# Serum Levels of Butorphanol by Radioimmunoassay

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**Abstract** □ A radioimmunoassay was developed for the analysis of butorphanol in human serum. The procedure involves extraction of serum with hexane containing 2% isoamyl alcohol, followed by evaporative solvent removal and radioimmunoassay of the reconstituted residue. The antibody significantly cross-reacts with unidentified butorphanol metabolites but not with two known metabolites, hydroxybutorphanol and norbutorphanol. Extraction eliminated interference from the butorphanol metabolites normally present in serum. The antibody also cross-reacts with pentazocine and cyclazocine but not with morphine, hydromorphone, oxymorphine, codeine, methadone, and meperidine. Butorphanol tartrate was administered intravenously (2 mg) to normal male volunteers. Serum butorphanol levels declined biexponentially with an average terminal half-life of 2.7 hr. Enzymatic serum hydrolysis prior to extraction yielded additional butorphanol, indicating the presence of butorphanol conjugates. The specificity of the assay for butorphanol was confirmed by GLC–mass fragmentography.

**Keyphrases** □ Butorphanol—radioimmunoassay in human serum, pharmacokinetics in human serum □ Pharmacokinetics—butorphanol in human serum □ Radioimmunoassay—butorphanol in human serum □ Narcotic agonist–antagonist—serum butorphanol pharmacokinetics by radioimmunoassay

Butorphanol (I), a totally synthetic morphinan (1) with narcotic agonist–antagonist properties, has been shown to be a potent analgesic in animals (2) and humans (3–5). Studies with $^3$H-butorphanol in normal human subjects demonstrated the presence of hydroxybutorphanol (II), norbutorphanol (III), and conjugates of I, II, and III as metabolites of I[1]. Additional unidentified metabolites were present. The plasma I levels indicated that conventional GLC or high-pressure liquid chromatographic procedures could not achieve the detection level necessary to define fully the serum concentration profile after parenteral administration. A specific radioimmunoassay procedure was needed to determine the pharmacokinetics and bioavailability of I.

Rabbit antibutorphanol antibody was prepared *via* I conjugation to bovine serum albumin, followed by injection of this antigen into rabbits to obtain the antiserum[2]. Radiolabeled antigen was prepared by a chloramine-T procedure for the introduction of iodine 125 into the aromatic ring of I, and this hapten was bound by the antiserum and displaced by comparable amounts of pure I. The conventional second antibody (goat antirabbit globulin) technique was utilized to separate free from bound antigen. No significant cross-reactivity with the butorphanol metabolites II or III was observed. Cross-reactivity from unknown metabolites, however, necessitated the development of a technique specific for I, so an extraction procedure prior to radioimmunoassay was included.

A combined GLC–mass fragmentography assay was developed to demonstrate that the final method was specific for I in the presence of its metabolites.

The purposes of these studies were to develop and validate a radioimmunoassay method for I and to demonstrate the utility of the method in the determination of serum levels of I after administration of therapeutic doses.

## EXPERIMENTAL

**Chemicals and Reagents**—Na$^{125}$I (carrier free)[3], *N*-chloro-4-methylbenzenesulfonamide sodium salt[4] (chloramine-T), diethylamine[4], normal human serum[5], normal rabbit serum[5], goat antirabbit γ-globulin antiserum[6], 3,14-dihydroxy-*N*-cyclobutylmethylmorphinan hydrogen tartrate[7] (butorphanol tartrate, I-tartrate, lot 75F1355-2), 3,14-dihydroxy-*N*-(*trans*-3′-hydroxycyclobutylmethyl)morphinan hydrogen tartrate[7] (hydroxybutorphanol tartrate, II-tartrate, lot 1099-21), 3,14-dihydroxymorphinan[7] (norbutorphanol, III, lot 923-19), 3,14-dihydroxy-*N*-cyclopropylmethylmorphinan hydrogen tartrate[7] (internal standard, IV, lot 75F1878), *N,O*-bis(trimethylsilyl)trifluoroacetamide[8] (V), *N,O*-bis(perdeuterotrimethylsilyl)trifluoroacetamide[9] (VI), and rabbit antibutorphanol antiserum[2] were used as received.

