Physicochemical Properties, Solubility, and Protein Binding of Δ^9-Tetrahydrocannabinol

EDWARD R. GARRETT[x] and C. ANTHONY HUNT

Abstract □ The rate and extent of glass binding of Δ^9-tetrahydrocannabinol in aqueous solution depend on the surface area and pretreatment of glass and the concentration of the drug. A total of 20 and 40% at 0.1 and 0.05 μg/ml, respectively, was bound to 50-ml volumetric flasks but could be minimized by silyl pretreatment of the glass. The drug rapidly diffused into plastics, and 70–97% was taken up by the rubber closures used for plasma vials. These bindings precluded classical methods of solubility determination, so spectral and particle-size counting determinations, which observed those concentrations at which true solution was terminated, were used. The aqueous solubility was a linear function of both the ethanol concentration (increasing) at constant ionic strength and the square root of the ionic strength (decreasing) at constant ethanol concentration. The salting-out coefficient was of high magnitude and typical solubilities were 2.8 mg/liter in water and 0.77 mg/liter in 0.15 *M* NaCl at 23°. The bindings also precluded the use of the classical methods of equilibrium dialysis and ultrafiltration to determine the protein binding of tetrahydrocannabinol. A method of variable plasma concentrations was devised, so protein binding was determined from the pseudoplasma concentrations of the drug after the separation of the pseudoplasma from the red blood cells added to form pseudoblood with known concentrations of Δ^9-tetrahydrocannabinol. This use of the competition between the high partitioning of drug between red blood cells with plasma water (D = 12.5) and the binding to plasma protein permitted an estimate of 97% binding which was not drug concentration dependent. The spectrophotometric pKa′ of Δ^9-tetrahydrocannabinol was 10.6. Δ^9-Tetrahydrocannabinol degraded readily in acid solutions. Subsequent to a rapid loss, the kinetics appeared to be first-order and specific hydrogen-ion catalyzed. Concomitantly, small amounts of Δ^8-tetrahydrocannabinol were produced, as were two GLC observable products, P_2 and P_3, and the rate of their appearance appeared to parallel the rate of Δ^9-tetrahydrocannabinol degradation. A peak, P_1, also appeared almost instantaneously but did not parallel drug degradation.

Keyphrases □ Tetrahydrocannabinol—physicochemical properties, solubility, and protein binding □ Marijuana—physicochemical properties, solubility, and protein binding of tetrahydrocannabinol □ Protein binding—tetrahydrocannabinol

Tetrahydrocannabinol is highly insoluble in water (1–3). This can be a critical factor in its bioavailability, pharmacokinetics, and pharmacological action. Large differences in the bioavailability of tetrahydrocannabinol from various solutions and administrative routes have been reported (2, 3). Metabolism is extremely rapid (4–6) and appears to be even faster than chloral hydrate (7) which has a half-life of 3 min. Nevertheless, tetrahydrocannabinol persists in the plasma for 72 hr after a single intravenous dose (4, 5). This is a possible consequence of a slowly releasing deep compartment (8). Evidence has been presented (1) that tetrahydrocannabinol's solubility may be exceeded in plasma, resulting in its possible precipitation and fortuitous localized accumulation in body organs which may act as such a deep compartment.

Stabilities have been reported for tetrahydrocannabinol on filter paper (9) and in various organic solvents (10), but no report is available on its aqueous stability or pKa′.

All relevant physicochemical properties of a drug should be obtained prior to initiating detailed pharmacokinetic investigations (8). This paper presents the results of studies on the solubility, pKa′, stability, partitioning, and protein binding of tetrahydrocannabinol and their biopharmaceutic and pharmacokinetic relevance. The fact that tetrahydrocannabinol is so highly bound to plasma proteins that its extent is difficult to determine by classical procedures has led to the development of a new method of variable plasma concentrations that permits estimation by extrapolation.

EXPERIMENTAL

Determination of Solubility—*Method A*—Twelve stock solutions of aqueous ethanol were prepared at varying ionic strengths. At each ionic strength the ethanol concentrations were 5.0, 7.5, 10.0, and 12.5% (v/v). The ionic strengths at each ethanol concentration were adjusted to 0.05, 0.1, and 0.15 with sodium chloride. Δ^9-Tetrahydrocannabinol[1] was dissolved in absolute ethanol to yield a stock solution containing 4 mg/ml.

Four milliliters of a stock solution was pipeted into matched 1-cm cells. A small volume (0.25–1.0 μl) of the tetrahydrocannabinol stock solution was added to one cell which was stoppered and shaken. The absorbance was then recorded[2] against the blank between 350 and 215 nm. This process was repeated until turbidity was clearly evident. The cell was washed and the process was repeated.

Beer's law plots were obtained by plotting the absorbance at one wavelength *versus* the amount of tetrahydrocannabinol added. The solubility of tetrahydrocannabinol in these solutions was assigned to that concentration at which deviation from Beer's law was observed.

Method B—The procedure of Saad and Higuchi (11) for determining solubility by using a particle-size counter[3] was modified. All solutions were filtered through a 0.45-μm pore size filter[4], and all samples were counted using a 30.0-μm aperture. The coincidence corrections for the counter were made on a calculator[5] programmed in accordance with the coincidence correction chart

[1] S.S.C. Lot 61591, furnished by the Department of Health, Education, and Welfare, U.S. Public Health Service, National Institute of Mental Health, Bethesda, MD 20014

[2] Cary model 15, Applied Physics Corp., Monrovia, Calif.

[3] Coulter counter, model ZBI, Coulter Electronics, Hialeah, Fla.

[4] Millipore Filter Corp., Bedford, Mass.

[5] Wang 700, Wang Laboratories Inc., Tewksbury, Mass.

supplied in the manufacturer's manual. The total counts were also corrected for background. The background for each sample averaged 5000/50 μl.

Twenty-four milliliters of filtered 0.9% NaCl was added to each of two 30-ml serum vials. Five microliters of a tetrahydrocannabinol solution (1.45 mg/ml in 95% ethanol) was added to each vial. The contents were stirred for 1 min with a 0.6-cm (0.25-in.) Teflon-coated magnetic stirring bar. Fifty microliters of the first vial was counted, and 50 μl from the second vial was then transferred to the first. Five microliters of the stock tetrahydrocannabinol solution was again added to each vial; the vials were counted and then the procedure was repeated until turbidity was clearly evident.

Spectrophotometric pKa′ Determination—The molar absorptivities of tetrahydrocannabinol and its anion were obtained between 210 and 350 nm using 0.5 *N* HCl and 0.5 *N* NaOH in 50% aqueous ethanol. A maximum difference in molar absorptivity of 8.5×10^3 occurred at 240 nm.

Buffer solutions with pH values between 7.1 and 13 (12) were adjusted to 0.1 ionic strength with sodium chloride. Buffer solution (29.5 ml) was added to each of two 10-cm cells. Tetrahydrocannabinol stock solution (5.00 μl) *(Determination of Solubility, Method A)* was added to one cell. The cell was stoppered and shaken, and the absorbance was recorded against the blank at 240 nm. This process was repeated until a valid Beer's law plot was obtained, and three such plots were obtained at each pH. The slopes of these plots (molar absorptivity) were used to calculate the pKa′.

Analytical Methods—The concentration of tetrahydrocannabinol was monitored by GLC. All quantitative analyses of Δ^9- and Δ^8-tetrahydrocannabinol, when at least 0.1 μg/sample was available for analysis, were performed with flame-ionization detection as described previously (14). When less than 0.1 μg was available, the pentafluorobenzoate ester of tetrahydrocannabinol was prepared and analyzed with electron-capture detection (13). This latter method does not differentiate between the Δ^8- and Δ^9-compounds.

Glass Binding—The binding of tetrahydrocannabinol to glass in various solutions was followed with time by analyzing the tetrahydrocannabinol concentration by GLC.

