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ON THE METABOLISM OF TRITIUM-LABELLED Δ1-TETRAHYDROCANNABINOL IN THE RABBIT*

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Abstract—Δ1-Tetrahydrocannabinol-^{3}H is almost completely metabolized to more polar metabolites after intravenous (i.v.) injection in the rabbit. About 35 per cent of the dose is excreted in the urine during the first 24 hr as compared to only 10 per cent in the faeces. A considerable amount (4–10 per cent) of the radioactivity excreted in the 24-hr urine is extractable with ether and upon acidification of the urine the extractable amount increases four times or more. At least three major metabolites of Δ1-THC-^{3}H are excreted in the urine. The half-life of radioactivity in blood after i.v. injection of Δ1-tetrahydrocannabinol-^{3}H ranged from 7 to 16 min. The compound is rapidly metabolized and 30 min after i.v. administration of Δ1-tetrahydrocannabinol-^{3}H, only 3–4 per cent of the radioactivity in the blood is due to unchanged compound. The blood level of an ether-soluble metabolite reaches its maximum 0·5–2 hr after injection. Thus, the formation of a pharmacologically active metabolite cannot be excluded at present. The distribution of radioactivity in tissues largely reflect the elimination of Δ1-tetrahydrocannabinol-^{3}H through liver and kidneys. Brain and spinal cord show the lowest activity levels of all the investigated tissues. Three days after administration high levels of radioactivity still persist in body fat and spleen.

DURING the last few years considerable progress has been made in the chemistry of cannabinoids and today, over a dozen of compounds of known structure have been isolated from *Cannabis sativa* L.[1] Still, the animal metabolism and pharmacological actions of pure cannabinoids have been little studied so far. Earlier pharmacological work has been carried out with Cannabis preparations of unknown compositions and consequently the interpretation of such data is difficult.

Δ1-Tetrahydrocannabinol (Δ1-THC)† is the major psychotomimetically active constituent of Cannabis.[2, 3] Except for our previous reports on the elimination of Δ1-THC-^{3}H in the rat,[4, 5] there is only one study on the distribution and excretion of Δ1-THC in the animal body. This study by Miras *et al.*[6] on the metabolic fate of biosynthetically ^{14}C-labelled Δ1-THC in the rat, is in good agreement with our data. The results may be summarized as follows: in the rat Δ1-THC is eliminated predominantly through the bile with 50–60 per cent being excreted in the faeces during 5 days, as compared to only some 10 per cent in the urine during this period. Further, Δ1-THC is completely or almost completely metabolized to more polar compounds.

Metabolic studies on Δ1-THC and other cannabinoids have previously been hampered by the lack of labelled compounds which are necessary for the investigation of

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† Designated as Δ9-THC by some authors.

the metabolism of these potent compounds. However, Miras[6] has produced THC-^{14}C by growing Cannabis plants in $^{14}CO_2$ and recently, Burstein and Mechoulam[7] prepared specifically tritium-labelled Δ1(6)-THC-^{3}H. We have described[4] the preparation of Δ1-THC-^{3}H of high specific activity (up to 140 μc/mg) and also the first synthesis[1] of specifically ^{14}C-labelled Δ1(6)-THC.

The present report is concerned with the distribution and elimination of Δ1-THC-^{3}H in the rabbit and the occurrence of metabolites of Δ1-THC-^{3}H in blood and urine.

