Pharmacokinetics of Morphine and Its Surrogates VI: Bioanalysis, Solvolysis Kinetics, Solubility, pK_a' Values, and Protein Binding of Buprenorphine

EDWARD R. GARRETT[x] AND V. RAVI CHANDRAN

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Abstract □ The 10-fold greater sensitivity of buprenorphine to fluorescence compared with morphine provides excellent detection for HPLC assay of buprenorphine in biological fluids with a 5-ng/mL sensitivity. Buprenorphine yields a stoichiometric final acid degradation product, a fluorescent-detectable, rearranged demethoxy analogue of buprenorphine, which serves as an excellent bioassay internal standard. Buprenorphine solvolysis is specific-acid and specific-base catalyzed. Alkaline hydrolysis produces no fluorescent products. Acid hydrolysis also produces a fluorescent-detectable, transient dehydro intermediate that is also completely transformed to the demethoxy analogue. The rate constants and Arrhenius parameters for these transformations have been determined. Estimated buprenorphine pK_a' values are 8.24 and 10 for the ammonium and phenol groups, respectively. The intrinsic aqueous solubility of neutral buprenorphine is 12.7 ± 1.2 μg/mL at 23°C. The red blood cell–plasma water partition coefficients of buprenorphine ranged between 6 and 15. Ultracentrifugation and the red blood cell partition methods led to an estimated 95–98% plasma protein binding. Ultrafiltration and equilibrium dialysis methods were inappropriate because of the high membrane binding of neutral buprenorphine.

The therapeutic potential of the morphine-type analgetic buprenorphine (**1**) is well documented.[1-18] Its main advantage over morphine is that the dose need not be increased during chronic administration. Buprenorphine is presently available in Europe for parenteral use.[16] The recommended dose is 0.3–0.6 mg by intramuscular or slow intravenous injection, repeated every 6–8 h as needed.

A radioimmunoassay[19] has been used to determine plasma levels of parenterally administered buprenorphine in dogs and humans.[20,21] A selective-ion monitoring method (SIM) of the silylated buprenorphine in GC–MS has been used to determine the plasma levels of buprenorphine over a 20–3000 ng/mL concentration range.[22] A GC assay with flame-ionization detection of silyl derivatives of buprenorphine was used in stability studies at 5–10 μg/mL of aqueous solutions.[23] These studies indicated only minor degradation in 10 weeks at pH 1–5 at 4°C and 27°C. Under the extreme conditions of autoclaving (10% HCl, 30 min at 112°C) buprenorphine was completely transformed to a rearrangement product (with a net loss of a molecule of CH_3OH), the demethoxy analogue, **3**. This acid-catalyzed rearrangement product of buprenorphine has been characterized, and the structure for **3** (Scheme I) has been assigned to this product by Cone et al.[23]

This paper reports buprenorphine studies conducted in preparation for future pharmacokinetic investigations. These studies include the determination of physicochemical properties such as solubility, pK_a' values and extractabilities, the development of HPLC assays in biological fluids with spectrophotometric and fluorometric detection, kinetics of solvolysis under acid and alkaline conditions, and the determination of plasma protein binding and red blood cell–plasma water partition coefficients.

Experimental Section

Materials—Analytical-grade solvents and reagents were used. Buprenorphine hydrochloride 21-cyclopropyl-7α-[(*s*)-1-hydroxy-1,2,2-trimethylpropyl]-6,14-*endo*-ethanotetrahydrooripavine (**1**), (NIDA, Rockville, MD)[22] and the demethoxy analogue of buprenorphine, **3**,[23] (Addiction Research Center, Lexington, KY) were used as received. A standard sample of 21-cyclopropyl-7α-[2-(3,3-dimethyl-1-butenyl)]-6,14-*endo*-ethanotetrahydrooripavine, **2**, was obtained from Dr. G. Lloyd-Jones of Reckitt and Colman, Pharmaceutical Division, Kingston-upon-Hull, England.

Apparatus—An HPLC (model M6000A pump, Waters Associates, Milford, MA), equipped with a variable-wavelength fluorescence detector (model 600S Fluoroscence Detector, Perkin-Elmer, Norwalk, CT), was used. Injections were carried out with an autosampler (WISP Autosampler, Waters Associates), and the data were analyzed by a microcomputer (Sigma 15, Data Station, Perkin-Elmer). A separate HPLC pump (series 3B, Perkin-Elmer) equipped with a variable-wavelength UV detector (model LC 75, Perkin-Elmer) was used in some studies. Plasma protein binding was determined with an ultracentrifuge (Beckman Ultracentrifuge model LS-50 with rotor Ti50, Beckman Instruments, Norcross, GA). A laboratory centrifuge was used in the separation of organic extract from plasma (Lab Centrifuge, International Centrifuge Equipment Co., Needham Heights, MA). MS was performed on an Associated Electronic Industries-MS530, data system Kratos DS55. NMR was performed on an EM-390 90-MHz spectrometer (Varian Associates, Palo Alto, CA).

Liquid Chromatographic Procedures—Aliquots (50–100 μL) of the solutions to be assayed were injected into the HPLC system equipped with a packed [packing material was C_{18} 5-μm reversed-phase μ-Bondapak (ODS-Hypersil), Shandon Southern Products Ltd., Cheshire, U.K.] reversed-phase 120-mm i.d. stainless-steel column [Knauer HPLC analytical column (unpacked), Berlin, F.R.G.] which was maintained at 40°C. The usual mobile phase flow rate was 1.5 mL/min of a 70:30 acetonitrile:acetate buffer (pH 3.75, 0.05 M) with the

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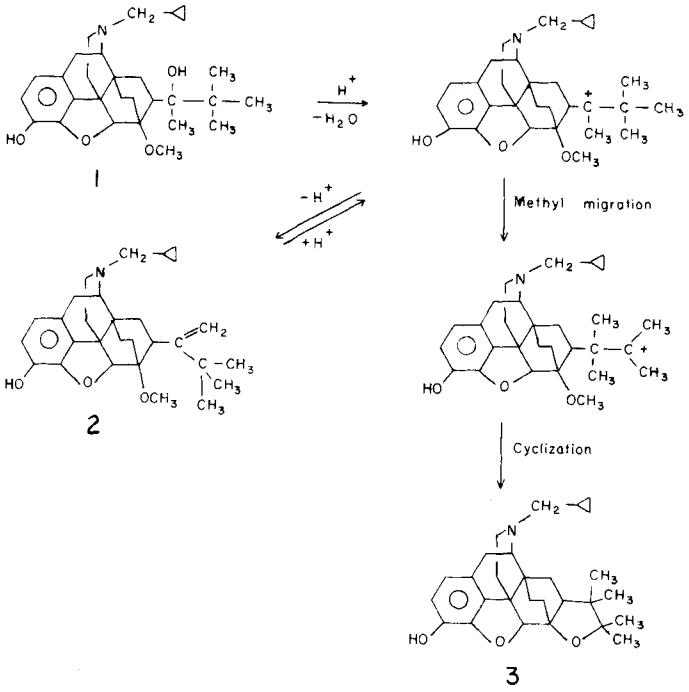