Method A—Clean volumetric flasks (50 ml) were filled with isotonic pH 7.4 phosphate buffer and binding was investigated. Two-tenths milliliter of ethanol, containing either 5 or 2.5 μg of tetrahydrocannabinol, was added to each flask to establish tetrahydrocannabinol concentrations of 0.1 and 0.05 μg/ml, respectively. The flasks were stoppered and shaken, and the contents were transferred to a 125-ml separator containing 15 ml of methylene chloride[6]. The extracting methylene chloride layer was transferred to a 15-ml glass-stoppered test tube and dried under nitrogen in a 55° water bath. The aqueous layer was extracted a second time with 10 ml of methylene chloride. The second extract was dried in the same tube, 100 μl of chloroform[7] with the internal standard was added, and aliquots were analyzed on the gas chromatograph. One hundred percent recovery was defined as the peak height ratio between tetrahydrocannabinol and the internal standard tetraphenylethylene obtained when 50 ml of an aqueous solution was extracted directly from a 100-ml volumetric flask.

In addition, similar studies were conducted using a water-soluble silicone concentrate[8] and trimethylsilyl-treated glassware. The former was used in accordance with the manufacturer's instructions (14) and the latter was effected by filling the glassware with a 1% solution of a silylating reagent[9] in chloroform and allowing this to stand for 30 min at room temperature. The silylating solution was then discarded and the glassware was rinsed once with chloroform and dried in an oven at 120° for 2 hr.

Method B—The extent of glass binding of tetrahydrocannabinol was also studied at low concentrations (1-100 ng/ml) of ^{14}C-Δ^9-tetrahydrocannabinol[10] in buffer, plasma, and whole blood. The ^{14}C-Δ^9-tetrahydrocannabinol and the unlabeled drug were combined in 50% aqueous ethanol to yield solutions with equivalent disintegrations per minute per milliliter and varying tetrahydrocannabinol concentrations. The details of the procedure are presented subsequently.

Method C—Serum vials[11] (5 and 10 ml) were washed, soaked in ethanol, and allowed to air dry. Four 10- and four 5-ml vials were filled with 9 and 4.5 ml, respectively, of pH 7.55 isotonic phosphate buffer. Three solutions of Δ^9-tetrahydrocannabinol and the ^{14}C-labeled compound were prepared to yield 0.0785, 0.785, and 24.8 ng/μl and 13.49 dpm/μl in each case. An aliquot (90 μl) of each solution was added to one each of the 5-ml vials, and 180 μl was added to one each of the 10-ml vials. The solutions were stirred for 5 min with a 0.6-cm (0.25-in.) Teflon stirring bar. The stirring bars were then removed (no significant radioactive residue remained on the stirring bar) and the solutions were allowed to stand for 30 min. Three milliliters was removed for analysis from the 10-ml vials and 2 ml from the 5-ml vials. These samples were extracted as described later for plasma samples. Each vial was then stoppered with its original rubber stopper and shaken slowly (60 rpm) on an automatic shaker for 1 hr. Duplicate volumes were again removed for analysis.

Protein Binding by Classical Methods—Equilibrium dialysis and ultrafiltration were used to determine the protein binding of tetrahydrocannabinol to plasma proteins. The details of these procedures were reported previously (15, 16). In both cases, isotonic phosphate buffer (pH 7.4) was used as a reference solution and drug concentrations of 0.05, 0.1, and 1.0 μg/ml were investigated.

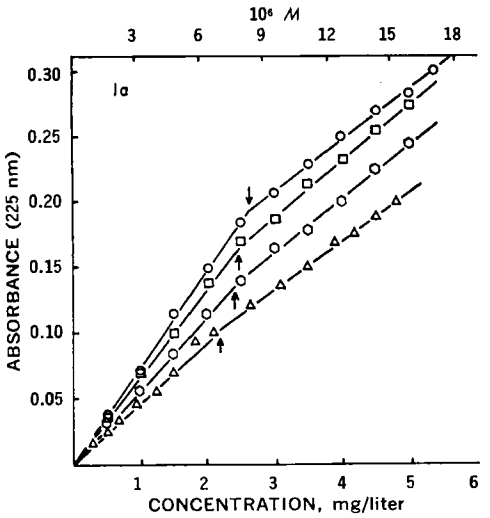
Protein Binding by Method of Variable Plasma Concentrations—Fresh, heparinized whole dog blood was centrifuged. The plasma was transferred to a glass-stoppered test tube; the red blood cells were washed with isotonic phosphate buffer and centrifuged, and the buffer phase was discarded. This procedure was repeated three times. The packed red blood cells were gently mixed and duplicate hematocrits (H_1) were taken.

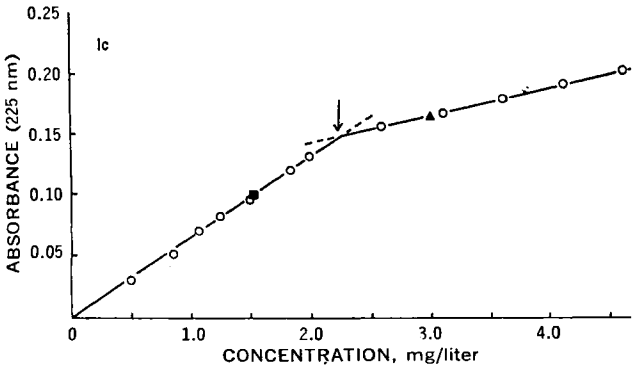
Appropriate amounts of plasma and isotonic phosphate buffer (pH 7.5) to give mixtures with 0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, and 100% plasma were prepared. The desired mixtures of ^{14}C-labeled and unlabeled drug in absolute ethanol were added to 50-ml glass centrifuge tubes and dried under nitrogen. The drug was reconstituted in 0.2 ml of isotonic 50% ethanol-water, and 3.00 ml of a particular plasma-buffer mixture was added so that concentrations of 1, 5, 10, 25, 50, 75, 100, 250, 500, and 1000 ng tetrahydrocannabinol/ml pseudoplasma could be studied. Five milliliters of 95% ethanol was added to one of these tubes to serve as a blank. All tubes but the blank were stoppered and placed in a 37.5° water bath for 10 min, at which time 2 ml of the packed red blood cells at 37.5° was added to each tube. Duplicate hematocrits (H_2) were made from each tube, which were then centrifuged at 3000 rpm for 10 min. An aliquot (2.5 ml) of plasma was removed for subsequent tetrahydrocannabinol analysis. The packed red blood cells were resuspended in the plasma residue, and a third hematocrit (H_3) was made on two samples of this resuspension. One milliliter of this suspension was transferred to a 50-ml glass test tube containing 1 ml of distilled water and hemolyzed.

One milliliter of a saturated sodium chloride solution was added to the 2 ml of the 2.5 ml of plasma previously removed and to the 2.0-ml sample containing the hemolyzed red blood cells. Heptane (15 ml) with 1.5% isoamyl alcohol was added to each. The tubes were stoppered and shaken on an automatic shaker for 20 min. The tubes were then centrifuged to separate the layers, and the heptane layers (14 ml) were transferred to liquid scintillation counting vials and dried under nitrogen. Ten milliliters of a liquid scintillation cocktail[12] was added to each vial, and each sample was counted[13] three times for 10 min. Addition of a quantitative amount of ^{14}C-toluene (904 dpm) indicated no significant quench.

An aliquot (2.00 ml) of the blank was transferred directly to a counting vial and dried to represent 100% recovery. Since the

[6] Nanograde dichloromethane, Mallinckrodt Chemical Works, St. Louis, Mo.
[7] Nanograde chloroform, Mallinckrodt Chemical Works, St. Louis, Mo.
[8] Siliclad, water-soluble silicone concentrate, Clay Adams, Parsippany, N.J.
[9] Regisil, Regis Chemical Co., Chicago, Ill.
[10] Lot CP-1, R.T.I., Research Triangle Park, N.C.
[11] Vacutainer, nonheparinized, Becton-Dickinson, Rutherford, N.J.
[12] Spectrafluor, Amersham Searle, Arlington Heights, Ill.
[13] Packard Tri-Carb, series 3000, Packard Instrument Co., Downers Grove, Ill.





