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ELIMINATION OF TRITIUM-LABELLED CANNABINOLS IN THE RAT WITH SPECIAL REFERENCE TO THE DEVELOPMENT OF TESTS FOR THE IDENTIFICATION OF CANNABIS USERS

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Abstract—A method is described for the preparation of tritium-labelled Δ9-tetrahydrocannabinol and other cannabinols. Intravenously injected Δ9-tetrahydrocannabinol-H^3 is eliminated very slowly by the rat; half of the administered dose still remained in the body after one week. About 80 per cent of the drug is excreted in metabolized form via faeces, the remainder being eliminated also as metabolites in the urine. During the first 24 hr 2–6 per cent of the injected activity appears in the urine, but less than 0·006 per cent of the dose, if any, is excreted unchanged. A considerable amount of the activity is readily extractable with ether and possibly an identification method for Cannabis-users may be based on the occurrence of this metabolite. Δ9-Tetrahydrocannabinol is apparently not excreted as a glucuronide.

CANNABIS (hashish, marihuana) is at present a widely abused drug and it has been estimated that 200–300 million people regularly use Cannabis.[1] Of several cannabinols present in the resin only Δ9- and Δ8-tetrahydrocannabinol* appear to be psychoactive.[2, 3] since the Δ9-isomer is the predominant compound in Cannabis, the psychotomimetic properties of Cannabis consequently can be largely attributed to Δ9-tetrahydrocannabinol.

The metabolic fate of the cannabinols and Δ9-tetrahydrocannabinol (Δ9-THC) are relatively unknown. The available information is limited to the observation by Miras[4] that the liver of rat 1½ hr after i.p. injection of a minute amount of Δ9-THC-^{14}C contained about 5 per cent of the administered activity and that also the urine contained a not determined amount of radioactivity.

There exists at present no test to identify Cannabis smokers. For other narcotic drugs (possibly except LSD) methods are available for the identification of the drug or its metabolites in biological samples. However, the possibility of developing a method to identify Cannabis users by the testing of biological fluids has attracted much interest.[4]

The present work was undertaken to investigate the distribution and elimination of cannabinols and Δ9-THC in the rat as well as the presence and structures of metabolites of Δ9-THC in tissues and urine. In this paper we report a method for the preparation of tritium labelled Δ9-THC and the results of elimination studies in the rat.

* By some authors designated as Δ1- and Δ6-tetrahydrocannabinol.

MATERIALS AND METHODS

Labelled materials

A purified extract of Cannabis, consisting of about 70 per cent cannabinols and containing tetrahydrocannabinol (71 per cent), cannabidiol (24 per cent) and cannabinol (5 per cent) was prepared by chromatographic fractionation on alumina.[5]

Cannabinols were tritiated by heating 150 mg cannabinols, 0·5 ml 100 per cent phosphoric acid, 2·5 ml tetrahydrofurane and 0·25 ml tritiated water (50 mc) to 80° for 2 hr in a sealed ampoule under nitrogen. The reaction mixture was then dissolved in ether (25 ml), washed once with an equal volume of saturated $NaHCO_3$ solution and twice with an equal volume of water. After drying (Na_2SO_4) the ether was evaporated *in vacuo*. Exchangeable hydrogens were removed by dissolving the residue in 2 ml of methanol and evaporating the methanol under a stream of nitrogen. This was repeated twice. Preparations containing tetrahydrocannabinol (84 per cent), cannabidiol (11 per cent) and cannabinol (5 per cent) with a spec. act. of 1·2–3·5 μc/mg were obtained.[6] With pure Δ9-tetrahydrocannabinol and tritiated water of high spec. act. (5c/ml), 97 per cent pure Δ9-tetrahydrocannabinol-H^3 with a spec. act. of 41 μc/mg was obtained. The remaining 3 per cent of the activity as shown by TLC (Fig. 1) and GLC was present in cannabinol.

