Pharmacokinetics of Morphine Injected Intravenously into the Anesthetized Dog

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This study determined the disposition of morphine in plasma and cerebrospinal fluid (CSF) and its relationship to ventilatory depression. ^{14}C-Morphine sulfate (0.3 mg/kg or 2.0 mg/kg as the base) was injected intravenously into dogs anesthetized with enflurane–N$_2$O–O$_2$. Arterial plasma, cisternal CSF, and urine were analyzed for unchanged morphine and morphine metabolites. By 10 min after injection, 97 per cent of the injected morphine had been cleared from plasma. The terminal elimination half-times for morphine in plasma were not significantly different for the two doses and averaged 75 ± 5 (SEM) min. The volumes of distribution were significantly greater for 0.3 mg/kg than for 2.0 mg/kg morphine; this difference appeared to be related to the lesser hypotension following the smaller dose. Metabolism of morphine was rapid and efficient; metabolites were apparent in plasma by 1.5 min. Morphine conjugates, primarily morphine glucuronide, accounted for more than 90 per cent of the radioactivity in plasma at two hours. By six hours, 66 ± 3 per cent of the administered ^{14}C radioactivity had been recovered in the urine, but only 13 ± 2 per cent had been excreted as unchanged morphine. Morphine concentrations in CSF did not peak until 15–30 min after injection, and the terminal half-time was significantly greater than that of morphine in plasma. Ventilatory depression was prolonged, and its abatement did not correlate with the decline of morphine concentrations in either plasma or CSF. It is concluded that morphine was rapidly cleared from the plasma by its uptake into tissues and by its efficient conjugation with glucuronic acid. Ultimate elimination of the drug was primarily by renal excretion of conjugated morphine. Uptake of morphine into CSF was delayed and its elimination from CSF was prolonged in comparison with its clearance from plasma. The ventilatory depressant effects of morphine were not directly related to its concentration in either CSF or plasma. (Key words: Analgesics, narcotic: morphine. Anesthetics, intravenous: morphine. Pharmacokinetics: distribution. Ventilation: depression. Cerebrospinal fluid.)

MORPHINE in small, analgesic doses has long enjoyed popularity as an anesthetic supplement, and in the past decade it has served as a primary anesthetic agent in doses of 1–3 mg/kg. The relationship of dose size to duration of action is of concern to the anesthe-siologist, especially in regard to ventilatory depression in the postoperative period. The effect of dose size on pharmacokinetics of morphine has not been determined. Also, the relationship between morphine concentration in plasma and the intensity of its ventilatory effect has not been characterized, while data relating analgesic effects to plasma concentrations of morphine after intravenous administration are contradictory.[1–8]

This study was designed to determine the pharmacokinetics of analgesic and anesthetic doses of morphine in the dog. Radioactively labelled morphine and solvent extraction were used to provide sensitivity and specificity in the analysis of biologic specimens for their content of the pharmacologically active, unchanged drug.

Methods and Materials

Ten mongrel dogs, weighing 10 to 21 kg ($\bar{x}$ 14 ± 3 SD), were divided into two groups, one receiving 0.3 mg/kg and the other receiving 2 mg/kg of ^{14}C-morphine (base) intravenously. Anesthesia was induced in all animals with enflurane, 3 per cent, in nitrous oxide (3 l/min) and oxygen (2 l/min) via a mask and Bain anesthesia circuit. Following induction, succinylcholine (0.1–0.3 mg/kg) and atropine sulfate (0.02–0.07 mg/kg, $\bar{x}$ 0.05 ± 0.02 SD) were injected intravenously and a cuffed oral endotracheal tube was inserted. Anesthesia was maintained with enflurane, 2.5 per cent (end-tidal concentration), in nitrous oxide (N$_2$O) and oxygen (3:2).

The six dogs given 0.3 mg/kg morphine were allowed to ventilate spontaneously. The endotracheal tube was connected by a Rovenstine connector to a Vortex respirometer¶ in series with the Bain circuit to measure tidal volume and minute ventilation. The dogs given 2 mg/kg were ventilated with a Harvard respirator (model 607) to maintain Pa$_{CO_2}$ at 41 ± 2 torr (range 35–45 torr for both Pa$_{CO_2}$ and PA$_{CO_2}$). In all dogs the end-tidal gas mixture was analyzed for carbon dioxide and enflurane by separate Beckman LB-2 infrared spectrometers. Atelectasis was minimized by intermittent sighing, and Pa$_{O_2}$ remained above 97 torr throughout the experiment.

A cannula was inserted in a foreleg vein and dex-

¶ Ohio Medical Products, Madison, Wisconsin.

trose, 5 per cent, in lactated Ringer's solution was administered at a rate of 15 ml·kg^{-1}·hr^{-1} for the first hour and then at 10 ml·kg^{-1}·hr^{-1} for the duration of the experiment. Bilateral femoral arterial cannulas were utilized for continuous blood pressure recording and periodic sampling of blood. When blood was withdrawn it was immediately replaced with an equal volume of Plasmanate** (5 per cent human plasma protein fraction), injected intravenously. The pharyngeal temperature was maintained at 37 ± 1° C. The electrocardiogram was monitored. Urine was collected from a transuretheral catheter. Cisternal cerebrospinal fluid was collected through a Hustead-Touhy needle inserted at the base of the skull.

N-methyl-^{14}C-morphine hydrochloride†† (57 mCi/mmol) and unlabelled morphine sulfate were dissolved in distilled water to produce a final concentration of 0.5 per cent morphine base (2 μCi/mg). All doses and concentrations are presented as morphine base (1 mg base = 1.33 mg sulfate salt). Radiochemical purity was determined to be at least 98 per cent by paper chromatography in two solvent systems: n-propanol, n-butanol, 0.1 N NH$_4$OH (2:1:1, v/v) and n-butanol, glacial acetic acid, distilled water (4:1:2, v/v). ^{14}C-Morphine sulfate was injected intravenously over a 30-sec period in a single dose (0.3 or 2 mg/kg). Samples of blood, cerebrospinal fluid (CSF), and urine were collected at specific intervals over the next six hours.

