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# CONTRIBUTION OF THE METABOLITE 7-HYDROXY-$\Delta^1$-TETRAHYDROCANNABINOL TOWARDS THE PHARMACOLOGICAL ACTIVITY OF $\Delta^1$-TETRAHYDROCANNABINOL IN MICE

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**Abstract**—Tritium-labelled 7-hydroxy-$\Delta^1$-tetrahydrocannabinol ($^3$H-7-hydroxy-$\Delta^1$-THC, specific activity 571 mCi/mmole) was prepared from $^3$H-$\Delta^1$-THC by oxidation with a rat liver microsome preparation. Brain levels of 7-hydroxy-$\Delta^1$-THC and $\Delta^1$-THC in mice were measured 20 min after intravenous injection of either $\Delta^1$-THC (2·0, 1·0 and 0·5 mg/kg) or 7-hydroxy-$\Delta^1$-THC (1·0, 0·5 and 0·25 mg/kg) and correlated with the inhibition of spontaneous motor activity. A theoretical dose–response relationship for $\Delta^1$-THC in the absence of the metabolite was derived on the assumption of additivity of the behavioural effects due to $\Delta^1$-THC and 7-hydroxy-$\Delta^1$-THC present together in the mouse brain. The theoretical dose–response line for $\Delta^1$-THC and that obtained experimentally for 7-hydroxy-$\Delta^1$-THC were parallel; on the basis of brain concentrations, 7-hydroxy-$\Delta^1$-THC was found to be more potent than $\Delta^1$-THC in producing behavioural changes and the calculated equipotent molar ratio was 7·1. The ratio of the concentrations of $\Delta^1$-THC and 7-hydroxy-$\Delta^1$-THC in the mouse brain 20 min after intravenous injection of $\Delta^1$-THC was 5·3 and the contribution of the metabolite to the overall behavioural effect was calculated as 55–63 per cent. Although metabolites of 7-hydroxy-$\Delta^1$-THC accounted for only about 10 per cent of the radioactivity present in the mouse brain 20 min after intravenous injection of $^3$H-7-hydroxy-$\Delta^1$-THC, about 50 per cent of the radioactivity in the blood was present as a chromatographically more mobile material which has not yet been identified.

(−)-$\Delta^1$-TETRAHYDROCANNABINOL ((−)-$\Delta^1$-THC), the major psycho-active constituent of cannabis,[1,2] is rapidly metabolized in the liver to 7-hydroxy-$\Delta^1$-THC by several species including man,[3–6] and it has been suggested[7,8] that the psychopharmacological effects of $\Delta^1$-THC are caused by the primary metabolite rather than by the parent compound. The pharmacological activity of 7-hydroxy-$\Delta^1$-THC has been established[3,5,9] but no clear estimate has been made of the contribution the metabolite makes towards the behavioural effects which follow administration of $\Delta^1$-THC to laboratory animals. Previous work in this laboratory[10] has shown that the reduction in spontaneous motor activity of mice injected intravenously with $\Delta^1$-THC correlates equally well with the brain concentrations of both $\Delta^1$-THC and 7-hydroxy-$\Delta^1$-THC and it is thus impossible to deduce the pharmacological significance of the metabolite from experiments with $\Delta^1$-THC alone. The use of metabolic inhibitors such as SKF 525A does not resolve the question since these do not eliminate the metabolite from the C.N.S.[10]

In this paper we describe the preparation of tritium-labelled 7-hydroxy-$\Delta^1$-THC and the correlation of brain levels of this compound with immobility index[11] after intravenous administration to mice. By comparison with results obtained after similar experiments with $^3$H-$\Delta^1$-THC, an estimate could be made of the true relative potencies

of $\Delta^1$-THC and its metabolite in the mouse brain and hence the degree to which 7-hydroxy-$\Delta^1$-THC is responsible for the behavioural changes produced by injection of $\Delta^1$-THC.

