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Pharmacokinetics of Morphine and Its Surrogates III: Morphine and Morphine 3-Monoglucuronide Pharmacokinetics in the Dog as a Function of Dose

EDWARD R. GARRETT [x] and ANDRÉ J. JACKSON [*]

Abstract □ The pharmacokinetics of morphine and its derived metabolite, morphine 3-monoglucuronide, were studied in normal and bile-cannulated dogs. High doses (7.2–7.7 mg/kg iv) caused renal and biliary shutdowns and time lags in urinary drug and metabolite excretion and in biliary secretion of the hepatically formed conjugate. Intermediate doses (0.41–0.47 mg/kg iv) inhibited urine flow but not renal clearance. Low doses (0.019–0.07 mg/kg iv) had no apparent effect. Dose-related effects on the total, metabolic, and biliary clearances imply saturable enzymes and/or dose-inhibited hepatic flows, accounting for the major elimination half-lives of 83 ± 8 and 37 ± 13 min at the high and low doses, respectively. The slow terminal phase in plasma morphine and metabolite elimination and urinary accumulation is due apparently to the entero-hepatic metabolite recirculation after biliary excretion, gastrointestinal hydrolysis, and hepatic first-pass reconjugation. Bile-cannulated dogs showed no fecal drug and no slow terminal plasma and urine elimination phases. Intravenous morphine 3-monoglucuronide was eliminated only renally and showed neither biliary excretion nor prolonged hepatically formed glucuronide elimination. Hepatic morphine clearances at normal therapeutic doses parallel hepatic blood flow and explain the lack of oral morphine bioavailability by anticipating complete first-pass liver metabolism. Renal morphine and morphine conjugate clearances were 85 (± 9 SEM) and 41 (± 4 SEM) ml/min, respectively, indicating glomerular filtration for the latter and glomerular filtration plus tubular secretion for the former. Urinary morphine and morphine conjugate excretion accounted for ~83% of the dose. Biliary secretion accounted for 11–14% of the dose. Morphine showed dose-independent plasma protein binding of 36 (± 1 SEM) % and a red cell–plasma water partition coefficient of 1.11 ± 0.04 SD. New equations were developed to model the discontinuous morphine and morphine metabolite pharmacokinetics.

Keyphrases □ Morphine—pharmacokinetics, dose related, dogs □ Morphine monoglucuronide—pharmacokinetics, dose related, dogs □ Pharmacokinetics—morphine, morphine monoglucuronide, dose related, dogs

Early studies on the distribution and disposition of morphine were largely descriptive (1–6). Rigorous pharmacokinetic studies were limited and usually only related to the parent compound at high doses. Early time course studies (7–10) in the dog were conducted at large time intervals and at high 30-mg/kg levels (human therapeutic dose is 0.14 mg/kg) due to color complex assay sensitivity limitations (10). Early morphine studies (9) estimated that 78–97% of the administered morphine was recoverable in urinary and fecal excretions, 14% as free morphine and 55% as presumed glucuronide conjugate in the urine, and 6–26% in the feces with less than 2% of the total drug as the conjugate. The plasma [N-^{14}C-$methyl$]morphine half-life was estimated at ~1 hr (11).

BACKGROUND

A fluorometric method with a sensitivity of 1 µg/ml of plasma was used by Kupferberg et al. (12) in a single rabbit study of six time samples at 15 mg/kg to demonstrate apparent biphasic morphine decay in plasma with a terminal half-life that could be roughly estimated as 1 hr.

Only in the last decade has the morphine time course been monitored with sufficient attention to warrant any pharmacokinetic data analysis.

A highly sensitive GLC method was used to monitor the time course of morphine at 2.5 mg/kg iv in the rat (13). At least three exponentials were needed to fit the plasma level–time curve.

Radioimmunoassay was used to monitor morphine, 0.14 mg/kg iv, in human plasma (14, 15), and the results indicated an apparent triphasic loss that included a rapid initial decline during the first 5–10 min, a subsequent slower but precipitous decline with a half-life of 1.0–3.1 hr, and a terminal slow disappearance of 10–44 hr at plasma levels of <10 ng/ml. A separate study (16) at 1 mg/kg infused over 12 min could only demonstrate a two-compartment body model when conducted for 2 hr after the completion of the infusion. Total clearance was 378 ± 63 ml/min. The apparent half-lives were similar to those of the previous studies; mean values were 1 ± 1 and 137 ± 14 min, with the latter phase ranging from 1.3 to 3.5 hr. These half-lives were similar to those for the lower 0.14-mg/kg dose and suggested that the assumption of a saturable or capacity-limited process for morphine was not warranted. Since different subjects were used for the different doses, this concept cannot be rigorously concluded. Mean apparent volumes of distribution of the central compartment and the total apparent distribution space were 9 and 102% of body weight, respectively.

Radiolabeled morphine at 0.14 mg/kg in humans (17, 18) showed an initially fast distribution phase and an apparent terminal half-life of 2.1–2.6 hr. The sensitivity of 20–30 ng/ml of plasma may not have permitted the observation of a slow terminal phase. Conjugate quickly appeared in the plasma close to its maximum value at ~40 min. Cumulative urinary excretion of free morphine and of the conjugate was 8.5–9.3 and 66–70% of the dose, respectively. The renal drug and metabolite clearances were given as 109–171 and 50–115 ml/min, respectively. The qualitative formation of N-demethylated morphine was observed by following the time course of expired $^{14}CO_2$.

Catlin (19) investigated morphine pharmacokinetics in the rabbit by morphine radioimmunoassay methods with the intent of confirming the presence of multiphasic pharmacokinetics as indicated by previous studies that apparently demonstrated a terminal half-life of many hours or days. He clearly demonstrated that a two- or three-compartment body model in the rabbit could be observed, dependent on antiserum specificity and on possible interaction with metabolites. He stated that he would reject the third compartment for the rabbit and retain only two half-lives of 13 and 72 min. No pharmacokinetic dose dependency was observed between the 1- and 10-mg/kg doses. Catlin (19) issued the caveat for the previously cited radioimmunoassay studies in humans (14, 15), where an apparently long terminal half-life was observed, that "providing the antiserum used . . . did not seriously cross-react with morphine metabolites, it appears that in man the two compartment model is not sufficient
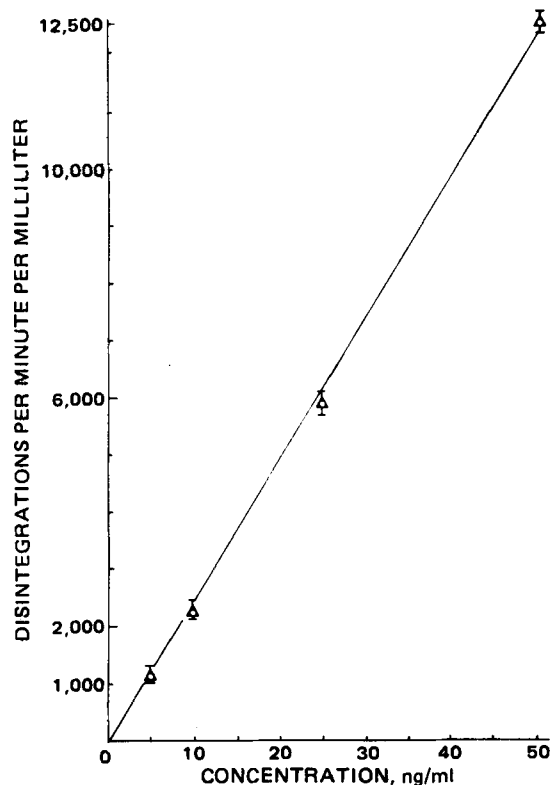
Figure 1—*Typical calibration curve for the assay of ^{14}C-morphine in dog plasma extracted with 10% butanol–chloroform after the addition of 0.5 g of $NaHCO_3$ to 2 ml of plasma by liquid scintillation spectrometry in the absence (△) and presence (○) of 100 ng/ml of ^{14}C-morphine-3-glucuronide. The vertical bars give the ± SD of four replicate determinations.*

to describe the data." Stripped of its niceties, the statement implies that radioimmunoassay assays for low morphine levels in the presence of metabolites are suspect and that the assumption of a deep compartment in humans is not assured.

Definitive pharmacokinetic studies in other animal species and humans still are needed to prove the presence of a deep compartment characterized by a slow terminal release of administered morphine from the body. The reported studies were designed to challenge the hypothesis of a deep morphine compartment with slow terminal release.

The only attempt to challenge systematically dose-dependent morphine pharmacokinetics was made by Catlin (19) in the rabbit. His conclusion of nondose dependence is surprising in the light of known morphine pharmacology. Although morphine is used primarily as an analgesic agent, it elicits many other pharmacodynamic responses (20) including cardiovascular and respiratory effects such as peripheral vasodilation, blood pressure decrease, blood compartment volume changes, and respiratory depression. Morphine increased biliary pressure and caused biliary tract spasms which could impede biliary excretion.

Handley and Moyer (21) showed that intravenous doses of 1 mg/kg or more to the dog produced not only a pronounced fall in mean blood pressure but also an abrupt and sustained depression of the glomerular filtration rate and renal plasma flow and a maximal rate of dextrose reabsorption by the renal tubules. Such pharmacological effects should have a decided "feedback" effect on morphine pharmacokinetics as a function of dose. This paper reports on the apparent perturbations of morphine pharmacokinetics by its dose-dependent pharmacological effects.

These potential problems offer a challenge in proper pharmacokinetic modeling. Not only must nonlinearities that may not conform to simple saturation models be taken into account, but discontinuities in pharmacokinetic processes must be considered. Such discontinuities can be caused by shutdowns in renal and biliary processes and the holdup of significant drug and metabolite quantities in the kidneys and bile.

Pharmacokinetic studies have not monitored the constancies of renal and metabolic clearances of morphine and its metabolites as a function of dose. These canine studies were designed to permit a pharmacokinetically unified appraisal of the time courses of intravenous morphine and its principal metabolite (2, 3), morphine-3-monoglucuronide, in plasma,

urine, bile, and feces over a wide spectrum of doses. It is believed (2, 3, 6) that the formation of other metabolites in the dog is negligible.

The separation techniques used here permit the specific monitoring of radiolabeled morphine and its conjugates in the various biological fluid studied (22). The analytical methods used were equivalent to several independent nonradioactive assays.

EXPERIMENTAL

Materials—The following were used: nanograde chloroform[1], analytical grade butanol[1], N-^{14}C-*methyl*-morphine hydrochloride[2], morphine sulfate USP[3], silicone solution[4], thin-layer plates[5], chromatogram sheets[6], a scintillant (Handifluor[1]), sodium bicarbonate[1], *tert*-butylhydroperoxide[7], heparin sodium[8] (10,000 units/ml), and pentobarbital sodium[9].

Purity of N-^{14}C-*methyl*-Morphine—Vendor analysis of the N-^{14}C-*methyl*-morphine hydrochloride (143 μCi/mg) (1 μCi = 2.2 × 10^6 dpm) stated a 98% radiolabeled purity in carbon tetrachloride–n-butanol–methanol–5 N ammonia (40:30:30:4). TLC[6] in the system of n-propanol-acetone–acetic acid–5% ammonium hydroxide–water (9:3:2:2:4) (23) separated morphine (R_f 0.67) from the other alkaloids. One-centimeter sections of the developed chromatogram were scraped into vials, dissolved in 1.0 ml of water, and counted in a scintillation counter[10].

^{14}C-Morphine and ^{14}C-Morphine Glucuronide Assay in Biological Fluids—All glassware was silylated because of morphine adsorption by glass at low concentrations (24). Sodium bicarbonate (0.5 g) was added to 2 ml of plasma, urine, or bile containing morphine. This solution was shaken with 9 ml of 10% butanol–chloroform for 10 min and separated by centrifugation[11] at 1000 rpm. The upper aqueous phase was removed by aspiration, and an appropriate organic phase aliquot was evaporated in a scintillation vial under nitrogen in a water bath at 50°. Scintillant (10 ml)[12] was added, and the resultant ^{14}C-morphine solution was counted.

The original plasma, urine, or bile ^{14}C-morphine concentration was calculated by dividing by the extraction efficiency and the specific activity. A typical linear calibration curve for ^{14}C-morphine in dog plasma in the absence and presence of ^{14}C-morphine-3-glucuronide is given in Fig. 1.

The morphine extraction efficiency from a buffered 2.0-ml plasma sample with 9 ml of 10% butanol–chloroform was 0.84 ± 0.03 (*SD*). The solvent–plasma partition coefficient was 1.17, calculated from:

$$K = \frac{A_o V_w}{V_o (A - A_o)} = \frac{C_o}{C_w} \qquad \text{(Eq. 1)}$$

where $A_o/(A - A_o)$ is the ratio of the organic phase concentration (0.84 A) to the aqueous plasma concentration (0.16 A); V_w (2 ml) and V_o (9 ml) are the plasma and organic solution volumes, respectively; and A is the total plasma concentration before extraction. Where the organic solvent volume (V_o) and/or the extracted plasma volume (V_w) were varied, the total extracted plasma morphine fraction (f_{ex}) was calculated from:

$$f_{ex} = A_o/A = 1/(1 + V_w/V_o K) \qquad \text{(Eq. 2)}$$

where $K = 1.167$.

The extraction efficiency of morphine from buffered urine (2 ml with 9 ml of organic solvent) was 0.87 ± 0.02 to give a partition coefficient from urine of $K = 1.49$ (Eq. 1). The fraction extracted from a different volume of urine was calculated from Eq. 2.

The plasma, urine, or bile concentration (disintegrations per minute per milliliter) of the unextractable morphine metabolite was determined from the difference between the experimentally determined total disintegrations per minute per milliliter of unextracted plasma, urine, or bile and the calculated disintegrations per minute per milliliter assigned to the morphine concentration in these biological fluids. Unextracted

[1] Mallinckrodt Chemical Works, St. Louis, MO 63160.
[2] Amersham/Searle, Arlington Heights, IL 60005.
[3] Merck Sharp and Dohme, West Point, PA 19486.
[4] Siliclad, Clay Adams, Parsippany, NJ 07054.
[5] Uniplate, Analtech Inc., Newark, DE 19711.
[6] Eastman Kodak Co., Rochester, NY 14650.
[7] Matheson Coleman and Bell, Norwood, OH 45212.
[8] The Upjohn Co., Kalamazoo, Mich.
[9] Nembutal Sodium, Abbott Laboratories, North Chicago, Ill.
[10] Beckman Instrument Co., Fullerton, CA 92634.
[11] International centrifuge, model K, International Equipment Co., Needham Heights, Mass.
[12] Handifluor, Mallinckrodt Chemical Works, St. Louis, MO 63160.

plasma, urine, or bile (0.2 ml) was counted by adding 1.0 ml of water plus 10 ml of scintillant.

All counts per minute were converted to disintegrations per minute by dividing by the counting efficiencies. Nanogram morphine base equivalents were determined by dividing the disintegration per minute values by the specific activity of the morphine free base. The counting efficiencies of plasma, urine, bile, fecal slurries (10% w/v homogenate in water), and their extracts were determined by adding a known standard ^{14}C-toluene amount to previously counted samples and recounting. The counting efficiency was the ratio of the difference in counts per minute values to the disintegrations per minute added. Blood and bile samples were digested with 0.5 ml of 1 N NaOH and bleached with 0.5 ml of *tert*-butylhydroperoxide prior to extraction and counting.

[14]C-Morphine-3-monoglucuronide Isolation—The metabolite was isolated from the urine of dogs administered ^{14}C-morphine. A literature procedure (24) was used, except that TLC was substituted for anionic-exchange separation. Urine (30 ml) was shaken with 2 g of charcoal in a 50-ml centrifuge tube for 1 hr and centrifuged for 3 min. The aqueous layer was decanted, and the residual charcoal was washed twice with 30 ml of water. The adsorbed morphine and metabolites were extracted from the charcoal by shaking it with 10 ml of acetic acid for 30 min and centrifuging for 10 min. This procedure was repeated three times.

The resulting extracts were collected and filtered through filter paper to remove the finer charcoal particles. The clear liquid was evaporated under reduced pressure[13], and the residue was dissolved in 10 ml of water. This solution was chromatographed[5] in n-propanol–acetone–acetic acid–5% ammonium hydroxide–water (9:3:2:2:4) to separate morphine from its metabolite. The chromatogram was developed for 15 cm, and the metabolite spot (R_f 0.48) was scraped from the plate and eluted with hot water. The silica gel slurry was filtered to give a metabolite solution.

Metabolite Purity—The purity of the radiolabeled morphine-3-monoglucuronide metabolite was based on the R_f values of the isolated compound *versus* an authentic nonradioactive standard[14]. The following systems were used to verify the purity: System I (R_f 0.48), n-propanol–acetone–acetic acid–5% ammonium hydroxide–water (9:3:2:4:4) (3); and System II (R_f 0.0), chloroform–ethanol–water–acetic acid (20:10:15:1) (4). The morphine-3-monoglucuronide was visualized under UV light, and 1-cm sections were scraped into scintillation vials and counted. The chromatographic purity was 96%.