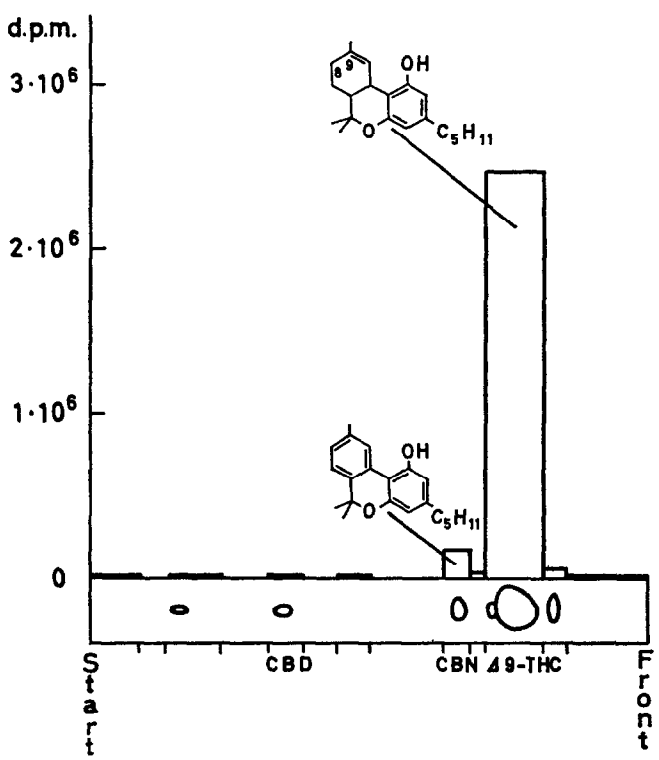

FIG. 1. Distribution of radioactivity, determined by liquid scintillation counting, on thin-layer chromatogram of Δ9-tetrahydrocannabinol-H^3 (Δ9-THC). Three per cent of the radioactivity present in cannabinol (CBN; cannabidiol (CBD).

Radioactivities were determined by liquid scintillation counting (Packard Tri-Carb Model 3375); of aqueous solutions in XDC solvent.[8] Faeces was counted after oxygen flask combustion.

Chromatographic methods

TLC was carried out on Silica Gel G plates impregnated with 20 per cent dimethylformamide in acetone[7] and developed in light petroleum (b.p. 40–60°) containing 20 per cent ether. The cannabinols were detected by spraying the plate with 0·2 per cent Echtblausaltz "Merck" in 2N NaOH. The distribution of radioactivity on thin-layer plates was determined by liquid scintillation counting of the scraped off adsorbent.

GLC was carried out with an Aerograph 204 (F.I.D.) gas chromatograph using a 6 ft × $\frac{1}{8}$ in. o.d. glass column packed with 5 per cent SE-30 on GasChrom P (AW-DMCS). Flow rate 25 ml N_2/min. Oven temp. 230°.

Glucuronidase treatment

Urine was adjusted to pH 7·0 and incubated with bacterial β-glucuronidase (Type 1, Sigma Chemical Co.) in phosphate buffer pH 7·0 at 37°, overnight.

Animal experiments

Female rats (200 g) were injected i.p. (non-labelled cannabinols) with cannabinols dissolved in olive oil or dimethylsulphoxide or i.v. (labelled compounds) with an emulsion. The emulsion was prepared from a solution of the cannabinols or Δ9-tetrahydrocannabinol in sesame oil, saline (proportions 1:9) and lecithin using ultrasound. Commercial chow diet and water were offered *ad libitum*. The animals were kept in metabolic cages and urine and faeces were collected separately every 24 hr.

Extraction and purification methods

Urine was saturated with NaCl and extracted three times with an equal volume of light petroleum and dried (Na_2SO_4). In *labelled experiments* the total amount of radioactivity in this extract was determined (Table 1). The amount of radioactivity present in any unchanged cannabinol in the extract was determined after separation by TLC (Fig. 4a). The remaining urine was then extracted three times with diethylether (Table 1).

