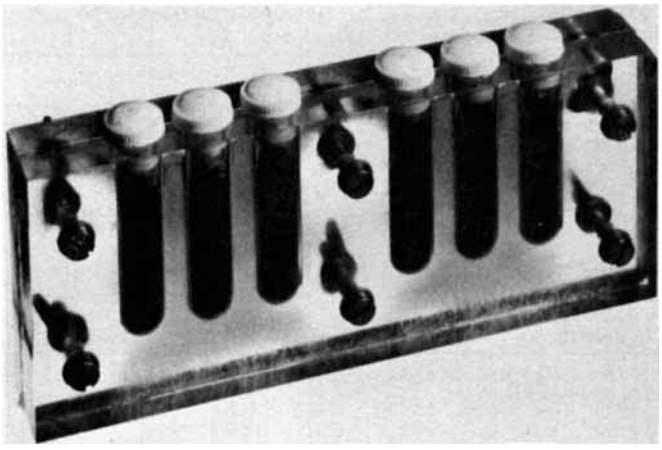

Figure 1—*Equilibrium dialysis units. (Reprinted with permission of the editors of Biochemical Society Transactions).*

Krieglstein *et al.* (11), who investigated the binding of 10 phenothiazines to bovine serum albumin, and Deutsch and Hansch (12), who studied barbiturate binding to the same protein. In each case, log (bound/free) was plotted; negative intercepts of 0.628, 0.578, and 1.22 were obtained, respectively. Penicillins, phenothiazines, and barbiturates apparently do not have as marked a binding site on albumin as do clorobiocins.

The difference in log P of 1.06 between VI and clorobiocin, which differ only by a chlorine atom, is only partially explained by the π value for aromatic chlorine of 0.71 (7), whereas the hydrophobic fragment constant of 0.92 (13) approaches it much more closely. Nevertheless, it seemed important to develop a mathematical relationship based on experimental results rather than on theoretical data for those complex molecules having a great deal of conformational interaction.

Salicylate binding to whole plasma gave a value of −1.32 for log P and 94% drug bound at a concentration of 13.8 μg/ml (6), a result that clearly does not fit the correlation obtained in this work. Other compounds with structures unrelated to either salicylate or clorobiocin also did not fit the correlation, which suggests that they might be binding at a different protein site. Sudlow *et al.* (14) reported that more than one binding site exists on human serum albumin for foreign compounds. The correlation obtained in this study applies to the clorobiocin derivative series. It also may apply to any structure that binds at the same site.

Use of the equilibrium dialysis units has facilitated accurate and reproducible work, which is difficult to carry out by dialysis in a large number of tubing bags. The alternative is to purchase an expensive instrument. The units described here combine the accuracy of the instrument with little more than the cost of the dialysis tubing. Used with the two computer programs, they estimate the binding to plasma albumin of drugs that are tightly bound and are not available in radioactive form. In addition, these units can be used to measure the binding of radioactive drug to whole plasma (6) and, potentially, the binding of any ligand to a macromolecule.

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Pharmacokinetics of Subcutaneous and Intramuscular Butorphanol in Dogs

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Abstract □ Butorphanol tartrate was administered intramuscularly and subcutaneously to adult male and female dogs at a dose of 0.25 mg/kg. No significant absorption lag time and no significant difference between peak intramuscular and subcutaneous serum concentrations were observed. The mean peak serum concentration was 29 ng/ml at mean times of 28 min after subcutaneous administration and 40 min after intramuscular administration. There were no significant differences in the pharmacokinetics of butorphanol in dogs with either route. The serum half-life was 1.62 hr, and the serum clearance was 3.45 liters/kg/hr. The apparent volume of distribution of butorphanol was 7.96 liters/kg. Although considerable inter- and intraanimal variation in C_{max} and AUC was observed, there was no significant difference in the area under the serum concentration *versus* time curves, and the two administration routes were considered bioequivalent.

Keyphrases □ Butorphanol—pharmacokinetics following subcutaneous and intramuscular administration, dogs □ Pharmacokinetics—butorphanol, subcutaneous and intramuscular administration, dogs □ Analgesics—butorphanol, pharmacokinetics following subcutaneous and intramuscular administration, dogs

Butorphanol tartrate [17-(cyclobutylmethyl)morphinan-3,14-diol tartrate], a new narcotic agonist–antagonist analgesic, is available[1] for use in humans and dogs (1). Previous studies indicated that loss processes account for a decrease in the extent of drug availability from extravascular parenteral injection sites (2). Deposition (3) and degradation (4) of the drug at the injection site can decrease both the rate and extent of absorption. The injection

[1] Stadol, Bristol Laboratories, Syracuse, N.Y.

