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Excretion of *trans*-Δ⁹-Tetrahydrocannabinol and Its Metabolites in Intact and Bile Duct-Cannulated Rats

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Abstract □ Tritiated Δ⁹-tetrahydrocannabinol (I) was administered both orally and intravenously to groups of bile duct-cannulated rats and those with their bile duct intact. From 55.8 to 66.9% of the total radioactivity was excreted during the 96-hr. period following administration. The excretion of radioactivity was minimal in each group beyond 48 hr. after drug administration. The major route of excretion following the intravenous administration in bile duct-cannulated rats was by way of bile (59.4%; feces, 2.7%), whereas more radioactivity was excreted in feces (41.5%) than bile (21.5%) when the drug was given orally. The radioactivity excreted in the feces of the orally medicated rats was mainly extractable in petroleum ether. This extract was found to contain I by TLC and GLC analysis. After intravenous administration, the radioactivity in the feces was not extractable in petroleum ether but appeared in ether, methanol, and water extracts. TLC confirmed that the radioactivity in these solvents was associated with metabolites of I. Bile contained mainly metabolites of I, as did the urine. Less than 10% of the radioactivity was excreted in the urine of each group of rats.

Keyphrases □ *trans*-Δ⁹-Tetrahydrocannabinol, radiolabeled, and metabolites—excretion kinetics after oral and intravenous administration, intact and bile duct-cannulated rats □ Excretion kinetics—radiolabeled *trans*-Δ⁹-tetrahydrocannabinol and metabolites after oral and intravenous administration, intact and bile duct-cannulated rats □ Cannulation *versus* noncannulation of bile duct—excretion kinetics of radiolabeled tetrahydrocannabinol after oral and intravenous administration, rats

The earliest reports describing the distribution of Δ⁹-tetrahydrocannabinol (I) were made by Miras (1) and Joachimoglu *et al.* (2). These investigators described the distribution of ^{14}C-Δ⁹-tetrahydrocannabinol, isolated from marijuana grown in a $^{14}CO_2$ atmosphere, in the rat. The findings of King and Forney (3) using a fluorometric method and those of Turk (4) using TLC were consistent with the earlier reports. Human studies by Miras and Coutselinis (5) indicated that only 5.2% of smoked radiolabeled hashish was secreted by the bile and 16.9% was excreted through the urine.

Due to the recent synthesis of highly purified tritium- and carbon-labeled I and their availability to the research community, more progress has been made concerning the metabolism and excretion of I. Lemberger *et al.* (6, 7) showed that radiolabeled I is mainly excreted by man in the feces and to a less extent in the urine, mainly as polar metabolites. Agurell *et al.* (8, 9) studied the metabolism and excretion of radiolabeled I in the rat and the rabbit. More recently, Klausner and Dingell (10) also reported the excretion of I in the rat. Their data agree with the excretion data reported by

Agurell *et al.* (8, 9); however, there was a difference in the rate of excretion between the two studies. The work of Klausner and Dingell (10) as well as that of Agurell *et al.* (8) showed that the radioactive material was mainly excreted in the feces and in a polar form. Liver perfusion experiments suggest that the main route of excretion is by way of the bile (10). This was also suggested in the early work of Joachimoglu *et al.* (2). To our knowledge, no one has reported the quantitative excretion of I and its metabolites in the urine, bile, and feces after oral and intravenous administration of radiolabeled I. Nor has the quantitative excretion of I and its metabolites been reported following cannulation of the rat bile duct *versus* noncannulation of the bile duct to determine the major route of excretion after oral and intravenous administration of the drug.

We have examined the excretion kinetics of intravenously and orally administered tritium-labeled I and its metabolites in the feces, urine, and bile of bile duct-cannulated rats and in urine and feces of noncannulated rats. The results of these experiments comprise the body of this report.