When *non-labelled cannabinols* had been administered, the light petroleum extract of the urine was subjected to further purification by TLC on Silica Gel H with 12

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN THE URINE OF RATS GIVEN 1·1–1·3 mg/kg (47·6–57·2 × 10^6 dpm) Δ9-TETRAHYDROCANNABINOL-H^3

	Percentage of injected radioactivity excreted:					
	In urine, total		In urine, extractable with: light petr.		ether	
	0–24 hr	24–48 hr	0–24 hr	24–48 hr	0–24 hr	24–48 hr
Rat No. 1	2·0	1·2	0·000	0·000	0·7	0·05
Rat No. 2	4·4	2·4	0·006	0·000	1·8	0·01
Rat No. 3	2·5	2·8	0·003	0·000	0·7	0·09
Rat No. 4	2·6	2·0	0·000	0·000	0·3	0·3

per cent ether in hexane as solvent. All three cannabinols have close R_f-values in this system. The area corresponding to the R_f of the cannabinols was removed from the plate and eluted with ether. The eluate was concentrated and analyzed by GLC. In model experiments with cannabidiol added to the urine the recovery from the extraction-TLC procedure was found to be 70–80 per cent.

RESULTS AND DISCUSSION

Studies with non-labelled cannabinols

The urine from ten rats given non-labelled cannabinols (50 mg/kg) i.p., were collected separately, purified and analyzed by GLC for cannabinols. No cannabinols could be detected in any of these experiments (Fig. 2). One per cent of the purified extract from the 24 hr-urine was analyzed in each injection. Estimating a lower detection limit of 2 ng for the major component (Δ9-THC) in the gas chromatograph and assuming a 70 per cent recovery in the purification process, it may be estimated that a maximum of about 300 ng (0·3 μg) of the major component Δ9-tetrahydrocannabinol may have remained undetected in the urine. The rats were given 10 mg cannabinols each and thus less than 0·005 per cent, if any, of the introduced cannabinols were excreted unchanged in the urine. This is in agreement with remarks made in passing in the literature, which show that no unchanged tetrahydrocannabinol or any other cannabinol has been unambiguously identified in urine from humans or experimental animals given Cannabis (ref. 4, pp. 89–94), although no experimental details have been given.

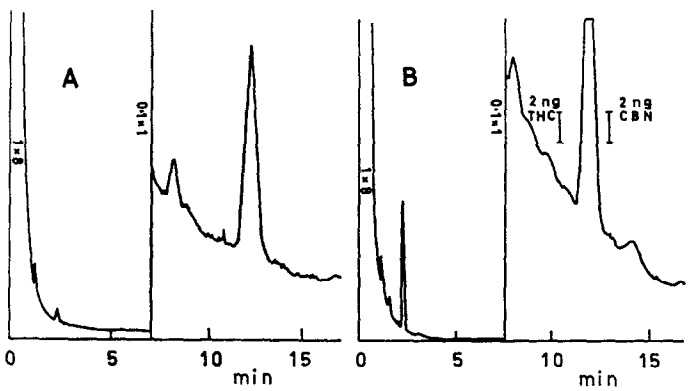

FIG. 2. Gas-chromatogram of purified light petroleum extract of urine from control rat (A); and rat injected with cannabinols (50 mg/kg) i.p. (B). Retention time and approx. peak height for 2 ng Δ9-tetrahydrocannabinol (THC) and cannabinol (CBN) indicated. For other details, see Experimental.

Studies with labelled cannabinols

Phenols are generally readily labelled in tritiated water by acid catalysis. The cannabinols however, are unstable towards acids but it was possible to devise conditions mild enough to prevent degradation of the molecule but still giving a high degree of labelling. Recent work[9] has shown that monohydric phenols and catechols do not exchange *ortho*- and *para*-tritium in biological systems. This was also found true for the cannabinols since part of the radioactive urine when distilled at atmo-

spheric pressure yielded no significant radioactivity in the distillate. Also the radioactivity in faeces was present in non-volatile form.