Scheme I

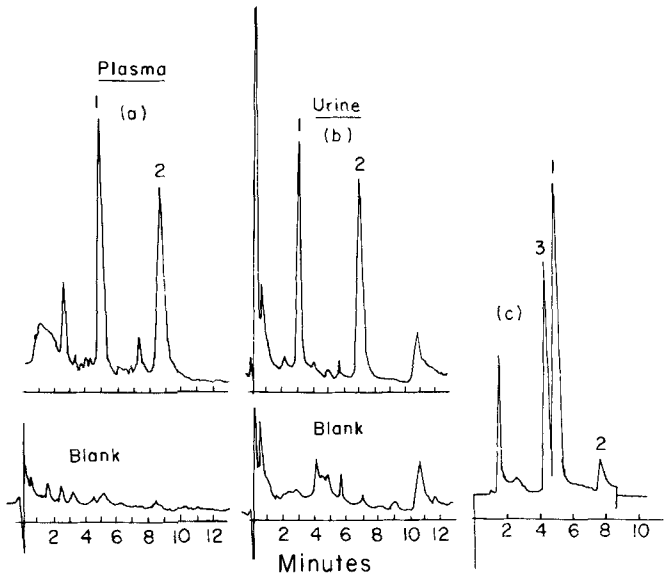


Figure 1—*Representative chromatograms after assay by method B of buprenorphine (**1**, 60 ng/mL) with internal standard (**2**, 100 ng/mL) from plasma (a) and urine (b). (The blank plasma and urine chromatograms without drug are given underneath). Mixture of 25 μg/mL of buprenorphine, **1**, with its products, **2** and **3**, after acid degradation in 1 M HCl for 3 min by method A (c).*

usual back pressure of 600 psi. The mobile phase for method B was 40:60 acetonitrile:acetate buffer (pH 3.75, 0.05 M) containing 0.004 M tetrabutylammonium phosphate. Fluorescence detection was effected at 285 nm excitation (slit 20 nm) and 350 nm emission (slit 15 nm) and was used unless stated otherwise. UV spectrophotometric detection was effected at 210 nm in some studies.

Calibration Curves for Assays in Biological Fluids—In method A, aliquots (1 mL) of plasma or urine in each of 10 15-mL centrifuge tubes were spiked with 100 μL of 100–1000 ng/mL of buprenorphine hydrochloride (**1**). Each solution contained 50 ng/mL of the final acid degradation product, **3**, of compound **1** (Scheme I) as the internal standard. The first sample contained no drug. Sodium borate–boric acid buffer (1.00 mL at pH 9.0, 1 M) and 4.2 mL of benzene were added to each tube. The tubes were shaken for 60 min, centrifuged at 3000 rpm for 20 min, and 4 mL of each benzene extract was transferred to another set of 10 15-mL centrifuge tubes. Hydrochloric acid (1.00 mL, 1.00 M) was added to each tube and the tubes were shaken for 30 min and then centrifuged at 3000 rpm for 20 min. After removal of the benzene layer by aspiration, 1 mL of both 1.00 M NaOH and pH 9.0 borate buffer (1.00 M) were added to each of the remaining aqueous phases. The pH values were confirmed or adjusted to be between 8.8–9.2. Benzene (3.2 mL) was added to each tube which was shaken for 30 min and centrifuged at 3000 rpm for 20 min. The benzene extract (3 mL) was transferred to a 5-mL Reacti-Vial and the benzene was evaporated under a stream of nitrogen at 55°C. Sodium acetate–acetic acid buffer (100 μL, pH 3.75, 0.05 M) was added to each of the Reacti-Vials and they were vortexed for 30 s, and then 75 μL of the solution was analyzed by HPLC. Method A was used in the studies reported herein unless stated otherwise.

The chromatograms were improved (Fig. 1a) and the peaks were further separated by modification of this procedure to method B where 100 ng of the acid degradation intermediate of **1**, compound **2**, was used as the internal standard in the 1.0 mL of biological fluid that was extracted twice at pH 9.0 with 4.2 mL of benzene. The combined benzene extracts were taken to dryness at 55°C under nitrogen and reconstituted in 1 mL of benzene that was extracted with 1.00 mL of 1 M HCl. The aqueous acid layer was washed with 5 mL of benzene, adjusted to pH 9 with 1.00 mL of 1 M NaOH plus 1.00 mL of 1.00 M borate buffer (pH 9.0). The compounds were extracted with 4.2 mL of benzene and the evaporated organic layer was reconstituted in 100 μL of acetate buffer (pH 3.75, 0.05 M) and analyzed by HPLC.

Kinetics of Hydrolytic Degradation of Buprenorphine—*Acid Hydrolysis*—Buprenorphine was dissolved in 0.05–1.0 M HCl solutions that were purged with nitrogen. Aliquots (1.00 mL) of the 10-μg/mL solutions were sealed under nitrogen in neutral glass ampules (Kimble Neutraglas, Kimble Products, Toledo, OH). The vials were placed in thermostatically controlled (90°C, 80°C, 62.5°C, and 60°C) water or oil baths and individual vials were withdrawn periodically. An aliquot (0.2 mL) of the immediately ice-cooled solution was diluted appropriately with water, and an aliquot was assayed by HPLC.

Alkaline Hydrolysis—Buprenorphine hydrochloride was mixed with thermally preequilibrated 1 M NaOH solutions. Aliquots (1.0 mL) of the 10-μg/mL solutions were transferred to sealed (under nitrogen) neutral glass ampules (Kimble Neutraglas, Kimble Products) which were thermostated in oil baths at 90°C, 80°C, 76°C, and 61°C. Individual ampules were withdrawn at intervals and immediately cooled with ice. An aliquot (0.2 mL) of the samples was neutralized with 1 M HCl, diluted appropriately with acetate buffer (pH 3.75, 0.05 M), and analyzed by HPLC.

Solubility and pK_a' Determination—The pK_a' of the tertiary amine was estimated from solubility studies. Buffer solutions[24] with pH values ranging between 6 and 13 were prepared. Powdered buprenorphine hydrochloride was added in excess of solubility to 3 mL of each buffer solution. The solutions were vortexed for 30 min and maintained at room temperature, 23.0 ± 0.3°C, overnight.

The solutions were filtered through 100-μm Millipore filters aided by reduced pressure. The clear filtrate was appropriately diluted and analyzed by HPLC using fluorescence detection. Calibration curves were prepared simultaneously.

Spectrophotometric Estimation of Phenolic pK_a'—Aliquots of aqueous buffer solutions[24] in the pH range from 6 to

12.5 were mixed with alcohol to obtain 10, 15, and 25% ethanol–buffer (v/v) mixtures. Aliquots (100 μL) of 1 mg/mL of ethanolic solution of buprenorphine hydrochloride were added to each buffer–ethanol mixture to give a 25-μg/mL solution of buprenorphine hydrochloride. The same systems were duplicated without buprenorphine to be used as spectrophotometric blanks. The apparent pH value of each alcohol–buffer mixture was determined (Fisher Pencil combination glass electrode no. E-5M containing Ag–AgCl used with a Fisher pH meter 125, Fisher Scientific Co.). The spectra of each solution was recorded between 230 and 330 nm in an automatic spectrophotometer (Cary 219 Spectrophotometer, Varian Associates).

Red Blood Cell Partitioning—Fresh heparinized dog blood was centrifuged for 20 min at 3000 rpm, and the plasma removed by aspiration. The packed red blood cells were washed twice with normal saline and once with plasma water with subsequent centrifugation. The plasma water had been obtained by filtration of plasma at 3000 rpm through a filter (100 Centriflo Ultrafiltration Membrane Cones, Amicon Corp., Lexington, MA).

The washed red blood cells were diluted with plasma water. The pseudoblood samples (3 mL) thus obtained were spiked with 0.2–1.4 μg of buprenorphine hydrochloride from a 10-μg/mL solution, and hematocrits were obtained using a microhematocrit (Damon/IEC Division, Needham Hts., MA) after equilibration for 60 min at 37°C. These pseudoblood samples were then centrifuged at 3000 rpm for 20 min, and 500 μL of collected plasma water was mixed with 1 mL of borate buffer (pH 9, 1 M). Hexane (3.2 mL) was added and the mixture was shaken for 30 min. It was then centrifuged at 3000 rpm for 20 min. The hexane layers (3 mL) were transferred to 5-mL vials and evaporated under a stream of nitrogen at 55°C. The residue was reconstituted in 100 μL of acetate buffer (pH 3.75, 0.05 M) and 50 μL was injected for HPLC assay. Compound **3** (50 ng/mL) was used as the internal standard.