Metabolite Purity by Extraction—One hundred nanograms (10 μl) of the radiolabeled metabolite was added to each of three test tubes containing 2 ml of water to give a final concentration of the metabolite of 50 ng/ml. Each tube was extracted three times after the addition of 0.5 g of NaHCO$_3$ with 9 ml of 10% butanol–chloroform. Then 8 ml of each total extract was collected, dried, and counted. Less than 1.4% (1.1, 1.4, and 1.2) was extracted by this procedure.

Attempted Identification of Additional Major Metabolites—Plasma ultrafiltrates and urine samples from several dogs administered morphine were analyzed by TLC (System I). Two compounds were visualized, and their R_f values were determined by radioactive analysis of scrapings. They were morphine glucuronide (R_f 0.48) and morphine (R_f 0.67). These plasma and urine samples were also extracted with tetrahydrofuran at pH 1, 8, and 10, and the extracts were chromatographed in System I. Each chromatogram was developed to 15 cm, and 1-cm sections were scraped into scintillation vials and counted after the addition of 1 ml of water. Additional compounds were not observed at other R_f values.

Protein Binding—The binding of ^{14}C-morphine and ^{14}C-morphine 3-monoglucuronide was studied by an ultrafiltration method (25). When these drugs were dissolved in buffer and ultrafiltered through the cone membrane[15], no significant cone binding of drugs was observed. Standard morphine solutions were prepared in plasma at 10 and 100 ng/ml and at 2.48 μg/ml. The ultrafiltration cones were soaked in water and spun dry prior to use. The dried cones were placed in ultrafiltration cone supports in centrifuge tubes.

One milliliter of 0.05 M phosphate buffer, at ionic strength 0.154 and pH values of 7.4, 7.5, and 7.8, was placed in each cone. The appropriate standard plasma solution concentration was added, with agitation to effect mixing, until a volume of 5.0 ml was obtained. An aliquot (0.2 ml) was analyzed for total radioactivity. The samples were allowed to stand

at room temperature for 3 min, 12 min, or 1 hr and then were centrifuged at 2000 rpm for 10 min to half-filtration. The ultrafiltrates were assayed by liquid scintillation spectrophotometry.

Similar studies were conducted with dog plasma at 100 ng of morphine-3-glucuronide/ml at pH values of 7.1 and 7.4.

Red Blood Cell Partition—The partition of ^{14}C-morphine and ^{14}C-morphine-3-monoglucuronide into the red blood cells was studied as a function of drug concentration and time. Fresh heparinized dog blood was added with gentle shaking to 10-ml volumetric flasks containing enough ^{14}C-morphine to achieve concentrations of 1, 10, 100, and 1000 ng/ml. Dog blood also was added to another set of volumetric flasks containing sufficient ^{14}C-metabolite to achieve concentrations of 50 and 500 ng/ml. Hematocrits were determined, and aliquots (3 ml) were taken after 30 sec and 30 min at room temperature and assayed for total radioactivity. The remaining blood was centrifuged at 1000 rpm for 10 min, and the plasma was analyzed for total radioactivity.

Pharmacokinetics of Morphine and Its Derived Metabolite—The dogs were fasted for 24 hr before each experiment and then weighed. Chronic jugular vein catheterization was done with medical grade tubing[16] at least 48 hr before the scheduled experiment. All blood sampling was done through this catheter.

On the day of the experiment, the dog was placed on a table in a standing position and restrained by separate straps around the fore- and hindlegs, which were fixed to a horizontal bar above. A water load of 15 ml/kg was administered through stomach tubing. A continuous 0.9% NaCl drip (1 ml/min) was maintained for 500 min. A transurethral catheter[17] was used to collect bladder urine for the first 12 hr. After 12 hr, urine was collected from the metabolic cages, with completeness achieved by acute catheterization. After anaerobic collection, the blood pH and P_{CO_2} were routinely determined on the gas analyzer[18]. Hematocrits were determined utilizing a microhematocrit centrifuge and reader[19].

Sterile procedures were used for blood handling when red blood cells were to be readministered. After separation from plasma, the packed cells were stored at 5°. At the end of the experiment, they were resuspended in normal saline and reinfused into the animal; 200,000 units of procaine penicillin G in dihydrostreptomycin sulfate[20] solution were administered intramuscularly.

Hematology showed negative results for microfilaria. Body weights and the intravenous morphine doses are listed in Table I. A minimum period of 2 weeks was allowed between any two experiments on the same dog.

[14]C-Morphine hydrochloride was diluted with morphine sulfate USP, and the specific activity of the final mixture determined. The morphine was administered intravenously, as freshly prepared solutions in plasma, into the jugular catheter over 10 sec for the 0.02- and 0.07-mg/kg doses; it was injected into the cephalic vein for the 0.4–0.5- and 7.2–7.7-mg/kg doses. Drug administration was followed by a 5-ml flush of normal saline to free the catheter of residual drug. At the highest dose, the dogs exhibited a phenomenon known as sham rage and had to be physically restrained so that blood could be sampled.

Blood samples were taken from the jugular catheter after the dead space volume was removed and the catheter was filled with fresh undiluted blood. Sterile disposable syringes containing approximately 50 μl of heparin (10,000 units/ml) were used to collect blood samples. The blood was transferred to a 15-ml centrifuge tube and centrifuged at 1500 rpm for 10 min, with 2 ml of the supernatant plasma being transferred to tubes containing 0.5 g of sodium bicarbonate for extraction. Blood samples (5 ml) were taken at 0.5, 1, 2, 5, 10, and 20 min after the morphine injection; 8-ml samples were taken at 30, 45, 75, 110, and 180 min; 1-ml samples were taken hourly thereafter to 12 hr and finally every 12 hr up to 50 hr. When pH and hematocrit values were to be evaluated, a small portion of the sample was left in the syringe.

Urine was collected every 10 min up to 1 hr, then hourly up to 12 hr, and then every 12 hr up to 100 hr. The volume was noted, and the urine was stored in bottles and refrigerated at 5°.

Feces were collected at 24-hr intervals.

Pharmacokinetics of ^{14}C-Morphine-3-monoglucuronide—The purified metabolite morphine-3-monoglucuronide was administered intravenously to Dog A, which had not been used in a previous phar-

[13] Büchi Rotavapor, Brinkmann Instruments, Westbury, NY 11590.
[14] National Institute of Mental Health, Center for Studies of Narcotic and Drug Abuse, Bethesda, Md.
[15] Centriflo CF 50 membrane filter cone with CSTL support, Amicon Co., Lexington, Mass.

[16] Silastic tubing, Dow Corning Corp. Medical Products, Midland, MI 48640.
[17] French size 6, Bard Inc., Murray Hill, N.J.
[18] Model 113-S-1 pH-blood gas analyzer, Instrumentation Laboratories, Boston, Mass.
[19] Damon/I.E.C. Division, Needham Heights, MA 02194.
[20] Distrycillin, 200,000 units, 0.25 g/ml, E. R. Squibb and Sons, New Brunswick, NJ 08903.

Table I—Pharmacokinetics of Intravenous Morphine in Dogs

Parameter	High Doses			Intermediate Doses		Low Doses		
Dose (D_0), mg	109.2	109.0	107.0	6.43	5.70	0.792	0.225	0.208
Dose (D_0), mg/kg	7.68	7.22	7.60	0.406	0.471	0.070	0.0191	0.0189
Specific activity, dpm/ng	1.17	0.624	0.792	5.99	14.92	128	416	438
Dog	C	C	E	C	D	B	B	A
Weight, kg	14.2	15.1	14.0	16.0	11.5	11.4	11.8	11.0
Bile cannulated	Yes	No	No	Yes	No	No	No	No
Parameters[a] from plasma data								
A, ng/ml	1200	5600	3700	82.8	111	40	12.5	17.5
B	2200	2200	2800	47	51	21.3	4.0	5.4
α, min^{-1} ($t_{1/2}$, min)	0.0974 (7.1)	0.175 (4.0)	0.0534 (13)	0.0344 (20)	0.0613 (11)	0.159 (4.4)	0.201 (3.4)	0.234 (3.0)
β	7.78×10^{-3} (89)	8.45×10^{-3} (82)	8.96×10^{-3} (77)	9.50×10^{-3} (73)	1.40×10^{-2} (50)	2.06×10^{-2} (34)	1.53×10^{-2} (45)	1.86×10^{-2} (37)
Parameters[a] from urine data								
β, min^{-1} ($t_{1/2}$, min)	7.73×10^{-3} (90)	9.4×10^{-3} (74)	8.6×10^{-3} (81)	1.03×10^{-2} (67)	9.14×10^{-3} (76)	2.84×10^{-2} (24)	1.41×10^{-2} (49)	1.80×10^{-2} (39)
γ	0.0	8.6×10^{-4} (808)	4.6×10^{-4} (1509)	0.0	5.04×10^{-4} (1375)	1.45×10^{-3} (477)	1.31×10^{-3} (531)	5.5×10^{-4} (1264)
Clearances, ml/min								
Cl_{tot}^M [b]	370	359	290	784	886	586	694	554
Cl_{ren}^M [c]	98	67	56	115	115	69	98	62
Cl_{met}^M [d]	272 (322)	292 (232)	234 (215)	669 (454)	771	517	596	492
$Cl_{met}^M - Cl_B^e$	—	205	182	—	256	341	417	207
Cl_B^f	—	87 (150)[g]	52	—	515	176	179	285
Cl_{ren}^{MG} [h]	57	45	38	50	34	48	33	24
Disposition, fractions of dose								
$\Sigma U_M^\infty/D_0$	0.148	0.099	0.070	0.139	0.151	0.124	0.145	0.123
$\Sigma U_{MG}^\infty/D_0$	0.785	0.674	0.794	0.508	0.620	0.813	0.736	0.720
$\Sigma B_{exp}^\infty/D_0$	0.143	—	—	0.109	—	—	—	—
Theoretical fraction of drug not in urine[i]	0.063	0.227	0.136	0.353	0.229	0.063	0.119	0.147
Theoretical fraction of drug excreted in bile j, $\dfrac{\Sigma B_{calc}^{tot}}{D_0}$	—	0.295	0.186	—	0.582	0.286	0.258	0.514
$\Sigma MG_{ent}^\infty/D_0$ [k]	—	0.156	0.145	—	0.333	0.258	0.129	0.367
$\Sigma MG_{feces}^{theo}/D_0 = B'_\infty$ [l]	0.0	0.139	0.041	0.0	0.249	0.028	0.129	0.147
$\Sigma MG_{feces}^{exp}/D_0$	0.0	0.087	0.053	0.0	0.135	—	—	—
Apparent volumes of distribution, liters								
V_c^{Mm}	32	14	16	19	5.2	13	14	9
V_d^{Mn}	48	42	66	23	63	30	46	31
V_{extrap}^{Mo}	50	50	38	50	35	37	56	39
V_{MG}^p	-(4.2)	5.05 (4.2)	4.23 (3.96)	-(2.85)	2.03	1.52	0.51	1.68
Time of commencement, min								
Renal function	60	65	105	0	0	0	0	0
Bile function	16	35	0–10	0	0	0	0	0
$[MG]_{max}$, ng/ml as morphine	8800	6400	10,300	360	330	92	50	26
$10^5 [MG]_{max}/D_0$	8.1	5.9	9.6	5.6	5.8	120	22	9.6

[a] Parameters estimated from best fit of $[M]_{exp}$ as morphine base in plasma against time to $[M] = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$ or from urine data where the γ parameter was determinable solely from slopes of plots of $\ln |\Sigma U_\infty - \Sigma U|$ versus time t and $\ln \Delta U_M/\Delta t$ versus time t_{mid}, where ΣU_M is the cumulative morphine base excreted into urine at time t and $\Delta U_M/\Delta t$ is the amount of morphine base excreted in a time interval of midtime, t_{mid}. A C and γ of 20 ng/ml and 8.7×10^{-4} min^{-1} ($t_{1/2} = 798$ min), respectively, could be estimated from plasma for Dog C, not bile cannulated, at the 109-mg dose in column 2. With the two intermediate doses, a quickly vanishing exponential $A'e^{-\alpha't}$ should also be added to best fit the plasma–time data where the values of A' and α' $(t_{1/2})\alpha'$ were 212 ng/ml and 0.242 min^{-1} (2.9 min), respectively, for the 6.43-mg dose in the bile-cannulated Dog C and 930 ng/ml and 0.921 min^{-1} (0.75 min), respectively, for the 5.70-mg dose in the non-bile-cannulated Dog D. [b] Total morphine base clearance estimated from ratio of dose to total area under plasma $[M]$ versus t curve, D_0/AUC^∞_M. [c] Renal morphine base clearance estimated from the slope of $\Delta U/\Delta t$ versus $[M]$ plots and the best fit of ΣU versus t data generated from Cl^t_{ren} $(AUC^t_M - AUC^{t_0}_M)$, where AUC_M is the area under the plasma $[M]$ versus t curve up to time t and $AUC^{t_0}_M$ is the area up to a time, t_0, where renal processes start at the high doses. [d] Estimated metabolic clearance of morphine base to morphine conjugate, MG, normally calculated from $Cl^M_{tot} - Cl^M_{ren}$. The parenthetical values were determined from appropriate plots of:

$$\frac{\Sigma U^{exp}_{MG} + \Sigma B^{exp}_{MG}}{[MG]_{exp}} = Cl^M_{met}\frac{AUC_M}{[MG]} - V_{MG} \qquad \text{or} \qquad \frac{\Sigma U^{exp}_{MG} + \Sigma B^{exp}_{MG}}{AUC_M} = - V_{MG}\frac{[MG]_{exp}}{AUC_M} + Cl^M_{met}$$

where the ΣB^{exp}_{MG} data were the cumulative excretions of the conjugate in the bile experimentally determined for the study in the bile-cannulated dog at the same dose. [e] Obtained from appropriate data plots in accordance with $\Sigma U^{exp}_{MG}/AUC_M = V_{MG}[MG]_{exp}/AUC_M + (Cl^M_{met} - Cl_B)$ or $\Sigma U^{exp}_{MG}/[MG]_{exp} = (Cl^M_{met} - Cl_B) AUC_M/[MG]_{exp} - V_{MG}$ on the assumption of a constant immediate biliary clearance, Cl_B, of the formed conjugate. [f] Calculated constant biliary conjugate clearance, immediately after formation, obtained from the difference between $Cl^M_{met} = Cl^M_{tot} - Cl^M_{ren}$ and the $Cl^M_{met} - Cl_B$ value defined in footnote e. [g] This parenthetical biliary clearance value is from the best fit of the ΣB_{exp} versus t data from the bile-cannulated study from $Cl_B(AUC_M - AUC^t_M)$, where AUC^t_M is the area up to a time, t_0, where biliary clearance is presumed to start at this high dose. [h] Renal conjugate clearance (as morphine base equivalents) from the slope of $\Delta U_{MG}/\Delta t$ versus $[MG]$ plots and the best fit of ΣU_{MG} versus t data generated from $Cl^{MG}_{ren} (AUC^t_M - AUC^{t_0}_M)$, where renal processes start at t_0. [i] $1 - (\Sigma U^\infty_{MG} + \Sigma U^\infty_M)/D_0 / \Sigma B^{tot}_{calc} = Cl_B AUC^\infty_M$ on the assumption of constant biliary clearance, Cl_B, and no enterohepatic recirculation. [k] Fraction of drug enterohepatically recirculated is calculated from $\Sigma MG_{ent} = \Sigma B tot - \Sigma B'_\infty$ where $\Sigma B'_\infty = Cl^M_{met} AUC^\infty_M - \Sigma U^\infty_{MG}$. [l] Theoretical fraction excreted elsewhere, most probably in feces, from $\Sigma MG^{theo}_{feces} = \Sigma B'_\infty = Cl^M_{met}AUC^\infty_M - \Sigma U^\infty_{MG}$. [m] Apparent central compartment volume referenced to morphine base concentration in plasma, $V^m_c = [(D_0)/(A' + A + B)]$. [n] Apparent overall distribution volume, referenced to morphine base concentration in plasma, $V^m_d = [(D_0)/(AUC^\infty_M)(\beta)]$. [o] Apparent volume of distribution on assumption of rapid equilibration so that α or α' is extremely fast, i.e., $V_{extrap} = D_0/B$, or for the intermediate doses where an α' existed, $V_{extrap} = (D_0)/(A + B)$. [p] Apparent overall distribution volume for the formed conjugate, MG, as determined from appropriate plots as specified in footnote e. The parenthetical estimates of this volume were determined from the appropriate plots as specified in footnote d.

