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Microelectrometric Titration Measurement of the pK_a's and Partition and Drug Distribution Coefficients of Narcotics and Narcotic Antagonists and Their pH and Temperature Dependence

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The pK_a's, partition coefficients, and drug distribution coefficients (apparent partition coefficients) have been investigated for a number of narcotics and, where possible, for their congener narcotic antagonists. These studies were carried out by a microelectrometric titration technique as a function of temperature and pH. This method enables one to determine not only the dissociation constants to deconvolute overlapping pK_a's but also to determine the solubilities and oil–water distribution of these various drugs. The drug distribution coefficients displayed marked sensitivity to pH at values which span the range of attainable human physiological pH values. This has significant pharmacological implications for proper choice and scaling of drug dosages under various clinical situations. The partition coefficients and drug distribution coefficients were noticeably different at 20° (where such measurements are customarily made) than at 37° (body temperature). Furthermore, various drugs exhibit very nonequivalent increases in drug distribution coefficients with increasing temperature, ranging from 21% for morphine to 200% for naltrexone. This nonregularity indicates that it will not be valid to extrapolate by any constant factor the measurements made at lower temperatures. Even the true partition coefficients increase with temperature from 20 to 37°. There is more of a difference in the drug distribution coefficients for naloxone and naltrexone than might have been expected from the similarities in their structures with naltrexone being significantly less lipophilic than naloxone. This would imply that this would lead to naloxone having a more rapid onset for antagonist activity and likewise a shorter duration of action than naltrexone.

Our primary emphasis in this study was on careful measurements of the pK_a's, partition coefficients, and drug distribution coefficients of the narcotic antagonists, almost none of which data were available when we initiated this study. We have measured these same properties also for their congener–narcotic agonists and for other representative narcotics.

Overall narcotic effectivity has previously been shown to depend partly on lipid solubility. In the work of Kutter et al. a series of narcotics of widely varying potency was injected both intravenously and intraventricularly.[1] The high analgesic activity of nonpolar analgetics following intravenous application was explained by a good penetration of these compounds through the blood–brain barrier and seemed not to be due to especially favorable drug–receptor interactions. Polar compounds, such as morphine, injected intraventricularly seemed to have a greater activity at the receptor than nonpolar analgetics. This work underlined the importance of passive penetration of narcotics through the blood–brain barrier. Good penetrability must depend both on whether the compound is charged by being protonated or an amine at important physiological pH's (these pH's may have a wide range near vital membrane surfaces) or whether the compound even if not charged is still polar by virtue of significant electronic charge redistribution and thus is still not prone to partition strongly into lipids. As an example, comparison of intravenous vs. direct intraventricular injection of dihydromorphine and etorphine indicated that the intravenous 3800-fold greater analgesic potency of etorphine compared to dihydromorphine dropped to only a 40-fold increase when each was injected intraventricularly.[1] Intraventricular injection bypassed the necessity of the narcotic to pass the blood–brain barrier—a phenomenon intimately connected to lipid solubility and partition coefficient. The high narcotic potency of etorphine arises in large part from its very high lipophilicity. These indications were given further experimental credence by the binding studies of these compounds to the "opiate receptor".[2] The inhibition of stereospecific naloxone binding by etorphine was only six times more effective as an analgesic (in the absence of sodium) or 23 times more effective (in the presence of sodium) compared to dihydromorphine, not the 3800-fold times etorphine is more potent an analgesic by intravenous injection.

Morphine and similar molecules should have two pK_a's, one for the proton on the nitrogen and one for the phenolic hydrogen. Yet often in the literature only one pK_a is reported.[3] Examination of the original experimental curves when available, supplemented by our rerunning some of those experiments under the reported conditions, indicated that often the reported value is some average of the two. It corresponds neither to the pK_a of the proton on the nitrogen nor to the pK_a of the hydrogen on the OH. This point is especially important because, in general, only an un-

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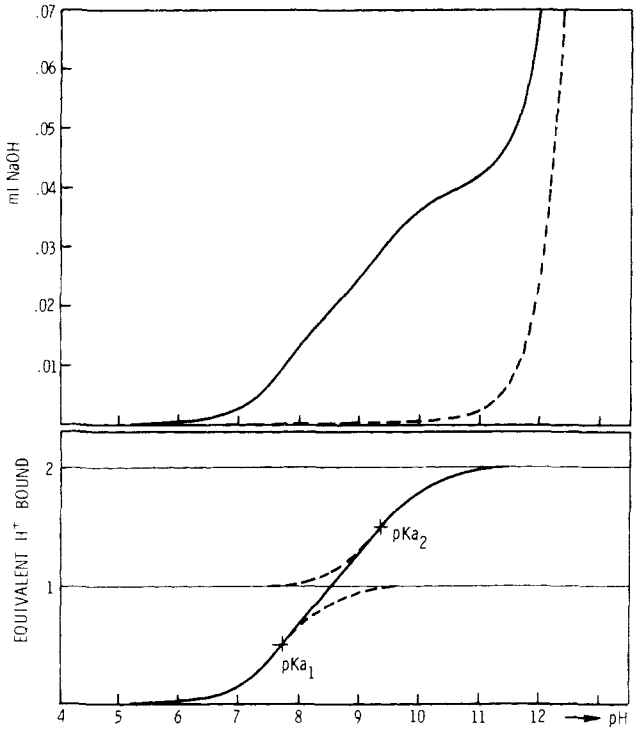

Figure 1. Aqueous titration of nalorphine hydrochloride. In the upper portion of the figure the solid line represents the actual titration curve. The dotted line is the blank and the lower curve show the results of the subtraction. Dotted lines in the lower figure show the fitting of the theoretical expression pH = pK_a + log $\alpha/(1 - \alpha)$.

charged species will tend to partition into lipids as indicated above; overall narcotic effectivity has been shown to depend partly on lipid solubility.