## MATERIALS AND METHODS

*Drugs and reagents.* The synthesis of $^3$H-$\Delta^1$-THC (571 mCi/mmole) has been described elsewhere.[12] Unlabelled $\Delta^1$-THC was isolated from the natural material.[13] Unlabelled 7-hydroxy-$\Delta^1$-THC was prepared as described below for the labelled material, confirmation of its identity being obtained by ultra-violet spectroscopy: $\lambda_{max}$ 282 nm ($\epsilon$ 1700), 276 nm ($\epsilon$ 1760) in carbon tetrachloride; and nuclear magnetic resonance spectroscopy (100 MHz): $\tau$ 9·21 (*t*, terminal methyl), 9·04, 8·72 (*s*, gem-dimethyl groups), 7·7 (*t*, methylene adjacent to aromatic ring), 6·87 (*d*, single proton at $C_3$), 6·07 (*s*, two hydroxymethyl protons), 4·02, 3·88 (*s*, aromatic protons), 3·39 (*s*, vinylic proton) in carbon tetrachloride. Di-isopropyl ether was purified by shaking with aqueous ferrous sulphate solution and distilled water, then dried with saturated brine and magnesium sulphate followed by distillation from sodium. Petroleum spirit (b.p. 60–80°) was redistilled before use and ethyl acetate was purified as described previously.[10]

*Liquid scintillation counting.* Tritium was measured with a Beckman LS 200B instrument using a scintillator solution of butyl PBD and naphthalene in dioxan.[10] Counting efficiencies were obtained using standard $^3$H-hexadecane from the Radiochemical Centre, Amersham.

*Gas–liquid chromatography.* Samples were run as their trimethylsilyl ethers, which were formed by standing the material with an excess of bis-(trimethylsilyl)-trifluoroacetamide[14] for 1 hr at room temperature after vigorous mixing, then removing the excess reagent in a stream of nitrogen. Analysis was carried out using a Pye 104 Gas Chromatograph, with a column consisting of 1% CDMS on 80–100 mesh siliconized diatomite C at a temperature of 225° with an argon flow rate of 20 ml/min.

When radioactive samples were chromatographed, the radioactivity (as tritiated water) was collected in fractions by passing the effluent gas through scintillator solution. When the flame-ionization detector indicated the presence of a peak, a fraction was taken to include the entire peak. All other fractions were of approximately the same length to mimimize baseline variation. A trapping efficiency of about 25 per cent was obtained by this method. Each fraction was then assayed by liquid scintillation counting.

*Preparation of $^3$H-7-hydroxy-$\triangle^1$-THC.* $^3$H-7-hydroxy-$\triangle^1$-THC was prepared from pure $^3$H-$\Delta^1$-THC by aerobic incubation with rat liver microsomal fraction in the presence of an $NADPH_2$ regenerating system.[9] No pretreatment with phenobarbitone was given, since small-scale trials indicated that this did not affect the yield of 7-hydroxy-$\Delta^1$-THC after 3 hr incubation.

Six male Wistar rats (obtained from Tuck, Rayleigh, Essex), each weighing 200–250 g, were sacrificed and their livers removed. The finely chopped liver (53 g) was homogenized with ice-cold 0·1 M phosphate buffer (530 ml, pH 7·4) containing 0·013 M magnesium chloride. The homogenate was centrifuged for 10 min at 10,000 *g*. To the supernatant thus obtained was added glucose-6-phosphate (1·36 g), nicotinamide–adenine dinucleotide phosphate (500 mg), glucose-6-phosphate dehydrogenase (125 units) and $^3$H-$\Delta^1$-THC (100 mg) as a suspension with Tween-80 (200 mg) in saline (20

ml). The mixture was incubated aerobically, with shaking, for 3 hr at 37°. The incubation mixture was then extracted with ethyl acetate (3 × 200 ml) and the combined extracts dried with saturated brine followed by magnesium sulphate. Chromatography of an aliquot of the dried extract on Whatman SG 81 paper in 1% v/v methanol in chloroform, followed by scintillation counting indicated that approximately 40 per cent of the radioactivity in the extract was present as $^3$H-7-hydroxy-$\Delta^1$-THC.