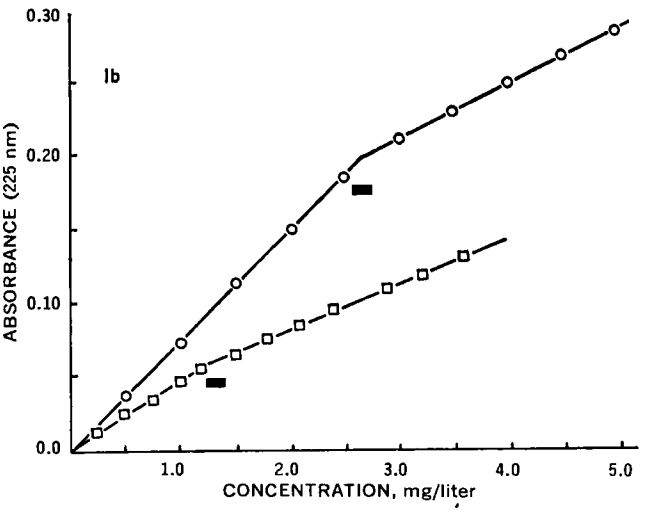


Figure 1—*Typical absorbances of tetrahydrocannabinol at 225 nm in various aqueous ethanol solutions as a function of concentration. (a) An ionic strength of 0.05 was used. Key: ○, 12.5% ethanol; □, 10% ethanol; ○, 7.5% ethanol; and △, 5% ethanol (v/v). The arrows indicate the concentrations where deviations from Beer's law occurred. Each point is the mean of two replicate studies. (b) The solid bars indicate the range of* ± **1 SD** *in the estimated concentration where deviations from linearity occurred. Key: ○, 12.5% ethanol (v/v) and 0.05 ionic strength; and □, 5% ethanol and 0.15 ionic strength. Each point is the mean of three replicate studies. (c) The absorbance (○) of tetrahydrocannabinol in 12.5% ethanol at 0.1 ionic strength where the arrow indicates the concentration at which the deviation from Beer's law occurred. The ▲ value was obtained by a 1:0.67 dilution of the final solution (4.6 μg/ml). The ■ value was obtained by a 1:0.33 dilution of the final solution.*

original packed and washed red blood cells obtained from whole blood retained a significant fraction of the washing buffer solution (12–22%), it was necessary to recalculate the percentage of plasma in the synthetic diluted plasma–buffer mixtures.

The extent of red blood cell-plasma distribution was monitored as a function of time.

Stability of Δ^9-Tetrahydrocannabinol in Acid Solutions—The stability of 1 liter of 1 μg/ml Δ^9-tetrahydrocannabinol was followed with time at 55° in 0.5, 0.1, 0.05, and 0.01 *N* HCl, with the ionic strength of 0.1 with sodium chloride in the last two cases. An aliquot of 1 ml of ethanol containing 1 mg of tetrahydrocannabinol was added to each liter of solution. Aliquots (48.0 ml) were sampled after vigorous shaking and transferred to a 125-ml separator containing 5 ml of enough sodium hydroxide to neutralize the hydrochloric acid. The solution was extracted twice with 15 ml of methylene chloride, and the pooled organic extracts were analyzed quantitatively by GLC.

RESULTS AND DISCUSSION

Determination of Solubility—Attempts to use classical methods of solubility determination were unsuccessful. When a saturated solution was filtered, the filtrate showed no detectable tetrahydrocannabinol due to the high affinity of the dissolved tetrahydrocannabinol for the filter paper. When a saturated solution was ultracentrifuged and the clear solution was decanted, this solution showed little and variable tetrahydrocannabinol due to the more rapid diffusion of solution tetrahydrocannabinol into the plastic (polycarbonate and nitrocellulose) of the centrifuge tube over the rate of reequilibration of the sedimented excess tetrahydrocannabinol with the solution. This hypothesis was validated further by the fact that the amount of tetrahydrocannabinol in the clear solution in the ultracentrifuged tube decreased with time of standing.

Typical data used to determine the solubility of tetrahydrocannabinol spectrophotometrically in various aqueous ethanol solutions at constant ionic strength are given in Fig. 1. The data initially followed Beer's law. Subsequent to the deviation from linearity, each addition of tetrahydrocannabinol resulted in increasing turbidity. The concentration where the deviation occurred was reproducible. The standard deviations (as percent of mean) of the concentrations at which such deviations occurred ranged from 6.32% in 5% ethanol at 0.15 ionic strength to 2.5% in 12.5% ethanol at 0.05 ionic strength. The dilution of a turbid solution below the apparent solubility limit resulted in the same absorbance obtained by the addition of increasing amounts of tetrahydrocannabinol. This reversibility was consistent with the assignment of the limit of the solubility of tetrahydrocannabinol to that concentration where the deviation from Beer's law occurred.

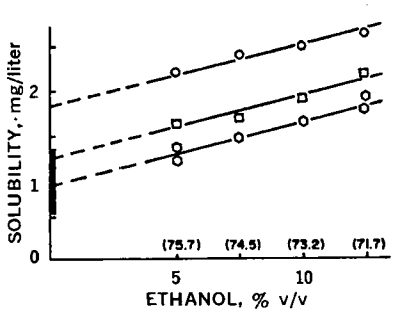


Figure 2—*Solubility as estimated from the observed deviations from Beer's law for tetrahydrocannabinol at various ionic strengths plotted as a function of percent ethanol in aqueous solutions. Key: ○, 0.05 ionic strength; □, 0.1 ionic strength; and ○, 0.15 ionic strength. The solid bar at the intercept includes the mean and the standard deviation for the determined solubility (Method B) in pure water at physiological ionic strength (0.9% NaCl). The numbers in parentheses are the dielectric constants for the four ethanol–water solutions used.*

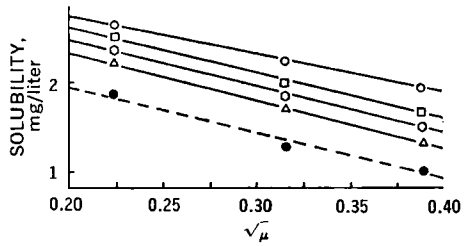


Figure 3—*Solubility of tetrahydrocannabinol in various aqueous ethanol solutions as a function of the square root of the ionic strength, $\sqrt{\mu}$. Key: ○, 12.5% ethanol; □, 10% ethanol; ○, 7.5% ethanol; △, 5% ethanol; and ●, solubility in pure water as obtained from extrapolation to 0% ethanol.*

The solubility was a linear function of both ethanol concentration (at constant ionic strength) (Fig. 2) and the square root of the ionic strength (at constant ethanol concentration) (Fig. 3). The solubility of tetrahydrocannabinol in water at various ionic strengths was estimated from the extrapolated intercepts of Fig. 2 and also are plotted in Fig. 3, where its extrapolated intercept estimates a solubility of 2.8 mg/liter for tetrahydrocannabinol in pure water at 23.0°. Although reasonable reproducibility in estimated solubilities was obtained with fixed ionic strengths, enormous variability in solubility estimates were obtained among replicate studies in pure water.

An often used empirical equation employed in the study of solubilities of unionized solutes in water (17) is:

$$\log S_0/S = k_s C + \log \alpha \qquad \text{(Eq. 1)}$$

where S_0 is the solubility of the solute in pure water, S is the solubility in a dilute salt solution of concentration C, k_s is the "salting-out coefficient," and the constant $\log \alpha$ is taken as zero.

The nonzero intercepts, $\log \alpha = 0.8$ and $\alpha = 6.3$, of such plots for tetrahydrocannabinol solubilities (Fig. 4) were reproducible on replication and when the solvent systems were carefully purified. A possible explanation may be that the observed solubility is not linearly related to ionic strength below the lowest ionic strength measured and it is invalid to accept the extrapolated intercept at zero salt concentration (Fig. 3) or that the solubility is overestimated since the solute does bind at the cell-solvent interface.

