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Pharmacokinetics of Δ9-Tetrahydrocannabinol in Dogs

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Abstract □ The pharmacokinetics of intravenously administered 14C-Δ9-tetrahydrocannabinol and derived radiolabeled metabolites were studied in three dogs at two doses each at 0.1 or 0.5 and 2.0 mg/kg. Two dogs were biliary cannulated; total bile was collected in one and sampled in the other. The time course for the fraction of the dose per milliliter of plasma was best fit by a sum of five exponentials, and there was no dose dependency. No drug was excreted unchanged. The mean apparent volume of distribution of the central compartment referenced to total drug concentration in the plasma was 1.31 ± 0.07 liters, approximately the plasma volume, due to the high protein binding of 97%. The mean metabolic clearance of drug in the plasma was 124 ± 3.8 ml/min, half of the hepatic plasma flow, but was 4131 ± 690 ml/min referenced to unbound drug concentration in the plasma, 16.5 times the hepatic plasma flow, indicating that net metabolism of both bound and unbound drug occurs. Apparent parallel production of several metabolites occurred, but the pharmacokinetics of their appearance were undoubtedly due to their sequential production during liver passage. The apparent half-life of the metabolic process was 6.9 ± 0.3 min. The terminal half-life of Δ9-tetrahydrocannabinol in the pseudo-steady state after equilibration in an apparent overall volume of distribution of 2170 ± 555 liters referenced to total plasma concentration was 8.2 ± 0.23 days, based on the consistency of all pharmacokinetic data. The best estimate of the terminal half-life, based only on the 7000 min that plasma levels could be monitored with the existing analytical sensitivity, was 1.24 days. However, this value was inconsistent with the metabolite production and excretion of 40–45% of dose in feces, 14–16.5% in urine, and 55% in bile within 5 days when 24% of the dose was unmetabolized and in the tissue at that time. These data were consistent with an enterohepatic recirculation of 10–15% of the metabolites. Intravenously administered radiolabeled metabolites were totally and rapidly eliminated in both bile and urine: 88% of the dose in 300 min with an apparent overall volume of distribution of 6 liters. These facts supported the proposition that the return of Δ9-tetrahydrocannabinol from tissue was the rate-determining process of drug elimination after initial fast distribution and metabolism and was inconsistent with the capability of enzyme induction to change the terminal half-life.

Keyphrases □ Δ9-Tetrahydrocannabinol—intravenous, radiochemical study of pharmacokinetics, dogs □ Pharmacokinetics—intravenous, Δ9-tetrahydrocannabinol, radiochemical study, dogs □ Radiochemistry—study of pharmacokinetics of intravenous Δ9-tetrahydrocannabinol, dogs

(−)-Δ9-Tetrahydrocannabinol is the major active component of marijuana. An essential prerequisite to understand its pharmacological action, presumably related to the plasma levels of the drug and its metabolites (1, 2), is the quantification of the time course of the drug and its metabolites in biological tissues to relate to the psychoactive effects.

Agurell *et al.* (3, 4) demonstrated that when tritium-labeled Δ9-tetrahydrocannabinol was intravenously administered to the mouse and rabbit, the radioactivity was slowly excreted as metabolites in the feces and urine with no unchanged drug observed in the urine. The relative amounts excreted biliary and renally varied with the species (3–5), and enterohepatic recirculation of metabolites was indicated (5, 6). The major studies to date with pharmacokinetic significance are those of Agurell *et al.* (4, 5) in rabbits and humans and Lemberger *et al.* (5, 7, 8) in humans. The general pattern appears to be a rapid initial fall of Δ9-tetrahydrocannabinol concentration in plasma with an apparent half-life of 12 min in rabbits (4) and 30 min in humans (8), with a slower decline to a terminal apparent half-life of 50–60 hr in humans (8). A similar pattern was observed in a preliminary experiment with dogs (9). Metabolite levels in human plasma increased rapidly to two to three times that of the drug (8). The slower terminal phase decline of the log metabolite level with time paralleled the similarly plotted decline of plasma Δ9-tetrahydrocannabinol.

It has been proposed (7, 10) that these facts can be explained by concomitant rapid hepatic metabolism and distribution to binding sites and other tissues such as fat (11) with a subsequent slow release of sequestered Δ9-tetrahydrocannabinol from these stores. The suggestion that induced metabolism significantly lessens the terminal half-life of Δ9-tetrahydrocannabinol in chronic users over that of naive individuals (2, 12) is inconsistent with this premise. The release rate from the "deep" tissues should be rate determining, not the metabolic rate. Changes in the latter would merely affect the relative plasma Δ9-tetrahydrocannabinol levels, not the terminal half-life.

The purposes of this paper are to present the results of studies designed to elucidate the rate-determining pro-

cesses for disposition of intravenously administered Δ^9-tetrahydrocannabinol in the dog and to give a detailed pharmacokinetic analysis and model to fit the data. The effects of dose and other variables on the metabolism of drug and biliary and renal excretion of metabolites are delineated. Sensitive analytical methods, previously developed (9, 13, 14) for Δ^9-tetrahydrocannabinol in biological tissues, were used. The metabolites were characterized by the partition properties of their radiolabels derived from ^{14}C-labeled drug and were monitored in all available biological fluids. In addition, radiolabeled metabolites were intravenously administered to challenge the pharmacokinetic model. The previously studied physicochemical properties and protein binding (13) were taken into account.

EXPERIMENTAL

Preparation of Δ^9-Tetrahydrocannabinol Intravenous Dosages—Labeled and unlabeled Δ^9-tetrahydrocannabinols were purified on the evening prior to a pharmacokinetic study by methods given previously (14). They were combined to give the desired specific activity, and amounts sufficient to dose at 0.1, 0.5, or 2.0 mg/kg were transferred to a 50-ml glass centrifuge tube and dried in a water bath (50°) under a nitrogen stream. The residue was dissolved in 1 ml of absolute ethanol. The solution and 3 × 1-ml ethanol rinsings were transferred to a 20-ml vial and reduced to dryness. The residue in the vial was dissolved in 0.5 ml of absolute ethanol, and the vial was capped and placed in a freezer. On the morning of the study, the vial containing a small stirring bar[1] was placed on a magnetic stirrer set at low speed and 1 ml of water was added dropwise over 2–3 min to form an emulsion.

Dog plasma, 10 ml taken 1 hr after the last meal, was added to ultrafiltration cones[2] and centrifuged (3000 rpm) until an ultrafiltrate (2–5 ml) was obtained. The concentrated plasma was delivered dropwise from a pipet to the aqueous ethanol emulsion of Δ^9-tetrahydrocannabinol. This method produced a true solution of drug in plasma protein which, within 10 min, was administered intravenously to the dog in a 10-ml silanized syringe. This method is the method of choice for intravenously administering highly protein-bound materials normally insoluble in water.

Treatment of Animals—Two weeks prior to the first pharmacokinetic study, each mongrel dog was transferred to a metabolic cage. The next day, the dog was weighed and anesthetized with pentobarbital (30 mg/kg iv). One external jugular vein of the neck was exposed and cannulated under sterile conditions with 51 cm of 0.32-cm (0.125-in.) o.d. tubing[3]; at least 20.3 cm was inserted into the vein. The incision was closed after verifying the patency of the cannula and filling with heparinized[4] saline.

On the following day, the dog was placed on a table in a standing position and restrained by two straps placed around the fore- and hindlegs and fixed to a horizontal bar above. After 8 hr, the dog was returned to the cage. This training procedure was repeated daily until the dog would remain on the table unattended and unrestrained. At the end of each day, the dog was walked briefly, the regularly deposited stool sample was collected uncontaminated on an aluminum foil sheet, and the patency of the jugular cannula was checked.

A laparatomy was performed on Dogs A and C at the same time as the jugular was cannulated. The gallbladder was tied off, and an incision was made in the common bile duct distal to the gallbladder. One end of a 1.22-m length of 0.32-cm (0.125-in.) o.d. tubing was inserted in the bile duct incision approximately 6 cm toward the liver, and the other end was inserted in the incision approximately 16 cm into the intestine. When bile flow was visibly observed through the tubing, an incision was made in the abdominal wall; the tube was then passed through the opening and subcutaneously to an exit incision on the back of the neck. All incisions were closed, and the exposed bile cannula was cut. Bile flow was checked, and unrestricted flow to the intestine was confirmed. The tube was reconnected with 14-gauge stainless steel tubing.

After a 1-day recovery period, the dogs were transferred to a table and

Table I—Doses and Biliary Modifications in the Pharmacokinetic Studies after Intravenous Administration of ^{14}C-Δ^9-Tetrahydrocannabinol

Dog	Weight, kg	Average Hematocrit	Dose, mg/kg	10^{-6} × Total, cpm	Bile Cannulation
A	12.4	0.30	0.1	145.2	Yes[a]
A	14.1	0.42	2.0	85.36	Yes[a,b]
B	12.7	0.31	0.5	76.22	No
B	14.4	0.38	2.0	116.3	No
C	13.3	0.39	0.5	229.4	Yes[c]
C	16.6	0.38	2.0	150.8	Yes[c,d]

[a] Bile was sampled, but all was returned to the intestine. [b] Bile flow was stopped at approximately 1300 min after dosing. [c] All bile was collected, and none was allowed to return to the intestine. [d] Metabolites were extracted from the bile of this dog, and 8.62 × 10^6 cpm was administered intravenously to this biliary-cannulated 14.1-kg dog.

conditioned as described. At the end of all surgical procedures, the dogs were given 8 × 10^6 units of penicillin G intravenously.

The dogs were fasted for 24 hr before each experiment and then weighed. A water load of 10 ml/kg was administered through a stomach tube, and a continuous 0.9% NaCl iv drip (0.5 ml/min) was started 30 min prior to dosing.

Experiments on a given dog were spaced at least 28 days apart. All dogs required transfer of the jugular cannula to the remaining jugular vein between the first and second pharmacokinetic experiments.

The bile from Dog A was collected over a specified time, sampled, and then returned *via* the intestinal tube. The bile from Dog C was collected but not returned *via* the intestinal tube. Bile flow in Dog C was maintained by four daily oral doses (with food) of 150 mg of taurocholic acid[5] and by incorporating taurocholic acid into the 0.5-ml/min iv saline drip at 0.833 mg/ml.

The weights, hematocrits, and intravenous doses administered to the dogs are listed in Table I, and their treatments for bile study are summarized.

Collection and Treatment of Samples—Δ^9-Tetrahydrocannabinol was administered intravenously as a freshly prepared suspension solubilized in doubled plasma protein concentration, prepared by plasma filtration through filters[6]. The drug was injected into the jugular catheter over 0.05 min. The dosing syringe and catheter were flushed with 10 ml of normal saline.

Blood was withdrawn from the jugular catheter, after filling the dead space of the catheter with fresh undiluted blood, into a sterile, disposable syringe containing 0.01 ml of heparin (10,000 units/ml). The blood sample was transferred to a sterile 15-ml centrifuge tube and centrifuged at 1500 rpm for 10 min. Plasma, 1–2 ml, was transferred to a 50-ml centrifuge tube with a sterile pipet, and the tube was quickly stoppered and frozen. A known volume (0.05–0.2 ml) of the remaining plasma was analyzed for total carbon-14 as described previously (14). The small amount of blood remaining in the syringe was used to determine blood pH in selected instances.

The separated red blood cells were refrigerated at 5°. Blood, 5 ml, was sampled at 0.5, 1, 1.5, 2, 3, 5, 7, 10, 13, 16, 19, 22, 27, 32, 37, 45, 55, 65, 80, 100, 130, 180, 210, 260, 310, 400, 500, 600, 700, and 1000 min and at 500-min intervals up to 7000 min (4.86 days). Sterile red blood cells were collected over the first 1500 min. They were pooled, washed once with an equal volume of sterile normal saline, resuspended in 50 ml of normal saline, and returned to the animal in equal portions after the next three blood samplings. Hematocrits were obtained on selected blood samples prior to centrifugation.

Urine was collected from the catheterized animal at approximately 20, 40, 60, 85, and 100 min; then every 50 min up to 500 min; then every 200 min through Day 1; then every 6 hr of Day 2; and then twice daily thereafter for 5–25 days. The volumes of each collection were measured. Urine pH was determined in selected instances, and an aliquot was removed to determine total carbon-14. A 2-ml aliquot of freshly collected urine was transferred to a 50-ml centrifuge tube, which was stoppered and frozen. The remaining urine (up to 50 ml) was stored frozen.