All other chemicals and reagents were USP, NF, or ACS grade or better and were used without further purification.

**Radioiodinated Butorphanol Hapten**—To a 1-dram vial with a stirring bar were added, with stirring, 20 mCi of Na$^{125}$I (200 μl), 3.5 μl of a solution of 1.46 mg of I-tartrate (1 mg of base)/ml of distilled water, and 100 μl of 0.25 *M*, pH 7.6 phosphate buffer. Then 10 μl of a fresh solution of 3 mg of chloramine-T/ml was added, followed within 5–10 sec by 10 μl of a fresh solution of 7 mg of sodium metabisulfite/ml. Carbonate buffer (50 μl) and hexane (2 ml) were added, and stirring was continued for 10 min.

The aqueous phase was frozen on dry ice; the hexane phase was removed, washed once with 1 ml of distilled water, and aliquoted into individual vials after its activity was determined. The iodinated butorphanol was stored at −20°, and aliquots were reconstituted with radioimmunoassay buffer just prior to use. After 1–3 months, the stock preparation becomes unsatisfactory due to the accumulation of degradation products.

**Solutions**—The butorphanol standard solution was prepared to yield 145.8 pg of I-tartrate (100 pg of base)/ml; 1-ml amounts were transferred to 0.5-dram vials and stored at −20°. The radioimmunoassay buffer was 0.05 *M*, pH 7.6 phosphate buffer. It was prepared fresh for use. The carbonate buffer was a saturated solution of sodium bicarbonate and sodium carbonate. The extraction solvent was 2 parts (v/v) of isoamyl alcohol to 98 parts (v/v) of hexane. The protein solution consisted of 4 parts (v/v) of normal rabbit serum diluted with 96 parts (v/v) of radioimmunoassay buffer. It was prepared from frozen (−20°) serum aliquots and kept on ice[6].

Rabbit antibutorphanol antiserum was diluted in protein solution in the ratio of 1 μl to 5 ml, respectively. It was made fresh from frozen (−20°) antiserum aliquots and kept on ice. [Other lots of antibody than the one used may require a different dilution to obtain useful standard curves (7).] The goat antirabbit γ-globulin solution was prepared by diluting the antiserum in radioimmunoassay buffer to obtain about 13 units/ml, *e.g.*, 8 ml of antiserum plus 22 ml of buffer. It was made fresh from frozen (−20°) antiserum aliquots and kept on ice[8].

To prepare the radioiodinated butorphanol hapten solution, the ethanolic stock solution of $^{125}$I-butorphanol was diluted to obtain (*a*) about 180,000 cpm/ml or (*b*) about 100,000 cpm, depending on whether

[1] R. C. Gaver, R. D. Smyth, M. Vasiljev, and D. R. Van Harken, Bristol Laboratories, Syracuse, N.Y., unpublished results.

[2] R. F. Mayol, Mead-Johnson Research Center, Evansville, Ind., unpublished results.

[3] Amersham Corp., Arlington Heights, Ill.

[4] Eastman Kodak Co., Rochester, N.Y.

[5] Glusulase, containing β-glucuronidase and arylsulfatase, Grand Island Biological Co., Grand Island, N.Y.

[6] Calbiochem, East Rutherford, N.J.

[7] Bristol Laboratories, Syracuse, N.Y.

[8] Pierce Chemical Co., Rockford, Ill.

[9] Regis Chemical Co., Morton Grove, Ill.