In time-dependent red blood cell partitioning studies the pseudoblood (3 mL) was equilibrated with buprenorphine (333 ng/mL of pseudoblood) for 1, 2, 3, 4, 5, 6, 8, and 10 min at 37°C before commencing centrifugation for 5 min at 4000 rpm. Calibration curves were constructed by mixing aliquots (100–1000 μL) of buprenorphine hydrochloride and 10 μg/mL of physiological buffer with borate buffer (pH 9, 1.0 M), extracting with 3.2 mL of hexane with shaking for 20 min, and centrifuging at 3000 rpm for 20 min. The hexane layer (3 mL) was evaporated under nitrogen at 55°C, the residue was reconstituted in 100 μL of acetate buffer (pH 3.75, 0.05 M), and 10 μL was injected into the HPLC. Compound **3** (500 ng/mL) was used as the internal standard.

Protein Binding by Ultracentrifugation—Aliquots (20–100 μL) of a standard solution of buprenorphine hydrochloride (10 μg/mL) in physiological buffer were added to tubes containing 3 mL of fresh dog plasma. These solutions were ultracentrifuged at 35,000 rpm for 18 h. Then, 400 μL of each of the supernatant plasma water samples were adjusted to pH 8.7 with borate buffer (pH 8.7, 1 M), extracted with 3.2 mL of hexane by shaking for 30 min, and centrifuged at 3000 rpm for 10 min. The hexane layers were evaporated under nitrogen and the residues were reconstituted in 100 μL of acetate buffer (pH 3.75, 0.05 M) with 50 μL of each being injected into the HPLC using fluorescence detection. The calibration curves were constructed from solutions of known concentrations of buprenorphine in 3 mL of physiological buffer that were treated similarly.

Protein Binding by Partition Into Red Blood Cells—This was based upon the method of Garrett and Hunt[25] to use red blood cell partitioning and continuous plasma variation to estimate protein binding.

Procedure 1—Buprenorphine hydrochloride (100 μL of 100-μg/mL of physiological buffer) was added to 3 mL of fresh dog blood with assayed hematocrit. After 1 h at 37°C, the blood was centrifuged at 3000 rpm for 10 min and 100 μL of plasma supernatant was analyzed by HPLC. The calibration curve was prepared from 100 μL of fresh dog plasma, to which 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 ng of **1** (100 μL of 200–2000 ng/mL of buprenorphine hydrochloride in physiological buffer) was added, and the pH was adjusted to 9 with borate buffer (1 mL, 1 M). Buprenorphine was extracted with 2.2 mL of hexane by shaking for 10 min and centrifuging at 2000 rpm for 10 min. The separated hexane layer was evaporated under nitrogen at 55°C and the residue was reconstituted in 100 μL of acetate buffer (pH 3.75, 0.05 M), and 80 μL was assayed by HPLC. The acid degradation intermediate of **1**, compound **2** in Scheme I, was used as an internal standard at 100 ng/mL.

Procedure 2—Two milliliters of pseudoplasma, containing various fractions (m) of true plasma admixed with isotonic saline with a range varying from 0.03 to 0.3 in 0.03 increments, was admixed with 1 mL of washed, packed red blood cells. To each tube in the set was added 100 μL of buprenorphine stock solution in normal saline. Two stock solutions of 10 and 100 μg/mL of buprenorphine hydrochloride were used. After 20 min at 37°C, hematocrits were taken and any tube showing hemolysis was discarded. The tubes were centrifuged at 3000 rpm for 20 min and the supernatant plasma samples were assayed for buprenorphine content. Simultaneously, similar studies were conducted with saline being substituted for plasma fractions to estimate the partition of unbound buprenorphine into the red blood cells.

Partition Studies as a Function of pH—Partitioning was studied between equal volumes of water-saturated benzene and buffer solutions of buprenorphine at pH values of 1–6. Aliquots of the separated organic layer were evaporated under nitrogen at 55°C, reconstituted in 100 μL of acetate buffer (pH 3.75, 1 mL, 0.05 M) and assayed by HPLC. An aliquot of the aqueous phase was adjusted to pH 3.75 and analyzed similarly. Concentrations were chosen so that the aqueous phase after equilibration had 10–40 ng/mL of buprenorphine.

Synthesis of 21-Cyclopropyl-7α-[2-(3,3-dimethyl-1-butenyl)]-6,14-*endo*-ethanotetrahydrooripavine(2)—Buprenorphine hydrochloride (25 mg) was dissolved in 2 mL of pyridine and cooled to 0°C. Thionyl chloride (800 μL), cooled to 0°C, was added slowly to the above solution, well mixed, and kept at 0°C for 5 min. Borate buffer (1 M, pH 9.10, 18 mL) was added, and the well-mixed solution equilibrated at room temperature. The solution was extracted twice with 20 mL of benzene, the combined organic layer was evaporated under reduced pressure, and the residue was recrystallized from hexane to give 20.74 mg (93% yield) of **2** as white crystalline solid, mp 156–157°C; ^{1}H NMR ($CDCl_3$): δ 1.11 (s, 9, *tert*-butyl), 3.31 (s, 3, 6-OCH_3), 4.35 (sharp m, 1, 7α-CH), 5.07 (d, 2, J = 8 Hz, C=CH_2), 6.65(AB_q, 2, J = 5 Hz, $AB_{\Delta\nu}$ = 10.9, ArH); MS: m/z 449 (M^+, 73.8%), 434 (40), 420(18), 408(19), 84(15), 57(22), 55(100), 41(22), and 29(32).

Anal—Calc. for $C_{29}H_{39}NO_3$: C, 77.47; H, 8.74; N, 3.12. Found: C, 77.38; H, 8.85; N, 2.67.

The white, crystalline solid material of **2** received from Reckitt and Colman for use as an analytical reference standard had an identical MS and NMR spectra as the aforementioned sample. The melting point was reported as 155.0–156.5°C with a found elemental analysis of C, 77.45, 77.30; H, 9.06, 9.01; N, 2.97, 2.97. They also reported 96.7% (w/w) by nonaqueous titration and an IR spectrum deemed consistent with structure **2**.

Isolation of 21-Cyclopropyl-7α-[2-(3,3-dimethyl-1-butenyl)]-6,14-(*endo*-ethanotetrahydrooripavine (2) from Hydrolysis of Buprenorphine—The intermediate **2** formed during acid hydrolysis of buprenorphine was collected by HPLC separation. Buprenorphine hydrochloride (25 mg) was treated with 1 M HCl at 90°C for 5 min, the mixture was cooled to 0°C, and the pH of the medium was adjusted to 9.0 with 1 M borate buffer. Compounds **1**, **2**, and **3** were extracted

three times with 50-mL portions of benzene and the combined organic layers were evaporated under nitrogen. The residue was reconstituted in pH 3.75 acetate buffer (0.05 M) and aliquots were repetitively injected into the HPLC. The mobile phases containing the intermediate **2** (retention time of ~7.8 min) were combined and the solvent was evaporated under a nitrogen stream until there was no detectable acetonitrile. The pH of the residual solution was adjusted to 9.0 with borate buffer (1.0 M) and then extracted into benzene. The organic layer was evaporated under nitrogen and the residual amorphous powder was checked for its chromatographic purity. This material had the same MS as the synthesized **2** and the same retention times in three different HPLC mobile phases.