EXPERIMENTAL

Tritium-labeled I (25.4 μc./mg.), labeled in positions 2, 4, 8, and 10, with radiopurity of 100% and GLC purity of 97%, was obtained from the National Institute of Mental Health. Male Sprague–Dawley rats, weighing between 230 and 260 g., were used in these experiments.

The tritium-labeled I was prepared for intravenous and oral administration by suspending it in bovine albumin (fatty acid poor) (11).

Excretion of Tritium-Labeled I Administered Intravenously—Tritium-labeled I (4 mg./kg., 5 μc./rat) was administered intravenously to each rat in a total albumin suspension volume of 0.2 ml. Two groups, consisting of four rats each, were used in this experiment. One group was bile duct cannulated and the other was not. All rats were housed individually in metabolism cages with water and food available *ad libitum*.

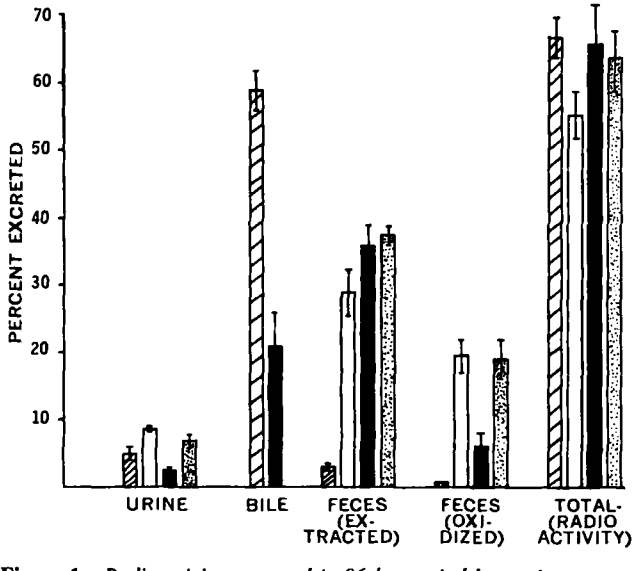

Figure 1—*Radioactivity excreted in 96-hr. period by various routes of excretion following intravenous injection of I (4 mg./kg., 4.7 μc./rat) or oral administration (100 mg./kg., 10 μc./rat); the bars represent the standard error of the mean. Key:* ■, *intravenous, bile duct cannulated* (n = 4); □, *intravenous, bile duct not cannulated* (n = 4); ■, *oral, bile duct cannulated* (n = 5); *and* ▣, *oral, bile duct not cannulated* (n = 5).

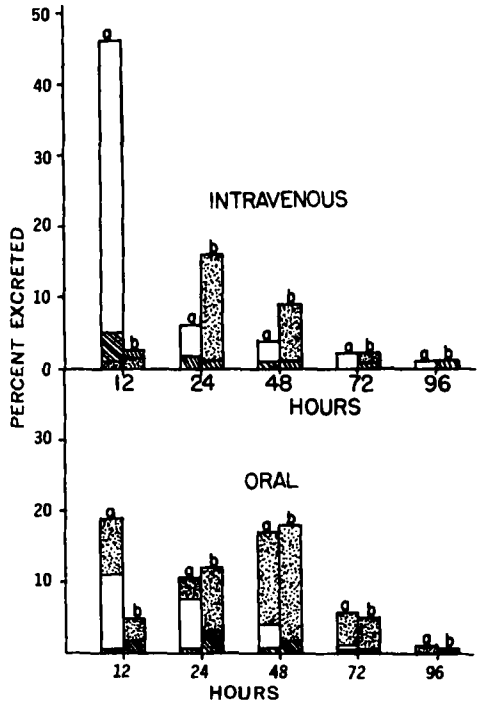

Figure 2—*Radioactivity excreted during various time periods following intravenous administration (4 mg./kg., 4.7 μc./rat) or oral administration (100 mg./kg., 10 μc./rat). These columns are not cumulative; for example, during the first 12 hr., 1% was excreted in urine, 10.5% in bile, and 19% in feces following oral administration in bile duct-cannulated rats. Key: (a) bile duct cannulated; (b), bile duct not cannulated;* □, *bile;* ■, *urine; and* ▣, *feces. As in Fig. 1, each group medicated intravenously contained four rats and each group medicated orally contained five rats.*