MATERIALS AND METHODS

Labelled materials

Δ1-THC-^{3}H with a specific activity of 140 μc/mg was prepared as described.[4] On account of the acidic conditions used to exchange the aromatic protons in the labelling procedure one could possibly expect an isomerization to the Δ1(6)-isomer to occur.[7] Since Δ1-THC and Δ1(6)-THC are well separated by GLC on XE-60 columns, (Fig. 1)

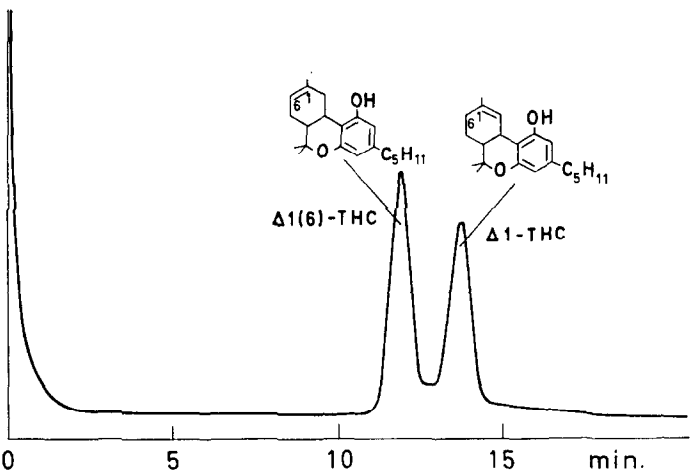

FIG. 1. Gas chromatogram of mixture of Δ1(6)-THC and Δ1-THC. Glass column 6 ft × ¼ in.; 5 per cent XE-60 on Gas Chrom P; 200°.

we could demonstrate that the radioactive compound was pure Δ1-THC. Radioactivities were determined by Liquid scintillation counting (Packard Tri-Carb Model 3375); aqueous solutions in XDC solvent.[4] The activities in blood and faeces were counted after oxygen flask combustion.

Chromatographic methods

TLC was carried out on Silica Gel G plates impregnated with 20 per cent dimethylformamide in acetone, and developed in light petroleum (b.p. 40–60°) containing 20 per cent of ether.[4] The cannabinoids were detected by spraying the plate with 0·2 per cent Echtblausalz "Merck" in 2 N aqueous NaOH. The distribution of radioactivity on thin-layer plates was determined by liquid scintillation counting of the scraped-off adsorbent.[4]

Animal experiments

Female albino rabbits (2 kg) were injected i.v. with an emulsion of Δ1-THC-^{3}H (0·1–0·6 mg/kg), prepared from a solution of Δ1-THC-^{3}H in sesame oil, saline (proportions 1:9) and lecithin using ultrasound.

Commercial chow diet and water were offered *ad lib.* The rabbits were kept in metabolic cages. Urine and faeces were collected separately every 24 hr.

Distribution studies

For tissue distribution studies the rabbits were sacrificed by injecting air intravenously. The whole organs were removed except for the following organs: proximal part of ileum (washed before activity determination); distal part of colon (faeces removed); fat was taken from perirenal depots; muscle tissue was taken from the lower part of the back in the latissimus dorsi region; spinal cord was taken from the cervical and upper thoraxical segments. The brain included the medulla oblongata. The organs were then homogenized separately and an aliquot (about 100 mg) of each homogenate was dissolved in 1 ml of NCS solvent (Nuclear Chicago Corp.) by shaking at 37° for 48 hr. The radioactivity was then determined by liquid scintillation counting.

Half-life of Δ1-THC-^{3}H in blood

Five rabbits were given Δ1-THC-^{3}H (0·1–0·6 mg/kg) i.v. in one ear vein and from the other ear blood samples were collected in heparinized tubes at certain time intervals. Fifty μl of each blood sample was dried on filter paper and the amount of radioactivity was determined after oxygen flask combustion. Blood samples were extracted with light petroleum and ether as described below, and the distribution of activity in the extracts was determined after TLC.

Extraction procedures

The following method was employed for the extraction of blood. An accurately measured blood sample of about 1 ml was shaken vigorously in a test tube with 10 ml of light petroleum. After centrifugation, the organic layer was aspirated and the extraction was repeated twice. The combined extracts were dried (Na_2SO_4) and an aliquot was used for the determination of radioactivity extractable with light petroleum. The residue was separated by TLC to determine the distribution of radioactivity in various compounds[4] e.g. Δ1-THC. The blood was then extracted using ether as organic solvent (3 × 10 ml). The combined ether layers were treated as described for the light petroleum extract.