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site also was demonstrated to be a critical factor in absorption rates due to differences in blood flow (5).

The present study was undertaken to examine and compare the pharmacokinetics and disposition of parenteral butorphanol following intramuscular and subcutaneous administration to dogs and to determine whether any observable differences were attributable to differences in the administration route.

EXPERIMENTAL

Animals—Six healthy adult beagles [three males (10.3 ± 1.8 kg) and three females (11.9 ± 1.9 kg)] were selected. The study was a complete, two-way crossover comparison of intramuscular and subcutaneous butorphanol tartrate at a nominal dose of 0.25 mg/kg (as butorphanol free base equi alent). There was a 2-week recovery period between successive doses.

Treatment—The intramuscular formulation was a solution of 5 mg of butorphanol base (7.29 mg of butorphanol tartrate)/ml of sterile water. The solutions were prepared immediately before administration. The dosing solution was administered into the caudal thigh muscle at a dose of 0.25 mg (0.05 ml)/kg. The subcutaneous formulation contained 1.38 mg of butorphanol base (2.01 mg of butorphanol tartrate)/ml of vehicle. The vehicle formulation was 3.300 g of citric acid USP, 6.400 g of sodium citrate USP, 6.412 g of analytical reagent grade sodium chloride, and water for injection USP to bring the solution to 1 liter. Butorphanol tartrate was stable in this formulation for a minimum of 1 year. Subcutaneous administration was made into the loose skin between the shoulder blades at a dose of 0.25 mg (0.18 ml)/kg.

The dogs had access to food overnight. A 10-ml/kg oral waterload was administered just prior to dosing. A continuous infusion of sterile 0.9% saline solution (25 ml/hr) was maintained for the first 6 hr of the study. The purpose of this waterloading was to eliminate the degree of hydration of individual animals as a variable since the volumes of distribution of solutes can vary with the degree of hydration (6).

Blood samples were collected from a catheterized saphenous vein immediately before the dose (zero time) and at 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 5, and 7 hr after injection. At least 4 ml of serum was prepared from each blood sample and frozen at −20° until it was assayed within 3 weeks of collection. Butorphanol was stable in frozen dog serum for a minimum of 5 weeks.

Serum Butorphanol Assay—All reagents were USP, NF, or ACS grade or better and were used without further purification.

Twenty-five microliters of a 10-ng/μl solution of codeine phosphate, the internal standard, in methanol was added to 3.0 ml of serum. The serum was alkalinized by the addition of 0.1 ml of 5.0 *N* NaOH and shaken[2] for 15 min with 10 ml of chloroform. The mixture was centrifuged[3] for 10 min at 3000 rpm to separate the aqueous and chloroform phases. The chloroform layer was transferred to a clean extraction tube, 4 ml of 0.1 *N* H_2SO_4 was added, and the mixture was shaken for 15 min. The phases then were separated by centrifugation at 3000 rpm for 10 min.

The aqueous acid layer then was transferred to a clean extraction tube and made basic with 0.2 ml of 5 *N* NaOH. Five milliliters of chloroform was added, and the mixture was shaken for 15 min. The chloroform extract was evaporated to dryness under a gentle stream of air[4], the dried residue was dissolved in 95 μl of acetonitrile, and 5 μl of heptafluorobutyric anhydride[5] was added to the acetonitrile solution. After incubation for 10 min at room temperature, the solvent and reagent were evaporated under a gentle stream of air, and the dried residue was reconstituted with 40 μl of acetonitrile.

A 1-μl portion of the final acetonitrile solution was injected[6] into a gas chromatograph[7] equipped with a scandium tritide electron-capture detector operated at 265° with a standing current of 3.2×10^{-8} amp and a nitrogen flow rate of 30 ml/min. The chromatographic column was 3% SE-30 on 100–200-mesh Gas Chrom Q in a 30.5-cm × 4-mm i.d. glass U tube[8]. The retention times of butorphanol and codeine were 6.6 and 3.7 min, respectively.

[2] Roto-Rack model 340, Fisher Scientific Co., Pittsburgh, Pa.
[3] Model UV, International Equipment Co., Needham Heights, Mass.
[4] Evap-O-Rac, Cole Parmer Co., Chicago, Ill.
[5] ICN Pharmaceuticals, Plainview, N.Y.
[6] Microliter syringe, Hamilton, Reno, Nev.
[7] Model 2100, Varian Associates, Palo Alto, Calif.
[8] Applied Science Laboratories, State College, Pa.