Bile was collected in glass tubes (containing 0.2–0.5 ml of cyclohexane) and pooled between the following times: 0, 1, 2, 3, 4, 8, 11, 14, 18, 22, 26, 30, 35, 40, 45, 50, 60, 70, 80, 110, 140, 170, 200, 250, 300, 350, and 400 min

and thereafter as plasma samples were taken. The volumes of the pooled bile samples were recorded, and aliquots were obtained for extraction and determination of total carbon-14.

The pooled bile samples from Dog A were returned through the intestinal side of the biliary cannula during the next collection period. Pooled bile samples from Dog C were stored frozen.

Biliary Elimination and Enterohepatic Recirculation of Δ⁹-Tetrahydrocannabinol and Its Metabolites—The collected bile fractions from Dog A were sampled prior to their intestinal return and analyzed for tetrahydrocannabinol and metabolites. Bile flow rates were determined at the start of each interval of bile flow collection, and the averages of the flow rates at the start and end of each interval were multiplied by the concentration of metabolites as equivalents of ^{14}C-Δ⁹-tetrahydrocannabinol in bile and by the time of collection to obtain the total biliary excretion of metabolites in that interval. No bile was collected from Dog B. No enterohepatic recirculation would be possible with Dog C. The feces of Dog C between 0 and 24 hr were collected and analyzed for total carbon-14 to confirm complete collection of all bile.

Pharmacokinetics of ^{14}C-Labeled Metabolites—The 0–300-min bile from the 0.5-mg/kg study in Dog C was pooled, the volume was measured, and the solution was adjusted to pH 10.0–10.5 with 0.5 M Na₂CO₃ and extracted twice with an equal volume of heptane (containing 1.5% isopentyl alcohol), which removed approximately 2% of the radioactivity. The remaining bile solution was adjusted to pH 2.0, saturated with sodium chloride, and extracted twice with three equivalent volumes of tetrahydrofuran, which removed over 90% of the original radioactivity.

An aliquot (30 ml) of the metabolites extracted into tetrahydrofuran at pH 2 was reduced to dryness, reconstituted in 1 ml of ethanol, and diluted with 9 ml of normal saline. Two 0.1-ml aliquots were counted for total carbon-14, and 9.5 ml of the solution was administered intravenously to Dog C. Blood, bile, and urine collections were the same as described for tetrahydrocannabinol.

Effect of Isotope Placement on Pharmacokinetics and Metabolism of Δ⁹-Tetrahydrocannabinol—Two types of ^{14}C-labeled Δ⁹-tetrahydrocannabinol were available: C-11 labeled[7] and aromatic ring labeled[8]. Metabolism at C-11 could result in loss of the ^{14}C-label. This hypothesis was tested by conducting the 0.5-ml/kg study in Dog C with the ring-labeled tetrahydrocannabinol and the 2.0-mg/kg study in Dog C with C-11 labeled agent. The null hypothesis was that no difference in metabolism or distribution would result.

Effect of Altered Bile Flow on Pharmacokinetics of Δ⁹-Tetrahydrocannabinol—After the 2.0-mg/kg injection of Δ⁹-tetrahydrocannabinol in Dog C, 150 mg of taurocholic acid was rapidly infused (30 mg/min and 6 mg/ml in normal saline) at 400 min over 5 min in lieu of the normally infused 0.417 mg/min. Additional blood samples (1 ml) were taken at 410, 420, 430, 460, 530, and 560 min; additional bile samples were collected between 400, 420, 430, 460, 480, 510, and 550 min. These samples were analyzed for total carbon-14 only. Oral taurocholic acid (150 mg four times a day) was reinstituted after 24 hr.

The taurocholic acid infusion was designed to increase bile flow sharply, which presumably would decline after cessation of infusion.

Analysis of Δ⁹-Tetrahydrocannabinol in Biological Fluids—A 2-ml plasma sample was adjusted between pH 9.5 and 11.0, extracted, treated, and analyzed as described by Garrett and Hunt (9, 14). It was shown (14) that the electron-capture GLC analysis of the high-pressure liquid chromatographic (HPLC) collected tetrahydrocannabinol gave the same assay results as the liquid scintillation analysis of the separated tetrahydrocannabinol when both methods of analysis were conducted in Dog A on administration of 0.1 and 0.5 mg of Δ⁹-tetrahydrocannabinol/kg iv. However, the sensitivity of the GLC analysis was approximately 1 ng/ml of plasma and that of the radiochemical analysis was 0.16 ng/ml. Since the former method only permitted plasma assays no more than 1000 min and the latter no more than 7000 min after the stated doses were administered (Table I), all other assays of plasma tetrahydrocannabinol in the remaining pharmacokinetic studies were obtained by the liquid scintillation analysis of the radiolabel after collection and separation of ^{14}C-tetrahydrocannabinol from plasma on the normal phase HPLC.

A 2-ml urine sample suspected of containing Δ⁹-tetrahydrocannabinol was adjusted to pH 9.5–11 by addition of 0.2 ml of 0.5 M Na₂CO₃. A bile sample was diluted with distilled water to yield a 5–25% (v/v) solution. A 2-ml aliquot of this solution was adjusted to pH 9.5–11 by addition of 0.2 ml of 0.5 M Na₂CO₃. Subsequent steps for urine and bile were the
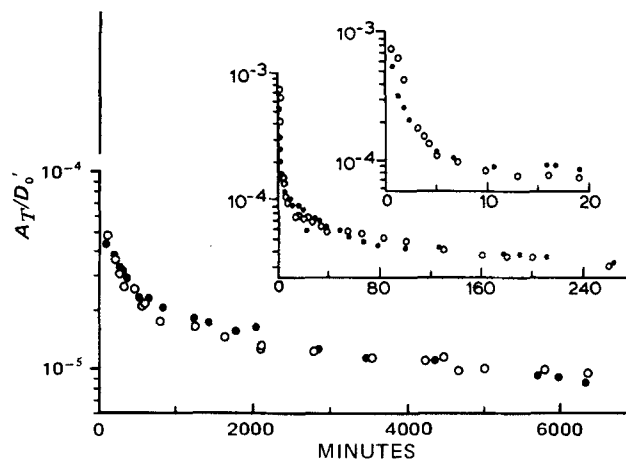

Figure 1—*Semilogarithmic plots of the fractions, A_T/D_0', of the total radioactive dose, D_0', per milliliter of plasma against time for the 2.0-mg/kg (●) (116.3 × 10⁶ cpm) and 0.5-mg/kg (○) (76.22 × 10⁶ cpm) doses of ^{14}C-Δ⁹-tetrahydrocannabinol in Dog B. The activities per milliliter corresponding to $A_T/D_0' = 10^{-4}$ are 11,630 (●) and 7620 (○) cpm and correspond to 2880 and 635 ng/ml as Δ⁹-tetrahydrocannabinol equivalents, respectively.*

same as described for the liquid scintillation analysis of tetrahydrocannabinol (14) in plasma.

Determination of Total Carbon-14 in Urine and Bile—An aliquot (0.2 or 0.4 ml) of freshly collected urine was transferred to a scintillation vial containing 0.1 ml of 0.1 N KOH. The sample was bleached to avoid quench by adding 0.2 ml of *tert*-butyl hydroperoxide[9]. The vial was tightly capped, placed in a water bath (50°), and reacted for 12–24 hr. Residual peroxide was hydrolyzed by the addition of 0.5 ml of water, and the capped vial was returned to the water bath for 1–3 hr. Six milliliters of scintillation solution was added to the vial, which was dark adapted for at least 6 hr before counting.

An aliquot of 0.05–0.2 ml of freshly collected bile was transferred to a vial containing 0.1 ml of 0.1 N KOH. Samples were bleached after addition of 0.1 ml of *tert*-butyl hydroperoxide/0.1 ml of bile. Subsequent manipulations were the same as for urine.

Samples with low counts (less than 400 cpm) were counted twice and averaged. This procedure resulted in negligible quench for 95% of all urine and bile samples.

Determination of Total Carbon-14 in Plasma—An aliquot of 0.05, 0.1, 0.2, or 0.5 ml of fresh plasma was transferred to a vial and 0.6, 0.5, 0.4, or 0.2 ml, respectively, of water was added. Six milliliters of scintillation solution was added to each, and the capped samples were dark adapted for at least 6 hr. Samples with low counts were counted twice and averaged. Selected plasma samples were checked for sedimentation error; if this error was observed, each sample was counted twice manually after vigorous shaking. Plasma samples showing evidence of hemolysis were treated as described for bile samples. This procedure resulted in negligible quench for all plasma samples.

Determination of Total Carbon-14 in Feces—The collected feces were transferred to a weighed sheet of aluminum foil, wrapped, weighed, and frozen. At the time of analysis, the frozen feces were transferred to a flask containing a measured volume of 15% ethanol in water. The volume of solvent was designed to give a 10–25% fecal suspension. The suspension was homogenized[10] for 15 min, and a 0.5-ml aliquot was transferred to a vial containing 0.2 ml of 0.5 N KOH and 0.5 ml of *tert*-butyl hydroperoxide. The vial was capped and placed in a water bath (50°) for 12–24 hr. One milliliter of water and 10 ml of scintillation solution were added, and the samples were dark adapted for at least 6 hr before counting. When an error due to sedimentation was suspected, each sample was manually counted twice after vigorous shaking. Quench corrections were required on all fecal samples.

Analysis of Metabolites in Plasma—The concentration of total metabolites in plasma, $[M_p]$, in terms of equivalents of Δ⁹-tetrahydrocannabinol was calculated from:

$$[M_p] = (A_T/S) - C \qquad \text{(Eq. 1)}$$

[7] Sample DW-III-23, 68 μCi/mg, R.T.I., Research Triangle Park, N.C.
[8] Sample 3168-145-37, 38.7 μCi/mg, R.T.I., Research Triangle Park, N.C.

[9] Mallinckrodt Chemical Works, St. Louis, Mo.
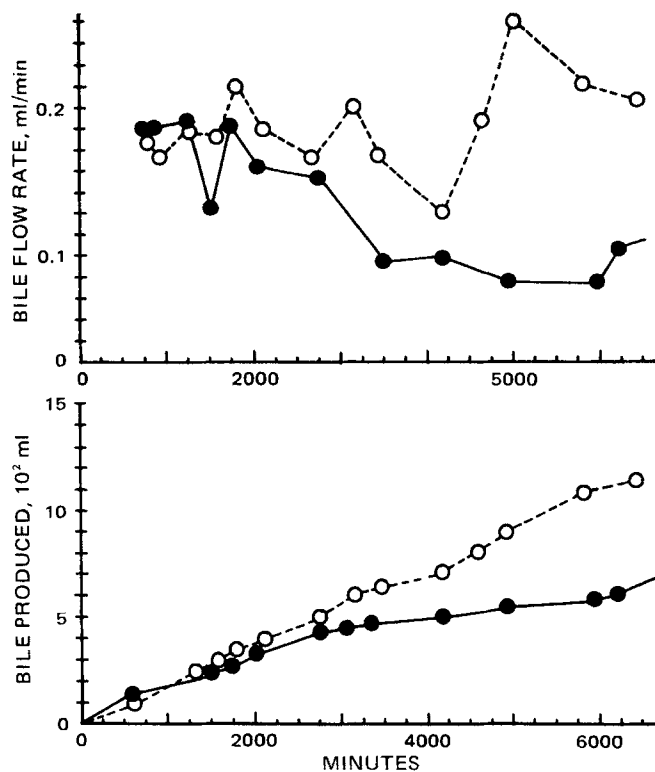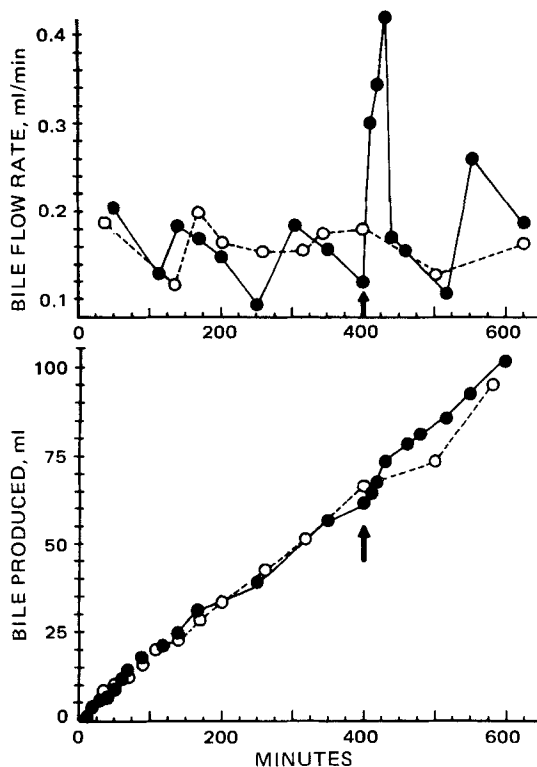[10] Polytron, Kinematica, Lucerne, Switzerland.