Data are expressed as the mean ± 1 standard error of the mean unless designated otherwise. Student's t test was used for group comparisons, with $P < 0.05$ as the minimal limit of significance. "Morphine" hereafter refers to the unchanged ^{14}C-labelled drug. "Total radioactivity" refers to both unchanged morphine and its ^{14}C-labelled metabolites. "Conjugated morphine" refers to the difference between morphine concentrations in samples analyzed for the unchanged drug before and after acidic hydrolysis, which releases morphine from its glucuronide and ethereal sulfate conjugates.[9] See the Appendix for a description of the analytical methods.

Results

Examination of the results of a typical experiment (fig. 1) reveals that 97 per cent of the injected dose had been cleared from plasma in the first 10 min after the injection. Thereafter, the decline of morphine concentrations was more gradual. The terminal elimination phase (indicated by the final linear portion of the curve and described by β) was not apparent

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†† Amersham Corp., Chicago, Illinois.

until two to three hours after injection, and had an average half-time of 75 ± 5 min for all ten dogs. The elimination of morphine from plasma of the dog following a single intravenous injection of either 0.3 or 2 mg/kg could be described by a triexponential equation. The exponential rate constants did not differ significantly for the two doses except for the α phase (table 1; $P < 0.05$ for 0.3 vs. 2 mg/kg).

Plasma concentrations of morphine were not proportional to dose; a 6.7-fold increment in dosage produced an approximately tenfold increase in plasma levels. This disproportionality is reflected in the calculated volumes of distribution (table 2). It should be noted that both the volume of the central compartment (V_1) and the overall volume of distribution (V_d) were smaller for 2 mg/kg than for 0.3 mg/kg morphine.

In relation to the differences in distribution volumes between 0.3 and 2 mg/kg morphine, it should be noted that the incidence and severity of hypotension were greater in the dogs given the larger dose (fig. 2). Those dogs given 2 mg/kg morphine sustained 72 ± 5 per cent reductions in mean arterial pressure during the first 5 min after morphine administration. By 10 min the pressure had returned to 60 per cent of that observed before morphine administration. By 60 min and thereafter, the pressure remained stable at a level approximately 20 per cent below pre-morphine levels. Dogs anesthetized with enflurane and receiving 0.3 mg/kg morphine had only a 12 ± 9 per cent decline in blood pressure (i.e., to 88 per cent of control), with blood pressure remaining at approximately that reduced level for the duration of the experiment.

The analytic procedure for unchanged morphine measured both free and protein-bound drug in plasma. Additions of ^{14}C-morphine to dog plasma in vitro followed by equilibrium dialysis for 20 hours at pH 7.3–7.5 resulted in the binding of 14.0 ± 0.7 per cent of the morphine in final concentrations of 20 to 2,000 ng/ml of plasma.

The results of this study can be summarized as a three-compartment model (fig. 3). In this model morphine is introduced directly into the central compartment (V_1) by intravenous injection. From the central compartment morphine is distributed to two peripheral compartments (V_2,V_3) at a rate represented by the rate constants k_{12} and k_{13}, respectively (table 2). Morphine is assumed to be eliminated from the body by way of the central compartment, primarily by biotransformation in the liver, at a rate represented by k_{el}. The rate constant k_{31} representing return of morphine from peripheral compartment V_3 to the central compartment was lower than the rate constant k_{el}, $P < 0.001$.

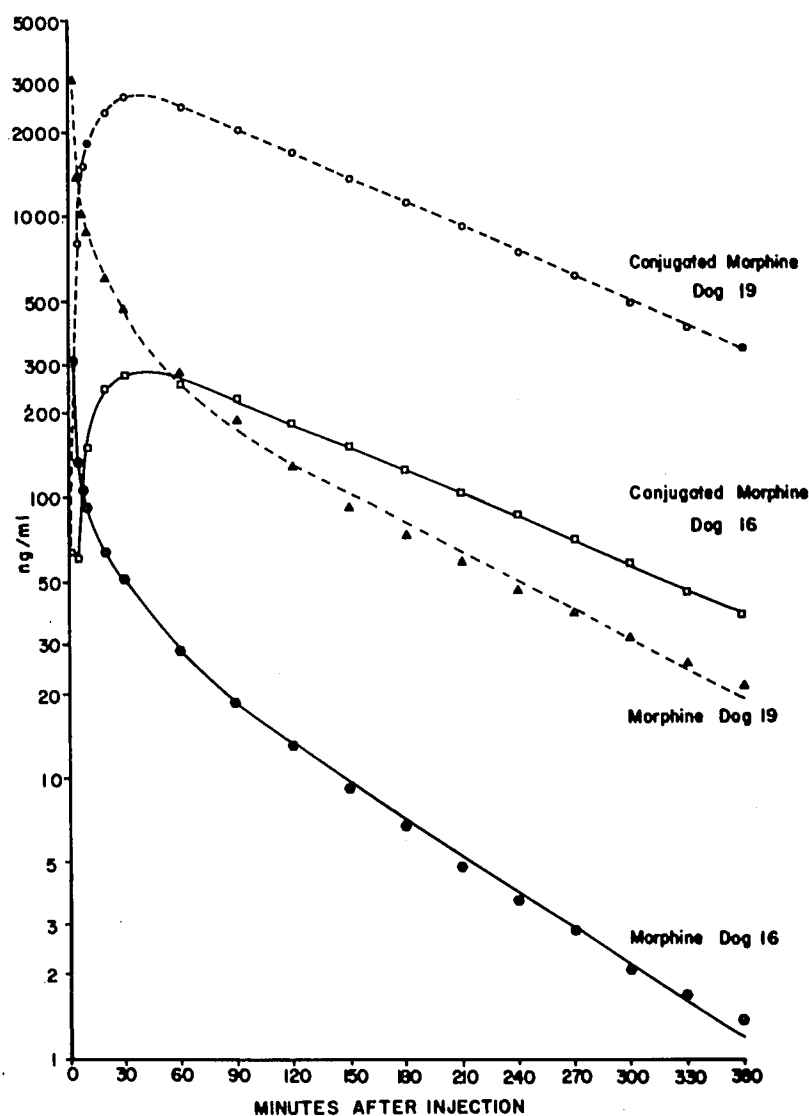Most of the radioactivity in plasma 10 min or more

FIG. 1. Plasma levels of unchanged and of conjugated morphine following the intravenous injection of 0.3 mg/kg (Dog 16) or 2.0 mg/kg (Dog 19). Lines were fitted to actual data points by least-squares analysis. Each data point represents the average of results of duplicate analyses of the sample. See Appendix.

after the intravenous injection of ^{14}C-morphine represented metabolites of the drug, primarily conjugated morphine (fig. 1). Conjugated morphine was present in the earliest samples of plasma, taken 1.5–2.5 min after injection, and its concentration increased rapidly to a maximum between 20 and 90 min after the injection. Subsequently, the elimination of conjugated morphine from plasma was monoexponential, with a half-time of 115 ± 21 min in the eight dogs for which the determinations were made. There was no difference between the rates of elimination of conjugated morphine from the plasmas of dogs receiving 0.3 and those receiving 2 mg/kg ^{14}C-morphine. Beyond two hours after the injection, conjugated morphine accounted for more than 90 per cent of the ^{14}C radioactivity in plasma.