Table I—Pharmacokinetic Parameters for Intramuscular and Subcutaneous Butorphanol in Dogs

Parameter	Intramuscular[a]	Subcutaneous[a]	Mean Difference[b]
C_{max}, ng/ml	25.1 ± 6.7	33.3 ± 16.9	8.2 ± 11.7
t_{max}, hr	0.7 ± 0.3	0.5 ± 0.3	−0.2 ± 0.2
AUC, (ng hr)/ml	67.7 ± 16.2	81.7 ± 34.3	14 ± 27.4
$t_{1/2}$, hr	1.53 ± 0.24	1.71 ± 0.40	0.18 ± 0.50
K_a, hr^{-1}	6.12 ± 3.79	7.30 ± 4.73	1.18 ± 2.14
K_{el}, hr^{-1}	0.466 ± 0.092	0.424 ± 0.094	−.042 ± 0.134
C_0, ng/ml	31.5 ± 9.3	34.0 ± 4.7	2.5 ± 10.3
t_0, min	1.3 ± 2.6	−3.8 ± 9.1	5.1 ± 5.4
V, liters/kg	7.51 ± 2.42	8.42 ± 3.08	0.91 ± 1.71
Cl_s, liters/hr/kg	3.41 ± 0.87	3.50 ± 1.32	0.09 ± 1.13

[a] Mean ± *SD*. [b] Mean difference between subcutaneous and intramuscular routes ±95% confidence limit.

A standard curve was prepared by plotting the peak area ratio, the area under the butorphanol peak divided by the area under the codeine peak, *versus* the concentration of butorphanol (as free base equivalent) in spiked serum standards.

Pharmacokinetic and Statistical Analysis—The serum butorphanol *versus* time data for the individual dogs conformed to the linear, open, one-compartment model with first-order drug absorption. The data are described by:

$$C = [K_a C_0/(K_a - K_{el})][\exp(-K_{el}t') - \exp(-K_a t')] \quad \text{(Eq. 1)}$$

where C is the serum concentration; K_a is the first-order absorption rate constant; K_{el} is the first-order elimination rate constant; $C_0 = D/V$, in which D is the dose and V is the volume of distribution; and $t' = t - t_0$, in which t is the time after drug injection and t_0 is the absorption lag time.

The area under the serum concentration *versus* time curve from $t' = 0$ to ∞ (AUC) was found by integrating Eq. 1 between these time limits and simplifying:

$$AUC = C_0/K_{el} \quad \text{(Eq. 2)}$$

The volume of distribution was $V = D/C_0$, and the serum clearance (Cl_s) was $Cl_s = VK_{el} = D/AUC$.

The serum half-life ($t_{1/2}$) was $t_{1/2} = 0.693/K_{el}$. The observed peak serum concentrations, C_{max}, and the time from the dosage to the peak concentration, t_{max}, also were tabulated and analyzed.

The individual serum concentration *versus* time data were optimized and fitted to Eq. 1 by nonlinear regression analysis. The digital computer program AUTOAN, which incorporates Hartley's modification of the Gauss–Newton method, was employed (7, 8).

The values for C_{max}, t_{max}, AUC, $t_{1/2}$, K_a, C_0, Cl_s, and t_0 for the intramuscular and subcutaneous dosing routes were statistically compared using an analysis of variance suitable for a complete, two-way crossover experimental design (9). The K_{el} value was not tested since $t_{1/2}$ was equivalent. The analysis of variance was used to determine whether there were significant differences, at the 5% level, between these parameters on the basis of study periods, interanimal variation, and differences in administration route. The 95% confidence limits for differences in administration routes were calculated using the error term from the analysis of variance and the standard formula (10).

RESULTS

Assay—The linear relationship between butorphanol concentrations and peak area ratios was highly significant ($p < 0.01$) by linear regression analysis. The lower limit of quantitative detectability was equivalent to a serum butorphanol concentration of 0.9 ng/ml. The linear range of the assay was 2–50 ng of butorphanol/ml of serum. The accuracy of the assay with serum standards at a concentration of 15 ng/ml was 97%; there was no significant difference ($p > 0.05$) between the observed and actual concentrations of serum butorphanol as determined by an independent t test.