Table II—Morphine Fraction Bound to Plasma Protein[a]

pH	Concentration, ng/ml									Average ± SD
	10			100			2,480			
7.4	0.37	0.26	0.33	0.33	0.36	0.36	0.40	0.35	0.34	0.344 ± 0.038
7.6	0.45	0.31	0.36	0.42	0.41	0.28	0.39	0.28	0.29	0.354 ± 0.066
7.8	0.40	0.36	0.37	0.37	0.40	0.35	0.38	0.37	0.33	0.370 ± 0.022
Average ± SD	0.357 ± 0.054			0.384 ± 0.028			0.328 ± 0.037			0.356 ± 0.046

[a] As determined at 3 min, 12 min, and 1 hr after addition to dog plasma.

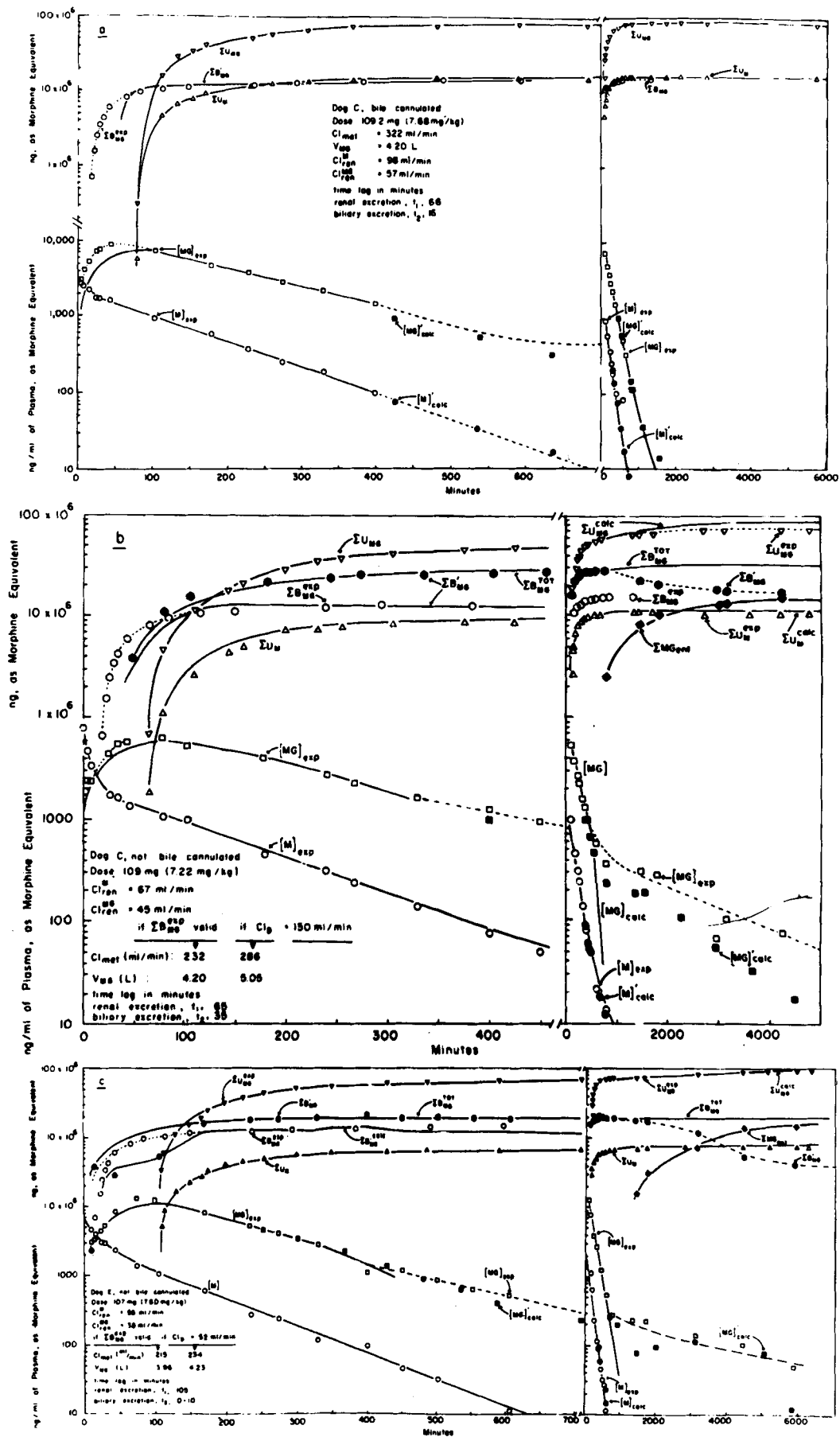**Figure 2**—*Plots of experimental values of morphine, $[M]_{exp}$ (☉), and the morphine conjugate, $[MG]_{exp}$ (⊡), in nanograms per milliliter of plasma as morphine equivalents against time at high morphine base doses, ~7.5 mg/kg iv. The solid line through the experimental [M] values was calculated*

from the polyexponential fit, $[M] = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$. (See Table I for parameters.) The solid line through the experimental $[MG]_{exp}$ values was calculated on the assumption of a constant metabolic morphine clearance, Cl^M_{met}, so that $[MG] = (Cl_{met}AUC_M - \Sigma B_{MG} - \Sigma U^{exp}_{MG})/V_{MG}$ as in Eq. A13. The Cl_{met} and V_{MG} values for each dog could be obtained from appropriate plots of the data in accordance with Eqs. A11 and A12 (Fig. 7) based on the assumed validity of the ΣB^{exp}_{MG} data (○) from the bile-cannulated dog for the normal dog. The $[MG]$ values were also calculated for the normal dog from estimates of $Cl_{met} = Cl_{tot} - Cl^{ren}_M$, where $Cl_{tot} = dose/AUC_M$, and $\Sigma B_{MG} = Cl_B AUC_M$, where $Cl_{met} - Cl_B$ and V_{MG} values were estimated from plots in accordance with Eqs. A16 and A17 (Figs. 8 and 9).

The experimental values of cumulative morphine, ΣU^{exp}_M (△), and its conjugate, ΣU^{exp}_{MG} (▽), in urine are also plotted against time. The solid lines through the data points are the theoretical values calculated from $\Sigma U_M = Cl^M_{ren} (AUC_M - AUC^{t_1}_M)$ and $\Sigma U_{MG} = Cl^{MG}_{ren} (AUC_{MG} - AUC^{t_1}_{MG})$, where $AUC^{t_1}_M$ and $AUC^{t_1}_{MG}$ are the respective areas under the $[M]$ and $[MG]$ versus time curves up to the time, t_1, of onset of renal excretion. The renal clearances, Cl^M_{ren} and Cl^{MG}_{ren}, were consistent with the values obtained from the slopes of the renal clearance plots of $\Delta U/\Delta t$ versus plasma level (Fig. 5).

The calculated values of $\Sigma B'_{MG} = Cl^M_{met} AUC_M - \Sigma U^{exp}_{MG} - V_{MG} [MG]_{exp}$ of Eq. A22 are represented by the solid line through the experimental values of ΣB^{exp}_{MG} (○), which were obtained from studies with the bile-cannulated dog at the same dose (Fig. 2a). The Cl_{met} and V_{MG} values were obtained from appropriate plots of the data in accordance with Eqs. A11 and A12 (Fig. 7).

Alternative estimates of cumulative conjugate in the bile, $\Sigma B'_{MG}$ (●), of the normal (not bile cannulated) dog (Figs. 2b and 2c) were based on the Cl_{met} and V_{MG} values obtained from appropriate data plots in accordance with Eqs. A16 and A17 (Figs. 8 and 9). The solid line through these data (●) was based on the assumed constant biliary clearance obtained from these plots and was calculated from $\Sigma B^{tot}_{MG} = Cl_B (AUC_M - AUC^{t_2}_M)$, where $AUC^{t_2}_M$ is the area under the $[M]$ versus time curve up to the time, t_2, of onset of biliary excretion. The difference between this total amount of conjugate excreted in the bile, ΣB^{tot}_{MG}, and the amount presumed to remain there, $\Sigma B'_{MG}$, estimates the amount of conjugate enterohepatically recirculated to the systemic circulation, $\Sigma MG_{ent} = \Sigma B^{tot}_{MG} - \Sigma B'_{MG}$.

Terminal values of $[M]_{exp}$ (○) and $[MG]_{exp}$ (□) tend to have larger errors since the liquid scintillation counts approach background. The respective solid symbols are the plasma level of $[M]'_{calc}$ (●) and $[MG]'_{calc}$ (■) calculated from the estimated renal clearances and the observed ΣU^{exp}_M and ΣU^{exp}_{MG} values, respectively (Eq. A27).

macokinetic study. A freshly prepared solution of the material containing 50% plasma was injected into the jugular catheter over 10 sec.

The same sampling schedule as for morphine was used. Urine was collected for 800 min. The dog was kept in the sling support for 8 hr and then placed in the metabolic cage.

Biliary Excretion—Dog C was anesthetized with 30 mg/kg of pentobarbital sodium, a laparotomy was performed, the cystic duct was tied and cut, and the common bile duct was cannulated with tubing[21] (0.2 cm i.d. and 0.3 cm o.d.). The other end was used to cannulate the caudal end of the common bile duct to provide for normal bile flow into the duodenum. The tubing loop resulting from the cannulation was pulled cephalically subcutaneously and brought to the surface through an incision in the skin at the back of the neck. This loop was cut, and a connecting piece of tubing was inserted which, when removed, allowed for continuous bile sampling.

The animal was allowed to recover from the surgical procedure and was administered a 7.7-mg/kg dose. After 3 weeks, this bile-cannulated animal was given a 0.41-mg/kg dose. Blood and urine were collected as in other experiments. Bile was collected under heptane at 4.7, 7.8, 10, 13, 16, 20, 25, 31, 43, 57, 86, 176, 290, 382, 520, 600, 730, and 800 min. The volume was measured, and the bile was stored at 0° until analyzed by liquid scintillation counting in the manner described previously.

Infusion Study—The procedure for this study was the same as for the other pharmacokinetic studies with the following changes. The infusion line was connected to the jugular vein catheter, using a 19-gauge needle introduced through the flash ball injection site of the Plexitron administration set[22] for the chloride drip. This apparatus was attached to the catheter by a 14-gauge needle[23] and a three-way stopcock. The drug (3750 μg of ^{14}C-morphine) was prepared in 286.6 ml of sterile 75% isotonic saline plasma solution. The infusion rate (10 μg/min) was attained using an infusion pump[24].

Blood (5 ml) was sampled at 30, 60, 90, 180, 280, 300, 310, 320, and 340 min during the infusion. Postinfusion samples were collected at 3 and 9 min, then at 30-min intervals up to 150-min postinfusion, and then at 90-min intervals up to 1200-min postinfusion. Urine was collected every 20 min during the infusion. Following the infusion, urine was collected every 40 min for the 1st hr, then at 2-hr intervals up to 700-min postinfusion, and subsequently every 8 hr up to 4000-min postinfusion.

RESULTS AND DISCUSSION

Protein Binding—The results of these studies in dog plasma are given in Table II. There was no significant concentration or pH effect on the $36 \pm 1\%$ (SEM) morphine binding to plasma protein. No significant differences were observed among the samples centrifuged at different times after the spiking of dog plasma, so equilibration at least within 3 min, the earliest time studied, must be concluded. No significant protein binding (<2%) in dog plasma of morphine-3-monoglucuronide (100

ng/ml) was observed at pH 7.1 and 7.4.

The protein binding of 32.8% at 2.5 μg/ml (Table I) is consistent with the estimates of 30% (26) for dog plasma studied in the ranges of 2.5–15 μg of morphine/ml and is not too different from the 35.7 ± 0.9% given for normal human plasma (27).

Red Blood Cell–Plasma Water Partition Coefficient—This coefficient, D, the ratio of the drug concentration in the red blood cells, $[A_{RBC}]$, to that in the plasma water, $[A^u_p]$, can be calculated (28) from the experimentally determined concentration, $[A_p]$, of drug in the plasma when the concentration in whole blood, $[A_B]$, the fraction bound to plasma protein, f, and the hematocrit, H, are known:

$$D = \frac{[A_{RBC}]}{[A^u_p]} = \left\{\frac{[A_B]}{[A_p](1-f)(1-H)} - \frac{f}{(1-f)} - 1\right\}\frac{1-H}{H} \quad \text{(Eq. 3)}$$

There was no apparent time or concentration dependence for the red blood cell–plasma water partition coefficient of morphine (1.11 ± 0.04 SD) or its glucuronide metabolite (0.66 ± 0.05) (Table III).

The following cited equations, labeled with the prefix A, are derived and given in Appendix I. The symbols used are defined in Appendix II. The experimental disposition of various morphine doses, the clearances, and the best fit pharmacokinetic parameters are given in Table I, and the fitting is demonstrated in Figs. 2–4.

Morphine Pharmacokinetics at High Doses—Plasma morphine levels, $[M]$, with time in the bile-cannulated dog, C, where the bile was completely collected, were best fitted by the two-compartment body model (Fig. 2a and Table I). There was no significant terminal plateau in $[MG]_{exp}$ or $[MG]'_{calc}$ values that could be assigned to the return of the conjugate or its precursor, M, from a deep compartment. Significant urine flow was not observed until after 53 min of drug administration at this 7.7-mg/kg dose when 1.5 ml was obtained.

Significant morphine, ΣU_M, and conjugate, ΣU_{MG}, in the urine were first observed in the interval of 53–80 min. The calculated amounts (Eqs. A6 and A8) of ΣU_M and ΣU_{MG} renally excreted (solid lines in Fig. 2a) were consistent only with the experimental data, the obtained renal clearances (Fig. 5) and areas, AUC_M, under the morphine and conjugate

[21] Tygon, Scientific Products, Chamblee, GA 30341.
[22] Travenol Laboratories, Deerfield, IL 60015.
[23] Becton Dickinson and Co., Rutherford, N.J.
[24] Model 600-950, Harvard Apparatus, Dover, Mass.

Table III—Dog Red Blood Cell–Plasma Water Partition Coefficients (D)[a] of Morphine and Its Glucuronide Metabolite

Time of Measurement	Morphine, ng/ml				Morphine-3-monoglucuronide, ng/ml	
	1	10	100	1000	50	500
30 sec	1.03	1.13	1.13	1.14	0.71	0.63
30 min	1.10	1.10	1.12	1.11	0.60	0.70
Average ± SD		1.11 ± 0.35			0.66 ± 0.53	

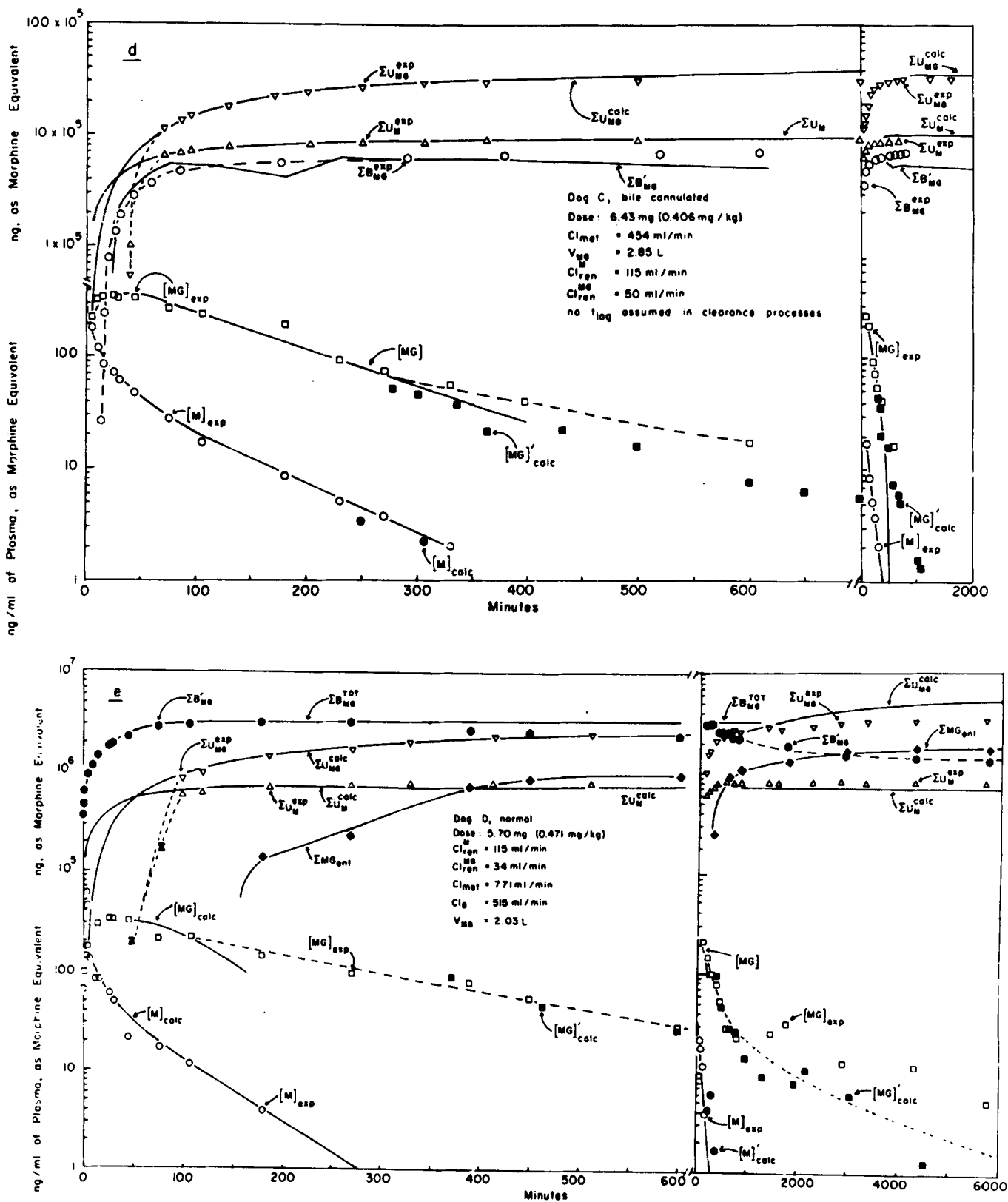[a] The values used in Eq. 3 for these calculations were $H = 0.35$ and $f = 0.32$ for morphine and $f = 0$ for the glucuronide.