Results and Discussion

Liquid Chromatographic Assays—In contrast with morphine which shows low natural fluorescence, both buprenorphine and nalorphine have relatively high fluorescence at 285 nm excitation and 350 nm emission. Their fluorescence in HPLC mobile phase A was 10-fold more sensitive than that of morphine. Typical statistics[26] for the calibration curves of concentration, C, against peak height ratio (PHR) of buprenorphine by fluorescence, using **3** as the internal standard by HPLC method A were, for plasma: range = 20–70 ng/mL, n = 5, r = 0.999, and $C \pm 1.14$ ng/mL = (44.4 ± 1.3)PHR − 0.6 ± 1.5. The sequential deviations in the equation were standard error of estimate of concentration on peak height ratio, standard error of estimate of the regression coefficient, and standard error of estimate of the intercept, respectively. Typical statistics[26] for a calibration curve in urine were: range = 40–90 ng/mL, n = 6, r = 0.998, and $C \pm 1.62$ ng/mL = (60.5 ± 1.9)PHR − 0.5 ± 2.4. The spectrophotometric detection of buprenorphine at 210 nm in HPLC method A using peak height was equally sensitive, but the background was greater and **3** could not be used as an internal standard since plasma components interfered.

The HPLC method B, which used a mobile phase containing tetrabutylammonium phosphate and **2** as the internal standard, had sharper and more widely separated peaks. The statistics of typical calibration curves for the HPLC assay of buprenorphine by method B were, for plasma: range = 10–100 ng/mL, n = 8, r = 0.9983, and $C \pm 1.95$ ng/mL = (64.7 ± 1.5)PHR(cm) + 2.2 ± 1.5; and, for urine: range = 5–60 ng/mL, n = 12, r = 0.9979, and $C \pm 1.23$ ng/mL = (156.8 ± 3.2)PHR − 0.6 ± 3.2.

Neutral buprenorphine is highly lipophilic and readily extractable into benzene. The apparent benzene–buffer partition coefficients for buprenorphine at room temperature were 0.2, 0.65, 5.2, 44, and 120 at pH values of 2, 3, 4, 5, and 6, respectively. No significant amounts of buprenorphine (<5 ng/mL) remained in the aqueous phase after extraction of pH 9 buffer containing 1 μg/mL of buprenorphine with an equal volume of benzene.

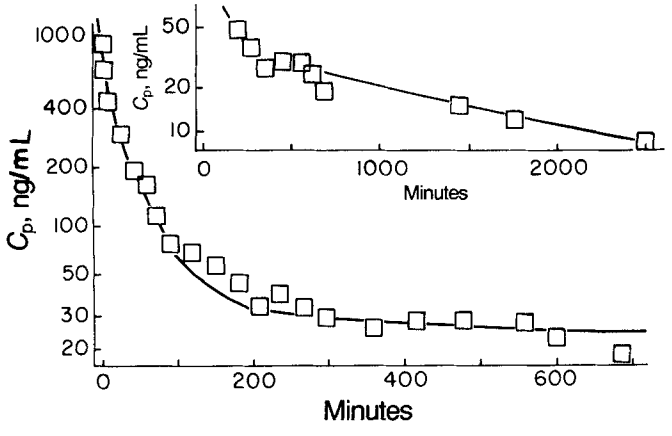


Figure 2—*Plasma concentration, C_p, against time after intravenous bolus administration of 32.4 mg of buprenorphine to a 22.85-kg dog.*

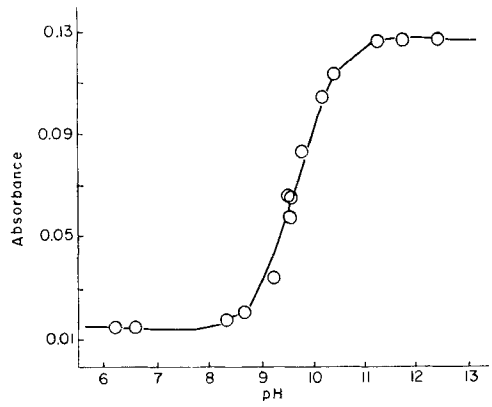


Figure 3—*Absorbances, A, at 300 nm of a 25 μg/mL solution of buprenorphine in 10% ethanol–buffer against pH. The curve through the data was generated by a nonlogarithmic form of eq. 1 for $pK'_{a2} = 9.7$.*

Preliminary Pharmacokinetic Study—The HPLC assay method B is applicable to the monitoring of buprenorphine in biological fluids. The time course of buprenorphine in plasma is shown in Fig. 2 for a 22.9-kg dog administered 1.417 mg/kg of buprenorphine. The curve through the data in Fig. 2 is the triexponential fit for concentration, C_p, in nanograms per milliliter:

$$C_p = 972e^{-0.68t} + 538e^{-0.0276t} + 35.4e^{-0.000593t} \quad (1)$$

The respective half-lives for the three phases are 1.02, 25.1, and 1170 min with a total body clearance, CL_{tot}, of:

$$CL_{tot} = \frac{\text{Dose}}{\text{AUC}} = \frac{32.4 \times 10^6}{80{,}480} = 402 \text{ mL/min} \quad (2)$$

Only 0.23% of the original dose was excreted in the urine as unchanged buprenorphine.

Estimation of the Phenolic pK'_{a2} of Buprenorphine—The low aqueous solubility of neutral buprenorphine prevented the facile determination of its pK'_{a2} values in aqueous solution by the classical methods of potentiometry and spectrophotometric titration. Classical UV spectra of aqueous solutions of 83 μg/mL of buprenorphine were not readily obtainable at pH values between 6.8 and 11.3 due to this poor solubility. The absorbances at the spectral maxima of the acidic (285 nm) and alkaline (300 nm) solutions of the chromophoric species did not vary greatly outside of these respective pH values.

Spectrophotometric titrations in 25, 15, and 10% ethanol–buffer systems (Fig. 3) gave midpoint pH values of their sigmoidal curves that corresponded to the apparent pK'_{a2} values of 10.16, 9.85, and 9.7, respectively. Semilogarithmic plots of $(A - A_B)/(A_A - A)$ against pH, in accordance with:[27]

$$\log[(A - A_B)/(A_A - A)] = pK'_{a2} - \text{pH} \quad (3)$$

were linear with negative slopes that were not significantly different than unity and with intercepts that confirmed the estimated pK'_{a2} values. In eq. 3, A was the absorbance at any pH value, A_B was the absorbance at the highest alkaline pH value, and A_A was the absorbance at the lowest acidic pH value.

A plot of these apparent pK'_{a2} values against percent alcohol in the titration media gave an intercept of 9.39 for 0% ethanol. This may be taken as an estimate of the pK'_{a2} of the phenolic hydroxyl group at 24 ± 0.3°C in aqueous solution. A normal caveat for such an extrapolation[28] is that selective solvation of the titrated species by the miscible organic solvent could invalidate such a prediction.

The spectrophotometrically determined pK'_{a2} must be assigned to the ionization of the chromophoric phenolic group. The fact that this pK'_{a2} is greater than the pH range where the neutral species has its highest insolubility supports the conclusion that the ionic equilibria through a zwitterionic intermediate (Scheme II) are improbable. In the following schemes, buprenorphine is represented by its functional groups:

$$R_3\overset{+}{N}H\} — OH \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} R_3\overset{+}{N}H\} — O^- \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} R_3N\} — O^-$$

Scheme II

Thus, the equilibria through an uncharged species (Scheme III) are favored since the spectrally observed phenolic dissociation has a higher pK'_{a2} than the pH range of relative insolubility associated with the neutral species:

$$R_3\overset{+}{N}H\} — OH \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} R_3N\} — OH \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} R_3N\} — O^-$$

Scheme III

As has been argued previously,[27] a linear plot in accordance with eq. 3 with a unit slope provides strong evidence that only one dissociating species, $R_3N\}$—OH (not $R_3N^+H\}$—O^-), is predominant in the pH region of least solubility.