Urine (5 ml) was similarly extracted with light petroleum (3 × 10 ml) and ether (3 × 10 ml). Further extraction with ether did not increase the yield of ether-soluble metabolites.

Glucuronidase treatment

Urine (5·0 ml) was adjusted to pH 3·8 with glacial acetic acid and incubated once with β-glucuronidase 1000 units/ml (Type L-1, Sigma Chemical Co.) and 3·0 ml 0·05 M citrate buffer pH 3·8 overnight at 37°.

Recovery of Δ1-THC from blood and urine

Δ1-THC-^{3}H (20–200 ng) dissolved in 10 μl propylene glycol was added to 1 ml of

blood. The mixture was incubated for 2 hr at room temperature and then extracted with light petroleum and the amount of radioactivity in the light petroleum phase determined. The recovery was 78 ± 5 per cent (five determinations).

Δ1-THC-^{3}H (2–50 ng) was similarly added to 5 ml of urine. Each sample was extracted with light petroleum and radioactivity recovered in the light petroleum was determined. The recovery was 90 ± 6 per cent (three determinations).

RESULTS AND DISCUSSION

Elimination in the rabbit

The elimination of Δ1-THC-^{3}H after i.v. injection in the rabbit differs quite markedly from that in the rat as shown in Fig. 2 where previous data[4] on the elimination of Δ1-THC-^{3}H in the rat has been included for comparison. In the rabbit, Δ1-THC-^{3}H is

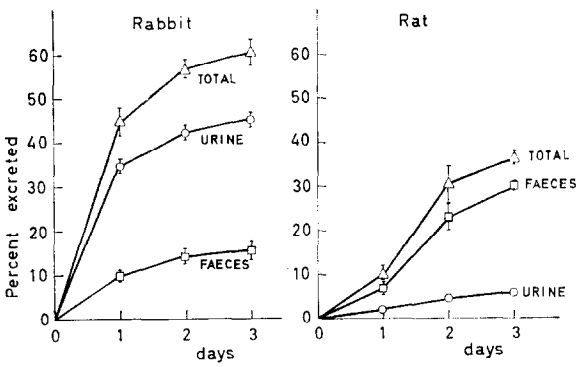

FIG. 2. Cumulative excretion of label after i.v. injection of Δ1-THC-^{3}H in (left panel) rabbit and (right panel) rat. Mean of four rabbits with standard deviations (vertical bars); 0·1–0·6 mg/kg; 72–157 × 10^6 d.p.m./kg. Mean of four rats with standard deviations; 1·3 mg/kg; 285 × 10^6 d.p.m./kg.

eliminated mainly in the urine with some 35 per cent of the drug being excreted during the first 24 hr. This in contrast to only a few per cent in the rat, where elimination *via* faeces was the major route. Δ1-THC-^{3}H is eliminated more rapidly in the rabbit than in the rat but still only 60 per cent is excreted during the first 72 hr. The effects of Δ1-THC-^{3}H (0·1–0·6 mg/kg) on gross behaviour have largely disappeared after 10 hr and completely after 20 hr.

Metabolites in urine

A detailed investigation was carried out on several urine samples, collected during the first 24 hr after i.v. injection of Δ1-THC-^{3}H in rabbits. Extraction of each urine sample with light petroleum and ether followed by TLC of the extracts and determination of the radioactivity of the area corresponding to Δ1-THC on each plate[4] showed that at most 0·01 per cent, if any, of the introduced Δ1-THC-^{3}H was excreted as such in the urine during the first 24 hr (Table 1). This is in agreement with studies in the rat[4, 6] showing at most negligible elimination of unchanged Δ1-THC in the urine. The negative results were not due to ineffective extraction techniques since 2–50 ng amounts of Δ1-THC-^{3}H added to urine were satisfactorily recovered.