Figure 2—*Bile flow rates and cumulative volumes of bile produced with time after the 0.5- (○) and 2.0- (●) mg/kg iv doses of Δ⁹-tetrahydrocannabinol in Dog C. The arrows indicate the time of increased infusion of taurocholic acid from 0.42 to 30 mg/min for 5 min.*

where A_T is the total radioactivity per milliliter of plasma, S is the activity of tetrahydrocannabinol in counts per minute per nanogram, and C is the experimentally observed plasma concentration of tetrahydrocannabinol. After extraction of plasma with heptane for determination of tetrahydrocannabinol and other heptane-extractable metabolites, the aqueous layer was adjusted to pH 2.0–2.3 by addition of 0.2–0.4 ml of a hydrochloric acid–acetic acid buffer [5.2 N HCl–1.0 M acetic acid–water (1:3:2)]. The final volume was either (a) adjusted to 3.0 ml by addition of 0.01 M acetic acid and then adding 15 ml of ethyl acetate or (b) prepared by adding 1 g of sodium chloride and 15 ml of tetrahydrofuran. The tube was stoppered, shaken for 15 min, and centrifuged.

In Case a, an aliquot (1–14 ml) of the organic layer was transferred to a scintillation vial and dried. The remaining organic layer was aspirated off, and 15 ml of tetrahydrofuran and 1 g of sodium chloride were added. This solution was shaken and centrifuged as before. An aliquot (1–14 ml) of the organic layer was transferred to a scintillation vial and dried. The contents of both vials were counted for total carbon-14 as described previously. In Case b, an aliquot (1–14 ml) of the organic layer was transferred to a scintillation vial, dried, and counted as described previously.

The concentration of metabolites in plasma in terms of tetrahydrocannabinol equivalents, extracted into heptane at pH 10.5, $[M_h]$, was calculated from:

$$[M_h] = (A_h/F_1 S) - C \qquad \text{(Eq. 2)}$$

where A_h is the activity extracted per milliliter of plasma, F_1 is the extraction efficiency of tetrahydrocannabinol (14), S is the specific activity of the dose, and C is the concentration of tetrahydrocannabinol in plasma. The calculation assumed that the metabolites extractable into heptane had extraction efficiencies equivalent to tetrahydrocannabinol. The plasma concentration of metabolites extracted into ethyl acetate, $[M_{ea}]$, or tetrahydrofuran, $[M_{thf}]$, under acid conditions following a heptane extraction was calculated from:

$$[M_{ea}] = \frac{A_{ea} - A_h/(1 - F_1)}{S} \qquad \text{(Eq. 3)}$$

and:

$$[M_{thf}] = \frac{A_{thf} - A_h/(1 - F_1)}{S} \qquad \text{(Eq. 4)}$$

where A_{ea} and A_{thf} are the activities of the extracts per milliliter of plasma and are corrected for the unextracted heptane-extractable metabolites; i.e., $A_h/(1 - F_1)$.

RESULTS AND DISCUSSION

Time Course of Total Radioactivity in Plasma—Semilogarithmic plots of the total radioactivity in plasma as A_T/D_0', where A_T is the total activity per milliliter of plasma and D_0' is the activity of the dose, are given in Fig. 1 for typical studies at two dose levels of ¹⁴C-Δ⁹-tetrahydrocannabinol for Dog B (Table I). The data plotted in this manner from the different doses were superimposable for the high and low doses in Dogs A and B, demonstrating no significant dose dependency. Differences in bile flow (Fig. 2) may have been responsible for the greater discrepancy between plasma levels for the two doses given to Dog C (Fig. 3).

An extended study of plasma levels of total carbon-14 was conducted in Dog C for 23 days at 2.0 mg/kg (Fig. 4). The data after 2000 min were
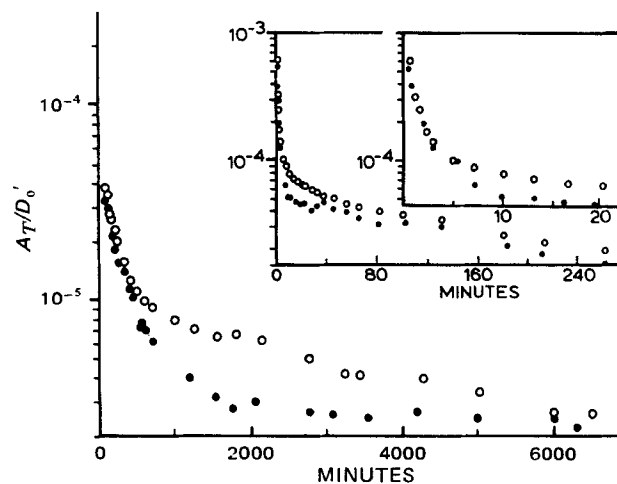

Figure 3—*Semilogarithmic plots of the fractions, A_T/D_0', of the total radioactive dose, D_0', per milliliter of plasma against time for the 2.0-mg/kg (●) (150.8 × 10⁶ cpm) and 0.5-mg/kg (○) (229.4 × 10⁶ cpm) doses of ¹⁴C-Δ⁹-tetrahydrocannabinol in Dog C. The activities per milliliter corresponding to $A_T/D_0' = 10^{-4}$ are 15,080 (●) and 22,940 (○) cpm and correspond to 3320 and 665 ng/ml as Δ⁹-tetrahydrocannabinol equivalents, respectively.*
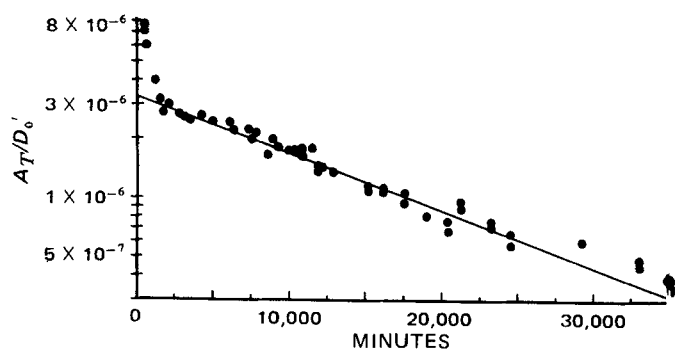
Figure 4—*Semilogarithmic plot of the fraction A_T/D_0' of the total radioactive dose, D_0', per milliliter of plasma against an extended time of study for the 2.0-mg/kg dose of Δ^9-tetrahydrocannabinol in Dog C. The apparent rate constant, k_5, obtained by least-squares regression was $6.74 \times 10^{-5} min^{-1}$.*

consistent with a single exponential of 171.4 hr half-life ($k_i = 6.7 \times 10^{-5}$ min). It was presumed that the terminal half-life for each study would be similar.

The variability of the data prior to 300 min was no different among dogs than within dogs. After 300 min, the total activity in plasma for both doses in Dog C (Fig. 3) dropped significantly below the levels in Dogs A and B (Fig. 1). The collection of total bile from Dog C (Table I), preventing any enterohepatic circulation of metabolites, could explain this difference.

Excretion of Total Radioactivity in Urine and Bile—No significant amount (<0.01%) of tetrahydrocannabinol was in the urine or bile on analysis of the HPLC collection (14). The total radioactivity as the percentage of dose recovered in urine was plotted against time (Fig. 5) and ranged from 14.7 to 16.6% at 7000 min for Dogs A and B.

The excretion rate of total radiolabeled metabolites in bile, dB_T/dt, in Dog C, where all bile was collected and bile flow was maintained by intravenous or oral solutions of taurocholic acid, was proportional to the radioactivity concentration in bile, $[B_T]$, and both values peaked between 25 and 45 min after drug administration. The concentrations of metabolites in bile were approximately 500 times the concentrations of total activity in plasma at this time and subsequently decreased to less than 50% of the plasma concentration at $t \geq 2500$ min.

Linear plots of the bile flow rates and the cumulative volume of bile produced as a function of time are given in Fig. 2 for the high and low dose studies in Dog C.

The percent of the dose recovered in bile for both doses in Dogs C (all bile collected) and A (bile sampled) was approximately 50% after 1 day or 1400 min (Fig. 5). Thereafter, only 5% of the dose was recovered.
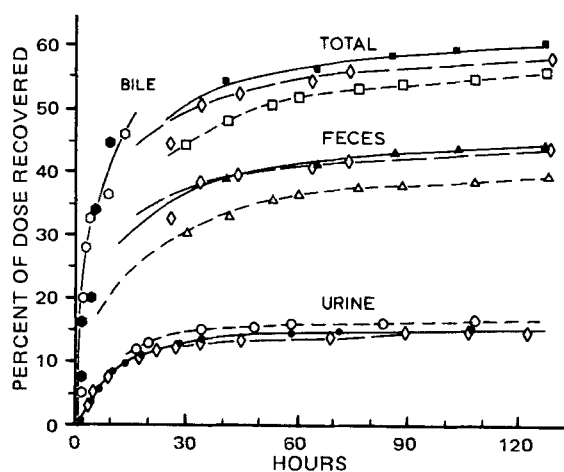

Figure 5—*Linear plots of the percent of dose recovered in urine and feces against time for the two intravenous doses in Dogs A and B. Key: Dog B urine, 0.5 (●) and 2.0 (○) mg/kg; Dog B feces, 0.5 (△) and 2.0 (▲) mg/kg; Dog B total (urine plus feces), 0.5 (□) and 2.0 (■) mg/kg; Dog A urine, feces, and total, 0.1-mg/kg dose (◇). The percent of dose excreted in the sampled bile of Dog A, corrected for the "dead" time of the cannula, is given as ○ for the 0.1-mg/kg dose and as ● for the 2.0-mg/kg dose. The percent of dose was calculated from [$\Delta t \times$ bile flow rate (mg/min) $\times$ 100]/dose, where Δt is the time of collection.*
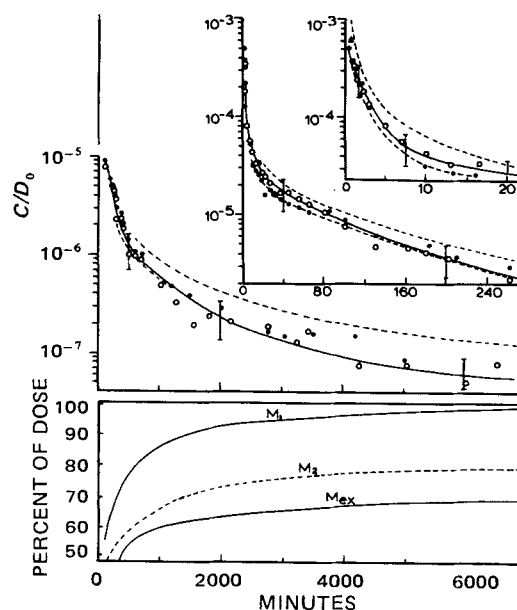

Figure 6—*Semilogarithmic plots of the plasma levels of Δ^9-tetrahydrocannabinol as C/D_0 against time for the 0.5- (○) and 2.0- (●) mg/kg iv doses to Dog C. The solid curve gives the calculated values for C/D_0, $\hat{Y}$, for the five-exponential fit of the 0.5-mg/kg data, and the vertical bars are the range for ± 2 SD of the weighted residuals (Table II). The dashed lines represent the range for the maximum and minimum of all $\hat{Y}$ values calculated. The concentrations of Δ^9-tetrahydrocannabinol in plasma corresponding to the fraction $C/D_0 = 10^{-4}$ are 665 (○) and 3320 (●) ng/ml. Curves M_1 and M_2 were calculated from Eq. 14 using the $\hat{Y}$ expressions of Eqs. 5 and 6, respectively, and represent the total metabolite in percent of dose formed with time, where M_{ex} is the experimentally determined metabolite in percent of dose excreted in both urine and bile.*

During this time, the biliary excretion rate decreased asymptotically to approximately 0.72%/week.

The rates of excretion of total metabolites per unit dose and their concentrations in bile were similar for both Dogs A and C. The consistency in the percent of radiolabeled dose recovered in bile for both doses in Dogs A (Fig. 5) and C demonstrated no significant dose dependency. The percentage recovered in feces ranged from 39.5 to 44.5% over 130 hr for Dogs A and B, whereas the percentage recovered in bile (Dog A) was always consistently higher at any given time (Fig. 5). When all bile was collected, as for Dog C, approximately 55% of the radioactive dose was excreted therein for the same time period. No radioactivity was found in feces when all bile was collected.