Figure 3 shows the elimination in the rat of radioactive metabolites of intravenously injected (1·3–3 mg/kg) cannabinols-H^3 (Fig. 3a) and Δ9-tetrahydrocannabinol-H^3 (Fig. 3b). The very slow elimination rate, with less than 50 per cent of the administered

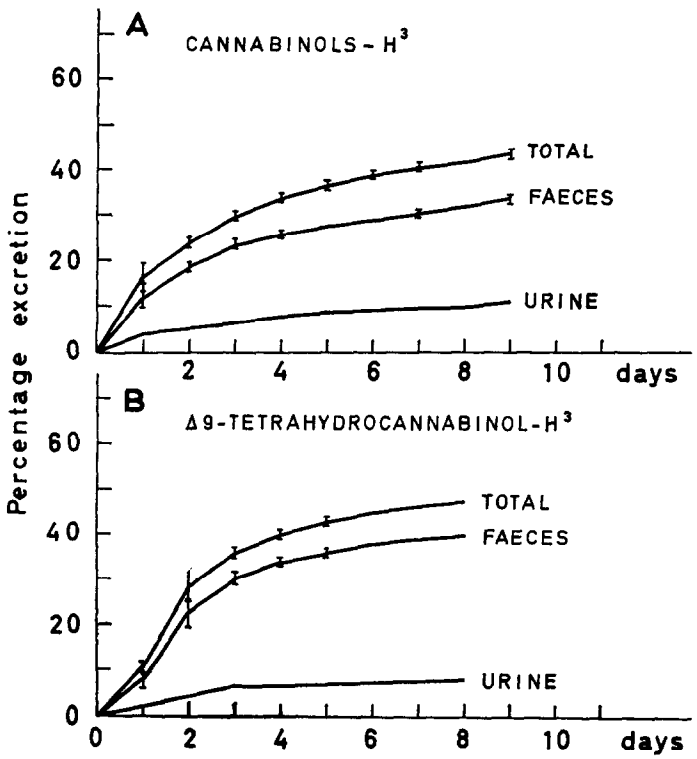

FIG. 3. Excretion of radioactive material in the urine and faeces of rats treated with H^3-labelled cannabinols and Δ9-THC. a: eight rats given 0·3–0·6 mg/kg (0·95–4·6 × 10^6 d.p.m.) of cannabinols-H^3 i.v.; b: four rats given 1·3 mg/kg (57 × 10^6 d.p.m.) of Δ9-THC-H^3 i.v. The results are expressed as averages with standard deviations.

drug eliminated the first week, is apparent. A substantial amount of the drug (7–12 per cent)* is excreted during that time in the urine and we have shown, as discussed below, this is only in metabolized form. The discrepancy between the shortlasting action of the drug with little effect visible 4 hr after i.v. injection and the very slow elimination is striking. The radioactive drug remaining in the body thus must exist in a modified form e.g. as metabolites or protein bound.

One interesting aspect in this connection, is the long-term effect, lasting for several days, which has been noted in performance tests with monkeys by Scheckel *et al.*,[2] thus may be explained by the slow elimination of the drug.

Previously we suggested[6] that the lipophilic Δ9-THC might be stored in fatty

* This amount may be higher if any tritium atom of the aromatic ring is replaced by e.g. a hydroxy group.

tissues, but preliminary tissue distribution studies (unpublished) does not support such an assumption. These preliminary distribution studies further indicate that the radioactivity (d.p.m./ml) in the blood is higher between 4–192 hr than during the initial excitation phase. This also suggests that the drug exists in a modified form.