Within the first six hours after an intravenous injection of either 0.3 or 2 mg/kg, 66 ± 3 per cent of the administered dose was recovered in urine as unchanged and conjugated morphine, which together accounted for all of the ^{14}C radioactivity in urine (table 3). It is evident that renal excretion of the unchanged drug contributed relatively little to the overall elimination of a single dose. Although the total recovery of morphine in urine did not differ significantly, the proportion excreted as the unchanged drug was less for the larger dose (table 3). Further analysis of urines from two dogs by paper chromatography and by incubation with β-glucuronidase showed that glucuronide conjugation was by far the principal pathway of biotransformation of morphine (table 4). Less than 5 per cent of the ^{14}C radio-

TABLE 1. Pharmacokinetic Parameters for Elimination of Intravenously Administered Morphine from Plasma of Anesthetized Dogs: $C_{p(t)} = P_{exp}^{-\pi t} + A_{exp}^{-\alpha t} + B_{exp}^{-\beta t*}$

	Weight (kg)	P (ng/ml)	π (min^{-1})	A (ng/ml)	α (min^{-1})	B (ng/ml)	β (min^{-1})	r^2†
Morphine, 0.3 mg/kg								
Dog 13	12.2	512	.58	52	.040	37	.0111	.999
Dog 16	15.0	577	.59	85	.052	45	.0101	.999
Dog 18	15.5	471	.51	79	.081	30	.0110	.999
Dog 23	11.0	354	.48	87	.060	22	.0066	.999
Dog 28	12.6	471	.60	92	.065	39	.0108	.999
Dog 29	13.4	260	.31	29	.044	21	.0089	.988
Mean		441	.51	71	.057‡	32	.0097	
SEM		47	.04	10	.006	4	.0007	
Morphine, 2.0 mg/kg								
Dog 10	9.6	5,067	.56	805	.046	341	.0115	.999
Dog 17	9.9	2,457	.41	426	.026	168	.0068	.999
Dog 19	21.0	5,818	.56	927	.055	363	.0082	.999
Dog 21	15.8	3,291	.71	623	.029	558	.0114	.999
Mean		4,159	.56	695	.039‡	358	.0094	
SEM		776	.06	109	.007	80	.0012	

* See Appendix.
† $r^2 = [\Sigma$ (observed)$^2 - \Sigma$ (deviation)$^2]/\Sigma$ (observed)^2.

‡ $P < 0.05$.

activity on the chromatograms was unaccounted for as either morphine or morphine glucuronide; of the total amount of conjugated morphine present in urine, all but 1.1–2.4 per cent was hydrolyzed by β-glucuronidase.

Morphine was detectable in CSF within 2–5 min after an intravenous injection (fig. 4). Peak concentrations were observed between 15 and 30 min after injection in all dogs. Thereafter, morphine levels in CSF declined at a slower rate than did those in plasma, so that morphine concentrations in CSF exceeded those in plasma 30 min after injection. The CSF-to-plasma concentration gradient increased progressively through the remainder of the experiment, and was approximately 3 to 1 after six hours. The half-time for the terminal elimination phase of morphine in CSF (121 ± 6 min, table 5) was significantly longer than that for plasma (75 ± 5 min; $P < 0.00005$).

The times to the first appearance of ^{14}C-metabolites

of morphine in CSF ranged from as early as the first samples drawn at 2.5–5 min to as late as 60 min, when all animals had detectable levels. Absolute concentrations of metabolites were extremely small. In terms of morphine equivalents, the metabolites had reached concentrations of 63 ± 7 and 5.5 ± 1.0 ng/ml six hours after 2.0 and 0.3 mg/kg, respectively, and represented 63 ± 4 per cent of the total ^{14}C radioactivity in CSF at six hours for all animals in which metabolites were determined.

Ventilatory depression was evident almost immediately after the intravenous injection of morphine, 0.3 mg/kg, in enflurane–N_2O-anesthetized dogs breathing spontaneously. Both tidal volume and ventilatory frequency decreased, and there was a 47 per cent reduction in minute ventilation 58 ± 9 min after injection. These changes in ventilation were reflected by a progressive rise of end-tidal carbon dioxide ($P_{ET_{CO_2}}$) through the first hour after the injection

TABLE 2. Kinetic Parameters Calculated for the Disposition of Intravenously Administered Morphine in Anesthetized Dogs*

	$t_{1/2}$ (min)			$C_{p(0)}$ (ng/ml)	V_1 (l/kg)	V_d (l/kg)	Cl_B (ml·kg^{-1}·min^{-1})	k_{el}	k_{12}	k_{21}	k_{13}	k_{31}
	π	α	β									
Morphine, 0.3 mg/kg (n = 6)												
Mean	1.4	13†	74	544	0.59	6.1†	57†	.100	.243	.135	.081†	.021
SEM	0.2	1	9	57	0.08	0.8	5	.007	.034	.014	.009	.001
Morphine, 2.0 mg/kg (n = 4)												
Mean	1.3	19†	77	5,212	0.43	3.8†	33†	.083	.319	.143	.046†	.017
SEM	0.2	3	10	904	0.08	0.9	4	.013	.047	.022	.017	.002