Since the percent of the dose recovered in urine was essentially the same for $t > 1000$ min between doses and among dogs, the 10–15% excess recovered in bile compared to feces can be attributed to the enterohepatic circulation of 15% of the biliary excreted metabolites.

Pharmacokinetics of Intravenously Administered Δ^9-Tetrahydrocannabinol—There may not be a unique linear sum of time exponentials, $\hat{Y}$, that best fits the actual data, $Y = C/D_0$, where C is the concentration per milliliter of Δ^9-tetrahydrocannabinol in plasma, and D_0 is the dose; instead, there may be several. A unique solution could only be obtained if the drug transferred into other compartments or transformed into metabolites was analyzed with time in its respective compartments. For example, the plasma data for Dog C at the dose of 0.5 mg of Δ^9-tetrahydrocannabinol/kg where the fraction of the dose per milliliter of plasma is plotted against time (Fig. 6) could be fitted by the method of residuals (15) to:

$$\hat{Y}_1 = \hat{C}/D_0 = 6.80 \times 10^{-4}e^{-1.01t} + 1.25 \times 10^{-4}e^{-0.202t} +$$
$$2.30 \times 10^{-5}e^{-0.0206t} + 7.65 \times 10^{-6}e^{-4.36\times10^{-3}t} +$$
$$5.30 \times 10^{-7}e^{-3.87\times10^{-4}t} \quad \text{(Eq. 5)}$$

or to:

$$\hat{Y}_2 = \hat{C}/D_0 = 5.02 \times 10^{-4}e^{-0.587t} + 4.25 \times 10^{-5}e^{-0.0877t} +$$
$$2.15 \times 10^{-5}e^{-1.16\times10^{-2}t} + 1.88 \times 10^{-6}e^{-1.28\times10^{-3}t} +$$
$$9.50 \times 10^{-8}e^{-4.98\times10^{-5}t} \quad \text{(Eq. 6)}$$

Table II—Parameters of the Best Fit Sum-of-Exponentials, $\hat{Y} = \Sigma_{i=1}^{5}A_i e^{-k_i t}$, Describing the Fraction of the Dose of Δ^9-Tetrahydrocannabinol per Milliliter of Plasma against Time in Minutes

	Dog A		Dog B		Dog C			Apparent
Parameter[a]	0.1 mg/kg	2.0 mg/kg	0.5 mg/kg	2.0 mg/kg	0.5 mg/kg	2.0 mg/kg	Mean[b]	$t_{1/2}$, min
$10^4 A_1$	6.40	5.40	13.7	8.0	5.02	6.70	7.54 (1.31)	—
$10^5 A_2$	7.45	3.93	11.3	14.0	4.25	3.80	7.46 (1.77)	—
$10^5 A_3$	2.10	1.30	2.70	2.35	2.15	1.50	2.02 (0.21)	—
$10^6 A_4$	1.48	2.06	1.90	2.05	1.88	2.45	1.97 (0.13)	—
$10^7 A_5$	1.92	1.92	2.70	1.95	0.95	1.15	1.77 (0.26)	—
$10\,k_1$	9.21	4.35	9.82	10.42	5.87	7.03	7.78 (0.99)	0.90 min
$10\,k_2$	1.26	0.37	1.43	1.31	0.88	0.90	1.03 (0.16)	6.8 min
$10^2 k_3$	1.09	0.70	1.16	1.01	1.16	0.93	1.01 (0.07)	1.15 hr
$10^3 k_4$	1.18	1.54	1.17	1.26	1.28	1.27	1.28 (0.05)	9.0 hr
$10^5 k_5$	5.0	5.0	6.80	6.60	4.98	6.74	5.85 (0.39)	197 hr
$10^2 \bar{\epsilon}$, mean of residuals[c]	0.96	0.40	−1.3	0.17	−0.16	2.1	—	—
$2s$[d]	0.273	0.191	0.284	0.162	0.363	0.289	—	—

[a]The A_i values are in fraction of dose per milliliter of plasma; k_i are in minutes^{-1}. [b]The parenthetical values are standard errors of the mean. [c]The average values of the weighted residuals, $\epsilon = (Y - \hat{Y})/\hat{Y}$, where $\hat{Y}$ is the predicted value and Y is the experimental, none of which is significantly statistically different than zero. [d]Twice the standard deviation of the weighted residuals.

where time, t, is in minutes. The terminal data of the 7000-min span in Fig. 6 were approximated by the best straight line for estimation of the parameters of the last exponential in Eq. 5. The parameters for the last exponential in Eq. 6 were based on the assumption that the terminal slope was close to the rate constant characteristic of the rate of decline of the total radioactivity in the plasma up to 35,000 min (Fig. 5).

The validities of both Eqs. 5 and 6 were confirmed by the demonstration that a plot of the weighted residuals:

$$\epsilon = \frac{Y - \hat{Y}_i}{\hat{Y}_i} \quad \text{(Eq. 7)}$$

for both $\hat{Y}_1$ and $\hat{Y}_2$ against log $Y' = \log C/D_0$ gave a calculated mean, $\bar{\epsilon}$, of zero and where the standard deviations of their ϵ values were practically the same, i.e., 0.13 for Eq. 5 and 0.12 for Eq. 6 (16).

The decision that a linear sum of five exponentials was necessary to fit the plasma tetrahydrocannabinol data for all studies was based on the fact that, when a linear sum of four or less exponentials was used, the plot of the weighted residuals (Eq. 8) against log C/D_0 demonstrated biases above and below the line of the mean of the n weighted residuals:

$$\bar{\epsilon} = \sum_{i=1}^{n}\left[\frac{(Y - \hat{Y})_i}{\hat{Y}_i}\right]\bigg/n \quad \text{(Eq. 8)}$$

which were not statistically significant from zero (Table II). In the particular case of the data of Fig. 6, the standard deviation for a best fit of a linear sum of four exponentials was higher, i.e., 0.21, than for five. The best fitting to a linear sum of six exponentials was similar to the sum of five and did not show significant nonrandom scatter about $\bar{\epsilon} \sim 0$, but the standard deviation (0.15) was not significantly improved.

When the fraction of the dose per milliliter of plasma as tetrahydrocannabinol or its logarithm (Fig. 6) was plotted against time for widely variant doses, the curves were superimposable, indicative of no dose dependency.

Pharmacokinetics of Intravenously Administered Biliary Metabolites of Δ^9-Tetrahydrocannabinol—The plasma levels of the intravenously administered radiolabeled metabolites were adequately described in Dog C by a sum of three exponentials (Fig. 7). These metabolites were those collected in the bile for 300 min after ^{14}C-Δ^9-tetrahydrocannabinol administration, and 98% of the collected radioactivity was retained after heptane extraction at pH 10 to remove highly lipid-soluble components. The metabolites administered were those extracted from the aqueous bile by tetrahydrofuran. This extract contained over 90% of the original radioactivity. The calculated terminal half-life for these metabolites in plasma was 230 min. At 500 min, a total of 90.7% of the administered dose was eliminated in bile and urine (Fig. 8). Ninety-six percent of the administered dose was eliminated by 24 hr, with the majority (73%) eliminated in bile. The final ratio of metabolites eliminated in bile to that in urine, 2.4, was slightly lower than the ratio, 3.2, for *in vivo* produced metabolites.

Evidence that Rates of Terminal Decline of Total Plasma Radioactivity Estimates Rates of Terminal Decay of Plasma Tetrahydrocannabinol Concentration and that the Ultimate Rate-Determining Step for Elimination is Return from Deep Tissues—Since no dose-dependent pharmacokinetics for tetrahydrocannabinol or its metabolites were observed, it can be postulated that the metabolism was first order and dependent only on the estimated concentration, $\hat{C}$, of tetrahydrocannabinol in the central compartment or:

$$dM/dt = k_m V_c \hat{C} \quad \text{(Eq. 9)}$$

where $k_m V_c$ is the metabolic clearance. Thus:

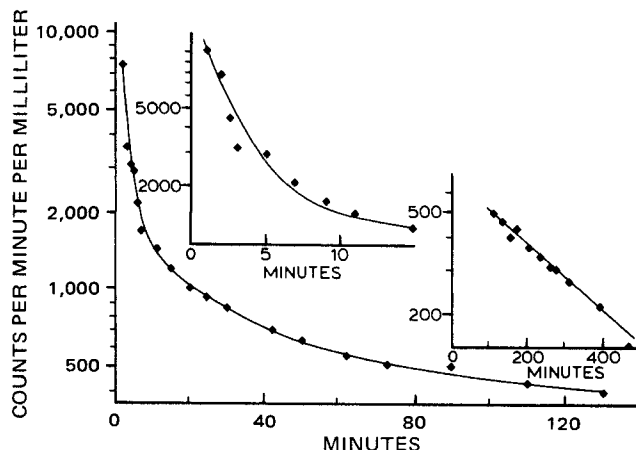$$M = \int_0^M dM = k_m V_c \int_0^t \hat{C}\, dt \quad \text{(Eq. 10)}$$

Figure 7—Semilogarithmic plot of the activity (counts per minute) per milliliter of plasma against time for the intravenous administration of biliary Δ^9-tetrahydrocannabinol metabolites to Dog C. The inserts are for expanded and extended time scales. The curves through the data are given by $\hat{Y}_m = \Sigma_{i=1}^{3} A_i e^{-k_i t}$, where $A_1 = 15{,}013$, $A_2 = 1330$, and $A_3 = 584$ cpm/ml and $k_1 = 0.542$, $k_2 = 0.0477$, and $k_3 = 3.005 \times 10^{-3}$ min^{-1}.
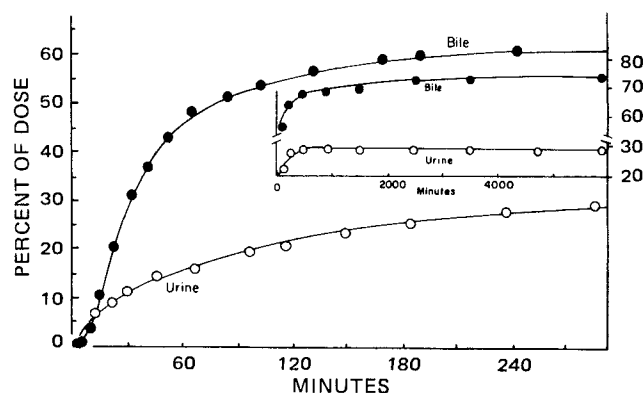

Figure 8—Linear plots of the percent of the radioactivity recovered in bile (●) and urine (O) against time for the intravenous administration of biliary Δ^9-tetrahydrocannabinol metabolites to Dog C.

Table III—Apparent Volumes of Distribution, Clearances, and First-Order Metabolic Rate Constants for Δ^9-Tetrahydrocannabinol

Parameter	Dog A		Dog B		Dog C		Mean (SEM)
	0.1 mg/kg	2.0 mg/kg	0.5 mg/kg	2.0 mg/kg	0.5 mg/kg	2.0 mg/kg	
10^4 $C_0/D_0{}^a$	7.37	5.95	15.1	9.66	5.68	7.26	8.50 (1.44)
$V_c{}^b$, liters	1.36	1.68	0.66	1.04	1.76	1.38	1.31 (0.17)
$Cl_p{}^c$, ml/min	120.4	106.9	98.9	114.3	152.2	151.0	124.0 (9.2)
$10^3 k_m{}^d$, min^{-1}	8.87	6.36	14.96	11.0	8.64	10.96	10.13 (1.19)
$V_B{}^e$, liters	0.77	0.95	0.90	0.90	0.811	1.05	0.90 (0.04)
$Cl_p{}^{uf}$, ml/min	4013	3563	3297	3810	5073	5033	4131 (307)
$V_{p_{ss}}{}^g$, liters	2405	2136	1454	1732	3055	2239	2170 (227)

[a] The fractions of the dose of tetrahydrocannabinol per milliliter of plasma at time zero estimated from $\hat{Y} = \Sigma_{i=1}^n A_i e^{-k_i t}$ for $t = 0$, where the A_i and k_i parameters are given in Table II. [b] Apparent volume of distribution of the central compartment referenced to total drug concentration in plasma, $D_0/10^3 C_0$. [c] Clearance referenced to total concentration in plasma, calculated from Eq. 14 where the parameters of Y are given in Table II. [d] The rate constant with respect to total concentration in plasma calculated from $Cl_p/10^3 V_c$. [e] The volume of plasma in the dog calculated from $(1-H) V_{B,true}$, where $V_{B,true}$ = dog weight in kilograms × 100 ml/kg (18), and H is the hematocrit (Table I). [f] Clearance referenced to unbound drug in plasma, calculated from $Cl^u = Cl_p/f_u$, where f_u is the fraction of the unbound drug in plasma and is 0.03 (13). [g] The apparent volume of distribution referenced to total plasma concentration in the ultimate pseudo-steady state of tetrahydrocannabinol elimination from the body calculated from $10^{-3} Cl_p/k_s$, where k_s is given in Table II.

where M is the amount of metabolite(s) produced in the first metabolic step from tetrahydrocannabinol, k_m is the first-order metabolic rate constant, and $V_c = D_0/C_0$ (Table III) is the apparent volume of distribution of the central compartment for tetrahydrocannabinol of dose D_0. The $\hat{C}_0$ is the estimated concentration of tetrahydrocannabinol in plasma at $t = 0$ and can be calculated from Eq. 5 or 6 with $t = 0$.