A larger amount (3–10 per cent) of the activity excreted in the urine could be

extracted by ether as shown in Table 1. We found that by acidifying the urine to pH 3·8 before extraction, the yield of radioactivity in the ether extract was increased four to ten times as compared to the original urine. This yield was approximately the same, if the acidification was made immediately before extraction, or if the urine was incubated, with or without β-glucuronidase, at pH 3·8 for 14 hr before extraction (Table 1). Thus, the amount of metabolite(s) extractable with ether are not increased appreciably by treatment with β-glucuronidase.

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN THE 24-hr URINE OF RABBIT GIVEN 0·18 mg/kg (49 × 10^6 d.p.m./kg) Δ1-TETRAHYDROCANNABINOL-^{3}H

Treatment of urine	Percentage of radioactivity extractable with:	
	light petr.	ether
Untreated (pH 7·8)	0·1*	9·9
Acidified pH 3·8	0·3*	43·3
Incubated pH 3·8 37°; 14 hr	0·1*	31·3
Incubated pH 3·8 37°; 14 hr β-glucuronidase	1·3*	35·3

Mean of 3 determinations.
* Mainly ether soluble metabolites.

The manifold increase in the amount of metabolites in the ether phase at pH 3·8 is probably not due to hydrolysis of labile conjugates. This was indicated by the fact that acidified urine (pH 3·8) readjusted to the original (pH 7·8) before extraction with ether, yielded no more radioactivity in the ether extract than the original, untreated urine. Conversely, this suggests the introduction in Δ1-THC-^{3}H of an acidic group, not affected by β-glucuronidase. Work on the structure of this metabolite is now in progress.

The β-glucuronidase treatment did increase the amount of radioactivity extractable with light petroleum. The distribution of radioactivity after TLC of this light petroleum extract showed that there might be some conjugated Δ1-THC in the urine hydrolyzed by β-glucuronidase. However, this amounted at most to 0·05 per cent of the introduced Δ1-THC and the increase in radioactivity in the light petroleum extract is in the main due to the release by β-glucuronidase of compounds other than Δ1-THC.

So far, paper chromatography with ethylacetate–ethanol (1:1) as solvent, has shown the presence of at least three major metabolites of Δ1-THC-^{3}H in the urine.

Distribution study

The relative distribution of radioactivity 2 hr and 72 hr after i.v. injection of Δ1-THC is shown in Fig. 3. The pharmacological effects of Δ1-THC are very pronounced after 2 hr whereas the animals have completely recovered after 72 hr.

Two hr after administration, the elimination of radioactive metabolites via liver and kidneys is reflected by the high radioactivities in these tissues and particularly so

in bile and urine (Fig. 3). A striking observation is the high amount of radioactivity in the lungs. This was also observed (unpublished) after i.v. injection of Δ1-THC-^{3}H in the rat. Brain and spinal cord contained the lowest levels of radioactivity of all the investigated tissues and thus show that there is no general affinity of Δ1-THC to the CNS.

Seventy-two hr after i.v. injection with Δ1-THC-^{3}H there is still evident the elimination of radioactivity by the kidneys and the liver. A relatively high level of activity still persists in the spleen and body fat.