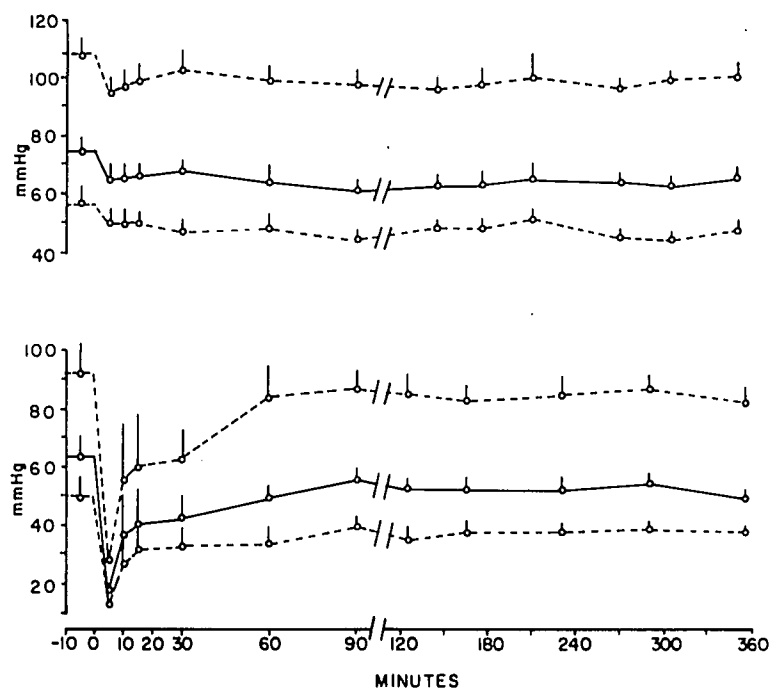* See Appendix for definition of symbols and equations for calculating parameters.

† $P < 0.05$.

FIG. 2. Systolic, mean, and diastolic blood pressures in dogs receiving a single intravenous injection of morphine, 0.3 mg/kg (upper, n = 6), or 2 mg/kg (lower, n = 4), injected at time 0. Each data point represents the mean + SEM for all animals at each dose.

(fig. 4). Subsequently, recovery from the ventilatory depressant effects of morphine was evident, but the rate of recovery did not parallel the decline of morphine in either CSF or plasma.

Discussion

Despite the importance of intravenously administered morphine as an anesthetic supplement and primary anesthetic agent, there have been few studies of its pharmacokinetics under anesthetic conditions. This study compared the pharmacokinetics of morphine administered intravenously in a range of doses (0.3–2.0 mg/kg) commonly used by anesthetists. The low dose, comparable to those used to supplement volatile

anesthetics, was chosen to demonstrate the relationship of the disposition of morphine to a measurable pharmacologic effect, respiratory depression, while the higher dose was chosen to represent "anesthetic doses" of morphine.

The elimination of morphine from plasma had three distinct phases in the six hours studied after its intravenous administration (table 1). The first (or "π") phase was evident in previously published studies of one dog[10] and two men.[11] It probably was missed in other studies because of the lack of frequent blood sampling at the early times after intravenous injection.[1,12-16] Both the first (π) and the second (α) phases
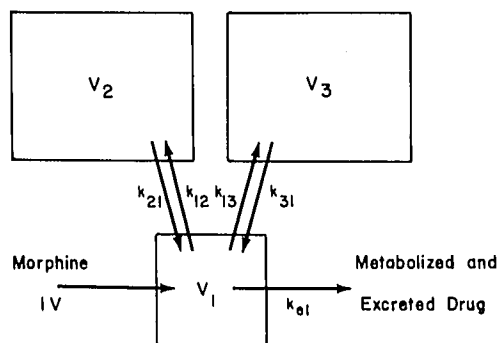

FIG. 3. Three compartment open model of morphine disposition. The intravenously administered drug equilibrates between the central (V_1) and peripheral compartments (V_2, V_3) but is eliminated only from the central compartment. See text for definition of abbreviations.

TABLE 3. Excretion of ^{14}C-Morphine in Urine Collected in the First Six Hours Following an Intravenous Injection in Dogs (Mean ± SEM)

	Percentage of Dose			
	Unchanged Morphine	Morphine Conjugates*	Total Morphine*	Total ^{14}C*
Morphine, 0.3 mg/kg (n = 6)	15.8 ± 2.3†	52 ± 3	68 ± 4	55 ± 7
Morphine, 2.0 mg/kg (n = 4)	6.9 ± 2.6†	56 ± 2	63 ± 3	61 ± 2

* Total morphine was determined by acidic hydrolysis followed by solvent extraction. Morphine conjugates were calculated as the difference between total morphine and unchanged morphine. Total ^{14}C radioactivity was determined by counting an untreated sample of urine and the counts converted to apparent morphine. The differences between total ^{14}C and total morphine are attributable to methodologic variations.

† p < 0.05.

represent primarily distribution of morphine to various tissues. It is expected that the distribution process would be nearly complete in four to five half-times. Thus, the earliest phase would be complete within 10 min. The second phase would be complete within one hour in the dogs receiving the low dose of morphine and in approximately one and a half hours in those receiving the high dose. The longer $t_{1/2\alpha}$ after the higher dose may reflect the alterations in tissue perfusion associated with hypotension, which was greater after 2 mg/kg than after 0.3 mg/kg. Specific experiments are needed to resolve this apparent discrepancy; however, it should be noted the blood pressure had normalized one hour after the 2 mg/kg dose (fig. 2), so the only apparent hemodynamic differences between the two doses occurred during the distributive phases.

The half-times of the third ("β") elimination phases were not significantly different for the two doses of morphine, and averaged 75 min. This value is close to those reported by Nishitateno et al.[12] (62 min) and by Finck et al.[13] (65 min) for dogs anesthetized with pentobarbital. Garrett and Gürkan[10] reported a half-time of 49 min after intravenous injection of approximately 1 mg/kg morphine into one unanesthetized dog. Terminal elimination half-times between 1.5 and 2.5 hours have been reported for human subjects,[1,11,14,15] including surgical patients studied under conditions comparable to those of the present study.[16]

TABLE 4. Characterization of ^{14}C-Radioactive Compounds in Urine Collected in the First Six Hours Following an Intravenous Injection of ^{14}C-Morphine*

Method	Percentage of Total Radioactivity in Sample		
	^{14}C-Morphine	^{14}C-Morphine Glucuronide	Unaccounted-for [14]C Activity
Dog 16 (0.3 mg/kg)			
Chromatography			
Acidic solvent	25.9	69.8	4.3
Basic solvent	24.2	71.8	4.0
Hydrolysis			
Acidic–autoclaving	26.0	74.0	—
β-Glucuronidase	27.1	72.9	—
Dog 19 (2 mg/kg)			
Chromatography			
Acidic solvent	17.0	77.7	5.3
Basic solvent	18.5	77.4	4.1
Hydrolysis			
Acidic–autoclaving	17.7	82.3	—
β-Glucuronidase	20.1	79.9	—

* See Appendix for details.