Substitution of Eq. 5 or 6 into Eq. 10 gives:

$$M = k_m V_c D_0 \int_0^t \hat{Y} \, dt \qquad \text{(Eq. 11)}$$

At $t = \infty$, $M = M_\infty = D_0$, since tetrahydrocannabinol was not excreted unchanged but was transformed to metabolites. Thus, the apparent metabolic rate constant is:

$$k_m = 1/V_c \int_0^\infty \hat{Y} \, dt \qquad \text{(Eq. 12)}$$

Substitution of Eq. 12 into Eq. 11 yields:

$$M = D_0 \frac{\int_0^t \hat{Y} \, dt}{\int_0^\infty \hat{Y} \, dt} = D_0 A_t / A_\infty \qquad \text{(Eq. 13)}$$

and the total metabolites, both excreted and unexcreted, produced at time t can be calculated from the pertinent fit, $\hat{Y}$, of the plasma tetrahydrocannabinol data against time or from the ratio of the area under the C versus t plot at time t, A_t, to the total area, A_∞. The values calculated for total metabolites with time, M_1 and M_2, as the percent of dose from the $\hat{Y}_1$ and $\hat{Y}_2$ obtained with Eqs. 5 and 6, respectively, are plotted in Fig. 6 against time. For comparative purposes, the experi-

mentally determined total amount of radiolabeled drug, M_{ex}, excreted into bile and urine as the percent of dose is also plotted against time for 0.5 mg/kg. At 7000 min, the total amounts of metabolites excreted by Dog C (Table IV), M_{ex}, comprised 68% of the dose, whereas the calculated amounts of metabolites formed, M_1 and M_2, were 98 and 79%, respectively (Fig. 8).

If the M_1 values, and thus the parameters of Eq. 5 with an apparent terminal half-life of 30 hr, were accepted as valid for tetrahydrocannabinol administration, it must be concluded that tetrahydrocannabinol metabolism was virtually concluded at 7000 min, that $98 - 68\% = 30\%$ of the formed metabolites was still in the body, and that this large amount of metabolites was very slowly eliminated well after 7000 min (Fig. 4) with an apparent half-life of 171 hr. The result implies that these metabolites are highly sequestered in, and/or bound to, body tissues so that they have extremely large apparent volumes of distribution. However, these hypotheses are inconsistent with the fact (Fig. 8) that 90.7% of intravenously administered biliary metabolites was eliminated in both bile and urine within 500 min of administration, with 96% eliminated by 1500 min. Also, the terminal plasma half-life of administered radiolabeled metabolites of 230 min (Fig. 7) supports the hypothesis that the rate-determining step for metabolite elimination on Δ^9-tetrahydrocannabinol administration is not the elimination of such metabolites.

The estimate of M_2 from Eq. 13 as the amount of total metabolites formed at any time is greater than M_1, since M_2 was obtained from the $\hat{Y}_2$ of Eq. 6 where the terminal slope was smaller and the plotted area, A_∞, was greater than would be obtained in Eq. 13 from the M_1 calculated from the $\hat{Y}_1$ of Eq. 5. This M_2 is more consistent than M_1 with the fraction of the dose excreted as metabolites, M_{ex} (Fig. 6). It follows that the ob-

Table IV—Percent Disposition of Δ^9-Tetrahydrocannabinol at 7000 min after Intravenous Administration

	Dog A		Dog B		Dog C[a]	
	0.1 mg/kg	2.0 mg/kg[b]	0.5 mg/kg	2.0 mg/kg	0.5 mg/kg	2.0 mg/kg
Δ^9-Tetrahydrocannabinol in central compartment[c]	0.018	0.023	0.011	0.013	0.012	0.010
Δ^9-Tetrahydrocannabinol in tissues[d]	29.2	29.0	24.4	21.3	20.4	16.1
Metabolized[e]	70.8	71.0	75.6	78.7	79.6	83.9
Excreted metabolites[f]	57.1	—	56.0	59.5	68.4	71.5
Metabolites in body[g]	13.7	—	19.6	19.2	11.2	12.4

[a] All bile was collected, and enterohepatic circulation was not possible. [b] Study terminated at 1300 min; all values given are extrapolated values, and no feces were collected. [c] $10^5 \times V_c \hat{Y}$, where $\hat{Y} = \Sigma_{i=1}^5 A_i e^{-k_i t}$ in fraction of dose per milliliter of plasma, and the A_i and k_i parameters of Table II were used. The V_c values in liters are listed in Table III. [d] $10^2 T/D_0$, where T is the amount of the dose, D_0, not in the central compartment, $V_c C$, or transformed to metabolite, M; i.e., $D_0 - V_c C - M$, where $V_c C = 10^2 V_c \hat{Y} D_0$. [e] The percent metabolized is $10^2 M/D_0$, where M was obtained from Eq. 13, $M = D_0 \int_0^t \hat{Y} \, dt / \int_0^\infty Y \, dt$. [f] Percent of dose excreted in urine plus bile (Dog C) or feces (Dogs A and B). [g] Difference of above two values.
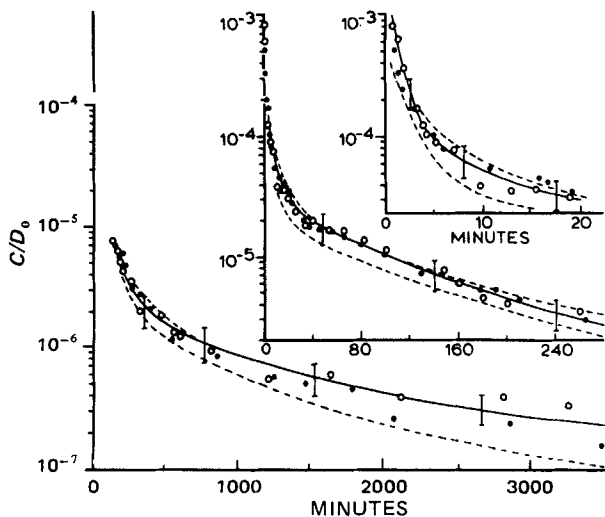
Figure 9—*Semilogarithmic plots of the plasma Δ^9-tetrahydrocannabinol levels as C/D₀ against time for the 0.5- (O) and 2.0- (●) mg/kg iv doses to Dog B. The solid curve gives the calculated values for C/D₀,$\hat{Y}$, for the five-exponential fit of the 0.5-mg/kg data, and the vertical bars are the range for ±2 SD of the weighted residuals (Table II). The dashed lines represent the range for the maximum and minimum of all $\hat{Y}$ values calculated. The concentrations of Δ^9-tetrahydrocannabinol in plasma corresponding to the fraction C/D₀ = 10⁻⁴ are 635 (O) and 2880 (●) ng/ml.*

Figure 10—*Percent of biliary metabolites in a given sample that were extracted into benzene (a) or ethyl acetate (b) at selected pH values plotted against time after the administration of 0.5 mg of Δ^9-tetrahydrocannabinol/kg iv to Dog C.*

served rate of terminal loss of plasma total radioactivity from 2000 to 35,000 min (Fig. 4) is consistent with the terminal loss of Δ^9-tetrahydrocannabinol in the plasma as characterized by the last exponential in Eq. 6, which cannot normally be observed since the available analytical sensitivity did not permit assay of plasma tetrahydrocannabinol for more than 5000–6000 min after administration of the given doses.

This terminal rate of the 171.4-hr half-life (Fig. 4) for the loss of total radioactivity provided initial estimates of the terminal half-life and the derived k_5 values for the plasma Δ^9-tetrahydrocannabinol data in each dog. The determined parameters for the linear sum of five exponentials used to fit the assayed plasma levels of Δ^9-tetrahydrocannabinol with time (Figs. 6 and 9) are given in Table II and are considered as the most appropriate parameters. An expression for $\hat{Y}$ using the mean values of the parameters obtained for all studies reasonably fit all plasma–time data for tetrahydrocannabinol. The data from each study lay within ±2 SD of the resultant common equation (Figs. 6 and 9).

The consistency of these terminal rates of plasma total radioactivity and Δ^9-tetrahydrocannabinol decay indicates a typical "flip-flop" (17), where the rate-determining step in plasma decay of both tetrahydrocannabinol and its formed metabolites is the slow return of the tetrahydrocannabinol in the tissues to the central compartment where it can be metabolized (Eq. 9). A logical consequence of this assertion is that the composition of the metabolites would remain constant and in pseudo-steady-state equilibrium when the rate-determining step is return of tetrahydrocannabinol from the tissues since metabolism is a relatively fast process. This was confirmed (Fig. 10) by the reasonable constancy of the percent of biliary metabolites extracted into benzene and ethyl acetate after 100–200 min. The similarity of data for the low and high doses of Δ^9-tetrahydrocannabinol confirmed the lack of dose dependency. Similarly, the percent of urinary metabolites extracted into various solvents at selected pH values did not significantly vary with time (Fig. 11), and the pseudo-steady state exemplified by these studies was reached prior to the collection of the 50–73-min urine.

The plasma levels of metabolites, $[M_p]$, in terms of tetrahydrocannabinol equivalents were calculated from Eq. 1. The fact that the ratio of plasma levels of total metabolites, M_p, to Δ^9-tetrahydrocannabinol, C, were constant after 3000 min subsequent to drug administration provided further evidence that the return of tetrahydrocannabinol from tissues was the rate-determining step for metabolism and subsequent elimination of metabolites.

Clearance and Apparent Volume of Distribution of Δ^9-Tetrahydrocannabinol—Tetrahydrocannabinol was cleared from the body solely by metabolism. This clearance, Cl_p, referenced to total plasma concentration is a product of the metabolic microscopic rate constant, k_m, and the apparent volume of distribution of the central compartment,
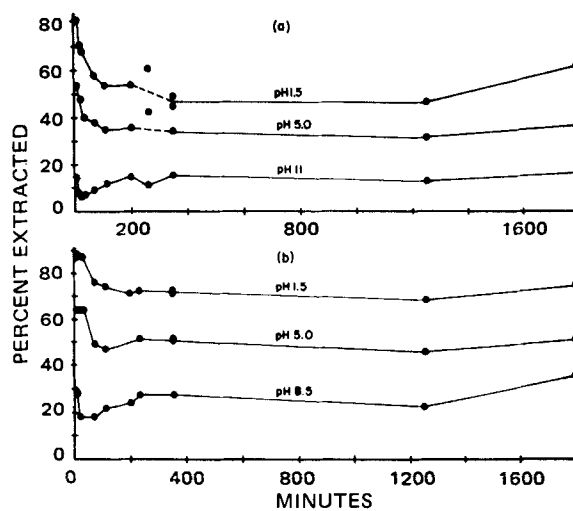
V_c, and can be obtained from a rearrangement of Eq. 12 to:

$$Cl_p = k_m V_c = 1 / \int_0^\infty \hat{Y}\, dt \qquad \text{(Eq. 14)}$$

where the values of $\hat{Y}$ can be obtained from the linear sum of five exponentials using the parameters of Table II.