The reproducibility of the assay was confirmed by replicate analyses of 15 serum samples in the range of 0–37 ng of butorphanol/ml. The mean absolute difference between replicates was 2 ng/ml with a standard deviation of 1.7 ng/ml. There was no significant difference ($p > 0.05$) between the replicate assays as determined by an independent t test.

Serum Butorphanol Concentration—The mean serum concentrations of butorphanol after intramuscular and subcutaneous adminis-

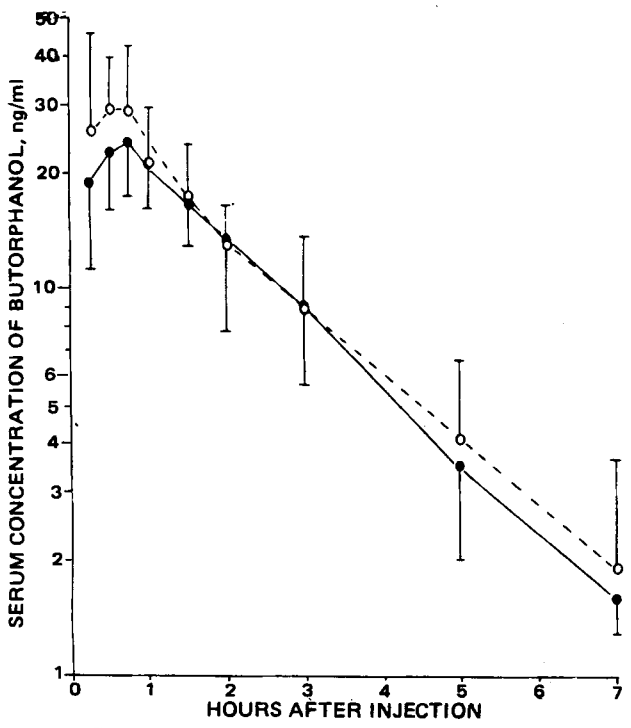

Figure 1—*Serum concentrations of butorphanol in dogs following intramuscular (●) and subcutaneous (○) administration. Mean values from six dogs with standard deviations (vertical bars) are given.*

tration are presented in Fig. 1. The mean pharmacokinetic parameters for intramuscular and subcutaneous administration for male and female dogs are listed in Table I. An analysis of variance indicated that there were no significant differences ($p > 0.05$) between the values of the pharmacokinetic parameters for different administration routes, dosing periods, and animals. There was significant interanimal variability ($p < 0.05$) in the absorption rate constant.

However, inspection of the 95% confidence limits of the mean difference between administration routes indicated considerable variability. This variability was due to both inter- and intraanimal variation. Determination of the bioequivalence of the two routes of administration by comparison of C_{max} and *AUC* within the individual animal demonstrated 85 and 97% relative bioavailability of the intramuscular dose as compared to the subcutaneous dose, respectively, with a coefficient of variation of 35% in both parameters. Although large differences could go undetected in the analysis of variance, the observed differences in the administration route in this study have no clinical, pharmacological, or toxicological significance (1).

DISCUSSION

The extent and time course of drug action can be affected significantly by the route of drug administration (2). There will be an initial lag between injection and the entrance of the drug into the circulation following extravascular parenteral administration. If drug partitioning from subcutaneous tissue and muscle to blood is similar, then the clearance of the drug from the injection site will depend primarily on blood flow. Since butorphanol is a basic (pKa 8.6), moderately lipophilic compound, differences in partitioning between the more lipophilic subcutaneous compartment and the intramuscular compartment could occur. The present studies indicated there were no significant differences in absorption, disposition, or clearance of butorphanol from these extravascular injection sites.

The absorption lag time for butorphanol tartrate in dogs on subcutaneous and intramuscular administration was -1.2 ± 6.9 min, indicating that absorption started immediately. The mean absorption half-times were 5.7 min for subcutaneous administration and 6.8 min for intramuscular administration. There should be a rapid onset of the effect of butorphanol in dogs following parenteral extravascular administration.

The disposition of butorphanol in dogs proceeded independently of the administration route. Although significant inter- and intraanimal variability was observed, the lack of a statistical or biologically significant difference between the *AUC* values indicated that the two routes were bioequivalent. The overall mean serum half-life of butorphanol was 1.62 hr, and the overall mean serum clearance was 3.45 liters/kg/hr. The mean volume of distribution of butorphanol was 7.96 liters/kg. When the apparent volume of distribution exceeds the true body weight in this manner, it usually is interpreted as indicating that the drug is distributed extensively to tissues.

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