Solubility and Amine pK'_{a1} of Buprenorphine—It follows that the total solubility of buprenorphine is the sum of concentrations of the neutral, bifunctional, undissociated phenol, $R_3N\}$—OH, and its conjugate base, $R_3N\}$—O^- and conjugate acid, $R_3N^+H\}$—OH, in equilibrium with the undissolved neutral species at a given pH. If it were assumed that the conjugate base would not be formed in significant amounts at low or moderate pH values, the equation representing the relationship between the conjugate acid and the concentration of neutral species would be:[29]

$$pK'_{a1} - pH = \log[(C - R_3N\}\text{—OH})/[R_3N\}\text{—OH}]] \quad (4)$$

where:

$$[R_3^+NH\}\text{—OH}] = C - [R_3N\}\text{—OH}] \quad (5)$$

and C is the total concentration of soluble buprenorphine at the measured pH.

At any two given pH values, pH_1 and pH_2, when the total concentration of buprenorphine is measured in the filtrate of pH-equilibrated excess drug, $C = C_S$ and $R_3N\}$—OH = C_{int}, the intrinsic solubility of neutral buprenorphine, and the total solubilities of buprenorphine are C_{S1} and C_{S2} at these pH values. Thus:[29]

$$\log[(C_{S1} - C_{int})/(C_{int})] = pK'_{a1} - pH_1 \quad (6)$$

$$\log[(C_{S2} - C_{int})/(C_{int})] = pK'_{a1} - pH_2 \quad (7)$$

Equations 6 and 7 can be solved simultaneously to permit the estimation of the intrinsic solubility of neutral buprenorphine, C_{int}, from experimentally determined values of C_{int} and pH:

$$C_{int} = [C_{S2}\ 10^{pH_2-pH_1} - C_{S1}]/[10^{pH_2-pH_1} - 1] \quad (8)$$

The experimentally determined solubilities of buprenorphine in various aqueous buffers are listed in Table I. The intrinsic solubilities of the neutral, uncharged buprenorphine species calculated by eq. 8 for several pairs of pH values are also given. The average of the listed intrinsic solubilities was 12.7 ± 1.2 μg/mL. A plot of the data in accordance with eqs. 4, 6, or 7, using the value of 12.7 for C_{int} and the listed total solubilities of Table I for C_S, is given in Fig. 4. The plot is linear with a slope of −1. The intercept of 8.24 estimates the pK'_{a1}, attributed to the dissociation of the protonated tertiary amine. The linearity of the data between 6 and 7.6 pH values is consistent with the premise of no significant phenolate anion or zwitterion in solution in that region and below a pH of 7.6. The isoelectric pH for the maximum concentration of the neutral species,

Table I—Total, C_S, and Intrinsic Solubilities, C_{int} (μg/mL), of Buprenorphine

pH_1	C_S at pH_1		pH_2	C_{int}[b]
	Exp.	Calc.[a]		
6	1401	2219	8.8	14.0
			8.6	14.4
6.4	692	891	8.8	13.0
			8.6	13.6
6.6	553	567	8.8	12.8
			8.6	12.5
			7.6	11.9
6.8	340	363	8.8	12.9
			8.6	12.6
			7.6	14.3
7	236	233	8.8	12.6
			8.6	12.2
			7.6	8.9
7.2	172	152	8.8	12.2
			8.6	11.4
7.6	66	68	8.8	12.8
			8.6	12.5
8.6	18	18	8.8	13.3
8.8	16	16		
9	10	15		
9.4	12	14		
10	18	13		

[a] Calculated from eq. 7 for C_{int} = 12.7 ± 1.2 μg/mL. [b] The intrinsic solubilities of $[R_3N\}$—OH] in μg/mL were calculated from eq. 6.

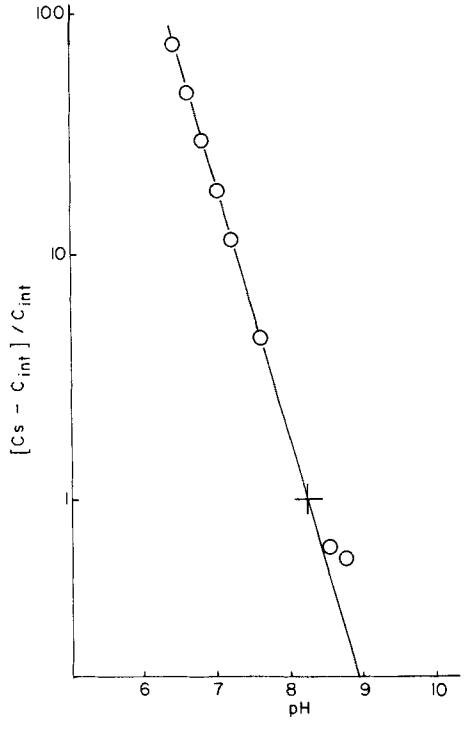


Figure 4—*Semilogarithmic plot of the ratios of concentrations of protonated, ($R_3N^+H\}$—OH) = $C_s - C_{int}$ to neutral buprenorphine, C_{int}, against pH in accordance with eq. 4 where C_s is the total solubility at the given pH. The cross designates the estimated pK'_{a1} = 8.24*

$[R_3N\}—OH]$, in solution can be estimated as $(pK'_{a1} + pK'_{a2})/2 = (8.24 + 9.39)/2 = 8.81$, a value that is one-half pH unit below the observed pH of minimum solubility in Table I.

The total aqueous solubilities of buprenorphine with acidic pH values can be estimated from a transformation of eq. 6:

$$C_S = C_{int}[1 + 10^{(pK'_{a1}-pH)}] \quad (9)$$

The solubilities calculated thusly agreed well with the available experimentally determined total solubilities (Table I).

A more accurate estimate of total solubility at any pH value would be:

$$C_S = C_{int}[1 + 10^{(pH-pK'_{a2})} + 10^{(pK'_{a1}-pH)}] \quad (10)$$

The estimated pK'_{a2} of 9.39 by extrapolation to 0% ethanol gave predicted values of total solubility that were not significantly different than those calculated for pH <9 from eq. 9 but gave larger values (20, 27, and 65 μg/mL at pH values of 9, 9.4, and 10, respectively) than observed. Thus, the aqueous pK'_{a2} of the phenol would have to be greater than the value of 9.39, obtained by extrapolation from organic aqueous solvent mixtures, to permit eq. 10 to calculate total solubilities in agreement with experimental values. Assumption of a $pK'_{a2} = 10$ gave calculated values of 14, 16, and 25 μg/mL at pH values of 9, 9.4, and 10, which are more reasonable approximations of the observed experimental values listed in Table I. This estimated pK'_{a2} would give a pH of minimum solubility of 9.1, a value closer to the experimental value than the 8.8 estimated from pK'_{a2} of 9.4.

Nevertheless, it is difficult to accept the fact that the aqueous pK'_{a2} of the phenol is greater than the 9.39 value obtained by extrapolation when selective solvation by ethanol should tend to give a higher estimated pK'_{a2} (rather than a lower). An uncharged acid such as phenol should have a higher, not lower pK'_a when the environment is of the lower dielectric (as favored by ethanol over water solvation). This should promote attraction rather than the repulsion that would have increased dissociation and lowered pK'_a.

Kinetics of the Degradation of Buprenorphine—Buprenorphine degrades by apparent first-order processes at fixed hydrogen and hydroxide ion concentrations. The first-order rate constants, k', (Table II) were determined from the slopes of the semilogarithmic plots (Figs. 5 and 6) of the HPLC peak heights of appropriately diluted aliquots of degrading buprenorphine solutions (Fig. 1c) against time. The first-order rate constants for buprenorphine degradation in 1.0 M NaOH were: [°C, k' (min^{-1})]; 90.0, 0.0042; 80.0, 0.00167; 76.0, 0.00128; 61.0, 0.000360. All studies but the 76.0°C study at 10 μg/mL were at 25 μg/mL.

There were no significant differences in rate constants at 90.0°C in 1.0 M HCl with buprenorphine HCl concentrations. They ranged between 0.196 and 0.217 with an average of 0.211 min^{-1} at 10, 50, 100, 500, and 1000 μg/mL.