Figure 3—*Plots of experimental values of morphine,* $[M]_{exp}$ *(⊙), and the morphine conjugate,* $[MG]_{exp}$ *(▫), in nanograms per milliliter of plasma as morphine equivalents against time at intermediate morphine base doses, 0.41 and 0.47 mg/kg iv. The solid line through the experimental* $[M]$ *values was calculated from the polyexponential fit,* $[M] = A'e^{-\alpha't} + Ae^{-\alpha t} + Be^{-\beta t}$. *(See Table I for parameters.) The solid line through the experimental* $[MG]_{exp}$ *values was calculated on the assumption of a constant metabolic morphine clearance,* Cl^M_{met}, *so that* $[MG] = (Cl_{met} \; AUC_M - \Sigma B_{MG} - \Sigma U^{exp}_{MG})/V_{MG}$. *The* Cl_{met} *and* V_{MG} *values for the bile-cannulated Dog C in Fig. 3d were obtained from an appropriate data plot in accordance with Eqs. A11 and A12 (Fig. 7) based on the assumed validity of the* ΣB^{exp}_{MG} *data (⊙). The MG values were calculated for the normal Dog D in Fig. 3e from estimates of* $Cl_{met} = Cl_{tot} - Cl^{ren}_M$, *where* $Cl_{tot} = dose/AUC_M$, *and* $\Sigma B_{MG} = Cl_B AUC_M$, *where* $Cl_{met} - Cl_B$ *and* V_{MG} *values were estimated from plots in accordance with Eqs. A16 and A17 (Figs. 8 and 9).*

The experimental values of cumulative morphine, $\Sigma U^{exp}_M(\triangle)$, *and its conjugate,* ΣU^{exp}_{MG} *(▽), in urine are also plotted against time. The solid*

lines through the data points are the theoretical values calculated from $\Sigma U_M = Cl_{ren}^M AUC_M$ and $\Sigma U_{MG} = Cl_{ren}^{MG} AUC_{MG}$. The renal clearances, Cl_{ren}^M and Cl_{ren}^{MG}, were consistent with the values obtained from the slopes of the renal clearance plots of $\Delta U/\Delta t$ versus plasma level (Fig. 5).

The calculated values of $\Sigma B'_{MG} = Cl_{met}^M AUC_M - \Sigma U_{MG}^{exp} - V_{MG}$ [MG]$_{exp}$ for the bile-cannulated dog (Fig. 3d) are represented by the solid line through the experimental values of ΣB_{MG}^{tot} (O). The Cl_{met} and V_{MG} values were obtained from appropriate data plots in accordance with Eqs. A11 and A12 (Fig. 7).

Estimates of cumulative conjugate in the bile, $\Sigma B'_{MG}$ (●), of the normal (not bile cannulated) dog (Fig. 3e) were based on the Cl_{met} and V_{MG} values obtained from appropriate data plots in accordance with Eqs. A16 and A17 (Figs. 8 and 9). The solid line through these data (●) was based on the assumed constant biliary clearance obtained from these plots and was calculated from $\Sigma B_{MG}^{tot} = Cl_B AUC_M$. The difference between this total amount of conjugate excreted in the bile, ΣB_{MG}^{tot}, and the amount presumed to remain there, ΣB_{MG}, estimates the amount of conjugate enterohepatically recirculated to the systemic circulation (◆), $\Sigma MG_{ent} = \Sigma B_{MG}^{tot} - \Sigma B'_{MG}$.

Terminal values of [M]$_{exp}$ (O) and [MG]$_{exp}$ (□) tend to have larger errors since the liquid scintillation counts approach background. The respective solid symbols are the plasma levels of [M]$'_{calc}$ (●) and [MG]$'_{calc}$ (■) calculated from the estimated renal clearances and the observed ΣU_M^{exp} and ΣU_{MG}^{exp} values, respectively (Eq. A27).

plasma level–time curves when renal processes were not considered operative until a time, t_0, of 65 min after drug administration.

The only major discrepancy in the fitting of the data for the bile-cannulated dog in Fig. 2a was that the calculated ΣB_{MG}^{calc}(Eq. A14) and [MG]$_{calc}$ (Eq. A13) values based on the 4200-ml value of V_{MG} underestimated the experimental values of ΣB_{MG}^{exp} and [MG]$_{exp}$, respectively, for the first 45 min, even when ΣU_{MG} was taken as zero for this time. The assumption of an average V_{MG} of 2500 ml for this interval improved the fit (dashed lines before 45 min in Fig. 2a) with the experimental data. This finding strongly indicates that the apparent conjugate distribution volume may have increased during this interval, which is not consistent with the assumption of a one-compartment body model for [MG] immediately after its production. A period of time may be necessary for the conjugate to equilibrate in its apparent overall distribution volume.

Biliary conjugate secretion also was inhibited at the high morphine doses. The use of a time lag of 16 min and the determined variable biliary clearance, Cl_B (Fig. 6), which depended on the bile flow, gave the fit to the ΣB_{MG}^{exp} data demonstrated by the dashed line before 45 min in accordance with $\Sigma B_{MG}^{calc} = \Sigma Cl_B(AUC_M^t - AUC_M^{tg=16 \, min})$ as described in Eq. A3.

The fact that no radioactivity was observed in the collected feces of this bile-collected dog shows that the fecal source of drug must be assigned to the biliary excretion.

Studies (Fig. 2b) of both the same Dog C at a similar dose, 7.2 mg/kg, and Dog E at 7.6 mg/kg (Fig. 2c) without biliary cannulation showed similar pharmacokinetic patterns, except for terminal elevations of plasma metabolite levels and pronounced drug and metabolite in the feces (Table I). Direct plasma conjugate measurements were consistent, [MG]$_{exp}$, with [MG]$_{calc}^{calc}$ values calculated by Eq. A27 from the presumed renal clearance, Cl_{ren}^{MG}, of the conjugate and the cumulative amount, ΣU_{MG}^{exp}, excreted in the urine.

This elevation in plasma and concomitant urine conjugate in the non-bile-cannulated dog can be assigned to material returned to the system by enterohepatic recirculation of the bile contents.

The cumulative amounts of enterohepatically recirculated drug in normal Dogs C and E, ΣMG_{ent}, are plotted in Figs. 2b and 2c as based on Eqs. A21–A23. The premise for the calculation is that the intravenously administered morphine was almost completely cleared by 100 min, as indicated by the data for bile-cannulated Dog C (Fig. 2a).

The data for the normal dogs in Figs. 2b and 2c were fitted in two manners. In one, the same biliary excretion, ΣB_{MG}^{exp}, was postulated as when the dog was biliary cannulated and the total bile was collected (Fig. 2a). The estimated V_{MG} and Cl_{met} values for the normal dogs at this dose (Eqs. A11 and A12) were 4.2 liters and 232 ml/min, respectively, for Dog C and 3.96 liters and 215 ml/min, respectively, for Dog E (Fig. 7). The solid lines ($\Sigma B'_{MG}$) in Figs. 2b and 2c through the ΣB_{MG}^{exp} data obtained from the bile-cannulated Dog C at the same dose were calculated from Eq. A22 with these parameters and fit well except at the early times. The solid lines for [MG]$_{calc}$ through the [MG]$_{exp}$ data were calculated from Eq. A13 with these parameters and were reasonable for all times up to when enterohepatic return from the bile was postulated, as indicated by the plotting of terminal dashed lines. A better fit of the initial [MG]$_{exp}$ data up to 45 min in Fig. 2b is given by the dashed lines when it was postulated that no drug was eliminated in the bile until ~50 min.

The alternative manner of fitting was to postulate a constant biliary clearance which only commenced a specified time after drug administration. This premise does not demand a specific knowledge of the amount of conjugate in the bile (Eqs. A16 and A17). The obtained respective values of V_{MG} and Cl_{met} were 5.05 liters and 292 ml/min for Dog C (Figs. 8 and 9) and 4.23 liters and 234 ml/min for Dog E. The calculated ΣB_{MG}^{calc} values (Eq. A22) based on these parameters are given in Figs. 2b and 2c. The solid lines through these data (ΣB_{MG}^{tot}) assumed a constant

biliary clearance, Cl_B (Eq. A3), and no enterohepatic conjugate recirculation in the bile. Obviously, when these values diverged from the ΣB_{MG}^{calc} values, the difference could be attributed to those amounts returned from the bile to the systemic circulation, i.e., ΣMG_{ent}.
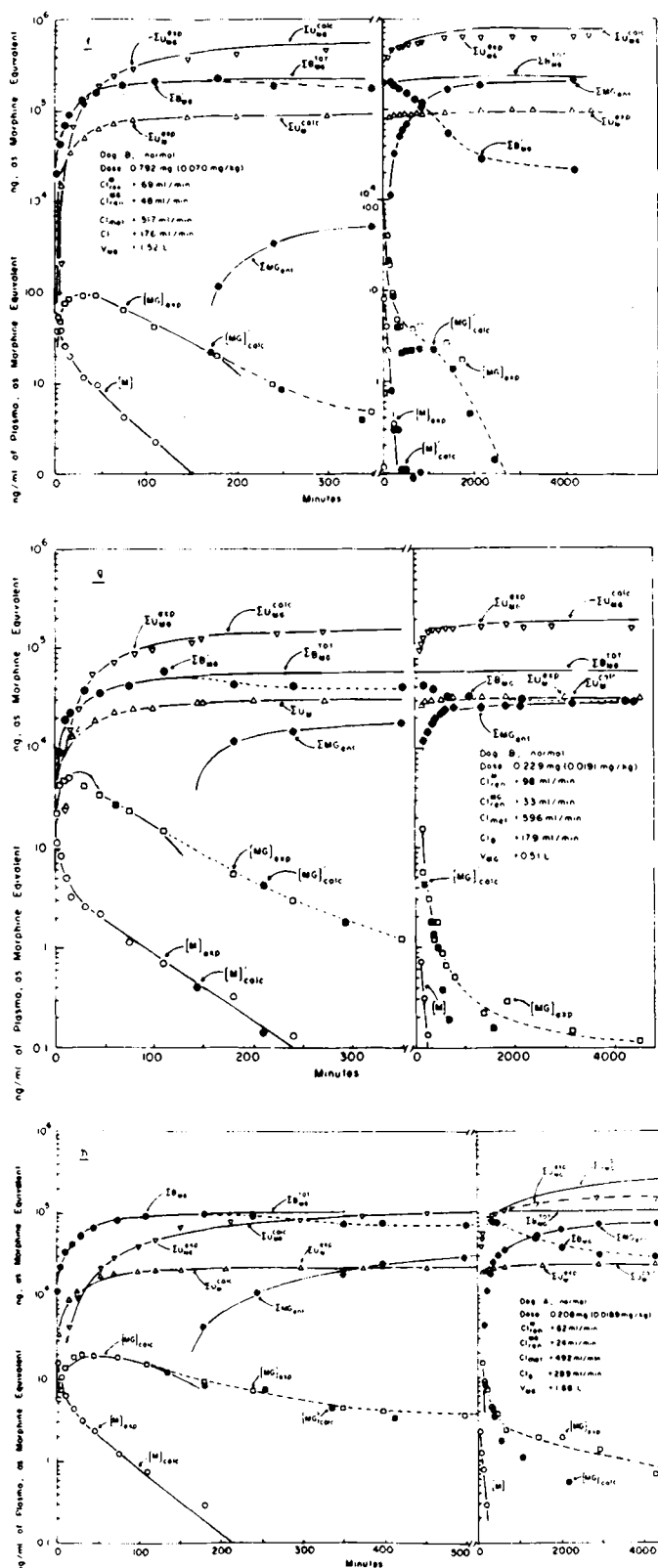
Morphine Pharmacokinetics at Intermediate Doses—The plasma morphine levels, [M], with time in the bile-cannulated Dog C at the 0.406-mg/kg dose were consistent with those observed with the high dose and were best fitted by the two-compartment body model (Fig. 3d and Table I). Again, no significant drug appeared in the feces, and there was no significant terminal plateau in [MG]$_{exp}$ or [MG]$'_{calc}$ values that could be assigned to the return of the conjugate or its precursor, M, from a deep compartment in the bile-cannulated dog. The non-bile-cannulated dog at the same dose (Fig. 3e) showed terminal plasma metabolite elevations and pronounced drug and metabolite in the feces (Table I).

Although the urine flow was negligible up to 75 min after morphine administration in both the bile-cannulated and normal dogs at this dose, the calculated amounts (Eqs. A6 and A8) of ΣU_M and ΣU_{MG} renally excreted after that time were consistent with the experimental data, the obtained renal clearances (Fig. 5) and the total areas (i.e., uncorrected for a time lag in commencement of renal processes) under the morphine and conjugate plasma level–time curves. Thus, in contrast with the higher dose, complete renal shutdown by the drug does not have to be postulated. The delay in renal morphine and conjugate excretion can be attributed solely to the lack of urine flow necessary to eliminate the morphine and metabolite trapped in the tubules.

The solid line, ΣB_{MG}^{calc}, in Fig. 3d through the experimentally obtained cumulative conjugate excreted in the bile, ΣB_{MG}^{exp} (dashed line), obtained from the bile-cannulated dog was calculated from Eq. A22 where the pertinent Cl_{met} and V_{MG} values were obtained by application of Eqs. A11 and A12 (Figs. 8 and 9). The solid line, ΣB_{MG}^{tot}, in Fig. 3e through the calculated (Eq. A22) cumulative conjugate in the biliary system of the noncannulated dog, ΣB_{MG}^{calc}, was estimated on the premises of a constant biliary clearance, Cl_B, of immediately formed metabolite and nonenterohepatic return to the system. The pertinent Cl_{met}, Cl_B, and V_{MG} values were obtained by application of Eqs. A16–A20; the values of the pharmacokinetic parameters are given in Table I. The difference between ΣB_{MG}^{tot} and ΣB_{MG}^{calc} (Eq. A23) estimates the cumulative amounts of drug returned to the systemic circulation, ΣMG_{ent}.

Morphine Pharmacokinetics at Low Doses—The pharmacokinetics at these doses in normal animals (Figs. 4f–4h) were similar to those for the normal animal at the intermediate dose, except that urine flow was not highly inhibited and no tubular entrapment of morphine and/or its metabolite had to be postulated. There was only one minor anomaly in the fitting of these data. Although the calculated (Eq. A8) and experimentally determined amounts of conjugate in the urine, ΣU_{MG}, were reasonably consistent until 500–700 min, less was excreted than anticipated with the presumption of the constant renal clearance, which conformed to the greatest extent of the clearance plots (Fig. 5). This finding could indicate a lessened renal clearance of ΣU_{MG} when the values of [MG] were less than 1.0–2.0 ng/ml of plasma. Alternatively, this result could be an artifact of studies at high morphine specific activities. The terminal values of unextracted radioactivity considered as [MG]$_{exp}$ after ~600 min could contain a higher quantity of radiolabeled impurities or other long-lived undefinable, unextractable morphine metabolites in plasma and could give an anomalous terminal radioactivity elevation that would overestimate the urine metabolite accumulation for a presumed constant clearance.

Renal Clearances and Eliminations of Morphine and Its Metabolite—The morphine and metabolite clearances showed remarkable constancies as long as the renal function was operative and there was adequate urine flow (Fig. 5). There were no significant dose effects on these clearances (Table I), which averaged to significantly different values

and $\Sigma B_{MG} = Cl_B AUC_M$, where $Cl_{met} - Cl_B$ and V_{MG} values were estimated from plots in accordance with Eqs. A16 and A17 (Figs. 8 and 9).