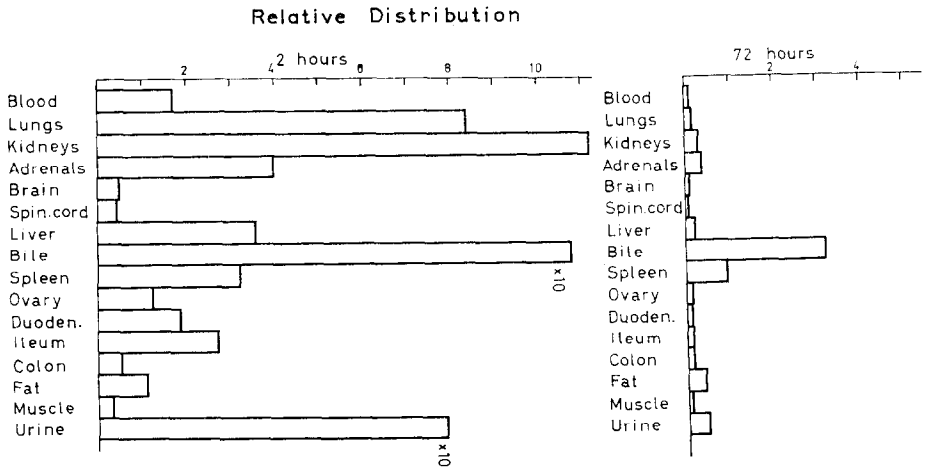

FIG. 3. Distribution of radioactivity in the tissues of rabbit 2 hr (left panel) and 72 hr (right panel) after i.v. injection of Δ1-THC-^{3}H. Two hr experiment: mean of three rabbits given 0·1–0·3 mg Δ1-THC-^{3}H/kg i.v. Seventy-two hr experiment: mean of four rabbits given 0·4–0·6 mg Δ1-THC-^{3}H/kg i.v. Relative distribution:

$$\frac{\text{the measured radioactivity of each tissue (d.p.m./g)}}{\Delta 1\text{-THC-}^3\text{H injected (d.p.m./g rabbit)}}.$$

Thus a relative distribution = 1 represents the calculated radioactivity at even distribution throughout the body.

Half-life in blood

The results in Fig. 4 indicate a half-life of Δ1-THC-^{3}H (i.v. injected) of about 14 min in the blood. The half-life values from five experiments ranged between 7–16 min, (mean 12 min) which is quite short relative to the behaviour of other drugs in the rabbit.

It should be noted (Fig. 4) that 30 min after injection only a few per cent (3–4 per cent, in three different experiments) of the radioactivity is present as Δ1-THC. Extraction of blood samples from different time intervals with light petroleum and ether, combined with TLC also showed a rapid formation of ether-soluble metabolite(s) as well as more water-soluble products (Fig. 4). The blood level of ether soluble metabolite reaches a maximum level between ½–2 hr after injection followed by a decrease. Since the action of the drug remains for a longer period of time than the

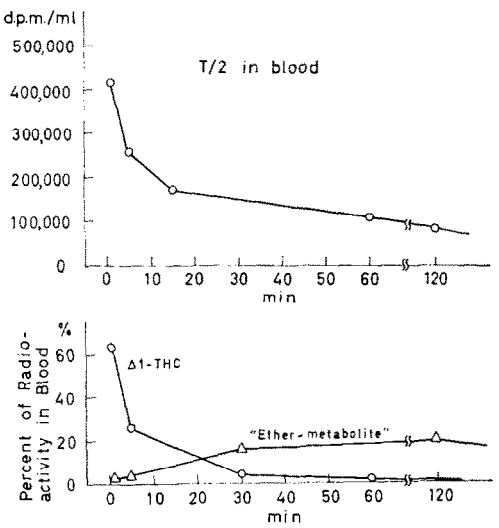

FIG. 4. Upper panel; Disappearance of radioactivity from blood of rabbit following administration of Δ1-THC-^{3}H. Lower panel: Amount of radioactivity in the blood (pH 7·8) appearing as Δ1-THC and as ether extractable metabolite(s) ("ether metabolite"). Determined by extraction with light petroleum (Δ1-THC) and ether followed by TLC and liquid scintillation counting.

Δ1-THC remains in the blood, the formation of active metabolite(s) cannot be excluded. The rapid disappearance of Δ1-THC in blood is not due to the fact that it cannot be recovered from plasma when bound to plasma proteins since recovery studies showed that Δ1-THC added to blood and incubated for 2 hr at room temperature can be recovered satisfactorily.

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