There were no significant differences between the rate constants obtained with bubbling oxygen and bubbling nitrogen through reacting 1.0 M HCl solutions at 90.0°C. Thus, oxidative enhancement or inhibition of buprenorphine degradation in acidic solutions does not occur.

In addition to the decreasing peaks due to the acid degradation of buprenorphine (**1**) the HPLC chromatograms showed the appearance of an apparent intermediate **2** with a detectable fluorescent HPLC peak (Fig. 1c) that went through a maximum (Figs. 7 and 8) and finally disappeared. The chromatogram also

Table II—Apparent First-Order Rate Constants for Buprenorphine[a] Loss (k') and the Demethoxy Analogue of Buprenorphine Gain (k'') in HCl Solutions

[HCl], M	90.0°C	
	k', min^{-1}	k'', min^{-1}
1.00	0.211	
0.75	0.102	0.102
0.3	0.032	0.0315
	0.0364	
0.20	0.0173	
0.10	0.010	0.0116
0.075	0.0068	
0.05	0.0046	0.0042
0.01	0.00087	
°C	1.00 M HCl	
	k'	k''
88.5	0.20	0.142
80.0	0.0644	0.044
	0.0631	
70.0	0.018	
	0.0157	0.0177
62.5	0.0066	0.0066
60	0.0045	
	0.0056	0.0057

[a] All studies were conducted at 25 μg/mL except at 90°C at 1.0 M HCl (10 to 1000 μg/mL), lower value at 0.3 M HCl (5 μg/mL), at 0.10 M (3.33 μg/mL), and at 0.01 M (10 μg/mL).

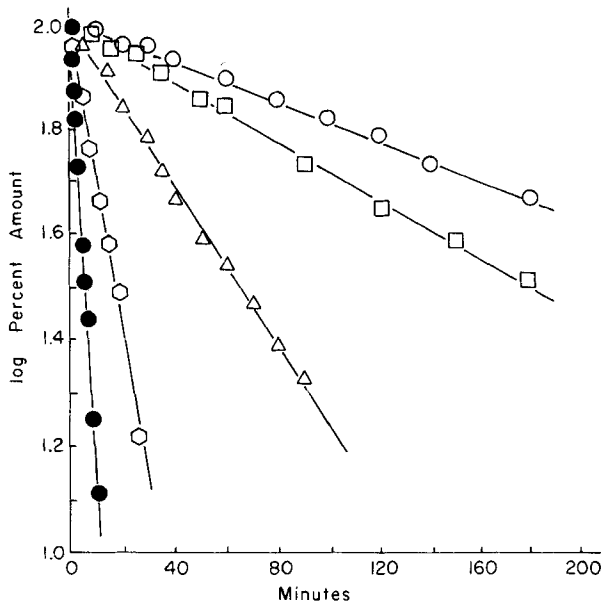


Figure 5—*Examples of semilogarithmic plots of percent of original buprenorphine concentration in 1.0 M HCl against time at several temperatures: (○) 60°C; (□) 62.5°C; (△) 70°C; (○) 80°C; (●) 90°C.*

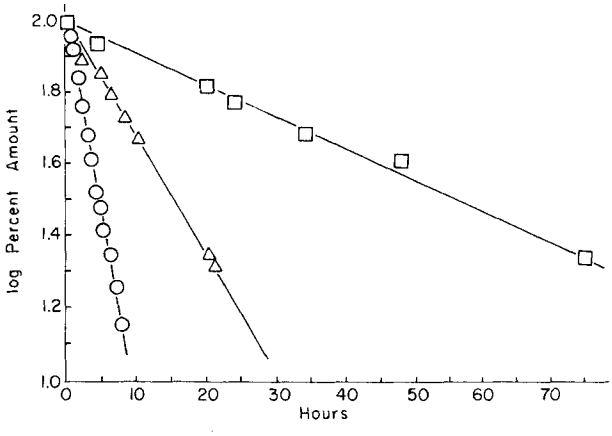


Figure 6— *Semilogarithmic plots of percent of original buprenorphine concentrations in 1.0 M NaOH against time. Key: (○) 90°C; (△) 76°C; (□) 61°C.*

demonstrated the appearance of a fluorescent final product **3** (Fig. 1c). Neither peaks for **2**, **3**, nor any other compound appeared in the alkaline degradation studies either on fluorescent or 210 nm UV detection. The peak heights, P_3, of the final product that appeared in acid degradation studies were fitted to a first-order rate of appearance and apparent first-order rate constants, k'', are listed in Table II. The k' and k'' values in each study were not significantly different.

Temperature, pH Dependency, and Prediction of Buprenorphine Stability—When the first-order rate constants, k', for the degradation of buprenorphine in 1.00 M HCl (Table II) were fitted to the Arrhenius equation:[30]

$$\log k' = \log A - (\Delta H_a/2.303R)(1/T) \qquad (11)$$

the estimated ΔH_a = 30.6 kcal/mol with log A = 17.83. The Arrhenius parameters for the first-order rate constants characterizing degradation in 1.00 M NaOH were ΔH_a = 20.5 kcal/mol and log A = 11.76.

Semilogarithmic plots of k' against pH with slopes of −1 are given in Fig. 9 in accordance with the expression for specific hydrogen-ion-catalyzed solvolysis:[30]

$$\log k' = \log k'_{a_{H^+}} - \text{pH} \qquad (12)$$

where $k'_{a_{H^+}}$ = 0.147 L mol^{-1}·min^{-1} at 90.0°C is the bimolecular specific hydrogen ion activity catalytic rate constant. The values of pH = $-\log f_{HCl}$[HCl] were calculated from the activity

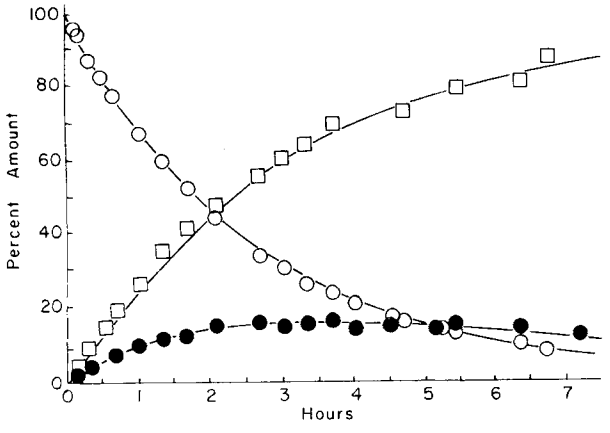


Figure 7—*Example of fitted data in accordance with Scheme IV and eqs. 12–17 for* k_1 = 0.0024, k_2 = 0.00407, *and* k_3 = 0.006 min^{-1}. *The data are from the degradation of 25 μg/mL of buprenorphine hydrochloride in 0.075 M HCl at 90°C. Key:* (○) **1**; (●) **2**; (□) **3**.

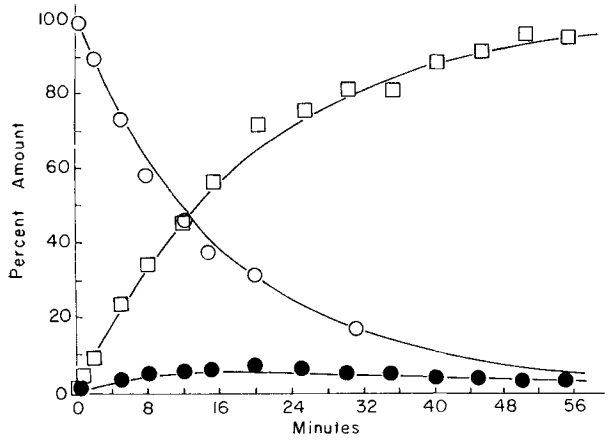


Figure 8—*Example of fitted data in accordance with Scheme IV and eqs. 12–17 for* k_1 = 0.0075, k_2 = 0.0520, *and* k_3 = 0.0580 min^{-1}. *The data are from the degradation of 25 μg/mL of buprenorphine hydrochloride in 1.0 M HCl at 80°C;* (○) **1**; (●) **2**; (□) **3**.