The experimental values of cumulative morphine, $\Sigma U_M^{up}(\triangle)$, and its conjugate, ΣU_{MG}^{up} ($\triangledown$), in urine are also plotted against time. The solid lines through the data points are the theoretical values calculated from $\Sigma U_M = Cl_{ren}^M AUC_M$ and $\Sigma U_{MG} = Cl_{ren}^{MG} AUC_{MG}$. The renal clearances, Cl_{ren}^M and Cl_{ren}^{MG}, were consistent with the values obtained from the slopes of the renal clearance plots of $\Delta U/\Delta t$ versus plasma level (Fig. 5).

Estimates of cumulative conjugate in the bile, $\Sigma B'_{MG} = Cl_{met}^M AUC_M - \Sigma U_{MG}^{up} - V_{MG}$ [MG]$_{exp}$, were based on the Cl_{met} and V_{MG} values obtained from appropriate data plots in accordance with Eqs. A16 and A17 (Figs. 8 and 9). The solid line through these data (●) was based on the assumed biliary clearance obtained from these plots and was calculated from $\Sigma B_{MG}^{tot} = Cl_B AUC_M$. The difference between this total amount of conjugate excreted in the bile, ΣB_{MG}^{tot}, and the amount presumed to remain there, $\Sigma B'_{MG}$, estimates the amount of conjugate enterohepatically recirculated to the systemic circulation (◆), $\Sigma MG_{ent} = \Sigma B_{MG}^{tot} - \Sigma B'_{MG}$.

Terminal values of [M]$_{exp}$ (○) and [MG]$_{exp}$ (□) tend to have larger errors since the liquid scintillation counts approach background. The respective solid symbols are the plasma levels of [M]$_{calc}$ (●) and [MG]$_{calc}$ (■) calculated from the estimated renal clearances and the observed ΣU_M^{exp} and ΣU_{MG}^{up} values, respectively (Eq. A27).

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for Cl_{ren}^M of 85 ± 9 and 41 ± 4 (SEM) ml/min, respectively, in contrast to the respective values of 66 and 80 ml/min cited previously for the dog (3). The morphine clearance with respect to drug unbound to plasma proteins (67%) was 127 ± 13 (SEM) ml/min; the morphine glucuronide showed negligible protein binding.

These differences in renal clearances imply different processes for the drug and its conjugate. Since the calculated renal clearance for inulin (glomerular filtration) for a 12-kg dog is 51 ± 12 ml/min (29), the glucuronide probably was eliminated solely by glomerular filtration and the intravenously administered morphine probably was eliminated by both filtration and tubular secretion.
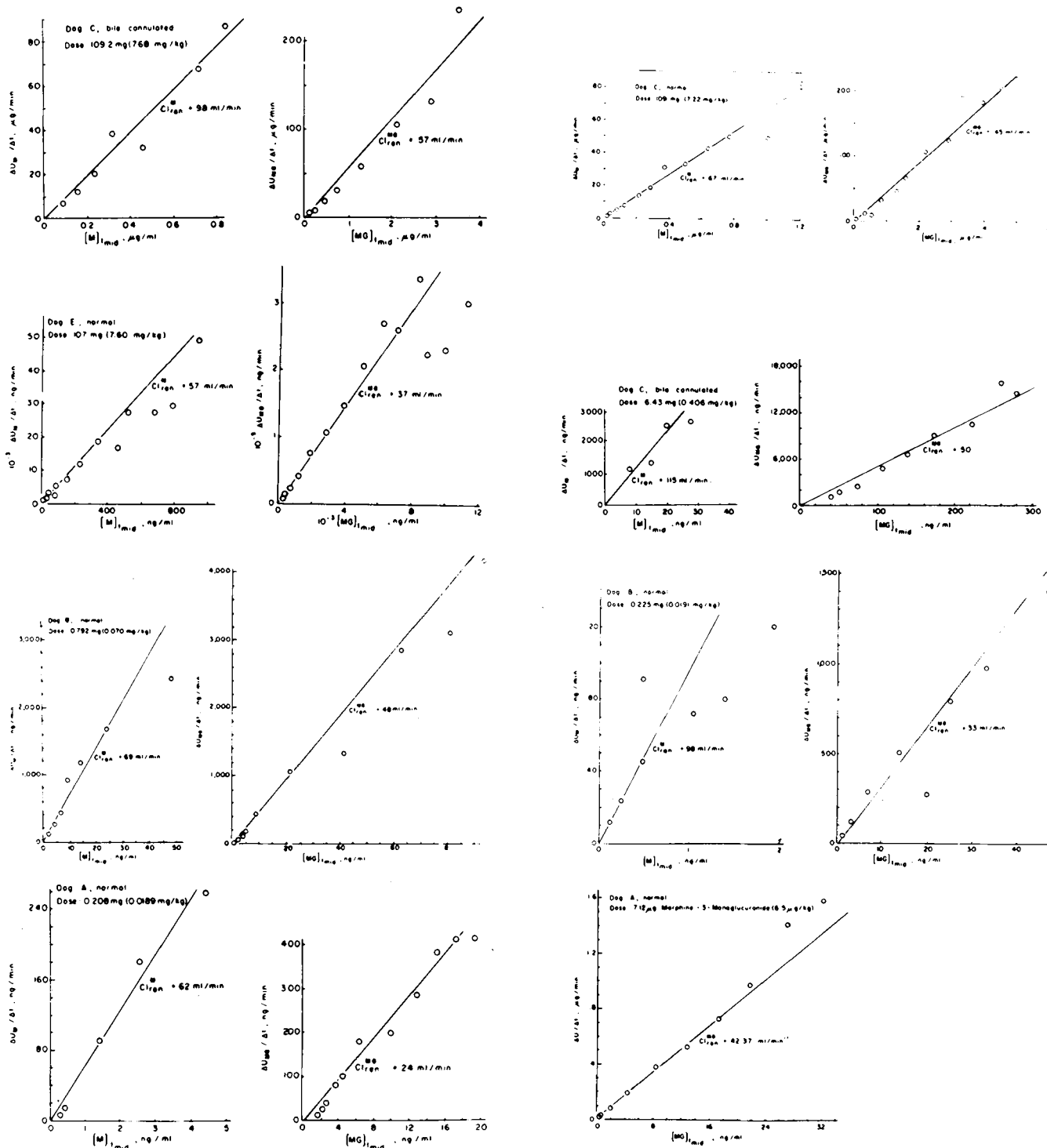
No pronounced differences in fractions of the intravenous dose excreted in the urine were observed among doses (Table I): 12 ± 3 (SD) % as unchanged morphine and 71 ± 10 (SD) % as morphine conjugate.

In the three noncannulated dogs (C, 109 mg; E, 107 mg; and D, 0.471 mg) where feces were collected, 9, 5, and 14% of the total radioactivity were found in the feces, respectively, with 47, 43, and 5% of the excreted amounts extractable as morphine, respectively. These values contrast with those previously reported (9), where negligible conjugate was observed in the feces of dogs. Unfortunately, the possibility of fecal conjugate hydrolysis or fecal morphine degradation does not provide incontrovertible proof of high degrees of GI hydrolysis of biliary secreted conjugate, the major drug form in bile. Less than 0.5% of the total drug excreted into bile was as free morphine in the two studies with the bile-cannulated dog.

The fraction of the administered radioactivity that appeared in the saliva drippings collected from Dog D at the 5.7-mg dose was an extremely low 0.087%.

"Deep" Compartment for Morphine—The plasma morphine levels with time generally could be fit to a sum of two exponentials. However, the data in the normal dog showed a terminal positive deviation in [M]$_{exp}$ from this fitting in some instances (Figs. 2b, 4g, and 4h), indicating the presence of a slower elimination process. The radioactive counts of extracted morphine were too close to background levels to make a definite conclusion. Conclusive demonstration of such a slower elimination process was clearly manifested in urinary morphine elimination, ΣU_M^{exp}. The semilogarithmic plots of amounts yet to be excreted against time (Figs. 10 and 11) showed a terminal phase, γ (Table I), whose half-lives averaged 994 ± 16 (SEM) min for all studies in the normal dog, with no apparent dose dependency. The fact that no such terminal phase was observable with the urinary data (Fig. 10) from the cannulated dog where total bile was collected is strong evidence that this slow morphine elimination is due to the recycling of amounts secreted into the bile. Since negligible free morphine is bile secreted, the conjugate must be gastrointestinally hydrolyzed, either in the lumen or gut wall, before reabsorption into the systemic circulation.

When 3.7 mg of morphine was infused at a constant rate of 10.7 μg/min into a dog, reasonable steady-state values of plasma morphine were achieved by 60 min and maintained during the 350 min of infusion at an average of 57 ± 4 (SEM) ng/ml. At cessation of infusion, there was a

Figure 4—Plots of experimental values of morphine, [M]$_{exp}$ (○), and the morphine conjugate, [MG]$_{exp}$ (□), in nanograms per milliliter of plasma as morphine equivalents against time at low morphine base doses, 0.07 and ~0.019 mg/kg iv. The solid line through the experimental [M] values was calculated from the polyexponential fit, [M] = $Ae^{-\alpha t}$ + $Be^{-\beta t}$. (See Table I for parameters.) The solid line through the experimental [MG]$_{exp}$ values was calculated on the assumption of a constant metabolic morphine clearance, Cl_{met}^M, so that [MG] = ($Cl_{met} AUC_M$ - ΣB_{MG} - ΣU_{MG}^{up})/V_{MG}. The [MG] values were calculated for the normal dog from estimates of $Cl_{met} = Cl_{tot} - Cl_M^{ren}$, where $Cl_{tot} = dose/AUC_M$,

Figure 5—*Renal clearance plots for morphine, M (left), and its conjugate, MG (right), when morphine was administered. Rates of drug amounts excreted versus plasma levels were obtained at the midtimes of the urine-collection intervals. The lower right clearance plot is for morphine-3-glucuronide administration.*

precipitous drop until plasma levels of both morphine [0.45 ± 0.03 (*SEM*) ng/ml of plasma, *n* = 7] and glucuronide [34.1 ± 0.9 (*SEM*) ng/ml of plasma] remained invariant over the studied period from 250 to 850 min after the cessation of the infusion. The plasma morphine levels monitored by liquid scintillation counting were four times the background radiation level. This evidence is considered valid for the slow enterohepatic return of both morphine and its conjugate from bile to show an apparent deep compartment in the dog. The total percent of the dose excreted in the urine was 85.8%, 68.5% as the conjugate and 17.3% as unchanged morphine.

The terminal elevation in plasma and urine morphine glucuronide levels in normal dogs (Figs. 2–4) was in decided contrast to the studies

in the bile-cannulated dog and is proper evidence for enterohepatic recirculation. The lack of morphine and the conjugate in the feces of the cannulated dog, but not in the normal, confirms this hypothesis and agrees with the observations of Woods (8). The conjugate, even when significantly hydrolyzed in the lumen or gut wall, must be largely reconjugated in the liver prior to its return to the systemic circulation for this hypothesis to be true. The return of small amounts of morphine could be responsible for the frequent observation of a slow terminal morphine loss in the plasma. It is estimated (Table I) that 27 ± 0.05 (*SEM*) % of the dose is enterohepatically recirculated at doses less than 0.5 mg/kg. It is possible that the 15% observed in the two studies at 7–8 mg/kg may signify a dose dependency.
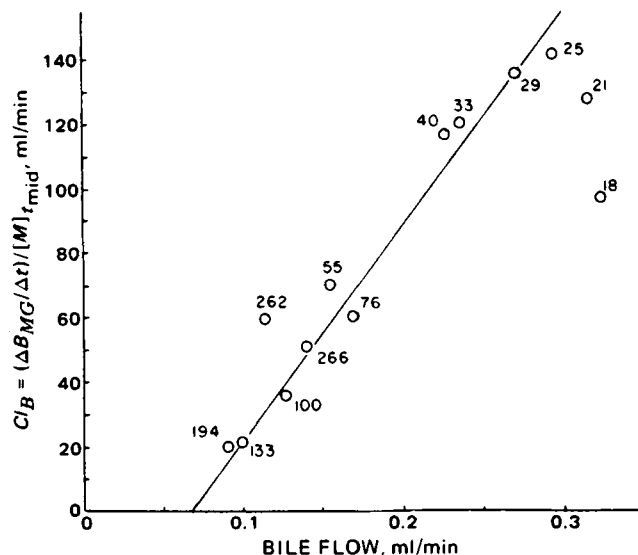
Figure 6—*Demonstration of the linear dependence of the apparent biliary clearance, Cl_B, of immediately formed metabolite, MG, on the rate of bile flow for the bile-cannulated Dog C at the 109.2-mg dose (7.68 mg/kg): Cl_B (ml/min) = 671 × bile flow (ml/min) − 45.6. The values are labeled with the measured times in minutes.*

Dose Effects in Morphine Pharmacokinetics—The anticipated "feedback" effects of morphine's pharmacology (20, 21) on its pharmacokinetics in the dog were observed. The 7.2–7.7-mg/kg doses of morphine caused an instantaneous cessation of renal processes (Figs. 2a–2c) that did not resume until 60–105 min after intravenous administration when the plasma morphine level reached 1000 ng/ml. At these doses, the dogs were completely unconscious for at least 3 hr after administration and lay supine upon the table.

The same doses inhibited biliary secretion for 10–35 min after intravenous administration and demonstrated uneven bile flows and concomitant uneven biliary clearances thereafter (Fig. 6), which readily confirmed the statements (20) that the drug causes biliary tract spasms and constrictions that can impede biliary excretion. The estimated average biliary clearances, Cl_B (Eqs. A16–A20), of formed metabolite were 52 and 87 ml/min at these high doses in the normal dog and 213 ± 36 (SEM) ml/min (range of 176–285) at the lowest doses of 0.019–0.07 mg/kg.

Morphine at the intermediate doses of 0.4–0.5 mg/kg (Figs. 3d and 3e) did not shut down the renal processes but did have a pronounced effect on urine flow. The time courses of the drug and its metabolite could best be fit on the premise of maintained viable renal processes with delayed elimination into the urine. The time courses at the lowest doses studied could be fit (Figs. 4f and 4g) without the postulations of such drug-induced discontinuities in pharmacokinetic processes.

Total morphine clearance is clearly dose related (Table I). At the highest doses of 7.2–7.7 mg/kg, Cl_{tot} averaged 340 ± 43 (SD) for the range of 290–370 ml/min in contrast to the average of 701 ± 138 (SD) for the range of 554–886 ml/min at doses below 0.5 mg/kg. Since renal clearances are relatively invariant with dose, this finding implies that the hepatic metabolism of morphine is dose dependent. The estimated average ± SD (SEM) (range) values for Cl_{met} were 261 ± 41 (17) (215–322) ml/min at the high dose and 609 ± 114 (51) (492–771) at the other doses. The fraction of formed metabolite partitioned into the bile, Cl_B/Cl_{met}, cannot be concluded to be dose dependent, however. About 25% was partitioned into the bile in two high-dose and two low-dose cases.

The hepatic blood flow in the 14-kg dog is in the range of 420–630 ml/min (30) and is the same as the range of metabolic clearances at the low doses. This finding could imply that the high dose of 7 mg/kg decreases blood flow to the liver and/or that the metabolizing enzymes are saturable. However, the fact that negligible plasma morphine levels occur in the dog orally administered 30 mg/kg (9) is indicative of the complete metabolism of orally administered drug by its first pass in the liver even at these enormous doses.

The dose dependencies of total and metabolic clearances are clearly reflected in the apparent rate constants and half-lives for the elimination of the major morphine fraction in the β-phase of the two-compartment body model (Table I). Excellent agreement was obtained with the urinary data for this phase when semilogarithmic plots of amounts yet to be ex-

creted (after correction for the slow terminal phase) and rates of urinary elimination were made against time (Figs. 10 and 11). The half-lives for the high dose (7–8 mg/kg) were 83 ± 6 (SD) min from plasma and 82 ± 8 (SD) min from urine. They were 39 ± 6 (SD) min from plasma and 37 ± 13 (SD) min from urine for the lowest doses.

There were no significant differences among the apparent morphine distribution volumes. The estimated central compartment volume is 15.3 ± 2.8 (SEM) liters, and the overall volume is 44 ± 6 (SEM) liters.

Pharmacokinetics of Morphine-3-monoglucuronide and Its Significance in Morphine Pharmacokinetics—The intravenous administration of ^{14}C-morphine-3-monoglucuronide to the 11-kg Dog A (71.2 μg, 6.5 μg/kg, specific activity of 273 dpm/ng) clearly demonstrated a two-compartment body model (Fig. 12), where the plasma level could be defined for time, t, in minutes as:

$$[MG], \text{ ng/ml} = 33.3e^{-0.245t} + 26.4e^{-1.46 \times 10^{-2}t} \quad \text{(Eq. 4)}$$

and the respective half-lives of the α- and β-phases were 2.8 and 47.6 min, respectively. The semilogarithmic plots of amounts yet to be excreted and amounts per unit of time against time showed respective rate constants of 56 and 51 min, in agreement with the terminal phase. The recovery of the total radioactivity in the urine was complete; the actual calculation was 116%. No free morphine was extractable either from the plasma or urine. The total clearance from the ratio of dose and the area under the curve was 37.1 ml/min, and the renal clearance from the classical plot (Fig. 5) was consistent at 42 ml/min. The conclusion is that renal excretion is the sole route of systemic glucuronide elimination. The apparent overall glucuronide distribution volume from the ratio of the total clearance and the terminal rate constant was 2541 ml, whereas the value from the quotient of the dose and the intercept of the extrapolated β-phase was 2696 ml.