The bile duct cannulation was performed 4 days prior to the injection. Bile and urine were collected separately at the following times: 3, 6, 12, 48, 72, and 96 hr. after administration of drug. Feces were collected at 12, 24, 48, 72, and 96 hr. after administration of drug.

Excretion of Tritium-Labeled I Administered Orally—Two groups of rats (one bile duct cannulated, the other not), consisting of five rats each, were used in this experiment. The only variation from the intravenous study was that these rats were administered 100 mg./kg. of Compound I (10 μc.) orally in a total albumin suspension volume of 2 ml.

Determination and Solvent Extraction of Radioactivity in Excreta—After collection of the excreta, its volume (bile and urine) or dry weight (feces) was recorded. The feces, after drying, were ground in a mortar to assure a uniform sampling. After the dried feces samples were ground, each sample was extracted three times for 15 min., first with petroleum ether (30–60°), second with ethyl acetate, third with methanol, and finally with water. Each solvent extract was pooled, evaporated to dryness, and made up to 10 ml.; then a 0.2-ml. aliquot of each was counted in a liquid scintillation counter using a scintillation fluid containing 1 l. of a purified

Table I—Comparison of the Radioactivity Excreted in Bile following the Oral or Intravenous Route of Administration

Collection Period, hr.	Route	Percent of Total Radioactivity Excreted, Mean ± *SE*
0–3	Intravenous	26.03 ± 1.47
	Oral	2.42 ± 0.63
3–6	Intravenous	10.82 ± 0.59
	Oral	1.98 ± 1.13
6–12	Intravenous	9.29 ± 0.71
	Oral	2.85 ± 1.16

Table II—Fecal Excretion of ^3H in Rats Treated with ^3H-Δ^9-Tetrahydrocannabinol

Route of Administration	Bile Cannulation	Percent of ^3H Excreted in Feces Extracted in Solvents: Petroleum Ether	Ethyl Acetate	Methanol	Water	Oxidized, Not Extracted
Intravenous	Yes	Negative	Negative	Negative	Negative	Negative
Intravenous	No	3	12	27	16	42
Oral	Yes	47	29	8	0	16
Oral	No	10	16	24	16	34

nonionic detergent[1], 8 g. of 2,5-diphenyloxazole, 200 mg. of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene, and 2 l. of toluene. After the last water extraction, the remaining fecal sample was dried and oxidized in a combustion flask and counted in the liquid scintillation counter to determine the remaining radioactivity. The amount of radioactivity in the oxidized sample and that found in each extraction solvent were combined to determine the total radioactivity excreted in feces for each time period.

Aliquots of bile (0.2 ml.) and urine (0.4 ml.) were counted directly in the liquid scintillation counter, and the total radioactivity excreted by each route was calculated for each collection time. All samples were corrected for quenching by internal standardization. The remaining bile and urine were each extracted three times with petroleum ether and ether as described for the fecal samples.

Identification of Radioactive Compounds Excreted—The various solvent extracts of the bile, feces, and urine were chromatographed on TLC System 1 reported by Turk *et al.* (4, 12, 13) and on the TLC system (System 2) reported by Lemberger *et al.* (6). This was done to identify the components present in each solvent extract. System 1 allows the separation of I from its metabolites, and System 2 separates the metabolites 8,11-dihydroxy-Δ^9-tetrahydrocannabinol (II) and 11-hydroxy-Δ^9-tetrahydrocannabinol (III). The presence of I was further identified by GLC (4).