Repeated distillation of a sample of the aqueous layer, each time from previously uncontaminated glassware, followed by scintillation counting of the water indicated that about 1 per cent of the tritium label had been converted to tritiated water by microsomal action on the 1- and 2-positions of the *n*-pentyl side chain.

After filtration and removal of the solvent the extractable material was purified by column chromatography on Florisil (23 g), eluting initially with 20% diethyl ether in petroleum spirit (b.p. 60–80°). Fractions of eluate were monitored for cannabinoids by spotting on filter paper and spraying with 1% aqueous Fast Blue B. Fractions which gave a positive reaction were analysed by thin-layer chromatography (Silica gel, diethyl ether) using authentic samples of $\Delta^1$-THC and 7-hydroxy-$\Delta^1$-THC as markers. The first component off the column was $^3$H-$\Delta^1$-THC, together with a large amount of Tween.

After all the THC had been eluted, the solvent was changed to 40% ether in petroleum spirit, when the $^3$H-7-hydroxy-$\Delta^1$-THC came off rapidly. Removal of the solvent *in vacuo* left 15 mg of a white, semi-crystalline solid which was stored in a brown bottle as a solution of 50 $\mu$g/ml in benzene. The concentration of this solution was checked by scintillation counting, the specific activity being that of the original $\Delta^1$-THC.

This material was shown to be identical with the unlabelled sample of 7-hydroxy-$\Delta^1$-THC by gas-liquid chromatography, and by bioassay using the mouse catalepsy test.[11] The radiochemical purity was checked by chromatography and scintillation counting. Both this method and the GLC indicated that the purity was greater than 95 per cent, the only detectable impurity being $^3$H-$\Delta^1$-THC.

The octanol–water partition coefficient was determined as described previously[10] for $^3$H-$\Delta^1$-THC.

*Correlation of the behaviour of mice with brain levels of cannabinoids.* The methods employed in this experiment were essentially the same as those reported in a previous paper.[10] The immobility indices of male albino mice (23–27 g) were determined by means of the ring test[11] about 1·5 hr before intravenous injection of either $^3$H-$\Delta^1$-THC (2·0, 1·0 or 0·5 mg/kg) or $^3$H-7-hydroxy-$\Delta^1$-THC (1·0, 0·5 or 0·25 mg/kg). Both drugs were dispersed in physiological saline by Tween-80; the dose of Tween in all cases being 10 mg/kg. The volume of the solution injected was ca. 0·2 ml. Fifteen min after injection the immobility index of each mouse was determined by an observer who was unaware of either the drug or the dose which the mouse had received. The mouse was then killed with carbon monoxide. After withdrawal of 0·5–1·0 ml of blood by intracardiac puncture, individual whole brains were removed, weighed and then rinsed and homogenized in 0·1 M phosphate buffer (pH 7·4). Determination of the total radioactivity in the brain was carried out by digestion of an aliquot of the homogenate with hyamine hydroxide and liquid scintillation counting. The remainder of the homogenate was extracted with purified ethyl acetate[10] and the extract was counted for radioactivity and chromatographed as described previously.[10] Blood levels of radioactivity were also measured.[10] In some preliminary experiments di-isopropyl ether was used as an alternative solvent in the extraction.

## RESULTS

*Dose–response relationships for $\Delta^1$-THC and 7-hydroxy-$\Delta^1$-THC.* A dose of 1 mg/kg of 7-hydroxy-$\Delta^1$-THC given intravenously produced a peak cataleptic effect about 15 min after injection; 2 mg/kg of $\Delta^1$-THC produced a somewhat greater peak response after the same time. The effect after 7-hydroxy-$\Delta^1$-THC declined rapidly, approximately paralleling the decline after $\Delta^1$-THC, although the variation between individual mice after the former treatment was greater, except at the peak response.