The apparent solubility in pure water may be corrected for this phenomenon so that $S_0' = S_0/\alpha$. The k_s values of Eq. 1 are plotted in the insert of Fig. 4 as a function of percent ethanol, and the

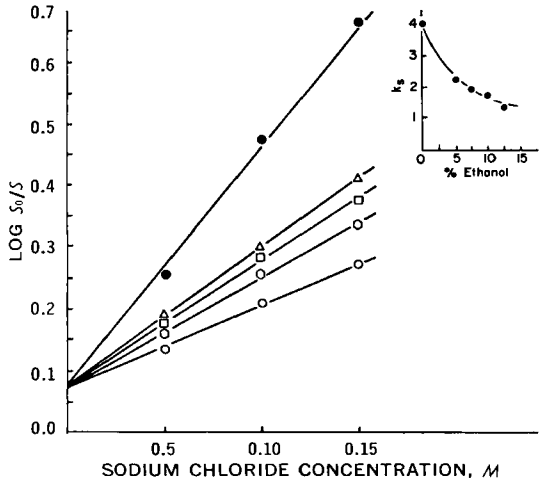


Figure 4—*Function log S_0/S plotted against the concentration of sodium chloride. S_0, the apparent solubility of tetrahydrocannabinol in pure water, was obtained from the extrapolation of the solubilities to 0% ethanol and zero ionic strength and S was the observed solubility. Key: ●, 0% ethanol; △, 5% ethanol; □, 7.5% ethanol; ○, 10% ethanol; and ○, 12.5% ethanol (v/v). The insert is a plot of the salting-out coefficients, k_s, as a function of percent ethanol (v/v).*

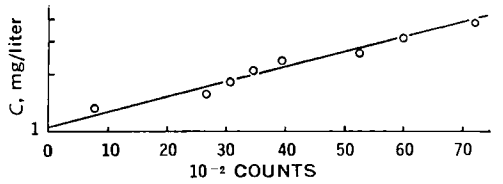


Figure 5—*Solubility of tetrahydrocannabinol in 0.9% NaCl estimated by the particle-size counting method on the postulate that the function log tetrahydrocannabinol concentration (C), against counts, corrected for background, is linear. The correlation coefficient for the data plotted in this manner is r = 0.986. The intercept, which estimates the solubility in 0.9% NaCl, is 1.05 mg/liter.*

log of the apparent solubility, S, at 23.0° may be expressed as:

$$\log S = \log S_0/\alpha - k_s C \qquad \text{(Eq. 2)}$$

for $\alpha = 6.3$ and for the salt concentration, C, in moles per liter.

These salting-out coefficients, k_s, calculated for tetrahydrocannabinol are larger than those reported for more polar neutral solutes such as phenol (0.22) and salicylic acid (0.196) (18).

The particle-size counting procedure of Saad and Higuchi (11) overestimated the true solubility since there is a limit below which particle size cannot be counted.

A plot of the corrected counts *versus* concentration of tetrahydrocannabinol after serial addition of the drug is given in Fig. 5. The intercept at zero net counts gives a mean solubility estimate of 1.05 mg/liter (σ = 0.38 mg/liter) for the solute in 0.9% NaCl (0.154 *M*). This is in good agreement with the water solubility of 0.77 mg/liter in 0.15 *M* NaCl calculated from Eq. 2.

As with cholesterol suspensions (11), size-distribution analyses by this method of the various concentrations of solutes gave no evidence of a preferred micellar size. The frequency of observable particles apparently increased semilogarithmically with size.

Spectrophotometric pKa'—A 1.19×10^{-5} *M* solution of tetrahydrocannabinol, more than three times the solubility of the drug in water at 0.1 ionic strength, is required for a 0.1 difference between the absorbances of completely ionized and unionized solutions at 240 nm.

The pKa' for tetrahydrocannabinol may be calculated by employing the modified form of the Henderson-Hasselbalch equation (19):

$$\log[(\epsilon_b - \epsilon)/(\epsilon - \epsilon_a)]_\lambda = \text{pH} - \text{pKa}' \qquad \text{(Eq. 3)}$$

where ϵ_a (5850) and ϵ_b (12,266) are the molar absorptivities of the unionized and completely ionized drug measured at a given wavelength, λ (240 nm), respectively, and ϵ is the apparent molar absorptivity of a solution measured at a particular pH.

The apparent molar absorptivity (ϵ) of the drug in various

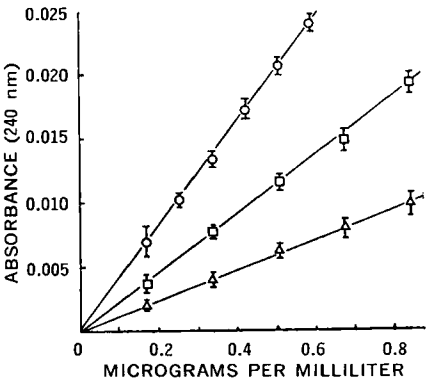


Figure 6—*Typical Beer's law plots for tetrahydrocannabinol in various aqueous buffer systems. A 10-cm cell was used (29.5 ml). The values shown are the mean and standard deviation of three determinations. Key: ○, pH 12.92; □, pH 10.35; and △, pH 7.15.*

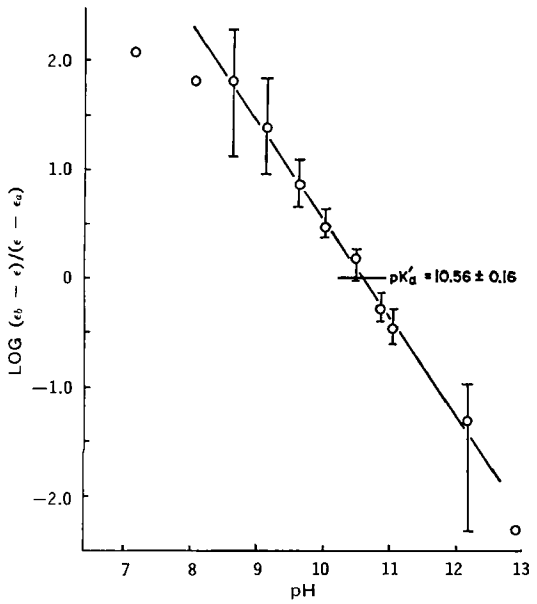


Figure 7—*Spectrophotometric determination of the pKa′ of Δ⁹-tetrahydrocannabinol in accordance with log* $[(\epsilon_b - \epsilon)/(\epsilon - \epsilon_a)] = pH - pKa'$, *where* ϵ_a *and* ϵ_b *are the molar absorptivities of the unionized and completely ionized drug at 240 nm, respectively, and* ϵ *is the apparent molar absorptivity at the stated pH value.*

buffer systems was calculated from the slopes of the plots of absorbance *versus* concentration at 240 nm; typical plots are given in Fig. 6. The absorptivities of the ionized and unionized species were estimated similarly using 0.5 *N* HCl and 0.5 *N* NaOH. The pKa′ was 10.56 ± 0.16 (σ), obtained from the plot of Fig. 7 in accordance with Eq. 3. This pKa′ is higher than would be anticipated for *ortho*- and *meta*-substituted phenols since *o*-methoxyphenol has a pKa′ of 9.98, *m*-methoxyphenol has a pKa′ of 9.65, and *m*-hydroxyphenol has a pKa′ of 9.8 (20).

A molecular model of the drug shows that the free rotation of the phenolic hydrogen is hindered by the Δ⁹-hydrogen and can explain Δ⁹-tetrahydrocannabinol's higher pKa′. Of course, if solvated dimers, trimers, *etc.*, exist with hydrophobic bonding, the observed pKa′ could be a hybrid pKa′ for a solution of such polymers.