There were differences in the distribution volumes of morphine observed in this study, as well as in those reported by other investigators. The basis of these differences is not certain, but the following points should be considered. The difference in V_d between dogs receiving 0.3 and those receiving 2 mg/kg morphine during enflurane anesthesia may reflect either dose-dependent kinetics (e.g., saturation of a tissue uptake

FIG. 4. Cerebrospinal fluid and plasma levels of morphine and end-tidal CO_2 (P_{ETCO_2}) in six dogs given a 0.3 mg/kg dose intravenously and allowed to ventilate spontaneously. Each point and vertical line represents the mean ± SEM.
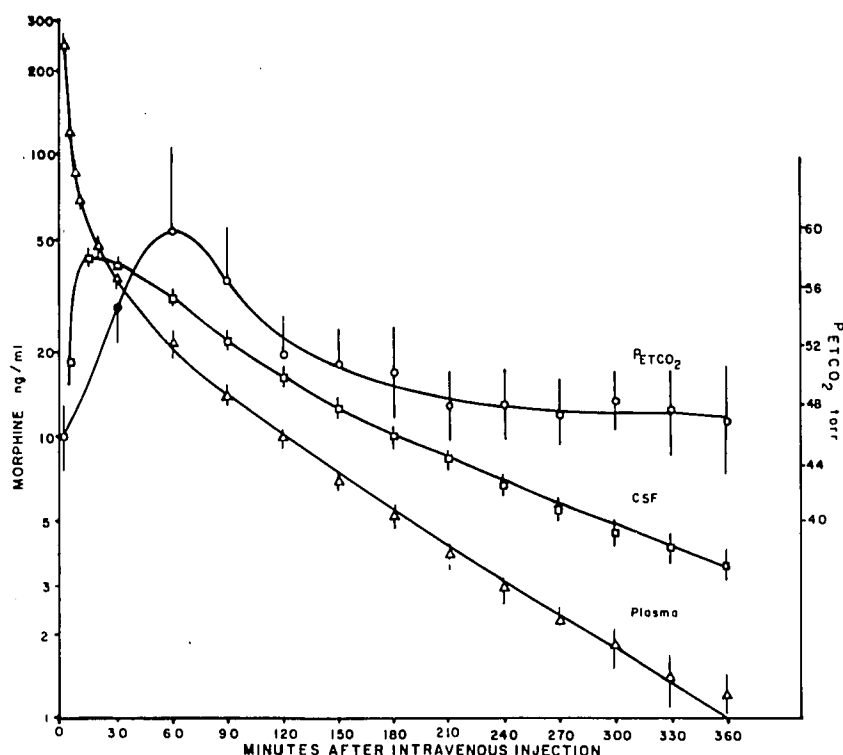
TABLE 5. Pharmacokinetic Parameters for Elimination of Intravenously Administered Morphine from Cerebrospinal Fluid of Anesthetized Dogs: $C_{CSF(t)} = A_{exp}^{-\alpha t} + B_{exp}^{-\beta t}$ *

	A (ng/ml)	α (min^{-1})	$t_{1/2\alpha}$ (min)	B (ng/ml)	β (min^{-1})	$t_{1/2\beta}$ (min)	r²†
Morphine, 0.3 mg/kg							
Dog 13	33	.017	40	18	.0046	149	.999
Dog 16	37	.019	36	24	.0048	143	.999
Dog 18	50	.030	23	21	.0073	94	.999
Dog 23	13	.023	30	23	.0052	134	.999
Dog 28	63	.030	23	28	.0059	118	.998
Dog 29	20	.025	28	44	.0069	101	.999
Mean	36	.024	30	26	.0058	123	
SEM	8	.002	3	4	.0005	9	
Morphine, 2.0 mg/kg							
Dog 10	98	.016	44	227	.0061	113	.999
Dog 17	44	.020	35	210	.0059	118	.999
Dog 19	162	.027	26	268	.0054	129	.999
Dog 21	32	.012	59	357	.0062	111	.999
Mean	84	.019	41	266	.0059	118	
SEM	30	.003	7	33	.0002	4	

* See Appendix.

† r² = [Σ(observed)² − Σ(deviation)²]/Σ(observed)².

mechanism) or an alteration in drug distribution to tissues as a result of hypotension in the animals receiving the larger dose. There is no evidence for saturable uptake of morphine by tissues other than the kidney[17] and possibly the brain,[17,18] and the amount of morphine accumulated in these tissues is small compared with amounts in other tissues such as skeletal muscle. Thus, the difference in distribution volumes is more likely to be related to the development of hypotension, which could elicit a reduction in tissue blood flow and decrease the rate and extent of morphine uptake by certain tissues. (This possibility seems more plausible in light of a further reduction in distribution volume in chloralose-anesthetized dogs, which experienced more profound hypotension [unpublished data.]) The range of V_d values in this study for the 2 mg/kg dose overlaps the values reported by others for dogs receiving 1 or 2 mg/kg.[10,12,13]

Clearance of a drug from the body depends on both distribution and elimination processes (Cl = $V_1 \cdot k_{el}$).[19] Hemodynamic changes can affect both processes. For example, blood flow to organs of elimination determines the rate of drug delivery, as well as influencing the functional activity of the elimination mechanisms.[20] In this study, clearances of morphine averaged 57 ml·kg^{-1}· min^{-1} with 0.3 mg/kg and 33 ml· kg^{-1}·min^{-1} with 2 mg/kg. The lower clearance after the higher dose may have been secondary to greater hemodynamic changes. The clearance of morphine was considerable (greater than or equal to hepatic blood flow), which suggests that its rate of biotransformation is dependent on hepatic blood flow. Anything that decreases splanchnic blood flow would be expected to

decrease clearance. Our clearance values are comparable to those found by Garrett and Gürkan[10] for 0.9 mg/kg (63 ml·kg^{-1}·min^{-1}) and by Nishitateno *et al.*[12] for 2 mg/kg (49 ml·kg^{-1}·min^{-1}). (These reports do not indicate the hemodynamic status of the animals.)