The average metabolic clearance, Cl_p, for all six studies was 124 ± 22.6 ml/min (Table III). The calculated hepatic blood flow for these dogs ranged from 372 to 747 ml/min, 30–45 ml/min/kg (18). Multiplication of the hepatic blood flow by $(I - \overline{H})$, where $\overline{H}$ = 0.363 was the mean hematocrit (Table I), gave a range for hepatic plasma flows of 237–476 ml/min and was approximately twice the metabolic clearance of both bound and unbound drug in plasma. Thus, Δ^9-tetrahydrocannabinol was not completely cleared in a single pass through the liver as previously reported (10). If only the unbound drug were cleared, then the mean clearance referenced to unbound drug, $Cl_p{}^u = Cl_p/f^u$ (Table III), would be 4132 ± 753 ml/min, an order of magnitude greater than hepatic plasma flow, where f^u = 0.03 (13), the fraction of unbound drug in plasma. Since the liver is the primary metabolizing organ (19), an apparent substantial fraction of the bound drug was cleared in a single pass through the liver.
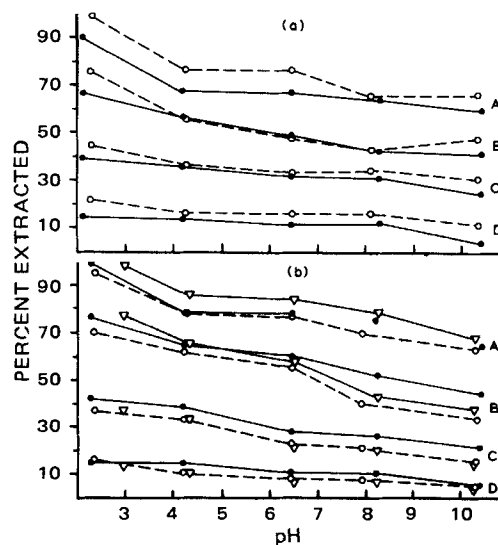

Figure 11—*Plots of the percent of urinary metabolites extracted into various solvents as a function of pH. Aliquots of the urine from the 2.0-mg Δ^9-tetrahydrocannabinol/kg study in Dog B were adjusted to the indicated pH and extracted with tetrahydrofuran (A), ethyl acetate (B), heptane containing 1% butanol (C), or heptane containing 1.5% isopentyl alcohol (D). Urine collection intervals in (a) were 50–73 (O) and 133–195 (●) min; in (b), they were 325–381 (●), 646–855 (O), and 1244–1474 (▽) min.*
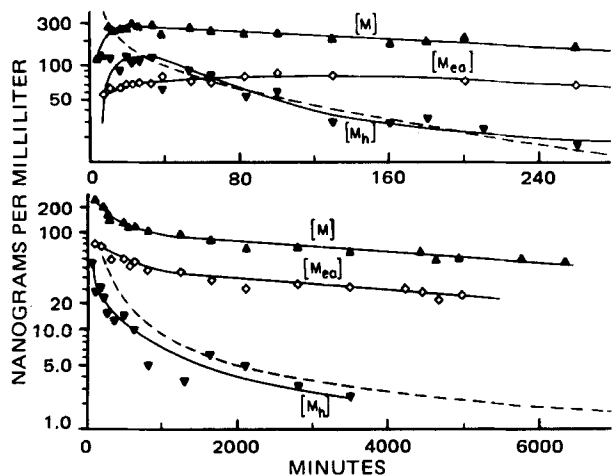
Figure 12—*Semilogarithmic plots of* Δ^9*-tetrahydrocannabinol metabolites in plasma against time for the 0.5-mg/kg iv dose of* Δ^9*-tetrahydrocannabinol in Dog B. The concentration of total metabolites in plasma [M] (▲), as nanograms per milliliter equivalents of* Δ^9*-tetrahydrocannabinol, was calculated from Eq. 1. The concentration of heptane-extractable metabolites (at pH 10.5) in plasma, [M_h] (▼), as nanograms per milliliter equivalents of* Δ^9*-tetrahydrocannabinol, is given by Eq. 2. The concentration of ethyl acetate-extractable metabolites (at pH 2.5; 5 ml of solvent/ml of plasma) in plasma, [M_ea] (◇), as nanograms per milliliter equivalents is given by Eq. 3. The best fit values for C/D₀,Ŷ (Table II), are given as concentration of* Δ^9*-tetrahydrocannabinol per milliliter of plasma in the dashed curve.*

The transit time of plasma in the liver may be sufficient for a series of reequilibrations between bound and unbound drug, with only the latter being actually metabolized.

The apparent first-order metabolic rate constants, k_m (Table III), calculated from the metabolic clearances (Eq. 14) ranged from 0.0636 to 0.1446 min^{-1} and were consistent with rapid metabolism ($t_{1/2}$ values from 4.8 to 10.9 min).

The high plasma protein binding (97%) and comparatively lower red blood cell partitioning (13) from plasma of Δ^9-tetrahydrocannabinol require that the expected apparent volume of distribution of the central compartment referenced to total plasma Δ^9-tetrahydrocannabinol concentration should be close to the plasma volume. Consistent with this premise, the apparent volumes of distribution, V_c, ranged from 0.66 to 1.76 liters, close to those of the calculated plasma volumes, V_p, which ranged from 0.77 to 1.05 liters (Table III).

Unfortunately, it is difficult to estimate accurately the apparent volume of distribution of the central compartment referenced to unbound drug in plasma when a drug such as Δ^9-tetrahydrocannabinol is highly bound, i.e., 97%. The equation (20) for the estimation of this volume depends on the fraction unbound, the apparent volume of distribution, and the true volume, V_p, of the plasma. When the latter two are similar, as in the present case, and literature values of V_p are used, large errors in such estimates are inevitable.

The apparent volume of distribution ($V_{p_{ss}}$ in Table III) of the pseudo-equilibrated fluids in the final pseudo-steady state of drug elimination referenced to the total drug concentration in plasma can be obtained from the quotient of the metabolic clearance, Cl_p (Table III), and the terminal apparent first-order rate constant of metabolism, k_5 (Table II). The value of $V_{p_{ss}}$ ranged from 1500 to 3000 liters. This apparent volume of distribution would be much higher (20) if it could be referenced to the unbound concentration in plasma. The high degree of sequestration and distribution of Δ^9-tetrahydrocannabinol in body tissues is apparent from the fact that dog weights ranged only from 12 to 17 kg.

Time Course of Plasma Levels of Total and Extractable Metabolites—Typical plasma levels of total metabolites, $[M_p]$, were calculated from Eq. 1 for the six studies (Table I) where Δ^9-tetrahydrocannabinol was administered intravenously and are given in Fig. 12 for the 0.5-mg/kg dose in Dog B. Peak concentrations, in terms of tetrahydrocannabinol equivalents, ranged from 1050 to 1450 ng/ml at 22–36 min in Dogs B and C for the 2.0-mg/kg doses and from 250 to 280 ng/ml at 22–28 min in Dogs B and C for the 0.5-mg/kg doses; a peak of 58 ng/ml occurred at 28 min for the 0.1-mg/kg dose given to Dog. A. The peak levels were proportional to dose and confirmed the lack of dose dependency.

The pH 10.5 heptane-extractable metabolites, $[M_h]$, from plasma (Eq. 2), corrected for any possible tetrahydrocannabinol in plasma (Fig. 12), increased rapidly to a broad maximum between 12 and 40 min and then decreased, paralleling the loss of tetrahydrocannabinol (Fig. 12) from plasma to indicate a similar metabolic clearance of these relatively nonpolar metabolites. At the earliest times, $[M_h]$ accounted for less than 50% of the concentration of $[M_p]$ in plasma, whereas at later times ($t > 2000$ min) $[M_h]$ accounted for less than 10% of $[M_p]$. The semilogarithmic plots of the pH 2.0 ethyl acetate-extractable metabolites, $[M_{ea}]$ (Eq. 3), which were identical with the tetrahydrofuran-extractable metabolites (Eq. 4), against time (Fig. 11) paralleled the total metabolite concentration in plasma $[M_p]$ after its maximum was achieved. This finding indicated that these more polar acidic metabolites maintained a constant ratio to the total metabolites in plasma subsequent to 70–100 min after tetrahydrocannabinol administration.

The simultaneous appearance in plasma of both nonpolar (heptane extractable, $[M_h]$) and acidic polar metabolites (ethyl acetate and tetrahydrofuran extractable, $[M_{ea}]$ and $[M_{thf}]$) may be explained by either parallel metabolic processes or by sequential metabolic processes during each pass through the liver.

This extremely rapid appearance of metabolites in the plasma, which peaked at 25–30 min, demonstrated the extremely rapid metabolism of tetrahydrocannabinol consistent with the metabolic half-lives of 4.8–10.9 min estimated from the derived k_m values (Table III). Since the experimentally observed plasma tetrahydrocannabinol values persisted for 7000 min, it must be stated that the nonmetabolized drug rapidly distributed into tissues concomitantly with its rapid metabolic clearance from the central compartment. Estimates of the disposition at that time are given in Table IV.

These phenomena can be dramatically demonstrated by analog computer fitting of the data obtained for the first 60 min after intravenous administration of Δ^9-tetrahydrocannabinol; the computer need only be programmed to a three-compartment body model, rather than the five needed for 7000 min, since negligible amounts of drugs were returned from the deeper third and fourth tissues during the 60 min. Programs, techniques, and procedures for fitting pharmacokinetic data by the analog computer were described previously (20).

The most highly divergent data from the various studies, the 0.1- and 0.5-mg/kg studies in Dogs A and C, were used for the analog computer fitting of the scheme in Fig. 13. They included the experimentally obtained plasma levels of Δ^9-tetrahydrocannabinol with time and the total amount of metabolites, M, produced with time as calculated from Eq. 13. The sets of microscopic rate constants are listed in Table V. The simultaneous rapid distribution of large amounts of drug into the tissues—viz., T_1 and T_2, and the transformation into metabolites, M, are apparent from the example of Fig. 13.

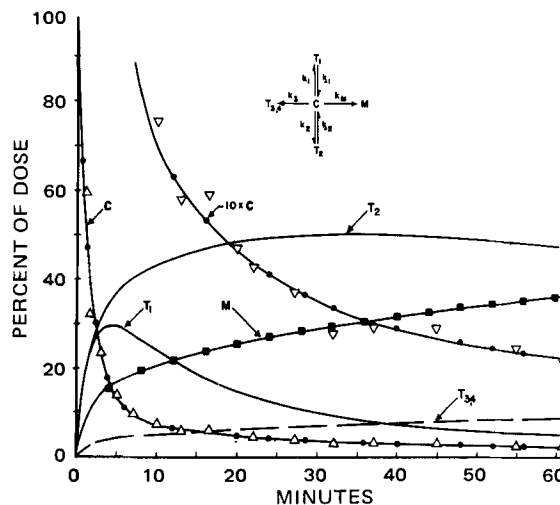**Pseudo-Clearances and Pseudo-Apparent Volumes of Distribu-**

Figure 13—*Analog computer fitting of plasma* Δ^9*-tetrahydrocannabinol data (△) and metabolites (■) produced, M (as calculated from Eq. 13), for 60 min after the 0.1-mg/kg study in Dog A for the scheme given. The plasma values (●) were calculated with the pertinent constants of Table II. The plasma values of* Δ^9*-tetrahydrocannabinol (▽) were also fit on a 10-fold expanded ordinate. The generated tissue levels of* Δ^9*-tetrahydrocannabinol, T₁, T₂, and T₃,₄ = T₃ + T₄, are also given.*

Table V—Pharmacokinetic Parameters after Intravenous Administration of Δ^9-Tetrahydrocannabinol Obtained by Analog Computer Fitting to the Scheme in Fig. 13[a]

Parameter	Dog A 0.1 mg/kg	Dog C 0.5 mg/kg
V_C, liters	1.35	1.77
$10^2 k_M$, min^{-1}	9.11	8.68
$10^2 k_1$, min^{-1}	34.62	21.83
$10^2 k_{-1}$, min^{-1}	20.11	11.38
$10^2 k_2$, min^{-1}	29.74	19.73
$10^2 k_{-2}$, min^{-1}	1.81	1.27
$10^2 k_3$, min^{-1}	7.59	2.11

[a]The fit was considered adequate when the experimental values of $10^5 \, V_C C/D_0$ in terms of percent of dose in plasma (where V_C is apparent volume of distribution of the central compartment in liters from Table III) and the $10^2 \, M/D_0$ values (from M calculated by Eq. 13) were reasonably fit.

tion of Metabolites of Δ^9-Tetrahydrocannabinol—Pseudo-clearances,

Cl_R and Cl_B, of total metabolites after tetrahydrocannabinol administration were estimated from the quotients of the rates of appearance of total radioactivity in the urine or bile, respectively, and the plasma concentrations of radiolabeled metabolites, $[M_p]$, where:

$$Cl_R = (\Delta U_T/\Delta t)/[M_p] \qquad \text{(Eq. 15)}$$

and:

$$Cl_B = (\Delta B_T/\Delta t)/[M_p] \qquad \text{(Eq. 16)}$$

where ΔU_T and ΔB_T are the amounts of radioactivity collected in an interval, Δt, and $[M_p]$ is the plasma concentration at the midpoint of the interval. These apparent clearances were plotted against the time at the midpoint of the collection interval, and typical examples are given for bile (Fig. 14) and urine (Fig. 15).