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**Table I—Accuracy and Precision of Butorphanol Radioimmunoassay**

| Parameter | Added Butorphanol, pg | | | | | |
|---|---|---|---|---|---|---|
| | 25 | 150 | 300 | 100 | 300 | 600 |
| | "Low" Standard Curve (0–300 pg) | | | "High" Standard Curve (0–600 pg) | | |
| Number of curves | 8 | 8 | 8 | 12 | 13 | 13 |
| Number of replicates per curve | 3 | 3 | 3 | 3 | 3 | 3 |
| Total number of samples | 24 | 24 | 24 | 36 | 39 | 39 |
| Mean predicted value | 26 | 154 | 295 | 95 | 308 | 567 |
| Intraassay variability, %[a] | 14 | 10 | 15 | 11 | 8 | 12 |
| Interassay variability, %[b] | 10 | 6 | 10 | 8 | 6 | 9 |

[a] Mean coefficient of variation for triplicate samples. [b] Coefficient of variation for all samples (three times number of curves).

a high or low standard curve was prepared. It was made fresh and kept on ice. The counts per minute may need to be adjusted if a different $\gamma$-counter is used.

**Radioimmunoassay**—For a given analytical run, a constant volume of serum was taken from all samples, and the same volume of human serum was used for the standard curve, because of the variable effect of serum volume on nonspecific and control binding values. Volumes of human serum from 0.1 to 0.5 ml were suitable; 0.2 ml is used as an example.

Into sets of triplicate extraction tubes[10] were pipetted[11] 0.25, 0.50, 1.0, 1.5, 2.0, 2.5, and 3.0 $\mu$l of butorphanol standard solution. Three additional sets of tubes were reserved for background (BKG), nonspecific binding (NSB), and maximum binding ($B_0$). Human serum (0.2 ml) was pipetted[12] into all of the tubes, and duplicate 0.2-ml portions of the desired test samples were pipetted into additional tubes. Carbonate buffer (50 $\mu$l) and the extraction solvent (2 ml) were added; then the tubes were racked, and each rack was shaken gently by hand for 1 min. The racks were centrifuged[13] for 10 min at 3000 rpm at −5° and then placed in a shallow bath of dry ice–ethanol for about 3 min.

Each organic phase was decanted into a clean tube[14] and reduced to dryness, using a manifold[15] supplied with dry, filtered nitrogen gas. All subsequent solutions were added to the tubes with a diluter–pipeter[16] set to deliver 0.1 ml of reagent solution followed by 0.4 ml of radioimmunoassay buffer. The final volume in each tube was 1.5 ml. Solutions were kept in an ice bath when not in use. Radioiodinated hapten was added to all tubes except the background tubes, to which extra radioimmunoassay buffer was added. Antibutorphanol antiserum was added to all tubes except the nonspecific binding tubes, to which protein solution was added. Antirabbit serum antibody was added to all tubes.

The tubes were centrifuged at 3000 rpm for 3 min at −5° and stored at 5–7° for 16 hr (overnight). Free and bound haptens were separated by centrifugation at 3000 rpm for 30 min, and 1.0 ml of each supernate was transferred to fresh tubes. The $^{125}$I-activity was determined in a $\gamma$-counter[17] with automatic correction for background. Calculations were carried out automatically in the system by a modified log–logit analysis (6). In this procedure, the bound radioactivity for each sample or standard ($B$) was estimated, and the ratio:

$$y = \frac{B - \text{NSB}}{B_0 - \text{NSB}} \qquad \text{(Eq. 1)}$$

was calculated and transformed to:

$$\text{logit } y = \ln\left(\frac{y}{1-y}\right) = \ln\left(\frac{B - \text{NSB}}{B_0 - B}\right) \qquad \text{(Eq. 2)}$$

The slope and intercept were obtained by linear regression for logit $y$ *versus* ln $p$, where $p$ equals the amount of standard in each tube. The amount of I in each sample tube was estimated by standard inverse prediction techniques.

**Cross-Reactivity**—Fifty micrograms each of II, III, morphine sulfate, hydromorphone, oxymorphone hydrochloride, codeine, pentazocine, cyclazocine, methadone hydrochloride, and meperidine hydrochloride were dissolved in 500 ml of 0.01 *N* HCl. Amounts of each of these solutions were added to control human serum to give concentrations of 0.2, 2, and 20 ng/ml, and the serum samples were carried through the radioimmunoassay as unknowns to determine the relative ability of the compounds to displace radioiodinated hapten from the antibutorphanol antibody.