The major mechanism of morphine elimination from the body is its biotransformation to morphine-3-glucuronide in the liver.[10,21–23] This mechanism is efficient, since metabolites of morphine were apparent in the first samples of plasma, drawn 2 min after injection; by 10 to 20 min, their concentration in plasma exceeded that of unchanged morphine. In only six hours, 66 ± 3 per cent of the injected dose of morphine had been recovered in urine, mostly as metabolites. More than 95 per cent of the ^{14}C radioactivity in urine was attributable to either morphine or morphine glucuronide (table 4).

For complete elimination of morphine from the body, drug contained in the peripheral compartments must return to the central compartment for elimination. Since the hepatic extraction and metabolism of morphine are relatively complete,[10,21,24] the rate-limiting step in the elimination of morphine may be its reuptake from peripheral tissues and delivery to the liver. This possibility is supported by the fact that the elimination constant for morphine, k_{el}, was greater than k_{31}, which reflects the rate of return of morphine from the third compartment of tissues (table 2). In most cases, the third compartment comprises tissues with low blood flows.[25]

Despite the fact that morphine is actively secreted by the proximal renal tubules,[14,26,27] the excretion

of unchanged morphine by the kidneys was limited. However, the kidneys are the primary avenue of excretion of morphine glucuronide, a much more polar and less lipophilic compound that is less likely to be reabsorbed by the renal tubules, and possibly may be secreted by them.[27,28]

Don et al.[29] reported prolonged ventilatory and CNS depression due to morphine in three patients without apparent hepatic impairment but with renal dysfunction. This depression was evident as long as six days after administration of the last dose of morphine and could be antagonized with naloxone. It was not determined whether the prolonged effects were due to accumulation of morphine or its metabolites. However, it is interesting to note that in our animals, morphine metabolites glucuronide?) penetrated into CSF, albeit at a slow rate. In the presence of renal failure, plasma morphine glucuronide would be expected to remain at a high level for a long time. This would favor greater penetration of this metabolite into the brain. Intracerebral injections of morphine glucuronide have been shown to produce analgesia and respiratory depression.[30]

Only 14 per cent of the morphine in dog plasma was bound to protein at pH 7.3–7.5, and the extent of binding was independent of morphine concentration (20–2,000 ng/ml). This extent of binding is somewhat less than those reported by other investigators for dogs (28 per cent)[31] and man (23–42 per cent).[32–34] Reasons for the differences are not apparent, since the variables known to affect morphine binding, that is, temperature and pH, were maintained in comparable ranges in all studies.

Although the penetration of morphine into some tissues was very rapid ($k_{12} \simeq 0.3$, $t_{1/2_{12}} \simeq 2.3$ min), the uptake of morphine into CSF was slower, and the peak concentration occurred 15–30 min after injection. The delay reflects the relative barrier imposed on drugs such as morphine with limited lipoidal solubility by biologic membranes, including the blood–brain barrier.[35–37] Once in the CSF, the elimination of morphine from CSF ($t_{1/2\beta} = 121$ min) did not parallel its elimination from plasma ($t_{1/2\beta} = 75$ min). These observations are similar to those of Mulé and Woods,[9] who injected 2 mg/kg morphine subcutaneously in dogs and found that morphine persisted in CSF, and especially in brain tissue, much longer than in plasma. The slower elimination of morphine from brain was also evident in the studies by Finck et al.[13] and Nishitateno et al.,[12] who suggested that in the relatively acidic milieu of the brain a greater portion of the morphine exists in the ionized form, which slows its return across the lipid barrier back into the circulation. Diffusion of morphine

from brain to CSF to plasma may occur and result in a CSF concentration that is intermediate between that in plasma and that in brain.

The concentration of morphine metabolites in CSF did not correlate with their concentration in plasma. The identity of the metabolites in CSF is not known.

Ventilatory depression, as measured by changes in end-tidal P_{CO_2} in enflurane–N_2O-anesthetized dogs breathing spontaneously following a single dose of morphine, 0.3 mg/kg, iv, could not be correlated with either plasma or CSF concentration of morphine (fig. 4). The end-tidal P_{CO_2} peaked between 30 and 60 min after morphine injection, probably reflecting both the slow entry of morphine into brain and the gradual accumulation of CO_2 in the presence of a reduced minute ventilation. The elimination of CO_2 appeared to be slower than the decline of the morphine concentration in either CSF or plasma. The lack of correlation between plasma morphine levels and respiratory depression may reflect retention of morphine within CNS tissue and its slow equilibration across biologic membranes, since a study of fentanyl, a much more lipophilic drug investigated under identical experimental conditions, showed a very close correlation between its concentrations in both plasma and CSF and the intensity of ventilatory depression.[38]

Existing reports in the literature both claim[1–6] and deny[7,8] a correlation between plasma levels of morphine and the intensity of its analgesic effects. Careful review of these studies shows only crude and indirect comparisons of concentration and effect data. While it is evident that there is a general trend of recovery from its pharmacologic actions as morphine concentrations decline in plasma and CSF, it is not possible to relate any specific concentration to a given intensity of ventilatory depression or analgesia. It is unlikely, therefore, that measurement of plasma levels of morphine in a clinical setting can provide more than a crude guide to either therapeutic effectiveness or the potential for toxicity. Certainly, changes in morphine levels in plasma do not indicate proportional changes in its concentrations in the central nervous system, which contains the sites of morphine's analgesic and respiratory depressant actions.

It should also be noted that none of morphine's metabolites is as potent as morphine itself, and except for the glucuronide, none appears in plasma in a concentration greater than that of morphine. There is no evidence for the formation of morphine metabolites within the central nervous system.

In conclusion, morphine was rapidly eliminated from plasma by uptake into tissues. ^{14}C-Morphine was metabolized efficiently, and morphine gluc-

uronide accounted for most of the radioactivity in plasma and urine; only small amounts of the unchanged drug appeared in urine. The rate-limiting step in morphine elimination was its re-uptake from tissues. Differences in kinetics between the two doses appeared to be related to hemodynamic changes produced by morphine. The intensity and duration of ventilatory depression were not directly related to the concentration of morphine in either CSF or plasma.