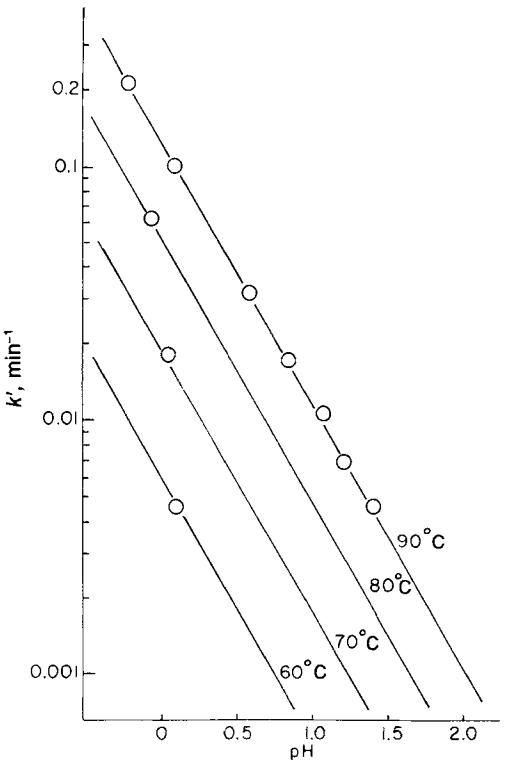


Figure 9—*Fitted and predicted log k'–pH profiles in accordance with eqs. 9 and 10 for the overall first order loss of buprenorphine in acid solutions at the designated temperatures (°C).*

coefficients available in, or extrapolated from, the literature.[31] A plot of k' against [HCl] was also linear and passed through the origin to indicate specific acid hydrolysis with no significant spontaneous or aqueous hydrolysis of buprenorphine.

The stabilities of buprenorphine can be predicted in acid solutions at all acidities and temperatures from eqs. 11 and 12. Thus, although the half-life of buprenorphine is 3.5 min in 1.0 M HCl at 90°C and is 11 min at 80°C, the half-lives at pH 2 and 3 are 12.5 and 125 years at 25°C, respectively. Complete stability can be anticipated in the neutral pH region even at elevated temperatures.

It is interesting to note that if one attempts to assay acid-hydrolyzable conjugates of buprenorphine by determination of the aglycones, there would be no buprenorphine per se to analyze since autoclaving of HCl solutions stoichiometrically transforms buprenorphine to **3**. Thus, the HPLC assay of acid-autoclaved buprenorphine conjugates should be based on the assay of **3** and not **1**. The compound **3** is stable and not a product of enzymatic transformation in biological fluids. Thus, it can be readily used as an internal standard in the HPLC assay of buprenorphine in plasma and urine.

Mechanism of Buprenorphine Transformation in Acid Solutions—The HPLC chromatograms (Fig. 1c) of acid-degrading buprenorphine clearly demonstrated the transient appearance of an intermediate **2** before the complete transformation of **1** to **3**. When the intermediate **2**, isolated from the HPLC from a reacting acidic solution, was reacted under the same acidic conditions, the associated HPLC peak **2** disappeared and a peak with the retention time of the final product **3** appeared. The same phenomenon with the same retention times occurred when synthesized **2** was treated with acid. The retention time of the material in three different mobile phases (isolated by HPLC separation of acid-degrading buprenorphine) as well as the mp, MS, and NMR spectra were identical with that synthesized by the action of thionyl chloride in pyridine on buprenorphine, and the compound assigned the structure, **2** (Scheme I), by Lloyd-Jones and associates.[32] This

provided further evidence of the identity of the compounds from these three sources. Thus, **2** in Scheme I is proposed as an intermediate in the production of **3** from **1** under acid hydrolysis.

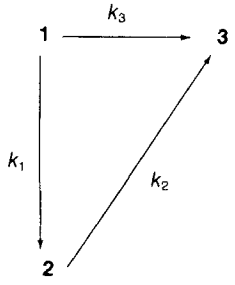
Since **2** is both formed from buprenorphine and transformed by first-order processes with respective rate constants, k_1' and k_2', the peak height, P_2, associated with **2** was fitted to:

$$P_2 = [(P_0 k_1')/(k_1' - k_2')](e^{-k_2' t} - e^{-k_1' t}) \quad (13)$$

where P_0 would have been the peak height of **2** if the concentration of **2** were the same as the initial concentration of the degrading buprenorphine.

The k_1' and k_2' were obtained by fitting the data by nonlinear least-squares regression. For five studies at 90°C in 1.0 M HCl, $k_1' = 0.325 \pm 0.018$(SEM) and $k_2' = 0.088 \pm 0.011$(SEM). Scheme I is in accordance with the above facts. Proton attack on the side chain produces the classical carbonium ion by the removal of water. The carbonium ion intermediate can form **2** by β-proton elimination or undergo methyl migration and cyclization to give **3**, the acid-rearranged demethoxy analogue of buprenorphine. The apparent intermediate can be conceived of as being susceptible to further proton attack and being in dynamic equilibrium with the carbonium ion reaction intermediate so that, although the **2** is kinetically formed, the parallel pathway to **3** is thermodynamically favored. Thus **3** is finally, stoichiometrically formed from **1**.

A kinetic model for Scheme I can be simplified to:



Scheme IV

Determination of Microscopic Rate Constants—The overall apparent first-order degradation rate constant, k', for the acid hydrolysis of buprenorphine is the sum of the microscopic rate constants k_1 and k_3 in Scheme IV. The differential equations for first order transformations are:

$$d[\mathbf{1}]/dt = -(k_1 + k_3)[\mathbf{1}] \quad (14)$$

$$d[\mathbf{2}]/dt = k_1[\mathbf{1}] - k_2[\mathbf{2}] \quad (15)$$

$$d[\mathbf{3}]/dt = k_3[\mathbf{1}] + k_2[\mathbf{3}] \quad (16)$$

If $[\mathbf{1}]_0$ is the initial concentration of buprenorphine, then the respective solutions are:

$$[\mathbf{1}] = [\mathbf{1}]_0 e^{(-k_1 - k_3)t} \quad (17)$$

$$[\mathbf{2}] = [(k_1[\mathbf{1}]_0/\{k_2 - (k_1 + k_3)\}]\,[e^{-(k_1 + k_3)t} - e^{-k_2 t}] \quad (18)$$

$$[\mathbf{3}] = [(k_3[\mathbf{1}]_0)/(k_1 + k_3)][1 - e^{-(k_1 + k_3)t}] + [(k_1 k_2[\mathbf{1}]_0)/\{k_2 - (k_1 + k_3)\}\,(k_1 + k_3)\}][1 - e^{-(k^1 + k^3)t} + (k_1[\mathbf{1}]_0/\{k_2 - (k_1 + k_3)\})\,(e^{-k_2 t} - 1) \quad (19)$$

Examples of the time courses of buprenorphine, and compounds **2** and **3** in different acid solutions, are given in Figs. 7 and 8. The data were fitted with an analog computer (Pace TR10 Analog Computer, Electronic Associates, Long Beach, NJ) using the programmed differential equations of eqs. 14–16. The microconstants obtained in this manner were used to simulate the data inserted into eqs. 17–19 to generate the curves plotted through the data in Figs. 7 and 8. The obtained microconstants are listed in Table III with their Arrhenius parameters determined from eq. 11.