**Enzymatic Hydrolysis**—To 1.0-ml samples of spiked or test human plasma were added 25 $\mu$l of enzyme solution[18], 5 $\mu$l of chloroform, and 4.0 ml of 0.1 *M*, pH 5.5 acetate buffer. The samples were incubated at 37° for 72 hr under a nitrogen atmosphere, and portions were carried through the radioimmunoassay.

**GLC–Mass Fragmentography**—To 3-ml samples of spiked or test human plasma was added 0.3 $\mu$g of IV. The samples were made basic and extracted with chloroform. The chloroform was extracted with 0.1 *N* $H_2SO_4$, which, in turn, was made basic and extracted with chloroform, which was reduced to dryness under a nitrogen stream. Compound V (25 $\mu$l) was added to each sample, and the samples were agitated and held at room temperature for 30 min to form the trimethylsilyl derivatives of I and IV. To each sample was added 2 $\mu$l of a solution containing 25 ng of I/ml of VI. This addition of the perdeuterotrimethylsilyl derivative of I provided a carrier for the picogram amounts of the trimethylsilyl derivative of I so that it was not lost in the chromatographic system.

The instrument used was a gas chromatograph[19] coupled to a quadrupole mass spectrometer[20] equipped with a chemical-ionization source. Conditions were: carrier–reactant gas, methane; injection port temperature, 250°; oven temperature, 235°; transfer line temperature, 250°; ion chamber pressure, 0.5–1 torr; and electron beam intensity, 100 ev. The methane flow rate was adjusted to maximize the mass 29 ion in the methane spectrum, resulting in a carrier gas flow rate between 10 and 20 ml/min. Four microliters from each sample was injected into a 91-cm × 2.5-mm i.d. column containing 3% OV-1 on Gas Chrom Q[21].

The column effluent was scanned continuously in the single-ion monitoring mode using a cycle time of 1 sec. Three mass numbers were monitored in each cycle, corresponding to the pseudomolecular ions ($MH^+$) of the trimethylsilyl derivative of IV (386.4 amu), the trimethylsilyl derivative of I (400.4 amu), and the perdeuterotrimethylsilyl derivative of I (409.4 amu). The derivatives of I eluted at 3.5 min, and the derivative of IV eluted at 2.0 min. A response ratio of the trimethylsilyl derivative of I to the trimethylsilyl derivative of IV was calculated, and the regression of the ratio on the I concentration in the plasma standards was used to calculate the I concentration in the test samples by inverse prediction.

**Subjects**—A signed informed consent was obtained from each normal, healthy, adult male subject. Complete physical examinations, hematology, blood chemistries, and urinalysis were performed on each subject on the day prior to the 1st study day. All parameters were within the normal range.

In Study 1, each subject in one group received 2 mg of butorphanol tartrate intramuscularly whereas each subject in the other group received 16 mg of butorphanol tartrate orally. Subjects were fasted overnight prior to drug administration in the morning. Blood samples were obtained at 0, 2, 4, and 8 hr.

In Study 2, subjects received 2 mg of butorphanol tartrate intravenously. Blood samples were obtained 0.5 hr prior to drug administration and at 5, 10, 15, 20, 30, and 45 min and at 1, 1.25, 2, 3, 4, 6, and 8 hr. Subjects abstained from solid food and liquid, except water, from midnight of the beginning of the test day. Subjects were instructed to walk around for at least 5 min after each blood collection. They received a light breakfast 4 hr after dose administration and were discharged after the