APPENDIX

Samples were analyzed for unchanged morphine by a modification of the method of Mulé and Woods.[9] Duplicate 1.0-ml samples of plasma or urine or 0.1-ml samples of CSF were brought to a volume of 4 ml with distilled water. Non-radioactive morphine sulfate (0.5 mg in 1 ml distilled water) was added as a carrier to decrease adsorption of the radioactive drug on glassware. The pH was adjusted to 9–10 with 2.5 N sodium hydroxide and the sample buffered with 3 ml of 1 M potassium phosphate (pH 10). Following saturation of the aqueous phase with 3–4 g of sodium chloride, 10 ml of 10 per cent (v/v) n-amyl alcohol in 1,2-ethylene dichloride was added and the samples were shaken for 20 min and centrifuged. An 8-ml volume of the organic (upper) layer was transferred to a glass liquid-scintillation vial and evaporated overnight at 60° C. The residue was dissolved in 0.8 ml n-amyl alcohol, and 10 ml of fluor (3 g PPO and 200 mg dimethyl-POPOP* per liter of scintillation-grade toluene) were added. The samples were counted in a Beckman LS-230 liquid scintillation spectrometer. Concentrations of morphine in biologic specimens were determined by comparing the radioactivity in the extract with those in extracts prepared simultaneously from solutions containing known concentrations of ^{14}C-morphine. Recoveries of known concentrations of morphine (1.6–1,000 ng/ml) averaged 90 ± 3 (SD) per cent.

Total ^{14}C-radioactivity (*i.e.*, unchanged morphine and its metabolites) was determined in 0.1-ml samples of plasma, urine, or CSF by the addition of 3 ml of absolute ethanol, U.S.P., followed by 7 ml of the toluene fluor. ^{14}C-Toluene was added as an internal standard in order to determine the extent of scintillation quenching in each sample. Standard solutions of ^{14}C-morphine were analyzed simultaneously and the quantity of total ^{14}C-radioactivity was expressed in terms of an equivalent concentration of ^{14}C-morphine.

Conjugated morphine was estimated by the acidic hydrolysis method of Mulé and Woods.[9] One-tenth milliliter of concentrated hydrochloric acid (12 N) was added to a 1-ml sample of plasma or urine and the solution was autoclaved at 121° C and 15 psi for 30 min. After the solution cooled to room temperature, the pH was adjusted to 9–10 with 2.5 N sodium hydroxide, the sample volume brought to 4.0 ml with distilled water, and 1 ml of morphine carrier solution was added. The sample was analyzed for unchanged morphine as described above. Conjugated mor-

phine was estimated as the difference between ^{14}C-morphine concentrations before and after acidic hydrolysis.

Chromatography of urine from dogs receiving ^{14}C-morphine was performed as follows. Samples of urine, with and without acidic or enzymatic hydrolysis, were applied to Whatman No. 3 paper along with an aliquot of a solution of authentic morphine sulfate. Chromatograms were developed in one of two solvent systems:

Acidic system:n-butanol, glacial acetic acid, water (4:1:2, v/v)

Basic system:n-propanol, n-butanol, 0.1 N ammonium hydroxide (2:1:1, v/v).

The strip of paper corresponding to the migration path of the sample of urine was cut out and divided into 1-cm segments, which were individually eluted for one hour with 4 ml of absolute methanol in a scintillation vial. The paper segment was removed, the methanol evaporated, and the residue dissolved in 0.5 ml methanol; 5 ml of toluene-phosphor were added and radioactivity determined in the liquid scintillation counter. The location of authentic morphine on the chromatogram was determined by spraying it with iodoplatinate reagent.[39]

The extraction procedure measured total unchanged morphine, including both free and protein-bound drug. Protein binding of morphine in dog plasma was studied *in vitro*. ^{14}C-Morphine was added to fresh plasma from a single dog (for each experiment) and aliquots were placed in cellophane bags. The total volume in each bag was 3 ml, consisting of 90 per cent plasma and with a final morphine concentration of 20 or 2,000 ng/ml. Equilibrium dialysis in 9 ml of Sorensen's buffer at various pH values (7.25–7.54) was accomplished over 18 hours at 37° C in a Dubnoff shaking incubator. Undialyzed plasma, dialyzed plasma, and the dialysate were analyzed for unchanged ^{14}C-morphine and albumin.

After intravenous injection, the decline of unchanged morphine concentrations in plasma was triphasic, and could be fitted to an exponential time function using the method of residuals as described by Gibaldi and Perrier[40] and modified for use as a computer program. The equation is:

$$C_{p(t)} = P_{exp}{}^{-\pi t} + A_{exp}{}^{-\alpha t} + B_{exp}{}^{-\beta t}$$

where

$C_{p(t)}$ = the concentration of morphine in plasma at time t,
P, A, B = the extrapolated zero intercepts computed from least-squares analysis of the data,
π, α, β = the first-order rate constants.

The apparent volume of distribution, V_d, the apparent volume of the central compartment, V_1, and the total-body clearance, Cl_B, were calculated by the following formulas:

$$V_d = \frac{dose}{area \cdot \beta}$$

$$V_1 = \frac{dose}{C_{p(0)}}$$

$$Cl_B = \frac{dose}{area}$$

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* Fisher Scientific Co., Pittsburgh, Pennsylvania.

$$\text{Area} = \frac{P}{\pi} + \frac{A}{\alpha} + \frac{B}{\beta}$$

$$C_{P(0)} = P + A + B$$

Following peak concentrations, the kinetics of decline of unchanged morphine in CSF were described by a biexponential equation using the method of residuals as above. Conjugated morphine concentrations, following their peak in plasma, declined in an exponential manner described by a single-function equation.