RESULTS

Intravenous Route of Administration—*Bile Duct-Cannulated Rats*—The excretion of tritium in the bile duct-cannulated rats is shown in Figs. 1 and 2. In the bile duct-cannulated rats, 66.9% of the radioactivity was excreted during the first 96 hr. Of this amount, 4.8% was excreted in the urine, 59.4% in the bile, and 2.7% in the feces. The major portion of the radioactivity that was excreted in the bile was excreted in the first 3 hr.; after 12 hr., excretion by the biliary route dramatically decreased (Table I).

Excretion of radioactivity in the urine was essentially complete within 48 hr. The excretion of radioactivity in the feces was detectable only during the first 24 hr.

Noncannulated Rats—In the noncannulated group of rats, 55.8% of the radioactivity was excreted during the first 96 hr. (Fig. 1). Of this radioactivity, 7.4% was excreted in the urine and 48.4% was excreted in the feces.

Excretion of radioactivity in the urine was greatest in the first 12 hr. and was negligible for the remainder of the 96-hr. period. Of the radioactivity excreted in the feces, the greatest excretion occurred between the 12- and 48-hr. interval and appeared to be complete by 72 hr. (Fig. 2).

Oral Route of Administration—*Bile Duct-Cannulated Rats*—The bile duct-cannulated group of rats excreted 65.5% of the radioactivity in 96 hr. Of this radioactivity, only 2.7% was excreted in the urine, 41.5% was excreted in the feces, and 21.5% was excreted in the bile (Fig. 1).

Noncannulated Rats—The noncannulated rats excreted 63.6% of the total radioactivity in the 96-hr. period. Of this amount, 6.9% was excreted in urine and 56.7% was excreted in the feces (Fig. 1).

Identification of Radioactive Compounds Excreted—After visualization with diazo blue B salt, both TLC Systems 1 and 2 indicated the presence of I in the petroleum ether extract of feces and no evidence for the presence of metabolites. The presence of I was further substantiated by GC and by zone scraping of the TLC plate. Counting of the zone scrapings confirmed that the major radioactivity was in the zone of the Compound I spot. The ethyl acetate, methanol, and water extracts of the feces were plated. Some of the components identified in these fractions had the same R_f values as II and III. There also existed, in these fractions, radioactivity of a more polar nature than II and III. The quantification of radioactivity in the various solvent extracts of bile, feces, and urine is given in Tables II–IV.

DISCUSSION

The results presented in this paper show that 55–67% of the radioactivity is excreted within 96 hr. after either an oral or intravenous injection of tritium-labeled I. These data do not agree with those reported by Agurell *et al.* (8), who found that the excretion of I was very slow in the rat and that less than 50% of the dose was excreted during the 1st week. However, the present data agree with those of Klausner and Dingell (10), who reported that the excretion of radioactivity was essentially complete within 72 hr. after an injection of I. The present data also show that the major route of excretion of I is by way of the bile, mainly as polar metabolites (II and III).

Analytical data indicate that when the bile duct was not cannulated, the radioactivity excreted in the feces following oral dosing represented mainly metabolites. However, when the bile duct was cannulated, these metabolites were found in the bile but not in the feces. Determination of radioactivity following intravenous administration showed that there was negligible excretion of radioactivity in the feces of bile duct-cannulated animals. However, ^3H excreted in the feces in the noncannulated rats was associated with metabolites II and III. On this basis, one might theorize that the long duration of action of I might be due to enterohepatic circulation of either one or more of its metabolites.

Table III—Biliary Excretion of Radioactivity in Rats Treated with ^3H-Δ^9-Tetrahydrocannabinol

Route of Administration	Excreted in Bile, d.p.m. × 10^6	Extracted in Petroleum Ether, d.p.m.	Extracted in Ether, d.p.m.
Intravenous	5.49	79,766 (0.64%)[a]	792,770 (7.17%)
Oral	4.82	71,414 (1.48%)	393,554 (8.17%)

[a] Percent of total excreted in bile that was extractable in the solvent.