[10] Screw-capped glass tubes (13 × 100 mm) with Teflon-lined caps (catalog No. 99447), Corning Glass, Corning, N.Y.
[11] With a 2-$\mu$l syringe, Hamilton Co., Reno, Nev.
[12] MLA micropipets, Fisher Scientific Co., Pittsburgh, Pa.
[13] Model PR-6000 refrigerated centrifuge equipped with IEC No. 418 radioimmunoassay head, Damon/IEC Division, Needham Heights, Mass.
[14] Catalog No. 2053 12 × 75-mm polypropylene tubes, Falcon Plastic Co.
[15] Evap-O-Rac, Cole-Parmer Instrument Co., Chicago, Ill.
[16] Model 25004 equipped with a 200-$\mu$l sampling pump and a 1-ml dispensing pump, Micromedic Systems, Horsham, Pa.
[17] PACE-1 $\gamma$-counter with PAC-5 programmable automatic calculator, Picker Corp., Northford, Conn.
[18] Endo Laboratories, Garden City, N.J.
[19] Model 2500, Finnegan Corp., Sunnyvale, Calif.
[20] Model 3300, Finnegan Corp., Sunnyvale, Calif.
[21] Applied Science Laboratories, State College, Pa.

**Table II—Butorphanol Concentrations as Determined by the Radioimmunoassay in Human Serum after Oral (16 mg) or Intramuscular (2 mg) Administration of Butorphanol Tartrate**

| Route of Administration | Subject | Butorphanol, ng/ml 0 hr | 2 hr | 4 hr | 8 hr |
|---|---|---|---|---|---|
| Oral | 1 | 0.14 | 0.80 | 0.82 | 0.36 |
| | 2 | 0.11 | 0.31 | 0.24 | 0.12 |
| | 3 | 0.12 | 2.68 | 2.99 | 1.51 |
| | 4 | 0 | 0.35 | 0.42 | 0.14 |
| Intramuscular | 5 | 0 | 1.46 | 0.96 | 0.21 |
| | 6 | 0 | 1.38 | 0.90 | 0.29 |
| | 7 | 0.10 | 1.13 | 0.63 | 0.10 |
| | 8 | 0 | 1.30 | 0.66 | 0.21 |

**Table III—Butorphanol Concentrations as Determined by GLC–Mass Fragmentography in Human Serum after Oral (16 mg) or Intramuscular (2 mg) Administration of Butorphanol Tartrate**

| Route of Administration | Subject | Butorphanol, ng/ml 0 hr | 2 hr | 4 hr | 8 hr |
|---|---|---|---|---|---|
| Oral | 1 | 0 | 0.32 | 0.87 | 0.09 |
| | 2 | 0 | 0.07 | 0 | 0 |
| | 3 | 0 | 2.97 | 2.27 | 0.74 |
| | 4 | 0 | 0.08 | 0 | 0 |
| Intramuscular | 5 | 0.07 | 1.63 | 0.98 | — |
| | 6 | 0.56 | 1.69 | 1.22 | 0.44 |
| | 7 | 0 | 1.04 | 0.47 | 0.18 |
| | 8 | 0.03 | 1.27 | 0.74 | 0 |

last blood sample was obtained.

Blood samples were obtained in plain evacuated tubes, allowed to clot at room temperature for 30 min, and centrifuged at 5°. Serum samples were removed and immediately flash frozen, stored, and shipped frozen; they were maintained frozen at −20° until analyzed.

**Pharmacokinetic Analysis**—The serum concentration data for each subject in Study 2 were plotted on semilog paper with time as the abscissa. Judgments then were made as to the nature and trend of the time course of serum concentration; also at this time, certain data points were judged to be outliers if they deviated widely (by ~25%) from the general trend. With the outliers excluded, all of the data sets were run through the program CSTRIP (7) to obtain initial polyexponential parameter estimates. These estimates were then passed, along with their associated data sets, to the program GUESS (8) for parameter optimization by a nonlinear technique. The optimized polyexponential parameters then were used to calculate pharmacokinetic parameters.