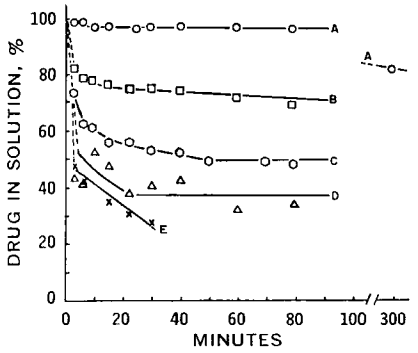


Figure 8—*Glass binding of Δ⁹-tetrahydrocannabinol showing the percent of drug added that remained in solution at various times for: (A) trimethylsilyl-treated 50-ml volumetric flasks from an aqueous drug concentration of 0.1 μg/ml, (B) an aqueous drug concentration of 0.1 μg/ml in untreated 50-ml volumetric flasks, (C) an aqueous drug concentration of 0.1 μg/ml in water-soluble silicone concentrate-treated 50-ml volumetric flasks, (D) an aqueous drug concentration of 0.05 ug/ml in untreated 50-ml volumetric flasks, and (E) an aqueous drug concentration of 0.1 ug/ml in a 20-ml stainless steel ultracentrifuge tube. Each point is the mean of four separate determinations.*

Table I—Percent Retention of Tetrahydrocannabinol from 0.1 mg/ml Solution after Various Seconds of Contact with Full Glass Pipets

Pipet Size, ml	Seconds of Contact		
	1	3	6
50	0.5	0	0.3
10	1	1	1
4	2.5	3	4
2	4	7	11
1	5	11	15

Table II—Binding of Δ⁹-Tetrahydrocannabinol to Serum Vials and Loss to Rubber Closures

Concentration, ng/ml	Vial Size, ml	Fraction Bound, *B*, to Glass[a]	Fraction Lost, *L*, to Closure[b]
1.57	5	0.48	0.71
1.57	10	0.26	0.98
15.7	5	0.30	0.80
15.7	10	0.26	0.92
494	5	0.24	0.85
494	10	0.22	0.75

[a] On the assumption that an equilibrium is established between drug in solution and drug bound to glass, a binding constant, K_B, may be defined for each vial: $K_B = B/V_0C = B'/V'C'$, where $B = (T - V_0C)/T$ is the amount bound prior to exposure to the closure, T is the total drug added, V_0 is the initial volume, and C is the observed concentration prior to exposure to the closure; B' is the amount bound after exposure, V' is the volume remaining after exposure, and C' is the concentration after exposure. [b] The total amount remaining, T', after the first sample is given by $T' = T - (V_0 - V')C$, and the amount bound to glass is given by $B' = K_BV'C'$. The fraction lost to the stopper, L, can be defined as $L = T' - B' - V'C'/T'$.

Glass Binding—The rate and extent of tetrahydrocannabinol in aqueous solution binding to glass depend on the surface area of the glass, the pretreatment of the glass, and the concentration of the drug. Curves B and D of Fig. 8 show the extent of binding, about 20 and 40%, respectively, of tetrahydrocannabinol as a function of time at concentration of 0.1 and 0.05 μg/ml in 50-ml volumetric flasks. At high drug concentrations (0.5–1.0 μg/ml) and after full equilibration, less than 7% of the amount in an aqueous solution was bound to glass from various flasks and test tubes.

Preconditioning glass with strong acid or alkali did not decrease the extent of binding. New, unused glassware bound the drug to a similar extent. Treatment of the flasks with a water-soluble silicone concentrate increased the extent of binding (Fig. 8, curve C). However, vigorous shaking immediately prior to sampling reduced the degree of binding, but no more than 50% in any case, although the fraction bound rapidly increased on further standing. All of the initial samples plotted in Fig. 8 were obtained immediately after vigorous shaking.

Trimethylsilyl treatment of glassware significantly reduced binding (Fig. 8, curve A) so that binding in 50-ml flasks and pipets was negligible for 80 min at 24° and for 24 hr at 0°. However, at room temperature the bound fraction of 0.1 μg/ml solution increased to the pretreatment level after 300 min. The equilibration binding of solutions to silyl-treated glassware with high concentrations of tetrahydrocannabinol (0.5–1.0 μg/ml) was negligible.

Polycarbonate, polypropylene, Teflon, and stainless steel (Fig. 8, curve E) containers showed more extensive binding than glass.

Aqueous solutions of tetrahydrocannabinol (0.1 and 0.05 μg/ml) were transferred by glass pipets. Significant retention of the pipeted tetrahydrocannabinol was observed, which increased with decreasing glass pipet size and with increased time of contact of the solution in a full pipet before delivery was permitted (Table I).

When aliquots of phosphate buffer solutions of tetrahydrocannabinol in serum vials were removed without prior shaking, significant amounts were bound to glass and the percent bound increased with decreasing concentration (Table II). However, of greater importance in light of the normal handling of samples of biological fluids is the fact that 70–97% of the drug in the vials

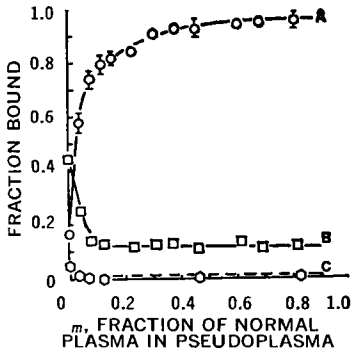


Figure 9—*Protein binding and glass binding of Δ⁹-tetrahydrocannabinol in pseudoblood for various fractions, m, of normal plasma in the pseudoplasma. (A) The fraction of drug in pseudoblood that is in pseudoplasma, A_P/A_T. The vertical bars give the mean ± 1 SD for 10 different drug concentrations. The open circles are for a concentration of 1.0 ng/ml in pseudoblood. (B) The fraction of drug added that is bound to glass, A_{BG}/A_0 for a concentration of 1 μg/ml in pseudoblood.*

was lost to the rubber stopper after 1 hr of shaking of the stoppered vials.

Tetrahydrocannabinol in whole blood or plasma also binds to glass (Fig. 9). However, since tetrahydrocannabinol protein binding is very large, glass binding is significantly reduced. In general, only one-fifth of the glass binding observed from saline solutions was observed from plasma solutions of tetrahydrocannabinol. The binding of drug from plasma to the silyl-treated glassware was negligible.

Protein Binding by Classical Methods—When tetrahydrocannabinol in isotonic phosphate buffer was dialyzed against isotonic phosphate buffer, 50–100% of the drug was bound to the tubing used as the membrane[14]. All of the drug was bound below 0.05 μg/ml. Ultrafiltration was equally unsuccessful, since only 0–5% of the drug in isotonic phosphate buffer was recovered in the ultrafiltrate. Attempts were made to saturate both dialysis membranes and ultrafiltration cones, but the membranes could not be saturated. The variability among repetitive samples with ultrafiltration was such that it could only be concluded that the fraction bound to plasma proteins was in the 0.85–1.0 range.

Protein Binding by Method of Variable Plasma Concentrations—The unbound, unionized fraction of a drug in whole blood may be presumed to partition into the red blood cells (7) in accordance with Scheme I. In Scheme I, the partition constant be-

$$A_{RBC} \overset{D}{\rightleftharpoons} A_P^u + P \overset{K}{\rightleftharpoons} AP$$

Scheme I

tween concentrations of drug in the red blood cells and unionized, unbound drug in plasma is:

$$D = [A_{RBC}]/[A_P^u] \quad \text{(Eq. 4)}$$

and the association constant for unionized, unbound drug, $[A_P^u]$, with the unoccupied protein binding sites, $[P]$, in plasma is:

$$K = [AP]/[P][A_P^u] \quad \text{(Eq. 5)}$$

where $[AP]$ is the apparent concentration of bound drug, on the presumption that the reactivity of binding sites is equivalent and that the occupancy of one does not modify the binding power of another.

If $[P_T]$ is the total concentration of binding sites, then:

$$[P_T] = m[P_1]_T = [P] + [AP] \quad \text{(Eq. 6)}$$

where the total concentration of binding sites may be expressed in terms of fractions, m, of the normal concentration of binding sites in plasma.