About 90 per cent of the radioactivity present in the brain 20 min after intravenous injection of $^3$H-7-hydroxy-$\Delta^1$-THC (1·0, 0·5 or 0·25 mg/kg) was extractable into ethyl acetate. In general, only one major peak was visible in the chromatogram of the extractable material, accounting for approximately 80 per cent of the total extractable activity (Fig. 1). This component, with the same $R_f$ value as that of authentic 7-hydroxy-$\Delta^1$-THC, was eluted from the chromatogram with diethyl ether and converted into its trimethylsilyl derivative. GLC of this compound gave only one peak, with the same retention time as the derivative of authentic 7-hydroxy-$\Delta^1$-THC. The peak contained more than 85 per cent of the radioactivity flushed from the chromatograph.

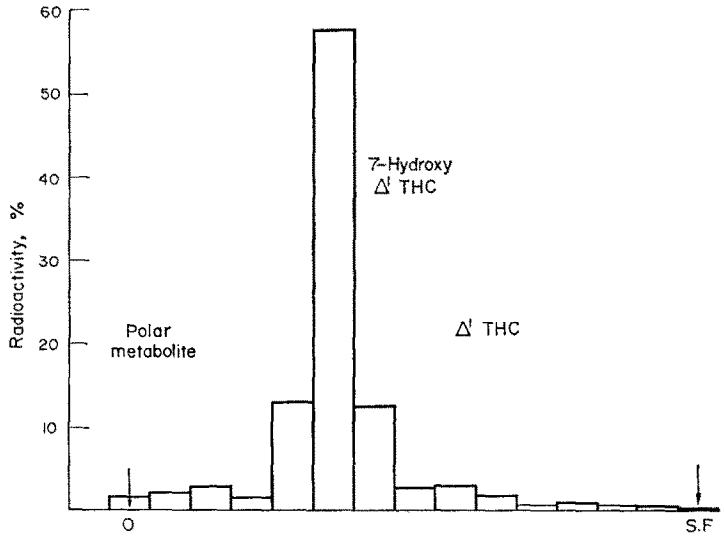


FIG. 1. Chromatogram of mouse brain extract 20 min after injection of $^3$H-7-hydroxy-$\Delta^1$-THC (1 mg/kg i.v.).

A second, less mobile, minor peak of radioactivity on the chromatogram (less than 10 per cent) was almost certainly inhomogeneous. No attempt was made to characterize this material, referred to as the "polar metabolite".

The chromatograms of the brain extracts after injection of $^3$H-$\Delta^1$-THC were similar to those reported previously[10] and had peaks of radioactivity corresponding to $\Delta^1$-THC, 7-hydroxy-$\Delta^1$-THC and the polar metabolite. In addition to those components, about 12 per cent of the total radioactivity in the brain was not extractable with ethyl acetate.

Figure 2 shows the immobility index of individual mice (determined immediately before measurement of brain levels of cannabinoids) plotted against the logarithm of