The fact that there was not the prolonged terminal phase in the plasma or urine for the administered glucuronide that appeared when morphine was injected (Figs. 2–4) demonstrates that its appearance is a consequence of a process unique to morphine metabolism. The hepatically conjugated morphine is partitioned into bile in the liver, whereas the systemic conjugate is not. This hepatically formed conjugate is gut hydrolyzed to be slowly reabsorbed as morphine, whereas the systemic conjugate is not. The time lag in the enterohepatic recirculation with concomitant gallbladder storage (3) postpones the return of biliary secreted drug and metabolite to maintain persistent levels in plasma and urine.

The calculated 2500 ml of apparent overall distribution volume in intravenous glucuronide administration, V_{MG}, is consistent with the volumes calculated for morphine doses below 0.5 mg/kg from Eqs. A11, A12, A16, and A17 and validate the premises upon which they were based. The significantly greater calculated volumes of 4200–5050 ml for glucuronide at 7.2–7.7 mg of morphine/kg must be related to the demonstrated extreme pharmacological effects at this dose.

APPENDIX I: METHODS AND PROCEDURES FOR PHARMACOKINETIC ANALYSES

Renal Clearances and Time Durations of Drug Inhibitions of Such Processes—When the drug concentration time course in the plasma, such as morphine [M], can be monitored, the cumulative drug excreted unchanged into the urine (ΣU_M) and bile (ΣB_M) and metabolized into a metabolite such as morphine glucuronide (ΣMG) at a time t can be estimated from the respective clearances Cl^M_{ren}, Cl^M_B, and Cl^M_{met}, provided that these clearances are not plasma level dependent. Thus, the cumulative amounts excreted at time t would be:

$$\Sigma U_M = Cl^M_{ren} \int_{t_0}^{t} [M]dt = Cl^M_{ren} AUC^{t-t_0}_M \quad \text{(Eq. A1)}$$

$$\Sigma MG = Cl^M_{met} \int_{t_0}^{t} [M]dt = Cl^M_{met} AUC^{t-t_0}_M \quad \text{(Eq. A2)}$$
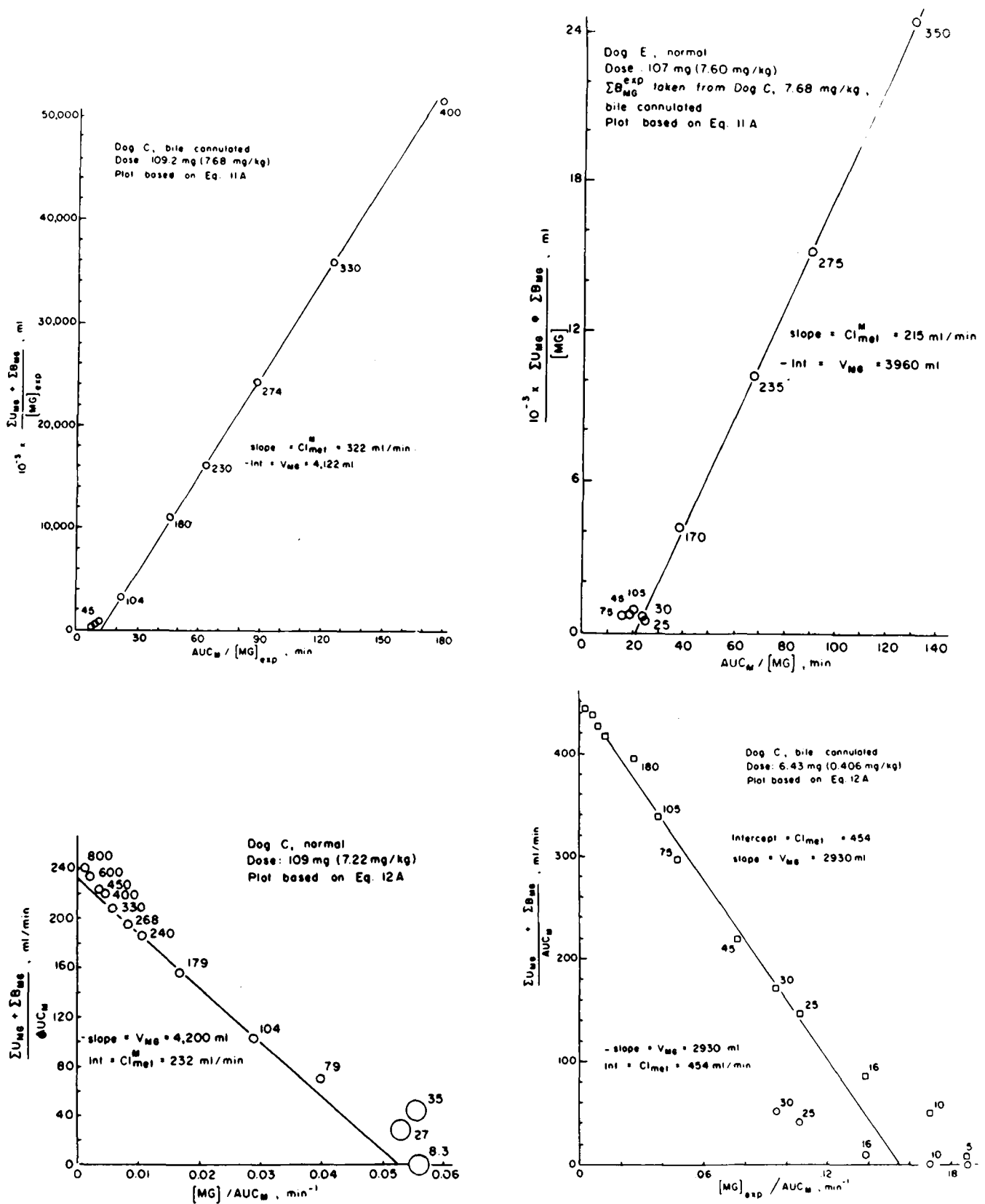
$$\Sigma B_M = Cl^M_B \int_{t_0}^{t} [M]dt = Cl^M_B AUC^{t-t_0}_M \quad \text{(Eq. A3)}$$

where AUC_M is the needed area under the morphine plasma level–time curve from t_0 to t, where t_0 is the time of onset of the specific clearance process. These equations are a consequence of first-order differential equations such as:

$$d(MG)/dt = k_{M,MG}V[M] = Cl_{met}[M] \quad \text{(Eq. A4)}$$

which, on integration, gives:

$$\Sigma MG = \int_0^{MG} d(MG) = Cl^M_{met} \int_{t_0}^{t} [M]dt$$

$$= Cl^M_{met} AUC^{t-t_0}_M \quad \text{(Eq. A5)}$$

Dog C, bile connulated
Dose 109.2 mg (7.68 mg/kg)
Plot based on Eq. IIA

$10^{-3} \times \dfrac{\Sigma U_{MG} + \Sigma B_{MG}}{[MG]_{exp}}$, ml

50,000

40,000

30,000

20,000

10,000

0

400
330
274
230
180
104
45

slope $= Cl_{met}^{M} = 322$ ml/min
$-Int = V_{MG}^{M} = 4,122$ ml

$AUC_{M} / [MG]_{exp}$, min

0   30   60   90   120   150   180

Dog E, normal
Dose 107 mg (7.60 mg/kg)
ΣB_{MG}^{exp} **taken from Dog C, 7.68 mg/kg,**
bile connulated
Plot based on Eq. IIA

$10^{-3} \times \dfrac{\Sigma U_{MG} + \Sigma B_{MG}}{[MG]}$, ml

24

18

12

6

0

350
275
235
170
105
48
75
30
25

slope $= Cl_{met}^{M} = 215$ ml/min
$-Int = V_{MG} = 3960$ ml

$AUC_{M} / [MG]$, min

0   20   40   60   80   100   120   140

Dog C, normal
Dose 109 mg (7.22 mg/kg)
Plot based on Eq. 12A

$\dfrac{\Sigma U_{MG} + \Sigma B_{MG}}{AUC_{M}}$, ml/min

240
200
160
120
80
40
0

800
600
450
400
330
268
240
179
104
79
35
27
8.3

$-$ slope $= V_{MG}^{M} = 4,200$ ml
$Int = Cl_{met}^{M} = 232$ ml/min

$[MG]_{exp} / AUC_{M}$, min^{-1}

0   0.01   0.02   0.03   0.04   0.05   0.06

Dog C, bile connulated
Dose 6.43 mg (0.406 mg/kg)
Plot based on Eq. 12A

$\dfrac{\Sigma U_{MG} + \Sigma B_{MG}}{AUC_{M}}$, ml/min

400
300
200
100
0

180
105
75
45
30
25
30
25
16
10
16
10
5

Intercept $= Cl_{met}^{M} = 454$
slope $= V_{MG} = 2930$ ml

$-$ slope $= V_{MG} = 2930$ ml
$Int = Cl_{met}^{M} = 454$ ml/min

$[MG]_{exp} / AUC_{M}$, min^{-1}

0   .06   .12   .18

Figure 7—*Data for dogs administered morphine in accordance with the premise that the amounts of cumulative conjugate excreted in the bile, ΣB_{MG}^{exp}, were monitored (Eqs. A11 and A12). The ΣB_{MG} values used in the normal dogs were taken from the studies on the bile-cannulated Dog C at the high dose. The values are labeled with the times at which the AUC_M and $[MG]_{exp}$ values were taken. The ΣU_{MG}^{exp} and ΣB_{MG}^{exp} values were estimated for these same times from interpolations in the respective plots of their values against time. The experimental values at the earlier times in these plots are expected to be aberrant because inadequate urine flow at the high systemic drug levels did not permit proper estimates of ΣU_{MG} values. Deviations of experimental values at the later times in the non-bile-cannulated dogs were due to the perturbations effected by enterohepatic recirculation that gave estimates of ΣU_{MG}^{exp} and $[MG]_{exp}$ in excess of those anticipated by the premises underlying the equation used, which did not take such delayed return to the system into account.*
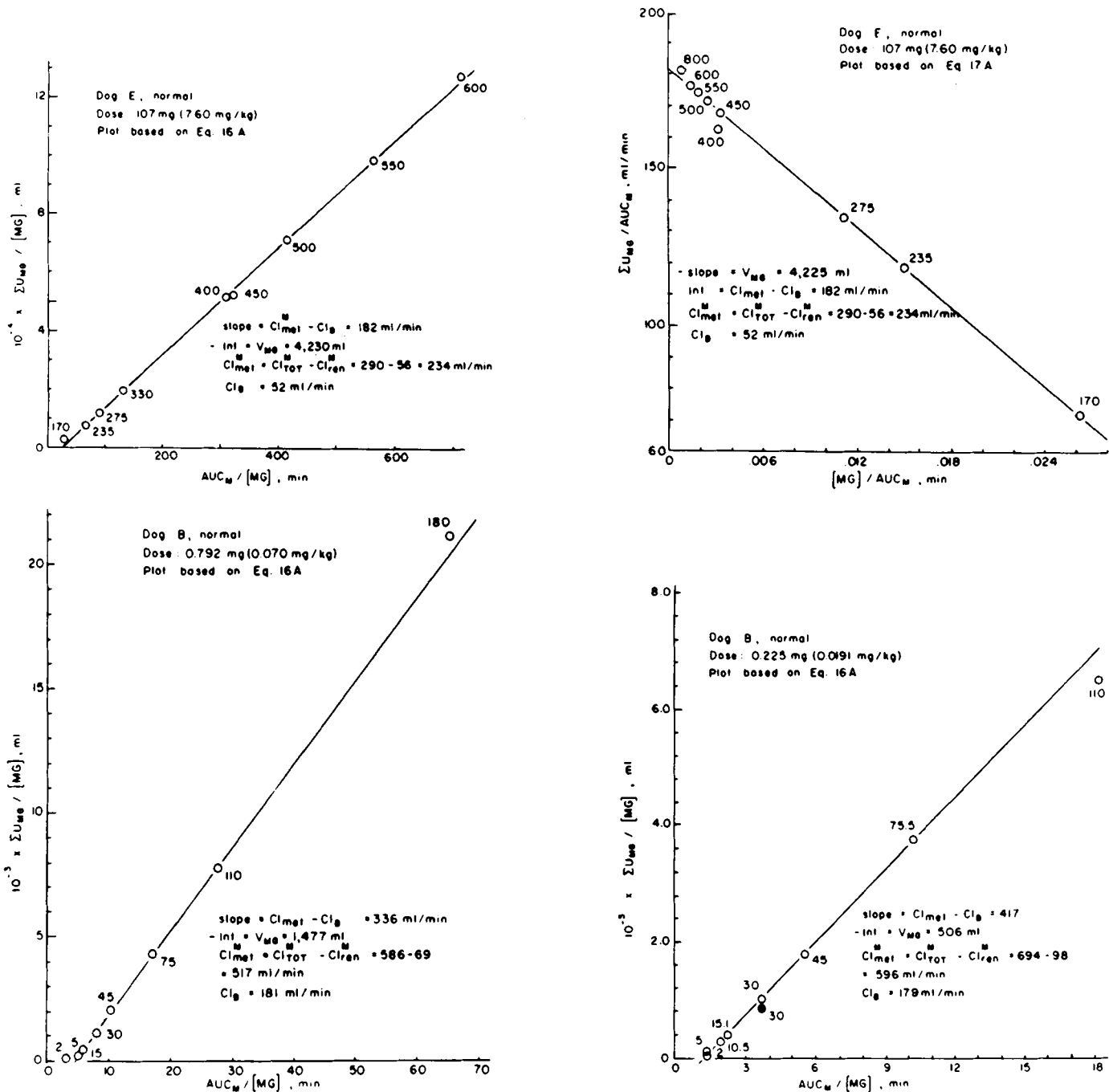
Figure 8—*Data for normal, non-bile-cannulated dogs in accordance with* $\Sigma U_{MG}^{exp}/[MG]_{exp} = (Cl_{met} - Cl_B) (AUC_M/[MG]_{exp}) - V_{MG}$ *(Eq. A16) or Eq. A17 on the premises that biliary clearance,* Cl_B, *of the metabolite is a constant fraction of the metabolic clearance,* Cl_{met}, *of morphine and that there is a negligible time lag in biliary process initiations. The values are labeled with the times at which the* AUC_M *and* $[MG]_{exp}$ *values were taken. The* ΣU_{MG}^{exp} *values were estimated for these same times from interpolations in plots of these values against time. Deviations of experimental values at the later times are due to the perturbations effected by enterohepatic recirculation that gave estimates of* ΣU_{MG}^{exp} *and* $[MG]_{exp}$ *in excess of those anticipated by the premises underlying Eq. A16, which did not take such delayed return to the system into account.*

The metabolism of morphine to the conjugate is apparently initiated at a time, t_0, of zero since there appears to be no lag in conjugate appearance, $[MG]$, in plasma at any studied dose (Figs. 2–4).