Red Blood Cell–Plasma Water Partition Coefficients—The red blood cell–plasma water partition coefficient,[33] D, of a drug can be calculated from:

$$D = C_{RBC}/C_{pw} = [A_{RBC}/(V_B - V_{pw})]/(A_{pw}/V_{pw}) = \frac{(A_{tot} - C_{pw}V_{pw})}{V_B - V_{pw}} \Big/ C_{pw} \quad (20)$$

where C_{RBC}, A_{RBC}, C_{pw}, and A_{pw} are concentrations and amounts in red blood cells and plasma water, respectively. V_B and $V_{pw} = (1 - H)V_B$ are the volumes of the original red blood cell suspension (in plasma water or buffer) and the plasma water in the mixture respectively. A_{tot} is the total amount of drug added to the 3.1 mL of red blood cell suspension (hematocrit 100H) studied.

There was no significant time-dependent partitioning of buprenorphine into red blood cells under the assay conditions. The red blood cell–plasma water partition coefficients, D, obtained in several studies for materials derived from different samples are listed in Table IV. The variation among the average values may be due to variability in blood components. However, in the one set of studies, performed at the lowest hematocrit and with the lowest plasma water concentration, the partition coefficient was significantly higher. In only one set of the studies indicated in Table IV was there a significant dependence of D on buprenorphine concentration in the equilibrated plasma water. The high red blood cell partition coefficient of 6–15 for buprenorphine in contrast to 1.11 for morphine,[34] 1.83 for naltrexone, and 1.49 for naloxone[35] supports the hypothesis that erythrocyte partitioning is related to lipophilicity.

Protein Binding—Determinations of the protein binding of buprenorphine by equilibrium dialysis and ultrafiltration

Table III—First-Order Rate Constants in 1.0 M HCl from Analog Computer Fitting to Kinetic Scheme IV[a]

Temp., °C	min⁻¹		
	k_1	k_2	k_3
60	0.014	0.0065	0.0032
70	0.0050	0.0192	0.013
80	0.0075	0.0520	0.058
90	0.0420	0.140	0.18
ΔH_a, kcal/mol	27.1	24.5	32.6
log A	15.0	13.9	18.9

[a] Additional analog computer fittings were effected in 0.075 M HCl at 90°C with $k_1 = 0.0024$, $k_2 = 0.00407$, and $k_3 = 0.006$; in 0.05 M HCl at 90°C with $k_1 = 0.00163$, $k_2 = 0.0056$, and $k_3 = 0.0049$ min⁻¹.

Table IV—Studies on Red Blood Cell–Plasma Water Partition Coefficients of Buprenorphine

Range p, ng/mL[a]	n[b]	100H	$D \pm$ SD	r[c]
127–866	12	46	7.9 ± 1.2	−0.87[d]
267–1466	12	27	6.0 ± 0.7	−0.22
129–1112	14	27–34	9.0 ± 1.5	−0.32
22–100	9	13.5	15.1 ± 1.0	−0.6
71–98[e]	4	26–29	11.8 ± 1.6	—
847–974[e]	4	23–27	11.5 ± 1.3	—

[a] Concentration in plasma water after 60-min equilibration with red blood cells of hematocrit, 100H. [b] Number of studies. [c] Correlation coefficient of p with D. [d] Regression equation: $D \pm 0.64 = -(41.0 \pm 7.4) \times 10^{-4}$p + 9.7 ± 0.4 where ± values are standard errors of the mean. [e] Buffer used in these two studies instead of plasma water.

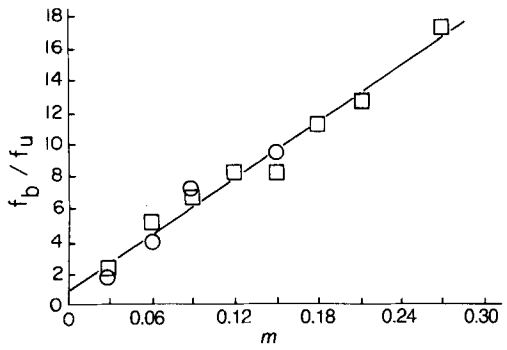


Figure 10—*Plot of the ratio of fraction bound, f_b, and unbound, f_u, to plasma protein of buprenorphine against the fraction of true plasma, m, in the pseudoplasma equilibrated with red blood cells. Key: (□) set 1; (○) set 2. The given line is the fitted regression equation to the set 2 data.*

were not possible. Buprenorphine concentration at pH 7.4 in dialysis decreased with time. When the pH was adjusted to 2 the original concentration was regained. This indicated that although the neutral species is bound to dialysis membranes, the protonated form is not. No drug was observed in the filtrate when 100 μg/mL of buprenorphine in physiological buffer was centrifuged through filter cones to show that neutral buprenorphine is highly bound to the filter cones.

The fraction of drug bound to plasma protein was determined by measuring drug concentration in plasma water after ultracentrifugation. The fraction bound, f_b, was computed from $(C_p - C_{pw})/C_p$ where C_p and C_{pw} are the buprenorphine nanogram/milliliter concentrations in the plasma and plasma water after ultracentrifugation, respectively. The C_p values were determined from assayed buffer to which the same amount of drug had been added as had been added to the same plasma volume. Values obtained in four studies were: [C_p(ng/mL), f], 132, 96.7; 196, 92.7; 260, 93.9; and 323, 94.8 for an average of 94.5 ± 1.7 (SD).

The method of determining protein binding by partitioning into red blood cells is based on the equilibria between drug in plasma water, red blood cells (RBC), and plasma protein where the fraction, f, of drug bound to plasma proteins can be calculated[33] from:

$$f_b = \frac{(1 - H)}{DH} + 1 - \frac{C_b}{DHC_p} \qquad (21)$$

where 100H is the hematocrit, D is the RBC/plasma water partition coefficient and C_b and C_p are the respective blood and plasma concentrations of buprenorphine. In one set of studies where C_p = 4.5, 4.0, 4.03, and 4.06 μg/mL, C_b = 3.3 μg/mL of blood, H = 0.335, and D = 9.4, the fraction bound, f, was estimated to be 0.95 ± 0.03(SD).

Also, two sets of protein binding studies at 0.323 (Set 1) and 3.23 μg (Set 2) of buprenorphine per milliliter of synthetic blood (erythrocytes + m fraction of normal plasma + buffer) were conducted by the red blood cell partition method. The fractions bound, f_b, were calculated from eq. 21 for each set using the individually observed hematocrit (range 27–30) and individually assayed concentrations in each of the equilibrated pseudoplasma samples (ranges: set 1, 158–315 ng/mL; set 2, 1880–3630 ng/mL). The partition coefficients, D, used and range of m fractions studied were, respectively: set 1 (n = 4), 11.8, 0.03–0.15 and set 2 (n = 8), 11.5, 0.03–0.27 where the partition coefficients had been determined between the same lot of red blood cells and buffer (Table IV).

It has been shown[25] for nonsaturable binding sites on plasma protein that:

$$f_b/f_u = mK[P]_T \qquad (22)$$

where f_b and f_u are fractions bound and unbound, respectively, m is the fraction of the normal concentrations of binding sites $[P]_T$ in the plasma (and thus the fraction of true plasma in the pseudoplasma), and K is the dissociation constant of each equivalent drug-bound protein-binding site. A plot of the ratio f_b/f_u against m should pass through the origin with a positive slope of $K[P]_T$.

The data in Fig. 10 are plotted at two different concentrations. The fact that the linear regression equations for both sets were not significantly different indicated that there was no significant concentration effect on protein binding. The linear-regression equation for set 2 was $f_b/f_u = (59.0 \pm 3.8)m + 0.83 \pm 0.60$ to indicate an intercept not significantly different from zero where the ± values are the calculated standard errors. As has also been shown previously:[25]

$$f_b = m[P]_T K f_u = m[P]_T K(1 - f_b)$$

$$= \frac{m[P]_T K}{1 + m[P]_T K} \qquad (23)$$

so that, in normal plasma where m = 1, the fraction of buprenorphine bound to plasma protein is estimated as 59/60 = 0.983. This relatively high plasma protein binding for the more lipophilic buprenorphine contrasts to the 26–36% plasma protein binding of morphine,[34] naloxone, and naltrexone.[35]

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