## RESULTS AND DISCUSSION

Preliminary experiments[22] showed that increasing amounts of human serum or of the residue of hexane extracts of human serum increasingly reduced the binding of radioiodinated hapten to the antibody. For a given serum volume, there was little variability in the reduction of this binding; for serum volumes of ≤0.5 ml, the effect was not of sufficient magnitude to interfere with the usefulness of the radioimmunoassay. Data on the accuracy and precision of the radioimmunoassay are presented in Table I. The data were obtained from the same standard curves used to estimate butorphanol concentrations in the discussed serum samples. As expected, the best precision was obtained for samples near the center of the range of the standard curve.

The potential interference of known[1] metabolites of butorphanol (II and III) and of various narcotics and agonist–antagonists (see *Experimental*) was tested at levels up to 20 ng/ml. Only pentazocine and cyclazocine cross-reacted; they gave apparent butorphanol values of 0.1–0.2 ng/ml at their highest level of 20 ng/ml. Therefore, misleading values for butorphanol may be observed in any subject who may have received large doses of pentazocine within 24 hr of assaying for butorphanol by the radioimmunoassay.

The plasma I concentrations as determined by the radioimmunoassay in human subjects after intramuscular and oral dosing (Table II) and those determined by GLC–mass fragmentography (Table III) were compared by least-squares analysis, assuming a linear relationship between the two analytical methods. The slope, $y$-intercept, and regression coefficient were calculated, and the null hypotheses that the slope was equal to unity and that the $y$-intercept was equal to zero were tested. The results (Table IV) indicate that the methods are equivalent and that the radioimmunoassay is specific for butorphanol in the presence of its metabolites.

**Table IV—Correlation of Radioimmunoassay and GLC–Mass Fragmentography Methods for Determination of Serum Butorphanol [a]**

| Sample Source ($n$) | Slope ± 95% Confidence Interval | Intercept ± 95% Confidence Interval | Correlation Coefficient |
|---|---|---|---|
| Oral (16) | 0.98 ± 0.18 | 0.24[b] ± 0.05 | 0.96 |
| Intramuscular (15) | 0.87 ± 0.19 | 0.01 ± 0.08 | 0.94 |
| Oral plus intramuscular (31) | 0.92 ± 0.14 | 0.13[b] ± 0.04 | 0.93 |

[a] Each value obtained by GLC–mass fragmentography was used as an independent variable ($x$ values), and the corresponding value obtained by radioimmunoassay was used as the dependent variable ($y$ value). [b] Significantly different than 0 ($p < 0.05$).

The slight, but significant, intercept in the radioimmunoassay results on subjects given I orally could indicate that the radioimmunoassay was measuring an immunoreactive moiety other than butorphanol, but one that was present in the serum in a constant amount. Or it could indicate that there was a slight positive bias in the radioimmunoassay for the analyses run on that day. Subsequent experience with many other subjects given oral butorphanol suggests that the latter explanation is