[1] Triton X-100.

Table IV—Urinary Excretion of Radioactivity in Rats Treated with ^3H-Δ^9-Tetrahydrocannabinol

Route of Administration	Bile Cannulation	Excreted in Urine, d.p.m. × 10^6	Extracted in Petroleum Ether, d.p.m.	Extracted in Ether, d.p.m.
Intravenous	Yes	0.56	498 (0.09%)[a]	26,112 (4.69%)
Intravenous	No	2.73	406 (0.01%)	31,232 (1.15%)
Oral	Yes	1.12	692 (0.06%)	5022 (0.45%)
Oral	No	2.18	1246 (0.06%)	99,426 (4.55%)

[a] Percent of total excreted in urine that was extractable in the solvent.

A considerable quantity of the radioactivity (47% in bile duct-cannulated and 10% in intact rats) excreted in the feces of the rats given tritium-labeled I orally was identified as the parent compound. This material probably represents Compound I, which was not absorbed from the GI tract.

Radioactivity in the urine was in the form of metabolites of I. These data agree with previous reports (6, 9) of the appearance of polar metabolites in the urine. Zone scraping of the TLC plates indicates the presence of metabolites other than 11-hydroxy-Δ^9-tetrahydrocannabinol and 8,11-dihydroxy-Δ^9-tetrahydrocannabinol. Identity of this large polar fraction has not been achieved. This may be accounted for, in part, by the recently described (14) diacetyl derivative found in bile. The identity of the material remaining in the feces after exhaustive extraction is also a mystery. Unfortunately, sufficient samples of material were not available for hydrolysis studies. These are planned.

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Interfacial Adsorption of a Psychotomimetic Drug Using Liquid Scintillation

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Abstract □ A study was conducted on the oil–water partitioning and interfacial adsorption of ^{3}H-2-pyrrolidylmethyl *N*-methyl cyclopentylphenylglycolate (I), an anticholinergic psychotomimetic agent. A new technique for radiotracer adsorption was developed involving the use of liquid scintillation counting. Among the physical parameters examined were partition coefficient, permeation constant, stability constant, and rate constant of I in a two-phase system of water and a lipid, didodecyl phosphate (II). II greatly accelerated the rate of oil–water partitioning of I and exhibited interfacial adsorption with I. The presence of polyanions, such as hyaluronic acid in the aqueous phase, promotes the transfer of drug from the oil to water phase. Equations found applicable to permeation of ions across membranes have been used to describe drug transfer through an oil–water interface.

Keyphrases □ Adsorption, interfacial, ^{3}H-labeled psychotomimetic agent—studied using liquid scintillation counting □ Interfacial adsorption and oil–water partitioning of radiolabeled compounds—studied using liquid scintillation counting □ 2-Pyrrolidylmethyl *N*-methyl cyclopentylphenylglycolate, radiolabeled—interfacial phospholipid adsorption and oil–water partitioning studied using liquid scintillation counting □ Anticholinergic psychotomimetic agents, radiolabeled—interfacial phospholipid adsorption and oil–water partitioning studied using liquid scintillation counting □ Liquid scintillation counting—used to measure interfacial adsorption and oil–water partitioning of radiolabeled psychotomimetic agent □ Partitioning (oil–water) of ^{3}H-2-pyrrolidylmethyl *N*-methyl cyclopentylphenylglycolate—studied using liquid scintillation counting

For a number of years, interest in this laboratory has focused on the mechanism of action of a group of anticholinergic psychotomimetic agents, particularly with regard to their ability to modify the physicochemical properties of excitatory membranes (1). A useful approach has been to examine the effect of the agents on the interfacial properties of surface films of phospholipids and membranous proteins using conventional interfacial techniques, including the measurement of surface adsorption with radioactive compounds (2).

The technique of surface adsorption involves the measurement of a radioactively labeled substance as it