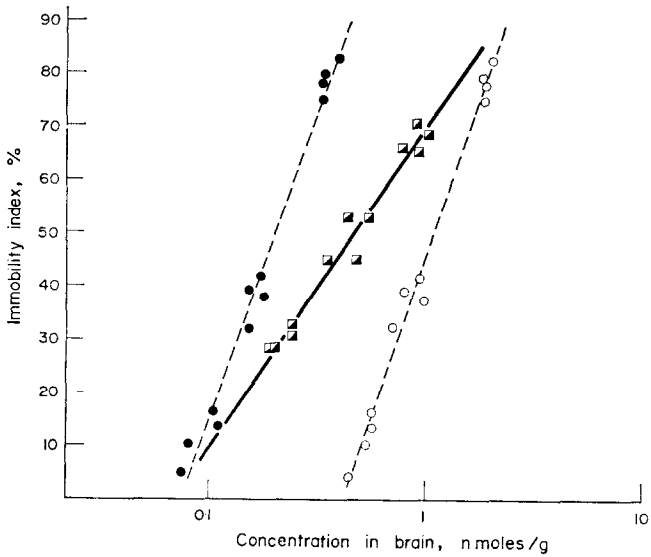


FIG. 2. Correlation of immobility index with whole brain concentration of 7-hydroxy-$\Delta^1$-THC (●– – –●) and $\Delta^1$-THC (○– – –○) after injection of $^3$H-$\Delta^1$-THC (2·0, 1·0 or 0·5 mg/kg i.v.), and of 7-hydroxy-$\Delta^1$-THC (◪——◪) after injection of that compound (1·0, 0·5 or 0·25 mg/kg i.v.). Each point represents a single mouse, the measurements being taken 20 min after injection; concentrations are plotted on a logarithmic scale.

the whole brain concentrations of (i) either 7-hydroxy-$\Delta^1$-THC or $\Delta^1$-THC after injection of $\Delta^1$-THC and (ii) 7-hydroxy-$\Delta^1$-THC after injection of that compound.

The regression lines which appear in Fig. 2 are those which give the best fit to the experimental points by the method of least squares. Only the full line (coefficient of linear regression 0·986), obtained after injection of 7-hydroxy-$\Delta^1$-THC, represents a true dose–response relationship. The dotted lines, representing immobility index against the logarithm of brain concentration of 7-hydroxy-$\Delta^1$-THC after injection of $\Delta^1$-THC (coefficient of lin. reg. 0·990) and immobility index against the logarithm of brain concentration of $\Delta^1$-THC after injection of $\Delta^1$-THC (coefficient of lin. reg. 0·994) are not true dose–response relationships, being in both cases uncorrected for the response produced by the second component. The dotted lines are parallel within the limits of experimental error and indicate that 20 min after injection of $\Delta^1$-THC, the mean molar ratio of $\Delta^1$-THC and the metabolite in the mouse brain is 5·3.

The true dose–response relation for the metabolite may be represented by the equation:

$$R = a \log [\text{7-hydroxy-}\Delta^1\text{-THC}] + b \qquad (1)$$

where $R$ is the pharmacological response in immobility index units; [7-hydroxy-$\Delta^1$-THC] is the whole brain concentration of the metabolite measured in nmoles/g of brain tissue; and $a$ and $b$ are constants. The regression gives the following equation for the dose–response relationship for the metabolite (the full line in Fig. 2):

$$R = 60{\cdot}9 \log [\text{7-hydroxy-}\Delta^1\text{-THC}] + 71{\cdot}0. \qquad (2)$$

A similar equation of the form:

$$R = c \log [\Delta^1\text{-THC}] + d \tag{3}$$

may be assumed for the dose–response relationship for $\Delta^1$-THC alone in the mouse brain. If it is also assumed that the pharmacological response to $\Delta^1$-THC and 7-hydroxy-$\Delta^1$-THC present together in the brain is simply the arithmetic sum of the cataleptic responses which would be produced by the single components, the total response is given by:

$$R_{\text{total}} = 60{\cdot}9 \log [\text{7-hydroxy-}\Delta^1\text{-THC}] + c \log [\Delta^1\text{-THC}] + 71{\cdot}0 + d. \tag{4}$$

Typical values of immobility index and brain concentrations may be calculated from the experimental regression lines and substituted into equation (4) to give the values of the constants $c$ and $d$. Thus the true dose–response relationship for $\Delta^1$-THC in the absence of the metabolite may be derived:

$$R = 59{\cdot}2 \log [\Delta^1\text{-THC}] + 19{\cdot}9. \tag{5}$$

This calculated dose–response line for $\Delta^1$-THC is parallel to that of the metabolite within the limits of experimental error (Fig. 3). If the mean gradient of 60·1 is assigned to both lines, the equipotent molar ratio of $\Delta^1$-THC and 7-hydroxy-$\Delta^1$-THC may be calculated:

$$60{\cdot}1 \log [\text{7-hydroxy-}\Delta^1\text{-THC}]' + 71{\cdot}0 = 60{\cdot}1 \log [\Delta^1\text{-THC}]' + 19{\cdot}9 \tag{6}$$

from which:

$$\frac{[\Delta^1\text{-THC}]'}{[\text{7-hydroxy-}\Delta^1\text{-THC}]'} = 7{\cdot}1$$

where $[\Delta^1\text{-THC}]'$ and $[\text{7-hydroxy-}\Delta^1\text{-THC}]'$ are brain concentrations of cannabinoids

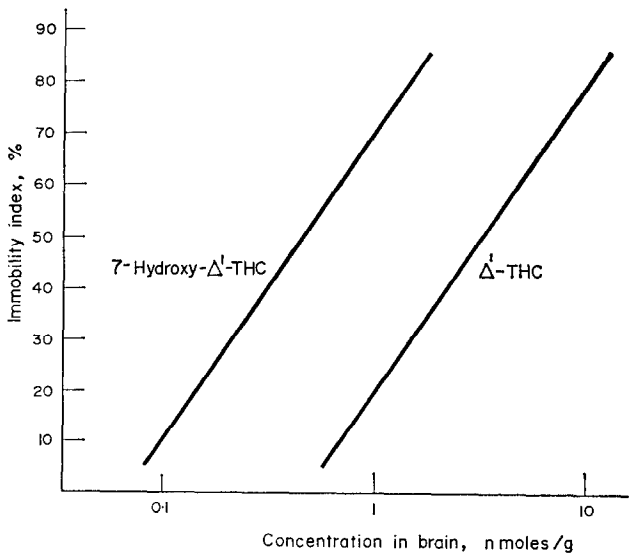


FIG. 3. Dose–response lines for 7-hydroxy-$\Delta^1$-THC (experimental) and $\Delta^1$-THC (calculated).

which produce the same degree of catalepsy. Thus in the mouse brain the metabolite is 7·1 times more potent than $\Delta^1$-THC.

*Blood levels after injection of $^3$H-7-hydroxy-$\Delta^1$-THC.* After injection of $^3$H-7-hydroxy-$\Delta^1$-THC intravenously, approximately 20 per cent of the radioactivity in the haemolyzed whole blood was not extractable with ethyl acetate. In contrast to the chromatograms of the brain extracts, three peaks were present in those of blood extracts (Fig. 4). In general the polar metabolite, and 7-hydroxy-$\Delta^1$-THC each accounted for about 17 per cent of the total extractable activity, while a more mobile material, henceforth referred to as the "mobile metabolite", constituted approximately 55 per cent of the activity. Such a non-polar substance would have been expected to cross the blood–brain barrier and the possibility arose that it could be an artefact in the extraction procedure; for instance di-acetyl-7-hydroxy-$\Delta^1$-THC has an identical $R_f$ to this material. However, extraction with di-isopropyl ether, in which acetylation could not occur, produced identical chromatograms, and the pattern of activity on the chromatogram was unchanged after the extract had been left at room temperature for two days, whether in di-isopropyl ether or ethyl acetate. (Despite the possibility of esterification of hydroxylated metabolites by ethyl acetate, this solvent appears to be superior to di-isopropyl ether in that the latter solvent extracts larger quantities of endogenous lipids which tend to interfere with the chromatographic separation of $\Delta^1$-THC and its metabolites. Thus, although $^3$H-7-hydroxy-$\Delta^1$-THC and $^3$H-$\Delta^1$-THC were detectable in brain extracts made with di-isopropyl ether rather than ethyl acetate, resolution after chromatography under otherwise identical conditions was much poorer.)