Detailed study of the excretion of metabolites of Δ9-THC-H³ in the urine

The excretion of radioactive metabolites in the urine from i.v. introduced Δ9-THC was further investigated by successive extraction of the urine with light petroleum, ether and finally *n*-butanol. Some of these results are reported in detail in Table 1.

During the first 24 hr 2–6 per cent of the administered dose (data from twelve rats) of Δ9-THC-H³ appeared in the urine. Less than 0·006 per cent of the dose was excreted as unchanged Δ9-THC-H³ which if present, could be extracted with light petroleum from the urine. The minute amount of activity in the light petroleum extract was apparently partly present as Δ9-THC-H³ since after TLC of the extract the activity coincided with authentic Δ9-THC (Fig. 4a). However, it cannot be excluded that in spite of considerable precautions these few hundred dpm of Δ9-THC-H³ may have leaked out after the injection of some 50 × 10⁶ dpm in a tail vein. These results, which corroborate the previously related data obtained with nonlabelled cannabinols, show that at most 0·006 per cent if any of introduced Δ9-tetrahydrocannabinol is excreted in unchanged form. Hence, attempts to devise a method for the identification of Cannabis users based on the excretion of unchanged cannabinols in the urine appears futile although our results should be checked in experiments with humans.

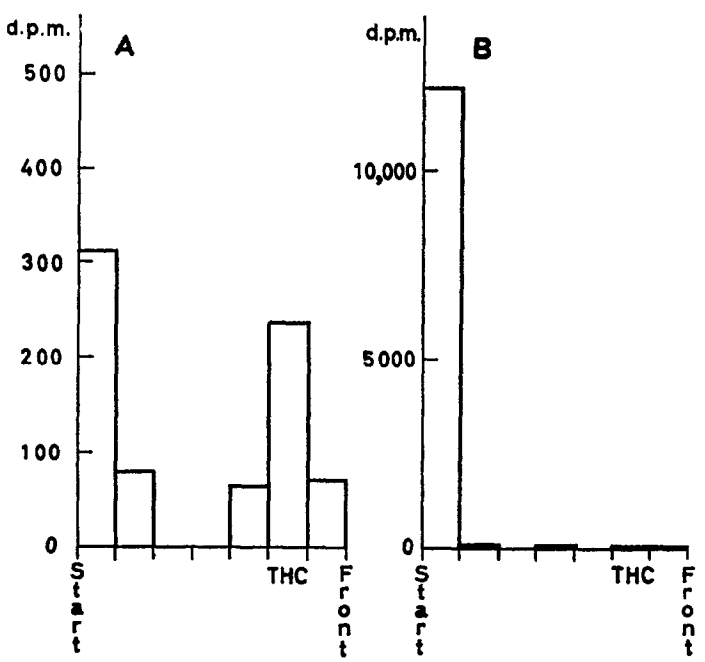

FIG. 4. Distribution of radioactivity in (a) light petroleum, (b) ether, extract of 24 hr urine from rat (Rat No. 2 in Table 1) given Δ9-THC-H³ i.v. TLC on dimethylformamide-treated Silica Gel G; eluent 20% ether in light petroleum. R_f value of reference Δ9-THC shown.

A considerable amount (17–40 per cent) of the radioactivity excreted in the urine during the first 24 hr can be extracted with ether. This fraction contains only metabolites of Δ9-THC-H^3 (Fig. 4b). If the human metabolism of Δ9-THC is similar to that in the rat, a method for the identification of Cannabis users might be based on the occurrence of this metabolite (or possibly several metabolites) which readily can be extracted from the urine. Since recent data[10] indicate that by smoking the active dose of Δ9-THC in the human body may be as low as 1–3 μg/kg, the demands on extraction, purification and identification techniques would thus be formidable.

The major part of the activity remaining in the aqueous phase after extraction with light petroleum and ether can be extracted with *n*-butanol. This more polar compound is probably not a glucuronide of Δ9-THC since treatment with a glucuronidase preparation failed to yield any free Δ9-THC.

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