However, the renal excretion of the drug and conjugate at high morphine doses (Figs. 2 and 10) may be inhibited by the action of the drug so that:

$$\Sigma U_M = Cl_{ren}^M \left(\int_0^t [M]dt - \int_0^{t_0} [M]dt \right)$$

$$= Cl_{ren}^M (AUC_M^t - AUC_M^{t_0}) \quad \text{(Eq. A6)}$$

where the parenthetical expression represents the difference between the total area under the plasma morphine concentration–time curve up
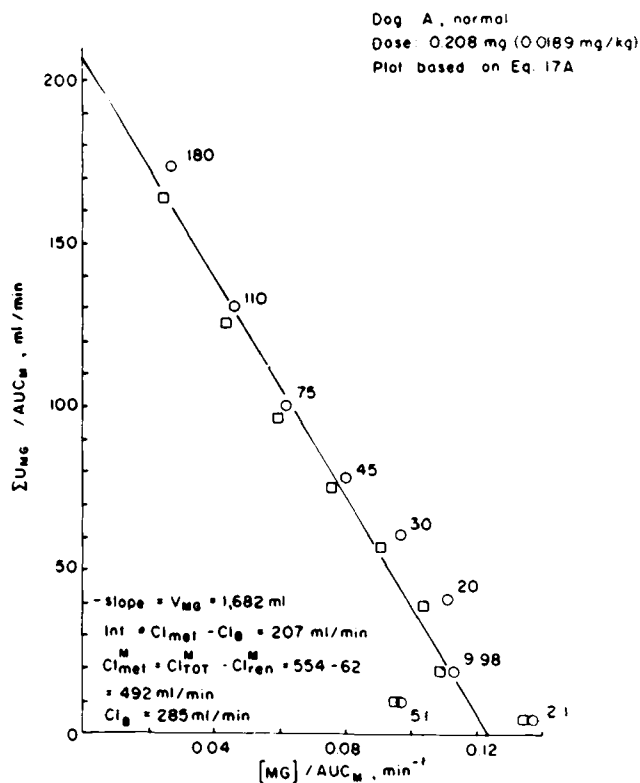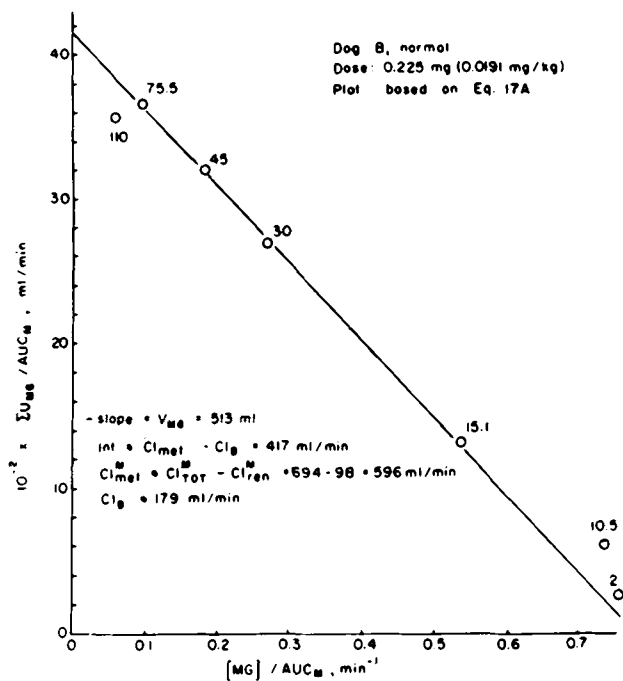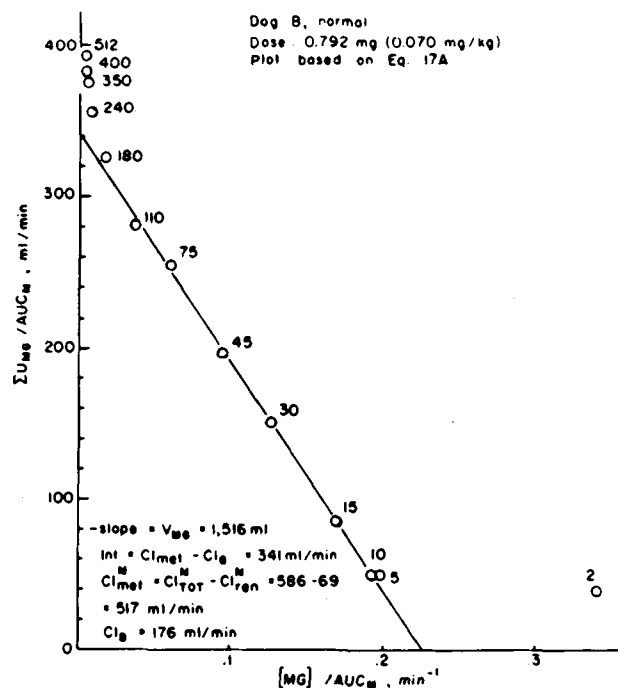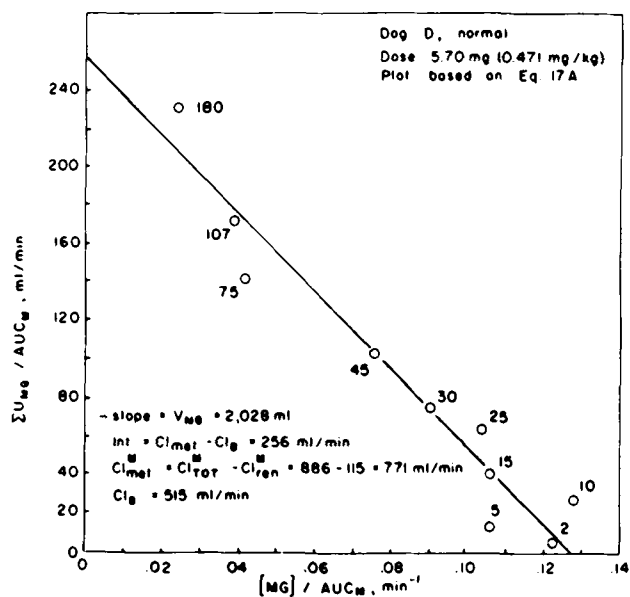
to time t and the area up to time t_0 when the renal process commenced.

As a consequence of:

$$dU_{MG}/dt = Cl_{ren}^{MG} [MG] \quad \text{(Eq. A7)}$$

the cumulative renal excretion of the metabolite (ΣU_{MG}) would be related to a similar difference in the areas under the plasma metabolite concentration–time curve:

$$\Sigma U_{MG} = Cl_{ren}^{MG} \left(\int_0^t [MG]dt - \int_0^{t_0} [MG]dt \right)$$

$$= Cl_{ren}^{MG}(AUC_{MG}^t - AUC_{MG}^{t_0}) = Cl_{ren}^{MG}(AUC_{MG}^{t-t_0}) \quad \text{(Eq. A8)}$$

Figure 9—*Data for normal, non-bile-cannulated dogs in accordance with $\Sigma U^{exp}_{MG}/AUC_M = V_{MG} ([MG]_{exp}/AUC_M) + Cl_{met} - Cl_B$ (Eq. A17) on the premises that biliary clearance, Cl_B, of the metabolite is a constant fraction of the metabolic clearance, Cl_{met}, of morphine and that there is a negligible time lag in biliary process initiation. The values are labeled with the times at which the AUC_M and $[MG]_{exp}$ values were taken. The ΣU^{exp}_{MG} values were estimated for those same times from interpolations in plots of these values against time. Deviations of experimental values at the later times are due to the perturbations effected by enterohepatic recirculation that gave estimates of ΣU^{exp}_{MG} and $[MG]_{exp}$ in excess of those anticipated by the premises underlying Eq. A17, which did not take such delayed return to the system into account. The values plotted for Dog A (0.208-mg dose) are based on two estimates of AUC_M: that calculated from the best-fit sum of exponentials (O) and that estimated by the trapezoidal rule (□) for $[M]_{exp}$ values with time.*

The clearances can be estimated from the slopes of $\Delta U_M/\Delta t$ ($\sim dU_M/dt$) versus $[M]_{t_{mid}}$ and $\Delta U_{MG}/\Delta t$ ($\sim dU_{MG}/dt$) versus $[MG]_{t_{mid}}$ in accordance with relations such as Eq. A7 where the plasma concentrations are taken at the midtime of the monitored excretory interval, Δt. Such anticipated linear plots were obtained (Fig. 5) and permitted the estimation of plasma level-independent renal clearances (Table I).

Thus, the values of ΣU_M and ΣU_{MG} could be generated from Eqs. A6 and A8. The appropriate choice of a time, t_0, for the renal process commencement should permit the generated values to agree with the experimental (Figs. 2-4).

It is possible that an amount of morphine in bile, ΣB_M, could be generated by a similar equation with a time of commencement of such a
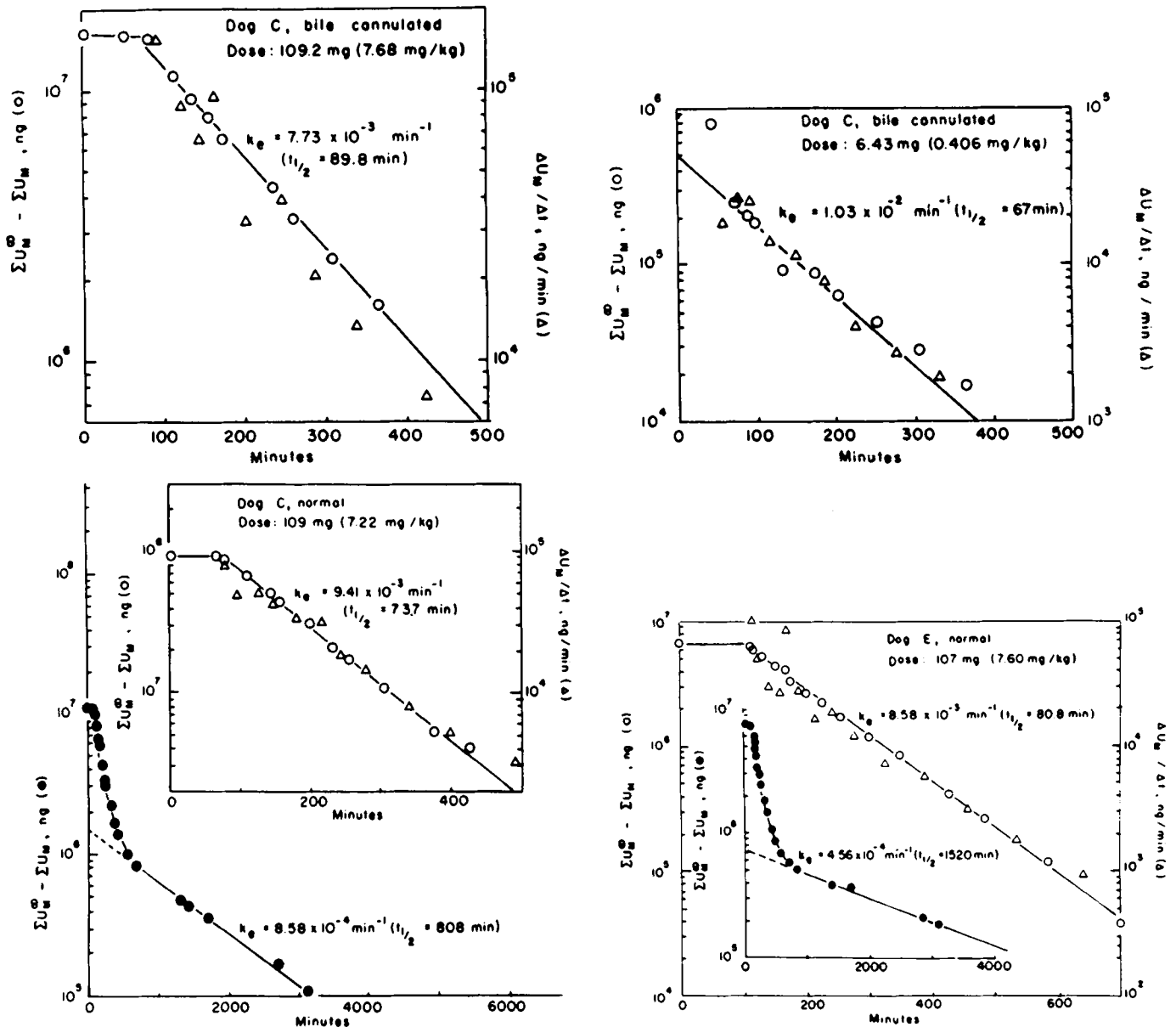
Figure 10—*Estimation of first-order rate constants, k_e, of urinary elimination from semilogarithmic plots of unchanged morphine not yet excreted, $\Sigma U_M^\infty - \Sigma U^M$ ($\bigcirc$, $\bullet$), and rates of elimination, $\Delta U_M/\Delta t$ ($\triangle$), against time in the bile-cannulated dog and at high doses in the normal dog. The former were plotted over the entire time interval ($\bullet$) and then plotted according to the method of residuals ($\bigcirc$). The time delay in elimination can be clearly noted.*

process at time t_0 (Eq. A3). However, the total amount of unchanged morphine that was experimentally determined in the bile was less than 0.005 of the dose in the two bile cannulation studies and was considered negligible.

Evaluation of Metabolic Clearances of Morphine in Bile-Cannulated Animal—The challenging of the validity of Eq. A2 for metabolite formation is not as simple. The metabolic clearance, Cl_{met}^M, is not readily obtained since the total metabolite produced, ΣMG, at a given time has to be constructed from the sums of amounts of the metabolite in the body, bile, and urine, respectively:

$$\Sigma MG = V_{MG}[MG]_{exp} + \Sigma B_{MG}^{exp} + \Sigma U_{MG}^{exp} \qquad (\text{Eq. A9})$$

where V_{MG} is the apparent metabolite distribution volume on the postulation of instantaneous equilibration in the body fluids on its formation.

In the intact animal, ΣB_{MG} at a given time and V_{MG} are not readily obtainable. The ΣB_{MG}^{exp} value can be estimated from studies on a bile-cannulated dog. Substitution of Eq. A9 into Eq. A2 on the presumption of a constant metabolic clearance starting at time $t_0 = 0$ gives:

$$V_{MG}[MG]_{exp} + \Sigma B_{MG}^{exp} + \Sigma U_{MG}^{exp} = Cl_{met}^M AUC_M \qquad (\text{Eq. A10})$$

which can be rearranged to:

$$\frac{\Sigma U_{MG} + \Sigma B_{MG}}{[MG]} = Cl_{met}^M \frac{AUC_M}{[MG]} - V_{MG} \qquad (\text{Eq. A11})$$

or:

$$\frac{\Sigma U_{MG} + \Sigma B_{MG}}{AUC_M} = -V_{MG}\frac{[MG]}{AUC_M} + Cl_{met}^M \qquad (\text{Eq. A12})$$

Thus, plots of the experimentally available $(\Sigma U_{MG} + \Sigma B_{MG})/[MG]$ or $(\Sigma U_{MG} + \Sigma B_{MG})/AUC_M$ against $AUC_M/[MG]$ or $[MG]/AUC_M$, respectively, can permit the estimation of V_{MG} and Cl_{met}^M values (Table I) from the slope and intercept of such plots (Fig. 7). The validity of these values can be ascertained by generating $[MG]_{calc}$ from a modification of Eq. A11 or A12:

$$[MG]_{calc} = \frac{\Sigma MG - \Sigma B_{MG}^{exp} - \Sigma U_{MG}^{exp}}{V_{MG}}$$

$$= \frac{Cl_{met}^M AUC_M - \Sigma B_{MG}^{exp} - \Sigma U_{MG}^{exp}}{V_{MG}} \qquad (\text{Eq. A13})$$

and the resultant calculated values of plasma metabolite concentration can be compared to those obtained experimentally, $[MG]_{exp}$ (Figs. 2–4).
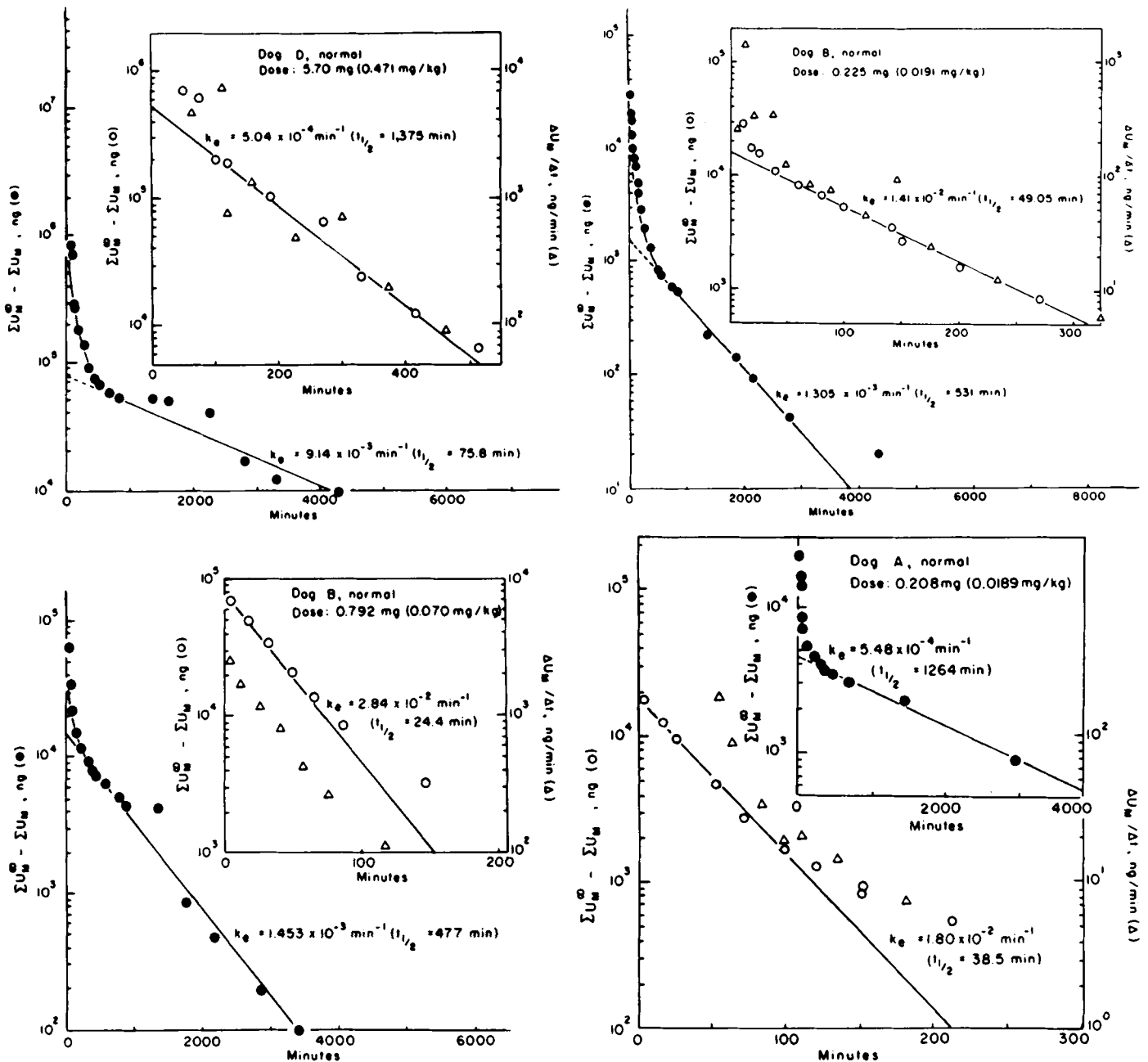
Figure 11—*Estimation of first-order rate constants, k_e, of urinary elimination from semilogarithmic plots of unchanged morphine not yet excreted, $\Sigma U_M - \Sigma U^M$ (O, ●), and rates of elimination, $\Delta U_M/\Delta t$ (△), against time at intermediate and low doses in the normal dog. The former were plotted over the entire time interval (●) and then plotted according to the method of residuals (O).*

Alternatively, the experimental values of metabolite concentration can be used to estimate the amounts of the metabolite, $\Sigma'B^{calc}_{MG}$, bile excreted from these estimates of Cl^M_{met} and V_{MG} (Eqs. A11 and A12) since:

$$\Sigma B^{calc}_{MG} = \Sigma MG - \Sigma U^{exp}_{MG} - V_{MG}[MG]_{exp}$$
$$= Cl^M_{met} AUC_M - \Sigma U^{exp}_{MG} - V_{MG}[MG]_{exp} \quad \text{(Eq. A14)}$$

Thus, the coincidences of $\Sigma'B^{calc}_{MG}$ with ΣB^{exp}_{MG} values and $[MG]_{calc}$ with $[MG]_{exp}$ can confirm or deny the validity of these postulates.