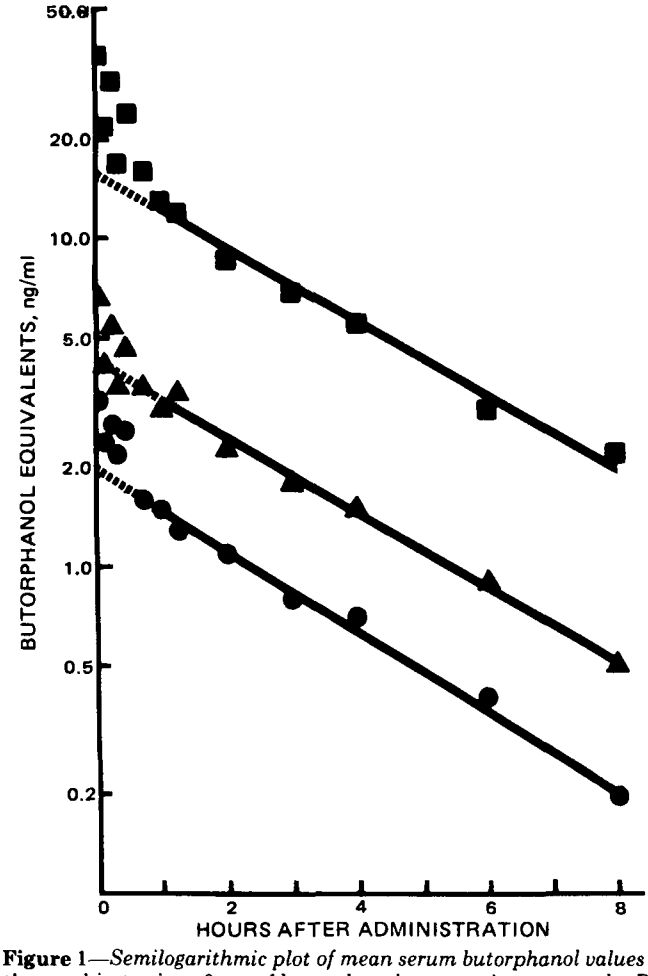


**Figure 1**—*Semilogarithmic plot of mean serum butorphanol values in three subjects given 2 mg of butorphanol tartrate intravenously. Butorphanol was determined by a complete radioimmunoassay (●) involving an extraction to separate butorphanol from its metabolites, by radioimmunoassay directly on serum (▲), and by radioimmunoassay after enzymatic hydrolysis to release butorphanol from conjugates (■), followed by extraction. Least-squares lines (solid and dashed) were calculated for log serum value* versus *time from 1 to 8 hr after dosing.*

[22] K. A. Pittman, unpublished data.

Table V—Serum Butorphanol in Subjects Given 2 mg of Butorphanol Tartrate Intravenously [a]

| Hours after Administration | Butorphanol Equivalents, ng/ml | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Subject 1 | | | Subject 2 | | | Subject 3 | | |
| | Complete RIA | Direct RIA | Hydrolysis RIA | Complete RIA | Direct RIA | Hydrolysis RIA | Complete RIA | Direct RIA | Hydrolysis RIA |
| 0 | 0.1 | 0.3 | 1.2 | 0 | 0.4 | 1.2 | 0 | 0.3 | 1.3 |
| 0.08 | 2.4 | 4.9 | 31 | 3.6 | 9.6 | 40 | 3.6 | 5.2 | 38 |
| 0.17 | 1.6 | 2.6 | 13 | 3.0 | 5.5 | 29 | 2.5 | 4.3 | 24 |
| 0.25 | 2.7 | 4.9 | 29 | 2.5 | 5.9 | 30 | 2.9 | 5.5 | 30 |
| 0.33 | 1.7 | 2.5 | 13 | 3.0 | 4.6 | 20 | 2.0 | 3.5 | 19 |
| 0.50 | 1.5 | 3.1 | 18 | 3.2 | 5.8 | 24 | 3.2 | 4.9 | 29 |
| 0.75 | 1.2 | 1.9 | 10 | 1.9 | 5.4 | 22 | 1.6 | 3.3 | 16 |
| 1.0 | 1.1 | 2.3 | 9.7 | 1.7 | 3.3 | 16 | 1.7 | 3.3 | 12 |
| 1.25 | 1.0 | 1.9 | 7.9 | 1.6 | 4.2 | 8.4 | 1.4 | 4.0 | 21 |
| 2.0 | 0.9 | 1.5 | 6.0 | 1.2 | 3.1 | 10 | 1.1 | 2.3 | 9.7 |
| 3.0 | 0.6 | 1.2 | 4.8 | 0.9 | 2.1 | 7.3 | 1.0 | 2.1 | 8.5 |
| 4.0 | 0.4 | 0.9 | 3.2 | 0.7 | 1.7 | 5.4 | 1.0 | 2.0 | 7.9 |
| 6.0 | 0.2 | 0.4 | 1.9 | 0.5 | 1.3 | 3.6 | 0.4 | 1.0 | 3.5 |
| 8.0 | 0.1 | 0.3 | 1.8 | 0.2 | 0.8 | 2.4 | 0.2 | 0.5 | 2.3 |

[a] Determinations were made by a complete radioimmunoassay (RIA) involving an extraction to remove butorphanol from its metabolites, by radioimmunoassay directly on serum, and by radioimmunoassay after enzymatic hydrolysis of conjugates and extraction of butorphanol. See *Experimental* for details.

correct. Regression, using the log–logit transformation of Hatch *et al.* (6), often leads to an overestimate of very low concentrations, especially those below the lowest standard. Particular caution must be taken when estimating values between zero and the low standard with this procedure.