The maximum pseudo-renal clearances (Fig. 15) for Dogs A and B (5.5–8.4 ml/min at about 300 min) were similar, but those for Dog C were higher (17–21 ml/min). The pseudo-renal clearances did plateau after their decrease from the maximum during the 400–1200-min interval and then gradually decreased toward zero over the remaining 5000 min. The pseudo-biliary clearances (Fig. 14) for Dog C (80–100 ml/min) showed a maximum slightly earlier at 60 min but did plateau after their decrease during the 100–500-min interval and then gradually decreased to an apparent asymptote of 1–3 ml/min by 3000 min.

The initially low, but subsequently increasing, pseudo-renal and biliary clearances are consistent with an initial production of nonpolar metabolites that can be extensively reabsorbed back to plasma in the kidneys and liver. The fact that peak renal clearances are low (Fig. 15) implies high plasma protein binding of metabolites and/or their large tubular reabsorption. A glomerular filtration rate of 80 ml/min (18) and a 92% plasma protein binding of such metabolites, a fact substantiated in pre-
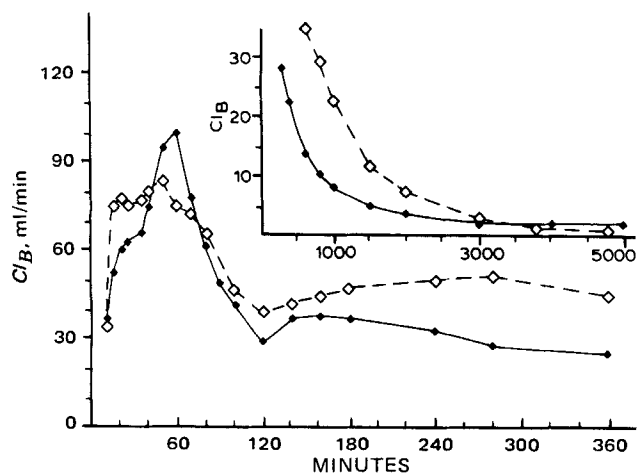

Figure 15—*Plots of the renal clearance,* Cl_R, *of total metabolites excreted in urine against time for the 0.5-* ($\triangle$) *and 2.0-* ($\blacktriangle$) *mg/kg doses in Dog B.* $Cl_R = (dU_T/dt)/[M_p]$, *where* dU_T/dt *and* $[M_p]$ *were estimated from data such as are given in Figs. 8 and 12, respectively.*
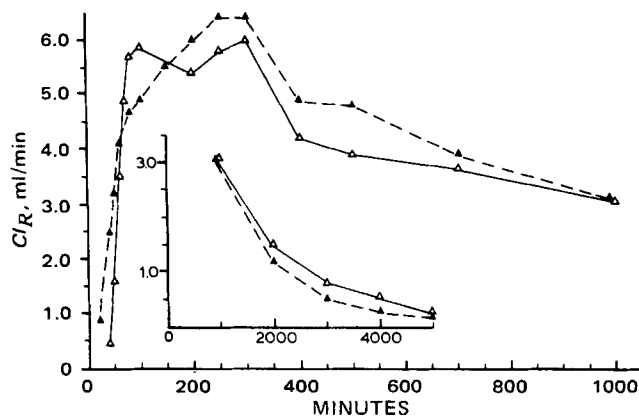
liminary binding studies, could account for the observed low peak pseudo-renal clearances of 6.0–6.4 ml/min in Dog B (Fig. 15).

These observed differences in pseudo-clearances with time undoubtedly reflected changes in the metabolite composition of plasma where the approach to the maximum resulted from the transition from production of less polar, reabsorbable metabolites to those of higher polarity. The subsequent decrease resulted from the relatively rapid elimination of these more polar compounds, with a subsequently relatively higher retention and accumulation in the circulating fluids of less rapidly eliminated metabolites. The fact that over 88% of the biliary metabolites intravenously administered was eliminated by 300 min (Fig. 8) is confirmatory. It should be remembered that material, <10%, had been extracted by heptane before these metabolites had been administered. The general invariance of extraction characteristics for the metabolites in bile (Fig. 10) and urine (Fig. 11) in the 200–1000-min interval was consistent with the reasonable constancy of the pseudo-clearances (Fig. 14) in the same interval.

The subsequent slow decay to the lower asymptotic value achieved after 2000–3000 min can only be explained by the formation of a metabolite that is not further metabolized easily which is initially a small percent of the dose but is not readily biliary or renally excreted. Thus, at a later time, it accounts for an increasing percentage of the radiolabeled metabolites in the plasma until a new pseudo-steady state among metabolite components is reached at 3000 min. At this time, sufficient amounts of this metabolite have accumulated so that its formation rate is approximately equal to its elimination rate from the body. This is the achievement of the final pseudo-steady state when the rate-determining step for the overall elimination of Δ^9-tetrahydrocannabinol from the body is the slow return of the drug from the deepest tissues where it has been sequestered.

Pseudo-apparent volumes of distribution, V_m, of the metabolite formed on intravenous administration of Δ^9-tetrahydrocannabinol as a function of time (Fig. 16) can be calculated from the quotient of the amount of metabolites in the body, M_{in}, and the observed concentration of metabolites in the plasma, $[M_p]$ (Fig. 12). These M_{in} values were estimated from the differences between the predicted amount, M, of total metabolites produced (Eq. 13 and Fig. 6) and the total amounts of radiolabeled metabolites, M_{ex}, excreted in the bile and urine at a given time (Fig. 8) so that:

$$M_{in} = M - M_{ex} \qquad \text{(Eq. 17)}$$

Thus:

$$V_m = M_{in}/[M_p] = (M - M_{ex})/[M_p] \qquad \text{(Eq. 18)}$$

These pseudo-apparent volumes of distribution as a function of time depend on the calculated M values (Eq. 13) and, thus, the set of parameters chosen to describe the time course, $\hat{Y}$, of tetrahydrocannabinol in plasma. Curves A and B in Fig. 16 were calculated on the premises of the validity of Eqs. 6 and 5, respectively, where the former was based on presumption that the half-life of tetrahydrocannabinol elimination was reflected by the terminal half-life for total radioactivity in the body (Fig. 4). Support for this presumption was afforded by the fact that the estimated apparent volume of distribution, $(V_m)_{est}$, of the intravenously administered biliary metabolites (curve C) was the same 6 liters between
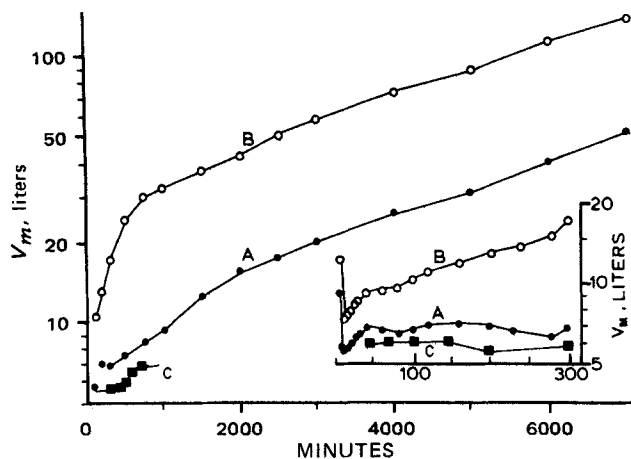

Figure 14—*Plots of the biliary clearance,* Cl_B, *against time for the 0.5-* ($\blacklozenge$) *and 2.0-* ($\lozenge$) *mg/kg iv doses in Dog C.* $Cl_B = (dB_T/dt)/[M_p]$, *where* dB_T/dt *and* $[M_p]$ *were estimated from data such as are given in Figs. 8 and 12, respectively.*

Figure 16—*Pseudo-apparent volumes of distribution of total metabolites for the intravenously administered biliary metabolites, $V_{m_{est}}$ (curve C), and those formed on intravenous administration of 0.5 mg of Δ^9-tetrahydrocannabinol/kg to Dog C, V_m. The calculations of V_m for curves A and B were based on the premise of the validity of Eqs. 6 and 5, respectively, for the Δ^9-tetrahydrocannabinol administration.*

Figure 17—*Semilogarithmic plots of the total metabolites, M, produced as percent of dose against time for the 0.5-mg Δ^9-tetrahydrocannabinol/kg dose in Dog C. The values were calculated from Eq. 16 using an estimated pseudo-apparent volume of distribution, $(V_m)_{est} = 6.0$ liters, and the drawn curve was calculated from Eq. 13.*

50 and 300 min as observed with curve A. These values were obtained from:

$$(V_m)_{est} = \frac{CPM_0 - CPM_{ex}}{[CPM]_p} \qquad \text{(Eq. 19)}$$

where CMP_0 is the total activity of the administered metabolites, CPM_{ex} is the total activity excreted in bile and urine at a given time, and $[CPM]_p$ is the concentration of activity per milliliter in plasma at that time.

The presumption of 6 liters as the apparent volume of distribution of metabolites should permit proper estimation of the cumulative total, M (Eq. 13), of metabolites produced:

$$M_{est} = (V_m)_{est}[M_p] + M_{ex} \qquad \text{(Eq. 20)}$$

if the composition of the metabolites comprising $[M_p]$ did not change significantly with time. The fact that M_{est} satisfactorily estimates M prior to 1000 min is clearly shown in Fig. 17. The deviations between them after 1000 min of approximately 10% at 7000 min are a consequence of the increase in the pseudo-apparent V_m (Fig. 16, curve A). This increase could have resulted from the accumulation in tissues of slowly eliminated nonpolar metabolites comprising less than 10% of the total metabolites formed, a fact consistent with: (a) the previous explanations of the observed declines in the apparent biliary and urinary clearances of total metabolites, (b) the fact that the biliary metabolites intravenously administered had 10% of the radiolabeled metabolites removed prior to administration and also had 88% of the administered dose eliminated by 300 min (Fig. 7), and (c) the fact that the fraction of the dose as metabolites in the body at 7000 min during the final pseudo-steady state was close to these 10% values (Table IV).

Significances and Implications of Observed Pharmacokinetics— The sequestration of administered Δ^9-tetrahydrocannabinol in deep tissues (Table IV) and its large retention over long periods produced a terminal half-life of approximately 8 days on acute dosing. Thus, there should be significant accumulation of drug on chronic administration. If daily chronic dosing is intravenously administered, a steady state of the amount of drug in the central compartment associated with the plasma results by approximately 7 days, oscillating between 0.03% of the daily dose at 3 hr after dosing and 2% at 24 hr after dosing. However, a steady-state oscillation of the amount of drug in the tissues is not achieved until after 30 days of chronic dosing and varies after each dose, from 450% of the single dose at 3 hr to 360% at 24 hr. The amounts in the tissues increase fivefold over that from a single dose at 7 days and 10-fold by 27 days. Thus, a pharmacodynamic response could increase with chronic dosing or be maintained for weeks after its cessation if tolerance did not develop.

The rate-determining step in drug elimination from the body, subsequent to the first several hundred minutes after administration, is not the rate of metabolism but the rate of return from deep tissues. Thus, it is unlikely that any claimed decrease (8, 12) in the apparent half-life for tetrahydrocannabinol in the plasma of chronic marijuana smokers rela-
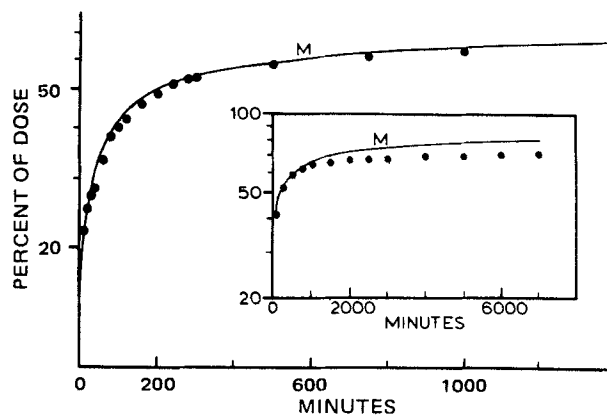
tive to naive smokers could be a consequence of marijuana-induced enzymes. If this were the case, the relative plasma levels would change but not the terminal half-life of decay.