When the numerator and the denominator of the second term of Eq. 5 are divided by the total concentration of drug in the plasma, $[A_P]$:

$$K = ([AP]/[A_P])/([P][A_P^u]/[A_P]) = f_b/[P]f_u \quad \text{(Eq. 7)}$$

since the fraction of drug in the plasma that is bound is $f_b = [AP]/[A_P]$ and the fraction of drug in the plasma that is unbound is $f_u = [A_P^u]/[A_P]$. If there are excess numbers of binding sites, $[P_T]$ is not significantly depleted and:

$$[P] \sim [P_T] = m[P_1]_T \quad \text{(Eq. 8)}$$

Substitution of the approximation of Eq. 8 into Eq. 7 results in:

$$m[P_1]_T K = f_b/f_u \quad \text{(Eq. 9)}$$

A constant volume, V_{PC} = 2.0 ml, of washed, packed red blood cells of hematocrit H_1 (usually in the 0.68–0.88 range) was added to a volume V = 3.0 ml, of plasma-buffer mixtures to give pseudoblood of volume V_{WB} = 5.0 ml, with the hematocrit H_2 (usually in the 0.26–0.38 range). The volumes of red blood cells, V_{RBC}, and plasma, V_P, are given by:

$$V_{RBC} = H_1 V_{PC} = H_2 V_{WB} \quad \text{(Eq. 10)}$$

and:

$$V_P = V + (1 - H_1)V_{PC} = (1 - H_2)V_{WB} \quad \text{(Eq. 11)}$$

The V_{RBC} and V_P values had to be the same when calculated from either the observed H_1 or H_2 values or hemolysis was assumed and the preparation was discarded. The total concentration of drug in the plasma, $[A_P]$, after centrifugation of the pseudoblood was determined radiochemically in V_P' = 2.5 ml of plasma based on the fact that 96% of the drug was extracted from *all* plasma-buffer mixtures. Therefore:

$$[A_P] = [A_P^u] + [AP] \quad \text{(Eq. 12)}$$

and apparently the salting-out procedure used minimized any variability in the extraction efficiency with variations in plasma protein concentration.

Hematocrit H_3 (usually in the 0.50–0.61 range) was taken on the red blood cells resuspended in the residual plasma. The concentration of the total drug in the plasma containing the resuspended red blood cells was determined radiochemically as C. Thus, the total amount, A_T, of drug in the original pseudoblood could be calculated from:

$$A_T = V_P'[A_P] + (V_{WB} - V_P')C \quad \text{(Eq. 13)}$$

Since:

$$C = \{A_{RBC} + (1 - H_3)(V_{WB} - V_P')[A_P]\}/(V_{WB} - V_P') \quad \text{(Eq. 14)}$$

the amount of drug in the red blood cell fraction, A_{RBC}, may be calculated from a combination of Eqs. 13 and 14:

$$A_{RBC} = A_T - V_P'[A_P] - (1 - H_3)(V_{WB} - V_P')[A_P] \quad \text{(Eq. 15)}$$

The red blood cell distribution coefficient, Eq. 4, was determined directly for red blood cell dispersions in buffer where P_T = 0 and was D = 12.5 for the dog red blood cells.

The fractions of drug unbound and bound are:

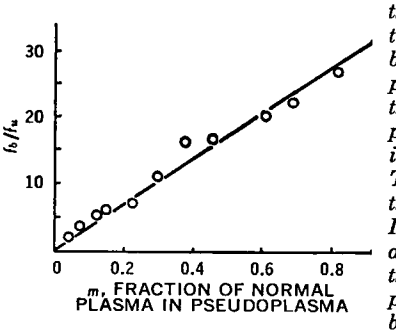


Figure 10—*The ratio of the fraction of drug bound to the fraction of drug unbound in the pseudoplasma as a function of the fraction, m, of normal plasma in pseudoplasma in accordance with Eq. 9. The intercept is zero and the slope, $[P_1]_T K$, is 34. In true whole blood with an equivalent hematocrit, the fraction bound to plasma proteins is given by 34 $(1 - f_b) = f_b$ and f_b = 0.97.*

[14] Visking.

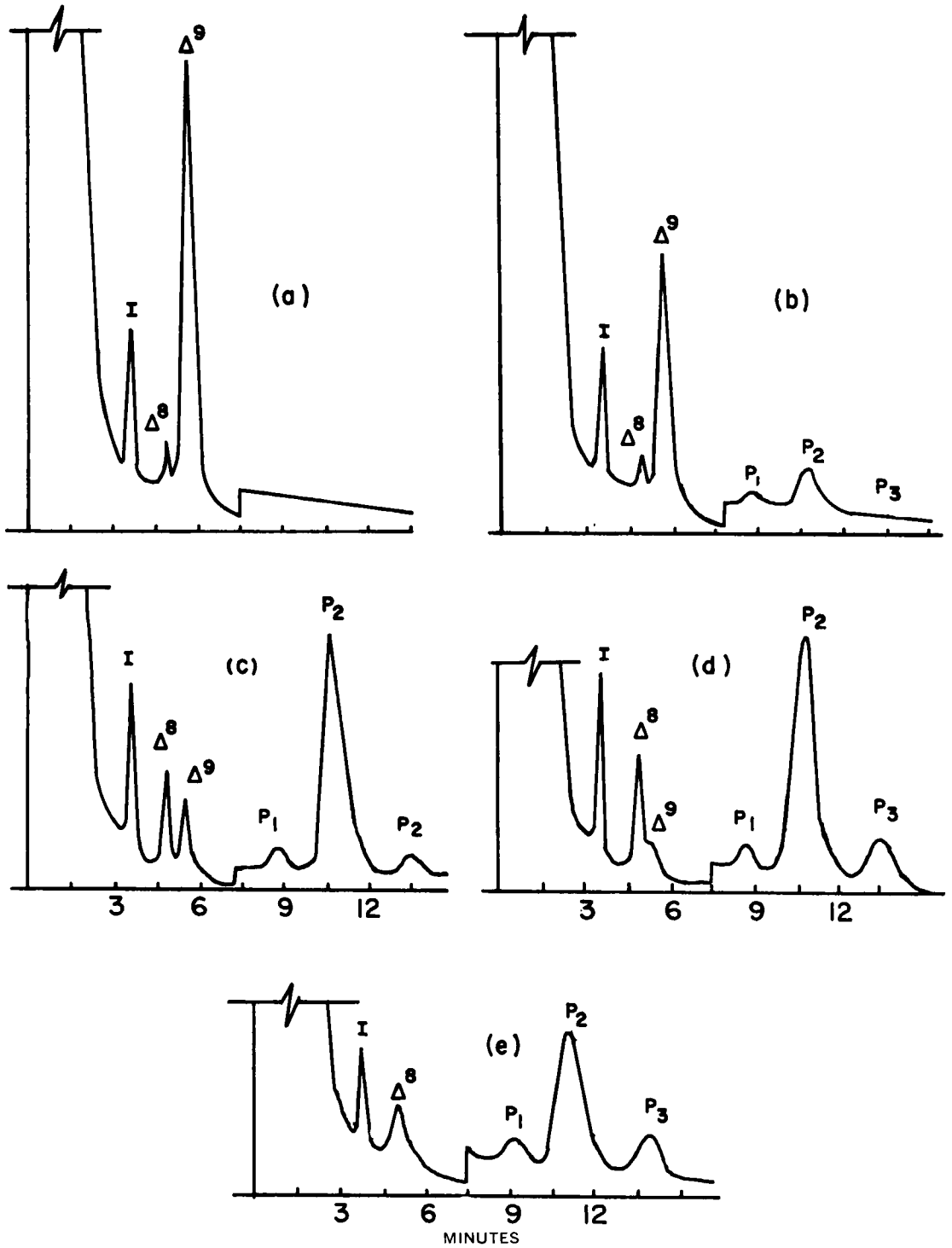


Figure 11—*GLC scan of organic extracts at various times of an aqueous 0.5 N HCl solution of Δ^9-tetrahydrocannabinol reacted at 55°. The first peak, I, following the solvent peak is the internal standard. The second and third peaks correspond to authentic Δ^8- and Δ^9-tetrahydrocannabinol, respectively. The last three peaks, P_1, P_2, and P_3, are for the three major degradation products. (a) Starting levels, t = 0, of Δ^8- and Δ^9-tetrahydrocannabinol. (b) A sample taken at 3 min shows loss of Δ^9-tetrahydrocannabinol and the appearance of P_1 and P_2. (c) A sample taken at 35 min shows an increase in Δ^8-tetrahydrocannabinol, $\Delta^8 > \Delta^9$, and shows P_2 to be the major product under these conditions. (d) A sample taken at 65 min shows that the loss of Δ^9-tetrahydrocannabinol is almost complete. (e) A sample taken at 780 min shows little change from the 65-min sample to suggest a final equilibrium among P_1, P_2, P_3, and Δ^8-tetrahydrocannabinol. The abscissas give the retention times.*

$$f_u = A_P^u/A_P = A_{RBC}V_P/DV_{RBC}A_P \quad \text{(Eq. 16)}$$

on consideration of Eq. 4, and:

$$f_b = AP/A_P = (V_P[A_P] - A_P^u)/A_P = 1 - f_u \quad \text{(Eq. 17)}$$

where the values of V_{RBC}, V_P, and A_{RBC} can be determined from Eqs. 10, 11, and 15, respectively, and where $A_P = V_P[A_P]$ was determined from the assayed total concentration of drug in plasma.

