRAIN MONOAMINE OXIDASE*

Imipramine conen (M)	Lung	Liver	Brain	% Inhibition		
	(nmoles 5-HIAA formed)			Lung	Liver	Brain
None	0·604	0·237	0·608			
1×10^{-5}	0·598	0·224	0·604	1·0	5·5	0·7
5×10^{-5}	0·518	0·226	0·600	14·2	4·6	1·3
5×10^{-4}	0·241	0·130	0·372	60·0	45·2	38·8

* Reactions were initiated with the addition of lung, liver or brain 600 *g* supernatant solution. Final protein concentration was 4·2, 11·0 and 11·8 mg protein/ml respectively. Reactions were incubated for 15 min at 37°.

The clinical relevance of the findings presented in this paper remains to be determined. However our data raise the possibility that tricyclic antidepressant drugs may act in man not only by preventing uptake of biogenic amines by brain neurones but may also prevent loss of these monoamines by inhibiting monoamine oxidase.

Departments of Anesthesiology and Pharmacology,
Yale University School of Medicine,
New Haven, Conn. 06510, U.S.A.

JEROME A. ROTH
C. N. GILLIS

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Blood and brain levels of Δ^1-tetrahydrocannabinol in mice
The effect of 7-hydroxy-Δ^1-tetrahydrocannabinol

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IN A PREVIOUS paper,[1] it was reported that after intravenous injection of ^{3}H-7-hydroxy-Δ^1- tetrahydrocannabinol (^{3}H-7-hydroxy-Δ^1-THC) into mice, a non-polar component was found in the blood extract, but to a much lesser extent in the brain extract. Both thin-layer and gas liquid chromatography indicated

that this material was a mixture of Δ^1-THC and cannabinol, but no specific identification was carried out. The possibility that this material originated from the injection solution was discounted because the ^{3}H-7-hydroxy-Δ^1-THC was shown to contain less than 3% of Δ^1-THC.

In the present work, the origin of this material was investigated further, and the major component was identified by combined gas–liquid chromatography and mass spectroscopy (GLC–MS).

MATERIALS AND METHODS

Drugs and reagents. The syntheses of both labelled and unlabelled 7-hydroxy-Δ^1-THC and ^{3}H-Δ^1-THC have been reported previously.[1,2] 7-Hydroxy-Δ^1-THC was repurified by the following method in order to remove any traces of Δ^1-THC. An aliquot (containing about 1 mg of 7-hydroxy-Δ^1-THC) of the stock solution was concentrated in a rapid stream of nitrogen and the resulting concentrate was streaked on to a strip of Whatman SG81 silica-impregnated paper (5 × 25 cm) followed by development in 1% v v methanol in chloroform. Visualisation was by spraying a 3 mm strip from each side of the chromatogram with *ca.* 1% aqueous Fast Blue B, and (for the tritiated sample) scanning the chromatogram for radioactivity on a Panax radiochromatogram scanner, type RTLS-1.

Liquid scintillation counting. Tritium was measured on a Beckman LS200B instrument using a scintillator of butyl PBD and naphthalene in dioxan.[2] Counting efficiencies were obtained using standard [^{3}H]-hexadecane from the Radiochemical Centre, Amersham.

Combined gas–liquid chromatography and mass spectroscopy. GLC–MS analyses were carried out on a Pye 104 gas chromatograph connected via a gas separator to an AEI MS9 mass spectrometer. A column consisting of 1% CDMS on 80–100 mesh siliconised diatomite C was used at an oven temperature of 190 with a helium flow rate of 40 ml/min.

Blood and brain levels of radioactivity. The methods employed in this work were essentially those reported in a previous paper.[3] Male mice (23–27 g, Tuck strain No. 1, obtained from Tuck, Rayleigh, Essex) were injected directly into the tail vein with ^{3}H-Δ^1-THC (10, 20, 100 or 1000 μg/kg), either with or without repurified 7-hydroxy-Δ^1-THC (1 mg/kg), as a suspension with Tween-80 in saline. Control mice were similarly injected with either "crude" or repurified ^{3}H-7-hydroxy-Δ^1-THC (1 mg/kg). The volume of solution injected was approximately 0·2 ml. Each mouse was killed with carbon monoxide 20 min after injection. A sample of blood was removed by intracardiac puncture, followed by removal of the whole brain, which was weighed, rinsed and homogenized in 0·1 M phosphate buffer (pH 7·4). An aliquot of the blood was lysed in the same buffer and extracted with purified ethyl acetate, while another was dried on filter paper, and combusted in oxygen over water. Samples of the ethyl acetate extract and the water from the combustion were assayed for tritium by scintillation counting. An aliquot of the brain homogenate was digested with hyamine hydroxide and the digestate counted for radioactivity. A second aliquot was extracted with purified ethyl acetate and the extract counted. Samples of both the brain and blood extracts were chromatographed as described previously.[3]

The blood extracts of the mice injected with "crude" ^{3}H-7-hydroxy-Δ^1-THC were pooled, derivatised[1] and the major radioactive component was identified by GLC–MS.