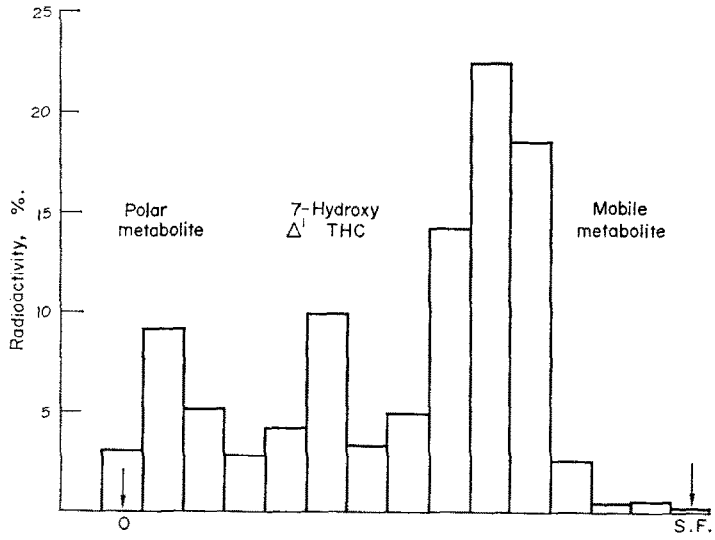


FIG. 4. Chromatogram of mouse haemolysed blood extract 20 min after injection of $^3$H-7-hydroxy-$\Delta^1$-THC (1 mg/kg i.v.).

The possibility that the $^3$H-7-hydroxy-$\Delta^1$-THC in the Tween–saline suspension decomposed, so that the mobile metabolite was injected into the mouse, was eliminated by chromatographing this suspension before and after the injections. Only a single peak

of radioactivity, corresponding to $^3$H-7-hydroxy-$\Delta^1$-THC, was present for at least 1 month from the time the suspension was made up, although it was kept in daylight at room temperature.

From the $R_f$ value on Silica-impregnated paper, this mobile metabolite could be $\Delta^1$-THC, cannabinol or an as yet unidentified further metabolite of 7-hydroxy-$\Delta^1$-THC. Elution of this material from the chromatogram with diethyl ether followed by gas–liquid chromatography of its trimethylsilyl derivative showed that it consisted of two components: the major having a retention time corresponding to that of $\Delta^1$-THC and the minor corresponding to cannabinol. About 75 per cent of the radioactivity came off the chromatograph within the duration of the $\Delta^1$-THC peak and about 10 per cent within the cannabinol peak.

*Octanol–water partition.* The octanol–water partition ratio of 7-hydroxy-$\Delta^1$-THC was found to be about 3500:1; for comparison, the corresponding value[10] for $\Delta^1$-THC is 6000:1. Thus the introduction of an additional hydroxyl group produces the expected increase in water solubility but nevertheless 7-hydroxy-$\Delta^1$-THC is still very lipid soluble and consequently would be expected to equilibrate rapidly across the blood–brain barrier.

## DISCUSSION

The work described in this paper is a continuation of that reported previously[10] and was designed to determine the extent to which the C.N.S. effects of $\Delta^1$-THC are attributable to 7-hydroxy-$\Delta^1$-THC, the primary metabolite produced *in vivo*.[3–6]

The results of the present experiment indicate that in the mouse brain, 7-hydroxy-$\Delta^1$-THC is 7·1 times more potent in producing catalepsy than $\Delta^1$-THC itself and that 20 min after intravenous injection of $\Delta^1$-THC, the molar concentration ratio in the brain of $\Delta^1$-THC and 7-hydroxy-$\Delta^1$-THC is 5·3. Because of the logarithmic form of the dose–response relationships, the relative contribution to the overall effect of each of the two active components present in the brain depends on the level of response. From equation (4) the contribution of the metabolite to the immobility index over the range 80–30 per cent is calculated to be 55–63 per cent.

In the previous work[10] the mice used appeared to have a slightly greater capacity for metabolizing $\Delta^1$-THC than those used in the present set of experiments: at the peak response the molar concentration ratio was 4·2. However, even with a ratio as low as this, the metabolite could account for only about 59 per cent of the total response. Furthermore, the mean molar ratio over the 4 hr time period studied was greater than 4·2 and the calculated contribution of the metabolite to the total response throughout this period is still within the range 50–60 per cent.