Due to the fact that the drug binds significantly to glass, the amount, A_0, of drug added and the total amount, A_T, calculated (Eq. 13) in the pseudoblood may not agree. The fraction of the total amount added that is bound to the glass container would be:

$$f_{BG} = (A_0 - A_T)/A_0 \qquad \text{(Eq. 18)}$$

The fraction of the amount of drug in the pseudoblood that is in the pseudoplasma, A_P/A_T, is plotted as curve *A* in Fig. 9 as a function of the fraction, *m*, of normal plasma in the plasma–buffer mixtures. The 10 concentrations of tetrahydrocannabinol studied were in the 1 to 1000-ng/ml range, and curve A showed no significant variation among such concentrations. The extent of the vertical lines through the mean values plotted represents the standard deviation of the values obtained for all such concentrations.

Curves B and C in Fig. 9 show the fractions of drug (Eq. 18) added that were bound to glass as a function of *m* for 1 ng and 1 μg, respectively, of tetrahydrocannabinol added to the various pseudoblood preparations. Since it is apparent that the fraction of tetrahydrocannabinol bound to plasma protein is independent of drug concentration (Fig. 9, curve A), the premise of Eq. 8 holds and the quotient f_b/f_u, with values calculated from Eqs. 16 and 17, is plotted against the fraction, *m*, of pseudoplasma that is normal plasma in accordance with Eq. 9. The resultant plot (Fig. 10) was completely linear with zero intercept in agreement with Eq. 9 and the slope estimated $K[P_1]_T = 34$. This permits extrapolation to true plasma conditions when $m = 1$ and the resultant $f_b/f_u = 34$, so that the fraction of tetrahydrocannabinol in plasma that is bound at normal protein concentrations is 0.972.

The only other known study on the binding of Δ^9-tetrahydrocannabinol to plasma proteins was performed with electrophoretic techniques on tritium-labeled material and human plasma (21). The compound was 80–95% associated with lipoproteins.

The apparent partition coefficient, *D* (Eq. 4), between buffer and red blood cells and of the fraction bound, f_b, to plasma proteins was determined by the described procedures at various times after the addition of drug to the preparation. The true equilibrium values were readily established within the 4.5 min used to add and suspend the red blood cells in plasma, centrifuge, and sample.

This procedure of estimating protein binding by the method of variable plasma concentrations permits estimates at high degrees of protein binding, which the normal errors in classical methods would not allow. The procedure can be used routinely to determine the protein binding of individual subjects.

Stability of Δ^9-Tetrahydrocannabinol in Acid Solutions—The degradation of Δ^9-tetrahydrocannabinol was followed by GLC with flame-ionization detection in aqueous hydrochloric acid solutions at 55°. Four major peaks could be detected in addition to that for Δ^9-tetrahydrocannabinol (Fig. 11).

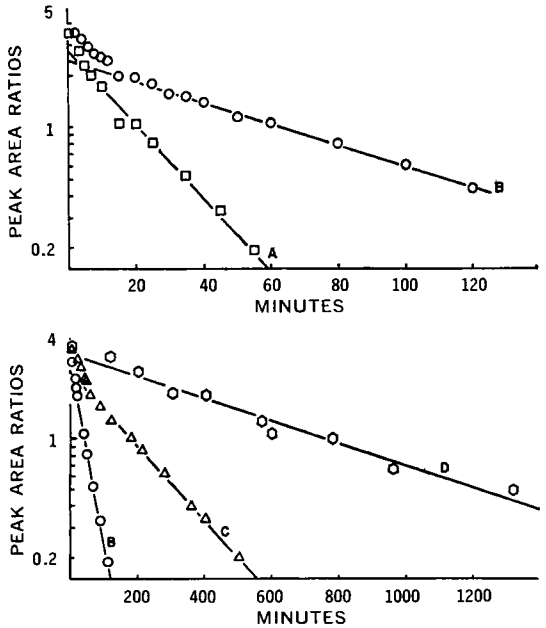


Figure 12—*Semilogarithmic plots of peak area ratios of Δ^9-tetrahydrocannabinol and internal standard as a function of time at 55°. Key: A, 0.5 N HCl; B, 0.1 N HCl; C, 0.05 N HCl (μ = 0.1 with sodium chloride); and D, 0.01 N HCl (μ = 0.1 with sodium chloride).*

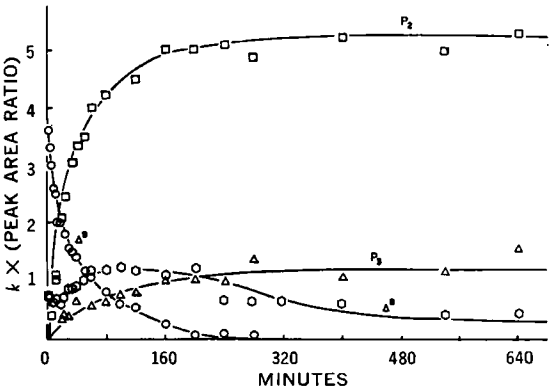


Figure 13—*Peak area ratios of Δ^8- and Δ^9-tetrahydrocannabinol and P_2 and P_3 plotted as a function of time for 0.1 N HCl at 55°. Key: ○, Δ^9-tetrahydrocannabinol, k = 1; ○, Δ^8-tetrahydrocannabinol, k = 3; △, P_3, k = 2; and □, P_2, k = 2.*

The semilogarithmic plots of peak area ratios of Δ^9-tetrahydrocannabinol to the internal standard, tetraphenylethylene, with time (Fig. 12) show an initial rapid drop with a subsequent linearity to indicate a possible rapid equilibration with one or more degradation products. The subsequent loss of Δ^9-tetrahydrocannabinol was apparent first order.

The retention time for one product corresponded to authentic Δ^8-tetrahydrocannabinol, and three others, P_1, P_2, and P_3, were observed with larger retention times (Fig. 11).

Plots of the peak area ratio of the products *versus* time (Fig. 13) showed that the peak corresponding to Δ^8-tetrahydrocannabinol increased rapidly and then decreased at a slower rate than that for the loss of Δ^9-tetrahydrocannabinol. Analysis of authentic Δ^8-tetrahydrocannabinol under similar aqueous conditions showed Δ^8-tetrahydrocannabinol to be more stable than Δ^9-tetrahydrocannabinol. Although this implicated the Δ^8-isomer as a product of Δ^9-tetrahydrocannabinol degradation, the facts that the retention time of the former was less and the peak area was also low indicated that it was not a major product.

Two of the other three products observed by GLC (Figs. 11 and 13), P_2 and P_3, increased slowly to a final equilibrium value. The rate of generation of these peaks appeared to parallel the apparent first-order loss of Δ^9-tetrahydrocannabinol within the experimental error, and the attainment of the final equilibrium value appeared to correspond to the complete loss of Δ^9-tetrahydrocannabinol. The other product, P_1, appeared almost instantaneously and gave a relatively small peak, which appeared to remain constant with time. The ratios of peak area values for the final equilibrium concentrations of P_2 and P_3 were independent of the hydrochloric acid concentrations studied, although the magnitudes varied.