RESULTS AND DISCUSSION

The major component of the "mobile metabolite" reported previously[1] was identified, after purification by paper chromatography, by GLC–MS as Δ^1-THC. The ^{3}H-7-hydroxy-Δ^1-THC which was injected contained less than 3% ^{3}H-Δ^1-THC, and if the percentage retention in the blood was the same as that after injection of 2 mg/kg Δ^1-THC, this small amount would have produced blood levels about 7–10 times lower than those found. However, although less polar metabolites of Δ^1-THC have been found in the mouse,[4] the reversal of the metabolism to 7-hydroxy-Δ^1-THC seemed unlikely as *in vivo* dehydroxylations are rare.[5] As a final check therefore, a batch of both labelled and unlabelled 7-hydroxy-Δ^1-THC was rechromatographed to remove all traces of Δ^1-THC. The resulting 7-hydroxy-Δ^1-THC contained no detectable Δ^1-THC (not more than 0·1 per cent estimated by GLC or by paper chromatography followed by scintillation counting). The chromatograms of the blood extracts of mice injected intravenously with this material (1 mg/kg) differed (Fig. 1) markedly from those obtained from mice injected with the same dose of the non-repurified material,[1] about 85 per cent of the "mobile metabolite" having been eliminated. The remaining mobile material was presumably that tentatively identified as cannabinol,[1] but insufficient was available for conclusive identification. The ratio of blood to brain levels of 7-hydroxy-Δ^1-THC was about 1:8, so that plasma levels of the metabolite must be regarded as only a poor measure of the brain levels.

In order to determine whether the high percentage of Δ^1-THC which remained in the blood of mice injected with "crude" 7-hydroxy-Δ^1-THC was due to the low dose ($\leqslant$3 μg/kg) or to the presence of the large amount of the primary metabolite, one group of mice was injected with ^{3}H-Δ^1-THC (20, 50, 100 or 1000 μg/kg) and another with ^{3}H-Δ^1-THC (10, 50, 100 or 1000 μg/kg) together with 7-hydroxy-Δ^1-THC (1 mg/kg) and their blood and brain levels were measured. For mice injected with ^{3}H-Δ^1-THC alone, the percentage of the injected dose which remained per gram of brain tissue 20 min after administration was

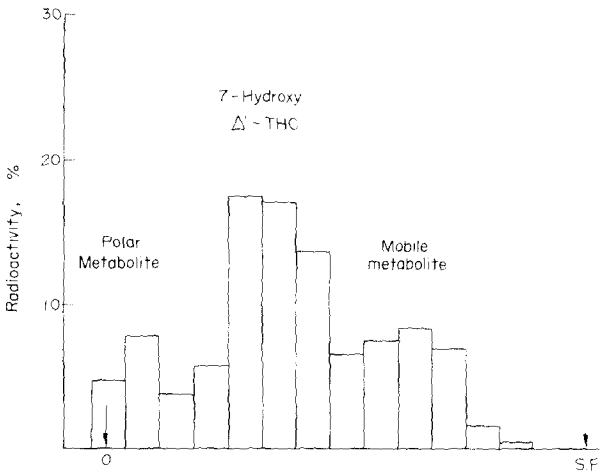

FIG. 1. Chromatogram of mouse blood extract 20 min after injection of ^{3}H-7-hydroxy-Δ^1-THC (Δ^1-THC-free, 1 mg/kg i.v.).

almost independent of the dose given, while the percentage remaining per gram of blood increased slightly with decreasing dose (Fig. 2). The magnitude of this effect was, however, insufficient to completely account for the high blood levels after administration of "crude" ^{3}H-7-hydroxy-Δ^1-THC. It may be speculated that the relative increase in the blood levels was due to the presence in the blood of two binding sites for Δ^1-THC: a high affinity, low capacity site and a lower affinity, higher capacity site. This has also been suggested[6] for synaptosomes, to explain the variation with concentration of Δ^1-THC of its partition ratio between synaptosomes and water. In contrast to these results, it was found that both the relative blood and brain levels of ^{3}H-Δ^1-THC were greatly elevated when that compound was injected together with a large dose (1 mg/kg) of 7-hydroxy-Δ^1-THC. The effect increased with decreasing dose, and at all doses the blood levels were higher than the brain levels (Fig. 2). In addition to the elevation of the Δ^1-THC