The calculated accumulation in tissues is consistent with the observations of Kreuz and Axelrod (11) that the drug accumulates in the body fat of rats after repetitive administration and implies that significant differences in the disposition and pharmacodynamics of tetrahydrocannabinol may be anticipated between lean and obese individuals. If the fat to lean ratio of the chronic marijuana smokers were higher than that of naive smokers, claimed apparent differences (12) in apparent plasma half-lives could be best explained as resulting from differences in the rates of return of plasma of the rate-determining release of drug from such lipophilic deep compartments.

11-Hydroxy-Δ^9-tetrahydrocannabinol, a metabolite of Δ^9-tetrahydrocannabinol (21–29), has similar pharmacological properties and could contribute to the total pharmacodynamic action. In fact, it was suggested (1, 30) that the action was primarily due to this metabolite, although other researchers did not reach the same conclusion (31–34).

Since the disposition and metabolism of the intravenously administered metabolite were claimed (32–34) to be similar to that of Δ^9-tetrahydrocannabinol, equivalent pharmacokinetic parameters may be assumed. It is on this premise that the scheme in Fig. 18 is based, where PI is considered to be the primary intermediate(s) in metabolism, including pharmacodynamically active metabolites such as 11-hydroxy-Δ^9-tetrahydrocannabinol that can be metabolized further. The analog computer-generated curves in Fig. 18 demonstrate the time course of the maximum amounts of PI as percent of dose in its central compartment
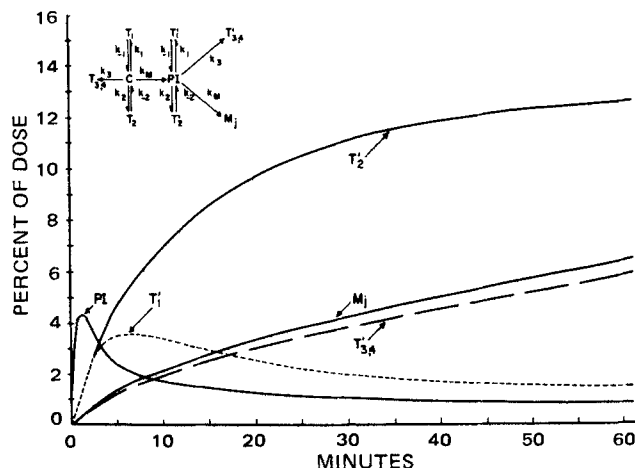

Figure 18—*Analog computer-generated curves of active metabolites, PI, which are primary intermediates in the metabolic sequence to M_j in the specified scheme for 60 min after intravenous administration of Δ^9-tetrahydrocannabinol in the 0.1-mg/kg study in Dog A. The generated M_j and tissue levels of PI in the tissues T_1', T_2', and $T_{3,4}' = T_3' + T_4'$ are also given.*

and in the various tissues, T_1', T_2', and $T_{3,4}'$, for 60 min after Δ^9-tetrahydrocannabinol administration. The time course of the amounts of the inactive metabolites, M_j, produced subsequently is also given. The ratios, R, of the amounts of PI to Δ^9-tetrahydrocannabinol in corresponding tissues approached asymptotes within these 60 min (compare Figs. 13 and 18). For example, T_1'/T_1 or PI/C approached one-half, whereas T_2'/T_1 or $T_{3,4}'/T_{3,4}$ approached lower values (one-fourth to one-third). Thus, PI would make the largest contribution to the observed activity if T_1 and T_1' were the biophases. Ryrfeldt *et al.* (6) observed R ratios in mice in blood and brain of this magnitude after intravenous administration of Δ^9-tetrahydrocannabinol.

Extant evidence from experimental animals (33) suggests that the relative potency, r, of 11-hydroxy-Δ^9-tetrahydrocannabinol to Δ^9-tetrahydrocannabinol ranges between 2 and 18; and if the central compartment and its rapidly equilibrating tissues, T_1 or T_1', are taken as the biophase, the contribution of PI to the observed pharmacological effect would be 50% at $r = 2$ and 90% at $r = 18$. If other tissues are the biophase, the contributions would be less.

However, these are maximum estimates since they are based on the premises that all PI produced in the liver is active and wholly returned to the plasma unchanged. This would not be so if PI were metabolized at the same rate as Δ^9-tetrahydrocannabinol. The fraction of the Δ^9-tetrahydrocannabinol entering the liver that left unmetabolized could be given by:

$$q = C_2/C_1 = e^{-\alpha T} \tag{Eq. 21}$$

where α is the intrinsic first-order metabolic rate constant, and T is the time required for complete replacement of the liver blood volume, *i.e.*, the time of blood transit through the liver. The C_1 and C_2 are the concentrations in the plasma entering and leaving the liver, respectively. If the active metabolites are further metabolized to M_j with the same rate dependency, the rate of change in the amount of PI in the liver could be given by:

$$d(PI)_L/dt = \alpha V_L C_1 - \alpha (PI)_L \tag{Eq. 22}$$

The fraction of the entering concentration, C_1, that retains its identity at the end of the interval, T, can be obtained by the integration (35) of Eq. 22 over the interval $t = T$ and is:

$$p = (PI)_L/V_L C_1 = \alpha T e^{-\alpha T} \tag{Eq. 23}$$

where V_L is the apparent volume of distribution of the liver.

If the logarithms of both sides of Eq. 19 are taken:

$$\alpha T = \ln q \tag{Eq. 24}$$

which may be substituted into Eq. 23. Equation 23 can be rearranged and divided by $1 - q$ to give the fraction of metabolites formed from Δ^9-tetrahydrocannabinol in the liver that remain as PI on exit from the liver:

$$p/(1 - q) = q \ln q/(q - 1) \tag{Eq. 25}$$

Since the apparent metabolic efficiency of tetrahydrocannabinol, $q = C_2/C_1$, is 0.5, only 69.3% of the PI formed in the liver would exit from this organ. Thus, if the central compartment is taken as the biophase for a relative potency of 2, the estimated ratio of amounts therein at 60 min becomes 0.35 rather than 0.5, and the contribution of PI to the observed pharmacological effect would be 41% rather than the 50% estimated if PI is not metabolized further during its passage through the liver prior to its return to the plasma.

On the basis of this discussion, an appropriate model for the pharmacokinetics of Δ^9-tetrahydrocannabinol is given in Fig. 18 on the premise that PI has pharmacokinetic properties similar to its precursor, where PI and M_j appear to be produced in parallel rather than sequentially when Δ^9-tetrahydrocannabinol is supplied continuously to the liver. When the hepatic efficiency, $q = C_2/C_1$, is small, negligible PI would be formed by the route designated with the microscopic rate constant $k_m[1 - p/(1 - q)]$. Even if PI were 20 times more potent than tetrahydrocannabinol, it could account for a maximum of only 30% of the observed response for a central compartment biophase when $q = 0.01$. The contribution of PI-type active metabolites, such as 11-hydroxy-Δ^9-tetrahydrocannabinol, to the observed pharmacological response of its precursor cannot be resolved until the true biophase is ascertained, their unique metabolic efficiencies are known, and the true relative potencies are determined.

Effects of ^{14}C-Label Position on Pharmacokinetics of ^{14}C-Δ^9-Tetrahydrocannabinol—Although the differently labeled ^{14}C-Δ^9-tetrahydrocannabinol in plasma, when radioactivity of the separated compound was monitored with time, showed the same pharmacokinetics

(Fig. 6), this was not so for total concentrations of radioactivity in plasma (Fig. 3) where the C-11-labeled drug showed significantly less plasma concentration per unit dose than the 1,3-ring-labeled drug at comparable times. A definite conclusion that metabolites tend to lose the C-11 group, as through decarboxylation of C-11 carboxylic acid metabolites, is clouded by the fact that the studies were conducted in biliary-cannulated Dog C where different bile flows had a pronounced effect on biliary excretions of the two studies (Fig. 2).

However, the total percents of dose excreted into bile for the differently labeled tetrahydrocannabinols were equivalent, although the metabolite derived from C-11-labeled drug showed less of a percentage excreted in the urine and totally excreted than the ring labeled. Nevertheless, the variation in total radioactivity as percent of dose excreted for the two differently labeled compounds in Dog C at the two different doses was not significantly different from the variation in total radioactivity as percent of dose excreted for the two studies at different doses of 1,3-^{14}C-Δ^9-tetrahydrocannabinol in Dog B (Fig. 5), where the relative amounts recovered in feces accounted for the latter's variability. Thus, the null hypothesis that there were no significant differences between the pharmacokinetics in the dog of the radiolabeled compounds derived from the two differently labeled Δ^9-tetrahydrocannabinols cannot yet be rejected.

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Determination of Porosity and Pore-Size Distribution of Aspirin Tablets Relevant to Drug Stability

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Abstract □ Total porosity and pore-size distribution of aspirin tablets prepared from aspirin, starch USP, and precipitated colloidal silicon dioxide were determined using mercury porosimetry. The model represented a hydrolyzable drug substance in combination with simple excipients. The role of starch and silicon dioxide on the microstructure of the tablets was investigated, as was the chemical stability of various systems. In general, the porosity of tablets containing a constant quantity of starch increased linearly with silicon dioxide concentration. Examination of the pore-size distribution, however, revealed that at low concentrations silicon dioxide functioned primarily to reduce the size and volume of coarse pores representing the spaces between the agglomerates of starch and aspirin particles. This effect was optimum at 3%. A further increase in silicon dioxide concentration produced tablets with relatively larger pore sizes. Studies of changes in the porosity characteristics of tablets as influenced by water vapor over time showed distinct differences in this complex parameter. A unique trend in the change of the pore-size distribution was noted with tablets containing 3% silicon dioxide. These observations are discussed relative to the stability of aspirin tablets in which this concentration of silicon dioxide produced a maximum stabilizing effect.

Keyphrases □ Aspirin—tablets, stability, porosity and pore-size distribution determined, effect of colloidal silicon dioxide □ Porosity and pore-size distribution—aspirin tablets, effect of colloidal silicon dioxide □ Stability—aspirin tablets, porosity and pore-size distribution determined, effect of colloidal silicon dioxide □ Silicon dioxide, colloidal—effect on stability of aspirin tablets, porosity and pore-size distribution determined □ Dosage forms—aspirin tablets, stability, porosity and pore-size distribution determined, effect of colloidal silicon dioxide

In any systematic and fundamental approach to the design of solid dosage forms, a comprehensive elucidation of the physical characteristics of the solid components is necessary and basic. Knowledge of the physical nature of solid pharmaceutical systems is often confined to determinations of such properties as particle size, particle-size distribution, and true and bulk density. To relate the disintegration, dissolution, and stability characteristics to meaningful physicochemical parameters, a direct examination of the porosity characteristics also may be essential.

The pharmaceutical and chemical literature clearly documents that the pore-size distribution of solids is fre-

quently among the controlling variables in processes involving disintegration, dissolution, adsorption, and diffusion. In tablet disintegration and dissolution, the initial stage is probably typically the penetration of the solvent medium into the tablet through the tablet pore system. The solvent penetration rate, in turn, was shown to be intimately related to the volume and size of tablet pores (1–6). From the standpoint of tablet stability, especially when dealing with hydrolyzable drugs, a physical description of the porous state of tablets may be important, since porosity and pore-size distribution affect the diffusion of water vapor and the accessibility of condensed vapor to the labile drug.

Studies on the porous nature of tablets have been conducted with increasing frequency recently (7). Porosity characteristics of tablets are primarily influenced by compression as a result of fragmentation, deformation, and consolidation of particles. Additional factors include particle size, particle shape, and the compressibility of particles (5, 6, 8).

As evident from review articles, the stability of aspirin in the solid state was the subject of numerous studies (9, 10). Many adjuvants have been proposed to enhance aspirin stability. Studies in these laboratories showed that inclusion of precipitated colloidal silicon dioxide into tablets prepared from aspirin and starch significantly improved aspirin stability. The maximum stabilizing effect of silicon dioxide occurred at 3%. Above this level, a continuous decrease in the stability of aspirin was observed. Although the major mechanism of this effect may be the large adsorptive capacity of silicon dioxide which functions as an internal tablet desiccant, an evaluation of tablet porosity characteristics may add significant dimensions in the interpretation of the physicochemical phenomena involved in aspirin decomposition.

The present study was undertaken to determine the influence of colloidal silicon dioxide on the porosity and pore-size distribution of aspirin tablets using mercury