References

1. Berkowitz BA, Ngai SH, Yang JC, et al: The disposition of morphine in surgical patients. Clin Pharmacol Ther 17: 629–635, 1975
2. Hipps PP, Eveland MR, Meyer ER, et al: Mass fragmentography of morphine: relationship between brain levels and analgesic activity. J Pharmacol Exp Ther 196:642–648, 1976
3. Patrick GA, Dewey WL, Spaulding TC, et al: Relationship of brain morphine levels to analgesic activity in acutely treated mice and rats and in pellet-implanted mice. J Pharmacol Exp Ther 193:876–883, 1975
4. Miller JW, Elliot HW: Rat tissue levels of carbon-14 labeled analgetics as related to pharmacological activity. J Pharmacol Exp Ther 113:283–291, 1955
5. Dahlström BE, Paalzow LK, Segre G, et al: Relation between morphine pharmacokinetics and analgesia. J Pharmacokinet Biopharm 6:41–53, 1978
6. Way EL: Distribution and metabolism of morphine and its surrogates, Proceedings of the Association for Research in Nervous and Mental Diseases 46:13–31, 1968
7. Johannesson T, Schou J: Morphine and normorphine in the brains of rats given identically analgaesic doses of morphine, codeine or normorphine. Acta Pharmacol Toxicol 20: 165–173, 1963
8. Mulé SJ, Clements TH, Layson RC, et al: Analgesia in guinea pigs: a measure of tolerance development. Arch Int Pharmacodyn 173:201–212, 1968
9. Mulé SJ, Woods LA: Distribution of N-C^{14}-methyl labeled morphine: I. In central nervous system of nontolerant and tolerant dogs. J Pharmacol Exp Ther 136:232–241, 1962
10. Garrett ER, Gürkan T: Pharmacokinetics of morphine and its surrogates. I: Comparisons of sensitive assays of morphine in biological fluids and application to morphine pharmacokinetics in the dog. J Pharm Sci 67:1512–1517, 1978
11. Stanski DR, Greenblatt DJ, Lowenstein E: Kinetics of intravenous and intramuscular morphine. Clin Pharmacol Ther 24:52–59, 1978
12. Nishitateno K, Ngai SH, Finck AD, et al: Pharmacokinetics of morphine: concentrations in the serum and brain of the dog during hyperventilation. ANESTHESIOLOGY 50:520–523, 1979
13. Finck AD, Berkowitz BA, Hempstead J, et al: Pharmacokinetics of morphine: effects of hypercarbia on serum and brain morphine concentrations in the dog. ANESTHESIOLOGY 47:407–410, 1977
14. Brunk SF, Delle M: Morphine metabolism in man. Clin Pharmacol Ther 16:51–57, 1974
15. Brunk SF, Delle M, Wilson WR: Morphine metabolism in man: effect of aspirin. Clin Pharmacol Ther 15:283–290, 1974
16. Murphy MR, Hug CC Jr: Pharmacokinetics of intravenous morphine in patients anesthetized with enflurane-nitrous oxide. ANESTHESIOLOGY: in press.
17. Hug CC Jr: Transport of narcotic analgesics by the choroid plexus and kidney tissue in vitro. Biochem Pharmacol 16:345–359, 1967
18. Scrafani JT, Hug CC Jr: Active uptake of dihydromorphine and other narcotic analgesics by cerebral cortical slices. Biochem Pharmacol 17:1557–1566, 1968
19. Greenblatt DJ, Koch-Weser J: Clinical pharmacokinetics. N Engl J Med 293:702–705, 1975
20. Wilkinson GR, Shand DG: A physiological approach to hepatic drug clearance. Clin Pharmacol Ther 18:377–390, 1975
21. Rowland M: Hemodynamic factors in pharmacokinetics. Triangle 14:109–116, 1975
22. Misra AL, Yeh SY, Woods LA: Morphine conjugates in the dog. Biochem Pharmacol 19:1536–1539, 1970
23. Way EL, Adler TK: The biological disposition of morphine and its surrogates. Bull WHO 25:227–262, 1961
24. Dahlström BE, Paalzow LK: Pharmacokinetic interpretation of the enterohepatic recirculation and first-pass elimination of morphine in the rat. J Pharmacokinet Biopharm 6: 505–519, 1978
25. Hug CC Jr: Pharmacokinetics of drugs administered intravenously. Anesth Analg (Cleve) 57:704–723, 1978
26. Baker WP, Woods LA: A study in the dog of the renal clearance of morphine and the effect of morphine on p-aminohippurate clearance. J Pharmacol Exp Ther 120:371–374, 1957
27. Hug CC Jr, Mellett LB, Cafruny EJ: Stop-flow analysis of the renal excretion of tritium-labeled dihydromorphine. J Pharmacol Exp Ther 150:259–269, 1965
28. Goldstein A, Aronow L, Kalman SM: Principles of Drug Action. Second edition. New York, John Wiley and Sons, 1974, p 227
29. Don HF, Dieppa RA, Taylor P: Narcotic analgesics in anuric patients. ANESTHESIOLOGY 42:745–747, 1975
30. Sasajima M: Analgesic effect of morphine-3-monoglucuronide. Keio Igaku 47:421–526, 1970
31. Blaney DJ, Woods LA: Plasma protein binding of morphine. J Pharmacol Exp Ther 116:7, 1956
32. Höllt V, Teschemacher H-J: Hydrophobic interactions responsible for unspecific binding of morphine-like drugs. Naunyn-Schmiedeberg's Arch Pharmacol 288:163–177, 1975
33. Olsen GD: Morphine binding to human plasma proteins. Clin Pharmacol Ther 17:31–35, 1975
34. Ganshorn A, Kurz H: Unterschiede zwischen der proteinbindung neugeborener und erwachsener und ihre bedeutung für die pharmakologische wirkung. Arch Pharmacol Exp Pathol 260:117–118, 1968
35. Herz A, Teschemacher H-J: Activities and sites of antinociceptive action of morphine-like analgesics and kinetics of distribution following intravenous, intracerebral and intraventricular application, Advances in Drug Research. Volume 6. Edited by NJ Harper, AB Simmonds. New York, Academic Press, 1971, pp 79–119.
36. Way EL: Brain uptake of morphine: pharmacologic implications. Fed Proc 26: 1115–1118, 1967
37. Oldendorf WH, Hyman S, Braum L, et al: Blood–brain barrier: Penetration of morphine, codeine, heroin and methadone after carotid injection. Science 178:984–986, 1972
38. Hug CC Jr, Murphy MR: Fentanyl disposition in cerebrospinal fluid and plasma and its relationship to ventilatory depresson in the dog. ANESTHESIOLOGY 50:342–349, 1979
39. Stahl E: Thin-layer Chromatography. New York, Springer-Verlag, 1965, p 493
40. Gibaldi M, Perrier D: Pharmacokinetics. New York, Marcel Dekker, 1975, pp 281–292