Evaluation of Metabolic Morphine Clearances in the Non-Bile-Cannulated Animal—If the amount of conjugate excreted in the bile is not specifically known, a constant biliary clearance, Cl_B, of hepatically formed conjugate can be postulated and Eq. A10 can be modified to:

$$V_{MG}[MG]_{exp} + Cl_B AUC_M + \Sigma U^{exp}_{MG} = Cl_{met} AUC_M \quad \text{(Eq. A15)}$$

where $Cl_B AUC_M/\Sigma MG = Cl_B/Cl_{met}$ is the fraction of conjugate formed in the liver that is partitioned immediately into the bile. If the biliary process is drug affected, a more accurate definition of the second term

would be $Cl_B(AUC'_M - AUC^{t_0}_M)$, where $AUC^{t_0}_M$ is the area under the plasma morphine level curve up to the time, t_0, of initiation of the biliary processes.

Thus, Eq. 15A can be rearranged to:

$$\frac{\Sigma U^{exp}_{MG}}{[MG]_{exp}} = (Cl_{met} - Cl_B)\frac{AUC_M}{[MG]_{exp}} - V_{MG} + \frac{Cl_B AUC^{t_0}_M}{[MG]_{exp}}$$

(Eq. A16)

or:

$$\frac{\Sigma U^{exp}_{MG}}{AUC_M} = - V_{MG}\frac{[MG]_{exp}}{AUC_M} + (Cl_{met} - Cl_B) + \frac{Cl_B AUC^{t_0}_M}{AUC_M}$$

(Eq. A17)

where the last term of both Eqs. A16 and A17 vanishes if there is no time lag in the initiation of the biliary process.

In the absence of such a time lag and the maintenance of a constant biliary partitioning of formed metabolite, V_{MG} and $I = Cl_{met} - Cl_B$ can
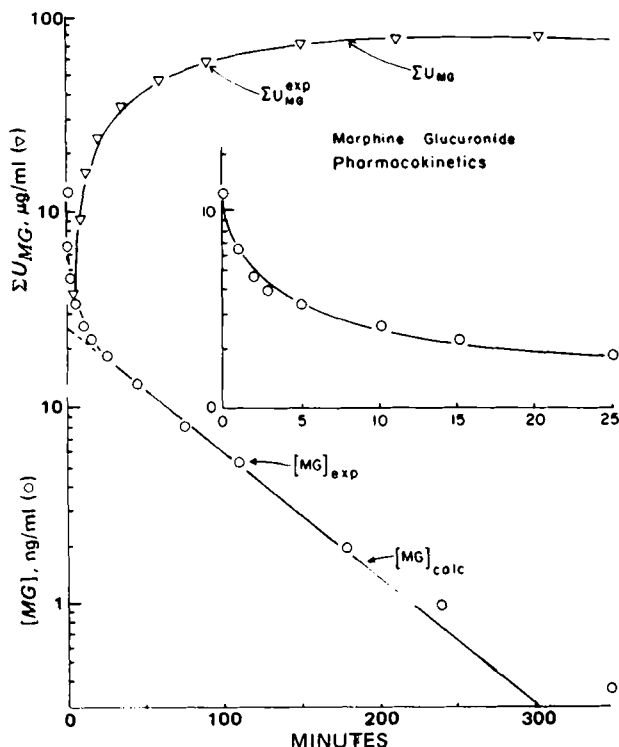
Figure 12—*Experimental values of morphine-3-monoglucuronide, [MG]$_{exp}$ (O), in nanograms per milliliter of plasma against time at an intravenous dose of 71.2 µg (6.5 µg/kg) to Dog A. The solid line through the experimental values was calculated from the polyexponential fit of Eq. 4. The solid line through the experimental values of cumulative conjugate in the urine, ΣU_{MG}^{exp}, are the theoretical values calculated from $\Sigma U_{MG}^{calc} = Cl_{ren}^{MG} AUC_{MG}$ for a renal clearance of 40 ml/min.*

be obtained from the slopes and intercepts of appropriate data plots in accordance with Eqs. A16 (Fig. 8) and A17 (Fig. 9), where $AUC_M^{t0} = 0$. If the total clearance of morphine can be estimated from:

$$Cl_{tot} = dose/AUC_M \qquad \text{(Eq. A18)}$$

then:

$$Cl_{met} = Cl_{tot} - Cl_{ren}^M \qquad \text{(Eq. A19)}$$

and:

$$Cl_B = Cl_{met} - I = Cl_{met} - (Cl_{met} - Cl_B) \qquad \text{(Eq. A20)}$$

The premises for the absolute validity of these relations of Eqs. A18–A20 demand constant metabolic, biliary, and renal clearances of the drug and metabolite and no time lags in their initiation.

The levels of the conjugate $[MG]_{calc}$ in the normal dog also can be estimated from Eq. A13. However, the cumulative amount of formed metabolite totally secreted in the bile must be calculated from:

$$\Sigma B_{MG}^{tot} = Cl_B AUC_M \qquad \text{(Eq. A21)}$$

The $[MG]_{calc}$ and $[MG]_{exp}$ values will diverge if and when there is significant enterohepatic recirculation of the conjugate secreted into the bile.

Evaluation of Extent of Enterohepatic Recirculation of Morphine Metabolite (MG)—The biliary partition of the hepatically formed conjugate can be calculated in the normal animal from the determined Cl_B value by Eq. A21, which gives the amount secreted into the bile.

On the postulation of stoichiometry being preserved, the *actual* amount of conjugate in the biliary system would be:

$$\Sigma B_{MG}^{calc} = \Sigma MG_{formed}^{tot} - \Sigma U_{MG}^{exp} - MG_{body}$$
$$= Cl_{met} AUC_M - \Sigma U_{MG}^{exp} - V_{MG} [MG]_{exp} \qquad \text{(Eq. A22)}$$

The difference between the calculated values of Eqs. A21 and A22 estimates the amount of conjugate at any given time that has been enterohepatically recirculated into the systemic circulation:

$$\Sigma MG_{ent} = \Sigma B_{MG}^{calc} - \Sigma B_{MG}^{'calc} \qquad \text{(Eq. A23)}$$

Estimation of Low Values of Morphine and Its Metabolite in Plasma

—The estimates of the experimentally determined terminal plasma values of morphine, $[M]_{exp}$, and its conjugate, $[MG]_{exp}$, tend to have large errors since their estimates by liquid scintillation counting approach background. Alternative estimates are $[M]_{calc}$ and $[MG]_{calc}$, which can be determined from the estimated renal clearances, Cl_{ren}^M and Cl_{ren}^{MG}, and the experimentally determined values of cumulative urinary excretions, ΣU_M^{exp} and ΣU_{MG}^{exp}.

The area under the plasma level–time curve at time $t_{(i+1)}$ can be estimated from the quotient of the cumulative drug in the urine at that time and the renal clearance:

$$AUC_{t_{(i+1)}} = (\Sigma U)_{t_{(i+1)}}/Cl_{ren} = AUC_{t_i} + \Delta AUC \qquad \text{(Eq. A24)}$$

This area is the sum of a known area, AUC_{t_i}, where the plasma concentration is C_{t_i} at time t_i and an increase in area, ΔAUC, which can be defined as:

$$\Delta AUC = AUC_{t_{(i+1)}} - AUC_{t_i} = \left(\frac{C_{t_i} + C_{t_{(i+1)}}}{2}\right) \Delta t$$
$$= \frac{C_{t_i} + C_{t_{(i+1)}}}{2} (t_{(i+1)} - t_i) \qquad \text{(Eq. A25)}$$

Thus, the plasma drug concentration at time $t_{(i+1)}$ can be estimated from:

$$C_{t_{i+1}} = \frac{2(\Delta AUC)}{t_{(i+1)} - t_i} - C_{t_i} \qquad \text{(Eq. A26)}$$

so that, on consideration of Eq. A24:

$$C_{t_{i+1}} = \frac{2[(\Sigma U)t_{(i+1)}/Cl_{ren} - AUC_{t_i}]}{(t_{(i+1)} - t_i)} - C_{t_i} \qquad \text{(Eq. A27)}$$

where $C_{t_{i+1}}$ can be $[M]_{calc}$ or $[MG]_{calc}$ plasma values at t_{i+1}. It is necessary to have one initial experimental set of values of C_{t_1} and AUC_{t_1} at time t_1 to substitute into Eq. A27 for the first calculation of a plasma concentration of C_{t_2} at t_2 from $(\Sigma U)_{t_2}$ and t_2. Subsequent calculations of $C_{t_3}, C_{t_4}, \ldots, C_{t_{(i+1)}}$ can then be made in accordance with Eq. A27 since prior $C_{t_2}, C_{t_3}, \ldots, C_{t_i}$ and AUC_{t_i} values were available. The new AUC_{t_i} can be estimated by adding the calculated ΔAUC obtained from Eq. A25 to the prior estimated AUC at a previous time, $AUC_{(t_{i-1})}$. Plasma values obtained in this manner tended to oscillate unless averaged. Thus:

$$\overline{C}_{t_i} = \frac{C_{t_{(i+1)}} + C_{t_i}}{2} \qquad \text{for } t_i' = \frac{t_{(i+1)} + t_i}{2} \qquad \text{(Eq. A28)}$$

$$\overline{C}_{t'_{(i+1)}} = \frac{C_{t_{(i+2)}} + C_{t_{(i+1)}}}{2} \qquad \text{for } t'_{(i+1)} = \frac{t_{(i+2)} + t_{(i+1)}}{2} \qquad \text{(Eq. A29)}$$

etc., can provide monotonically decreasing values of $[M]_{calc}$ and $[MG]_{calc}$ with time with minimum scatter about a smooth curve drawn through the points.

APPENDIX II: GLOSSARY OF SYMBOLS

Concentrations of morphine, M, and morphine conjugate, MG, are given as nanograms of equivalent morphine base in plasma, whereas amounts in urine or bile are given as nanograms of equivalent morphine base.

$[M]_{exp}$—experimental morphine concentration per milliliter of plasma.

$[M]_{calc}$—morphine concentration per milliliter of plasma calculated from experimental values of $\Sigma U_{M_{(i+1)}}$ at a time, $t_{(i+1)}$, from a known renal clearance, Cl_{ren}^M, and the known AUC_M and $[M]$ values of previous time, t_i (Eq. A27).

$[M]$—morphine concentration per milliliter of plasma calculated at a time, t, from a best-fitted linear sum of exponentials, $[M] = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$.

$[MG]_{exp}$—experimental morphine conjugate concentration per milliliter of plasma.

$[MG]_{calc}$—morphine conjugate concentration per milliliter of plasma as calculated from experimental values of $\Sigma U_{MG_{(i+1)}}$ at a time, $t_{(i+1)}$, from a known renal clearance, Cl_{ren}^{MG}, and the known AUC_{MG} and $[MG]$ values at a previous time, t_i (Eq. A27).

$[MG]$ or $[MG]_{calc}$—morphine conjugate concentration per milliliter of plasma as calculated from the difference between the cumulative amount of conjugate generated, ΣU_{MG}, and the experimental cumulative amounts of conjugate in bile and urine and divided by a volume of distribution, V_{MG} (Eq. A13). In the non-bile-cannulated dog, the

cumulative amount of conjugate in the bile must be calculated on the assumption of a constant biliary clearance of metabolized morphine (Eq. A21).

ΣMG or ΣMG^{tot}—cumulative morphine conjugate produced by metabolic morphine clearance, Cl_{met}^{M} (Eqs. A2, A5, and A9).

ΣU_{M}^{exp}—cumulative experimental morphine excreted in urine.

ΣU_{M}^{calc}—calculated cumulative morphine excreted in urine, based on renal clearance, Cl_{ren}^{M}, of plasma morphine (Eqs. A1 and A6).

ΣU_{MG}^{exp}—cumulative experimental morphine conjugate excreted in urine.

ΣU_{MG}^{calc}—calculated cumulative morphine conjugate excreted in urine, based on renal clearance, Cl_{ren}^{MG}, of plasma conjugate (Eq. A8).

ΣB_{MG}^{exp}—experimental cumulative morphine conjugate excreted in bile in the bile-cannulated dog.

ΣB_{MG}^{calc} or ΣB_{MG}^{tot}—calculated total cumulative morphine conjugate excreted in bile, based on biliary clearance, Cl_B, of metabolized morphine (Eqs. A3 and A21).

$\Sigma B_{MG}^{'calc}$—actual morphine conjugate in the biliary system calculated from the difference in the total formed, ΣMG, and the amounts of conjugate experimentally determined in the urine and in the body (Eq. A22).

ΣMG_{ent}—conjugate presumably enterohepatically recirculated from the bile to the systemic circulation as estimated from the difference between ΣB_{MG}^{calc} and $\Sigma B_{MG}^{'calc}$ (Eq. A23).

ΣMG_{feces}^{exp}—experimental conjugate excreted in feces.

ΣMG_{feces}^{theo}—conjugate calculated to be in feces based on the difference in calculated total conjugate formation and total amount excreted in urine.

ΣU^{∞} or ΣB^{∞}—total drug or metabolite in urine or bile at infinite time.

Cl_{tot}^{M}—total morphine clearance, in milliliters per minute (Eq. A18).

Cl_{ren}^{M}—renal morphine clearance, in milliliters per minute, basically estimated from $\Delta\Sigma U_M/\Delta t/[M]_{t\,mid}$.

Cl_{ren}^{MG}—renal morphine conjugate clearance, in milliliters per minute, basically estimated from $\Delta\Sigma U_{MG}/\Delta t/[MG]_{t\,mid}$.

Cl_{met} or Cl_{met}^{M}—metabolic morphine clearance, in milliliters per minute, which can be estimated directly in the bile-cannulated dog (Eqs. A11 and A12) and indirectly in the non-bile-cannulated dog (Eq. A19).

Cl_B or Cl_B^{M}—biliary clearance of a morphine conjugate fraction formed by morphine metabolism, in milliliters per minute, which can be estimated from $\Delta\Sigma B_{MG}^{exp}/\Delta t/[M]_{exp}$ in the bile-cannulated dog and indirectly estimated on the assumption of dose independence in the non-bile-cannulated dog (Eqs. A16, A17, and A20). The biliary clearance of systemic morphine conjugate is not presumed.

V_C^{M}—apparent distribution volume, in liters, of central compartment referenced to morphine base concentration in plasma.

V_d^{M}—apparent overall distribution volume, in liters, referenced to morphine base concentration in plasma.

V_{extrap}^{M}—apparent distribution volume, in liters, referenced to morphine based concentration in plasma on assumption of instantaneous equilibration in body fluids.

V_{MG}—apparent overall distribution volume, in liters, for the formed conjugate, MG, of morphine (Eqs. A11, A12, A16, and A17).

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