Serum samples for three subjects who received intravenous butorphanol were analyzed directly by the radioimmunoassay by omitting the extraction procedure. The values obtained (Table V) for serum butorphanol were 1.5–3 times higher (average 2.1 ± *SE*) than the values obtained (Table V) by including hexane extraction in the radioimmunoassay. The same samples also were analyzed by the radioimmunoassay after being subjected to enzymes ($\beta$-glucuronidase and arylsulfatase), which hydrolyze common conjugates of phenolic drugs. The values obtained (Table V) were 5–15 times higher (average 9.5 ± *SE* = 0.4) than those obtained without enzymatic hydrolysis.

Semilog plots of the mean serum butorphanol concentrations for those three subjects as determined by the complete radioimmunoassay, the radioimmunoassay without the extraction step, and the complete radioimmunoassay preceded by enzymatic hydrolysis are shown in Fig. 1. Lines of best fit were calculated for each set of data from 1 to 8 hr after dose administration. The slopes were not significantly different, suggesting that butorphanol elimination may be rate limiting in the clearance of it and its metabolites from the body.

When the data for each of the 18 subjects given butorphanol intravenously (Study 2) were plotted as log concentration *versus* time, log serum concentration in most subjects appeared to decline biexponentially, showing a rapid distributive phase ($\alpha$-phase) and a slower postdistributive phase ($\beta$-phase). When the data for each subject were analyzed using CSTRIP, the decline in serum concentration was biexponential for 17 of the data sets and monoexponential for one in which the distributive phase was missed entirely. The equation used for biexponential decline was:

$$C_s = Ae^{-\alpha t} + Be^{-\beta t} \quad \text{(Eq. 3)}$$

where $C_s$ is the serum concentration in nanograms per milliliter and $A$ and $B$ are the $y$-intercepts and $\alpha$ and $\beta$ are the slopes of the fastest declining and the slowest declining exponentials, respectively; $A$ and $B$ are in nanograms per milliliter, and $\alpha$ and $\beta$ are in reciprocal hours. Similarly, the equation used for the monoexponential decline observed for one subject was:

$$C_s = Be^{-\beta t} \quad \text{(Eq. 4)}$$

where $B$ and $\beta$ describe the same exponential ($\beta$-phase) as in Eq. 3.

The initial estimates for $A$, $B$, $\alpha$, and $\beta$ otained by CSTRIP were optimized to obtain a curve of best fit by GUESS. The average values and their standard errors for $\alpha$, $\beta$, and the serum half-life (0.693/$\beta$) were 7.7 ± 2.5 $hr^{-1}$, 0.262 ± 0.009 $hr^{-1}$, and 2.70 ± 0.09 hr, respectively. As the standard errors indicate, the values for $\alpha$ were quite variable from subject to subject while those for $\beta$ were consistent. Pharmacokinetic studies of narcotics and other narcotic agonists and antagonists have revealed polyexponential declines in plasma concentration following intravenous administration. Meperidine (9, 10), naloxone (11), and pentazocine (12, 13), for example, declined exponentially while fentanyl (14) and morphine (15, 16) declined triexponentially. Terminal plasma or serum half-lives for codeine (17), meperidine (9, 10), morphine (15, 16), and pentazocine (12, 13, 18) fell in the 2–4-hr range, as did butorphanol. In pharmacokinetic terms, the behavior of butorphanol after intravenous dosing appears to be typical of the narcotic and narcotic agonist–antagonist drugs.

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