This treatment adopts a necessarily simplified approach to the problem of the C.N.S. activity of cannabinoids. The assumption has been made that $\Delta^1$-THC and 7-hydroxy-$\Delta^1$-THC are the only active cannabinoids present in the mouse brain after injection of $\Delta^1$-THC and that the whole brain concentrations are a meaningful measure of the concentrations available at the site of action. In addition, the small number of data points available makes a refined statistical analysis impossible and it is difficult to obtain values for the limits of error on the potency ratio derived by this method. However, it is reasonable to conclude from these results that the metabolite, 7-hydroxy-$\Delta^1$-THC, although making a major contribution towards the central

effects in mice of intravenously administered $\Delta^1$-THC, cannot account entirely for those effects.

Christensen *et al.*[3] have reported that 7-hydroxy-$\Delta^1$-THC is 18 times more potent than $\Delta^1$-THC when administered to mice intracerebrally. However, they also found that $\Delta^1$-THC was about equipotent whether injected intravenously or intracerebrally. If equal amounts of $\Delta^1$-THC are injected intracerebrally and intravenously to produce the same pharmacological response, the brain concentration after the former treatment will be approximately 160 times that after the latter, since only 0·6 per cent of intravenously injected $\Delta^1$-THC reaches the brain.[10] This high concentration ratio suggests that permeation of highly lipid-soluble cannabinoids throughout the brain takes place very slowly after intracerebral injection. Under these conditions, potency ratios may be governed more by rates of diffusion than by intrinsic activity at the site of action.

The presence of the "mobile metabolite" in the chromatograms of the ethyl acetate extracts of the haemolysed whole blood of mice which had received 7-hydroxy-$\Delta^1$-THC is interesting. This mobile component appears to be a genuine metabolite of 7-hydroxy-$\Delta^1$-THC. Wall *et al.*[9] obtained mono-acetylated 7-hydroxy-$\Delta^1$-THC using ethyl acetate as an extracting solvent and deduced that the esterified compound was an artefact. However, in our experiment, the mobile material was not an artificially acetylated derivative of 7-hydroxy-$\Delta^1$-THC since the ethyl acetate used as the solvent was free of acetic acid and, in addition, the mobile material was present in extracts made with purified di-isopropyl ether. The unknown component was not present in the aqueous suspensions of $^3$H-7-hydroxy-$\Delta^1$-THC with which the mice were injected, nor was it present to any extent in the extracts of brain homogenates.

The rapid conversion of 7-hydroxy-$\Delta^1$-THC to the mobile metabolite could account for the apparent absence of 7-hydroxy-$\Delta^1$-THC from the blood[10] of mice injected with $\Delta^1$-THC. The $R_f$ value of the mobile component on the single TLC system reported here is similar to that of both $\Delta^1$-THC and cannabinol, and it is possible that it is one or both of these compounds. Preliminary gas chromatographic evidence supports this identification. However, the technique of trapping and counting the radioactive effluent from a gas chromatograph is not of high resolution and it would not be possible to distinguish between $\Delta^1$-THC and a compound of similar retention time. Previous work has shown that $\Delta^1$-THC equilibrates rapidly between blood and brain. If this unidentified component is indeed $\Delta^1$-THC, then the fact that corresponding amounts were not found in the brain suggests that when produced by dehydroxylation of 7-hydroxy-$\Delta^1$-THC it is more firmly bound to some plasma protein and thus unable to equilibrate into the C.N.S. If the mobile material is not $\Delta^1$-THC, the peak of radioactivity in mouse blood extracts previously attributed[10] to $\Delta^1$-THC is probably a mixture of compounds of similar chromatographic mobility. Until the identity of this material has been established conclusively, it will be necessary to interpret radiochromatograms of biological extracts of labelled cannabinoids with caution.

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