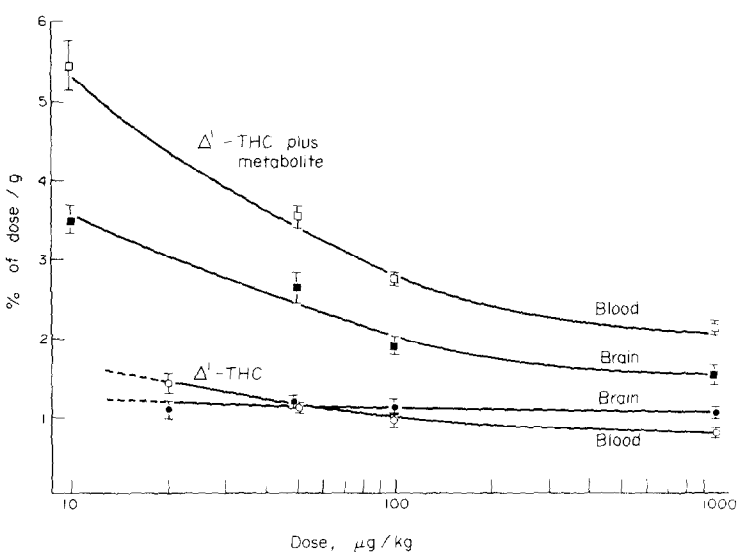

FIG. 2. Blood and brain levels (expressed as the percentage of the injected dose per 1 g material) of Δ^1-THC as a function of the dose of ^{3}H-Δ^1-THC i.v., either administered alone (○ or ● respectively) or together with 7-hydroxy-Δ^1-THC (1 mg/kg, □ or ■ respectively). All measurements were taken 20 min after injection, and are the mean readings from three mice (± S.E.M.).

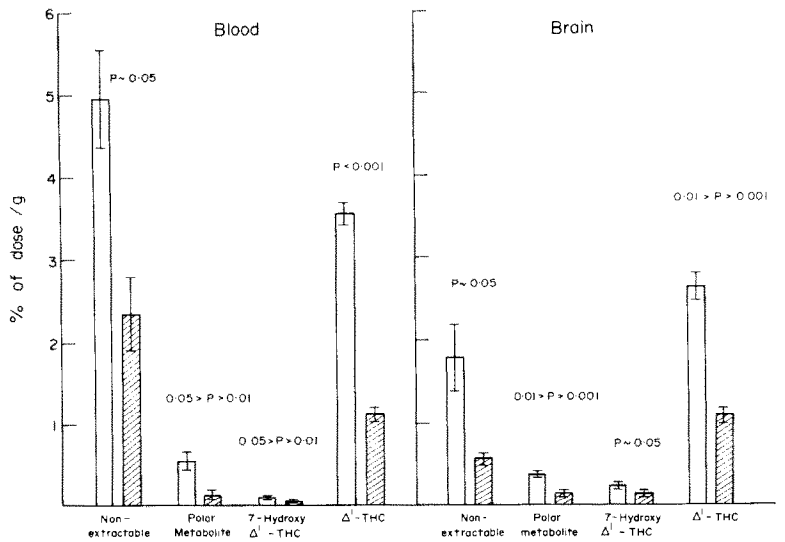

FIG. 3. Blood and brain levels (expressed as the percentage of the injected dose per 1 g material) of all components after injection of ^{3}H-Δ^1-THC (50 μg/kg i.v.) either administered alone (hatched) or together with 7-hydroxy-Δ^1-THC (1 mg/kg, open). The values are the means from three mice ($\pm$S.E.M.). P values were obtained by Student's *t*-test.

percentages, the levels of all other radioactive materials present in the brain and blood were increased to a significant degree (Fig. 3). Although the inhibition of the metabolism of the Δ^1-THC by the 7-hydroxy-Δ^1-THC would have produced an elevation in the Δ^1-THC levels, the increase in the levels of the polar metabolite and non-extractable material show that either the inhibition is at a very late stage in the metabolism chain, or that there is competition for a common tissue binding site. The elevation of the brain concentrations of both active cannabinoids (Δ^1-THC and 7-hydroxy-Δ^1-THC) at doses within the normal effective range suggests that if a non-psychoactive drug with similar tissue binding characteristics were administered together with Δ^1-THC, the effect of the latter might be potentiated. Such a situation might arise in the context of multiple drug abuse.

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University Department of Pharmacology,
South Parks Road,
Oxford, England

EDWARD W. GILL
DAVID K. LAWRENCE

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