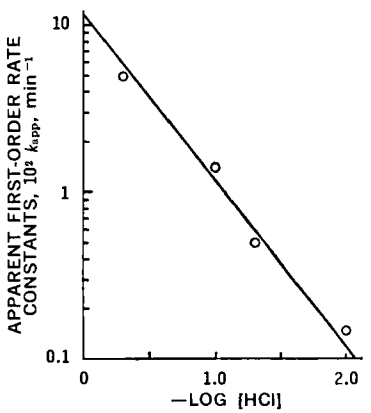


Figure 14—*Linear plot with slope of unity of the terminal apparent first-order rate constants, k_{app}, for loss of Δ^9-tetrahydrocannabinol (Fig. 12) as a function of −log [HCl].*

A plot of the apparent first-order rate constants obtained from the terminal slopes of Fig. 12 for the loss of Δ^9-tetrahydrocannabinol against $-\log[HCl]$ (Fig. 14) demonstrated a straight line consistent with a slope of unity to indicate hydrogen-ion-catalyzed degradation and an intercept $\log k_{H^+}$ that may characterize a bimolecular rate constant, k_{H^+}, of 0.002 liter/mole/sec. This observed instability of Δ^9-tetrahydrocannabinol in acid solution can account in part for the poor and variable availability of the drug when given orally. Studies are continuing to establish the complete log k *versus* pH profile for Δ^9-tetrahydrocannabinol and to identify the various degradation products.

Biopharmaceutical and Pharmacokinetic Implications of Physicochemical Properties of Δ^9-Tetrahydrocannabinol—The extremely low solubility of Δ^9-tetrahydrocannabinol (2.8 mg/liter in water and 0.77 mg/liter in 0.15 *M* NaCl at 23°) most certainly affects its bioavailability on oral dosing of amounts wherein the solubility is exceeded (2, 3). The premise that its solubility in the plasma is readily exceeded certainly is not inconsistent with the observation of its possible precipitation and localized accumulation in body organs (1). Tetrahydrocannabinol in excess of its solubility instantaneously forms a stable emulsion or micellar dispersion.

The rapid diffusion of Δ^9-tetrahydrocannabinol into the plastic of containers and into the rubber stoppers normally used as closures for plasma vials (70–97%) and the significant binding to glass at low tetrahydrocannabinol concentrations (20 and 40% at 0.1 and 0.05 μg/ml, respectively, in 50-ml volumetric flasks) definitely demand careful techniques in the handling, storage, and assay of this compound from aqueous and biological fluids. In fact, the results of any pertinent study where these conditions were not held in account should be quantitatively suspect.

The high degree of partition into lipid phases and of adsorption to all and any surfaces by tetrahydrocannabinol (lipoprotein binding may be included in these categories) implies that oral administration of the drug in lipid vehicles that are relatively immiscible with aqueous fluids would drastically reduce the bioavailability of the drug. A common practice of administering tetrahydrocannabinol admixed with lipid-containing feed in animal experiments would cast suspicion on the dose-response relationships proposed from the results of such studies.

The high sequestering and binding of Δ^9-tetrahydrocannabinol are consistent with the proposal of a pharmacokinetically deep compartment, especially with the known rapidity of its metabolism. The prolonged but lowered blood levels of administered drug (13) must be rationalized by its slow rate-determining release from such compartments. The lack of significant renal excretion of unchanged drug (13) is readily understandable since its high lipophilicity should result in complete tubular reabsorption.

The partitioning of tetrahydrocannabinol from plasma water into red blood cells is enormously high since $D = 12.5$. This may also be ascribed to the high surface affinity of the drug. However, the competition of a large degree of unsaturable binding (97%) to plasma proteins minimizes the amounts in the red blood cells of whole blood, although possibly large adherence of the drug to the walls of blood vessels and to the surfaces of tissues must be anticipated. The fact that the plasma binding is largely assigned to the lipoprotein fraction (21) may result in large individual variability in binding dependent upon individual and species variations in lipoprotein and fat content. Thus, the proposed method of variable plasma concentration may be useful in the routine determination of the protein binding of individual subjects.

The large instability of Δ^9-tetrahydrocannabinol in acid solution, which can be estimated minimally as $t_{1/2} = 1$ hr at pH 1.0 (55°) *after* an initial rapid drop in tetrahydrocannabinol content, implies that the drug may be significantly degraded in the normal stomach. Again, this intimates that oral administration may not be an optimum route on which to establish dose-response correlations.

REFERENCES

(1) R. I. Freudenthal, J. Martin, and M. E. Wall, *Brit. J. Pharmacol.*, **44**, 244(1972).

(2) L. A. Borgen and W. M. Davis, *J. Pharm. Sci.*, **62**, 479(1973).

(3) M. Perez-Reyes, M. A. Lipton, M. C. Timmons, M. E. Wall, D. R. Brine, and K. H. Davis, *Clin. Pharmacol. Ther.*, **14**, 48(1973).

(4) L. Lemberger, S. D. Silberstein, J. Axelrod, and I. J. Kopin, *Science*, **170**, 1320(1970).

(5) L. Lemberger, N. R. Tamarkin, J. Axelrod, and I. J. Kopin, *ibid.*, **173**, 72(1971).

(6) Å. Ryrefeldt, C. H. Ramsay, I. M. Nilsson, M. Widman, and S. Agurell, *Acta Pharm. Suecica*, **10**, 13(1973).

(7) E. R. Garrett and H. J. Lambert, *J. Pharm. Sci.*, **62**, 550(1973).

(8) E. R. Garrett, *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **4**, 6(1970).

(9) R. K. Raxdan, A. J. Puttich, B. A. Sitko, and G. R. Handrick, *Experientia*, **28**, 121(1972).

(10) R. F. Twik, J. E. Manno, N. C. Jain, and R. B. Forney, *J. Pharm. Pharmacol.*, **23**, 190(1971).

(11) H. Y. Saad and W. I. Higuchi, *J. Pharm. Sci.*, **54**, 1205(1965).

(12) "Handbook of Chemistry and Physics," 50th ed., Chemical Rubber Co., Cleveland, Ohio, 1969, p. D102.

(13) E. R. Garrett and C. A. Hunt, *J. Pharm. Sci.*, **62**, 1211(1973).

(14) "Siliclad," Clay Adams, Division of Becton, Dickinson and Co., Parsippany, NJ 07054

(15) H. J. Lambert, "Trichloroethanol Pharmacokinetics," Ph.D. dissertation, University of Florida, Gainesville, Fla., 1969, p. 12.

(16) E. R. Garrett, J. Tsau, and P. H. Hinderling, *J. Pharm. Sci.*, **61**, 1411(1972).

(17) S. Glasstone, "Textbook of Physical Chemistry," 2nd ed., Van Nostrand, New York, N.Y., 1952, p. 700.

(18) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd ed., Reinhold, New York, N.Y., 1958, p. 737.

(19) A. N. Martin, J. Swarbrick, and A. Cammarata, "Physical Pharmacy," Lea & Febiger, Philadelphia, Pa., 1969, p. 237.

(20) G. Kortüm, W. Vogel, and K. Andrussow, "Dissociation Constants of Organic Acids in Aqueous Solution," International Union of Pure and Applied Chemistry, Butterworth, London, England, 1961.

(21) M. Wahlquist, I. M. Nilsson, F. Sandberg, and S. Agurell, *Biochem. Pharmacol.*, **19**, 2579(1970).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 9, 1973, from the *College of Pharmacy, University of Florida, Gainesville, FL 32610*

Accepted for publication February 25, 1974.

Abstracted in part from studies intended to be submitted by C. A. Hunt to the Graduate School, University of Florida, in partial fulfillment of the Master of Science degree requirements.

Supported in part by Grants 1R03 MH 19268 and DA-00743-01 from the National Institute of Mental Health, Bethesda, MD 20014

The technical assistance of Mrs. Kathleen Eberst is gratefully acknowledged.

[x] To whom inquiries should be directed.