One must be careful not to imply for these types of species that lipid solubility is equatable with partition coefficient. Partition coefficients, precisely defined, refer to the partitioning between oil and water of the *same* species. In the case of basic compounds such as morphine and most narcotics and narcotic antagonists, the partition coefficients, strictly speaking, refer to the partitioning of the *free base* between lipid and water. Thus, in Hansch's excellent review of partition coefficients,[4] the figures he quotes are for the partition coefficients of the free bases. For example, he lists the partition coefficient of morphine as log P (octanol–water) = 0.76. This indicates that the free base is more soluble in lipid than in water. However, the distribution coefficient, which is pertinent for understanding of the differences in pharmacological potency, is the ratio of

$$\frac{[\text{free base} + \text{acid salt}]_{\text{lipid}}}{[\text{free base} + \text{acid salt}]_{\text{water}}} \sim \frac{[\text{free base}]_{\text{lipid}}}{[\text{free base} + \text{acid salt}]_{\text{water}}}$$

In our laboratory we have been using and exploring the adequacy and limitations of a titration technique reported by Davis et al.[5] for the determination of dissociation constants, solubilities, and oil–water (octanol–water) distribution coefficients of various drugs.[6-8] In this report we give the detailed results of this study.

Experimental Section

A. Apparatus. The electrometric microtitration apparatus is an adaptation of the conventional assemblies. It consists of a small sample cell in which 0.04 mmol of the sample is dissolved in 5 ml of CO_2-free triply distilled water. A constant temperature circulator is employed to circulate water continuously through a jacket surrounding the titration cell. A blanket of N_2 gas is maintained over the sample so as to prevent any CO_2 absorption from the air. A pH meter type PHM64 radiometer utilizing calomel and glass electrodes is used for pH measurements. A microburet attached to a microdelivery tip enables one accurately to add small quantities of titrant (2 N NaOH). A constant speed magnetic stirrer enables proper agitation of the sample solution and an externally mounted light source enables one to observe the first signs of any light scattering which occurs in the titration sample due to the formation of a precipitate.

B. Procedures. 1. pK_a Determination. In titrating molecular solutions which may have more than one pK_a one makes a conventional titration curve with volume of titrant plotted against pH (upper curve in Figure 1). Then a blank titration curve is prepared by the same procedure but the sample is omitted. When these two curves are subtracted volumewise the resulting curve (lower curve in Figure 1) is then analyzed for its pK_a's. This difference curve represents the hydrogen ion-binding capacity of the sample as a function of pH. When aqueous titration curves of equivalents of bound hydrogen ion per mole of sample vs. pH are plotted on a uniform scale, any single titratable group produces an inflection having the same shape regardless of its position on the pH scale. The midpoint on the inflection is the pK_a of the dissociating group. The theoretical curve for this inflection is given by the following expression

$$\text{pH} = pK_a + \log(\alpha/(1 - \alpha))$$

where α is the fraction of sample in the dissociated state for acids or the associated state for bases. The use of this theoretical expression enables one to determine the pK_a under conditions where only a portion of the complete titration curve can be observed. Such conditions arise when two inflections corresponding to two pK_a's are not completely resolved, or when a precipitate is formed, or at the extremes on the pH scale where only a portion of the titration curve is observed.

It was found that this technique when average precautions were taken gave very reproducible results. Each pK_a reported is the average of at least five determinations and the maximum deviation from the given values is ±0.02. (The values reported are the measured customary macroscopic pK_a's as opposed to microscopic pK_a's.[9])

As in all electrometric titrations difficulties are experienced when precipitation takes place during titration. This study indicates that if one can realize a significant portion of the titration curve before precipitation one can extrapolate this curve with some confidence since its theoretical shape is known and a reliable value of the pK_a can be obtained. In cases where a sufficiently large portion of the titration curve, before precipitation occurred, was not realized we applied the somewhat questionable but frequently used[10] procedure of using a 50% ethanol–water mixture as a solvent and then applying a correction factor to the pK_a so obtained to extrapolate it to the pK_a in H_2O. These data have been tabulated separately to emphasize this fact and so that the reader can consider them accordingly.

2. Determination of Aqueous Solubility. Many compounds are salts of acids or bases which have a limited solubility in water. When such soluble salts are titrated in aqueous solution precipitation occurs at some pH near the pK_a. At this point in the titration a sudden break occurs in the titration curve, an excess phase occurs, and a drastic pH shift occurs (upscale for an acid and downscale for a base). The initial part of the hydrogen ion binding curve before precipation was encountered conforms to the standard theoretical curve and the comparison of this curve with the experimental curve permits a determination of the aqueous pK_a even though the midpoint of the titration curve has not been reached. If the titration is continued at a slow rate through the insolubility range of pH, the hydrogen ion binding curve follows a course which can be shown to be of the form[11]

$$\text{pH} = \log(1 - \alpha) - \log K_{sp}/C$$

where K_{sp} is the solubility product which for an acid, HA, is $[H^+][A^-]$ (and for a base, B, is $[BH^+]/[H^+]$) and C is the concentration.

Applying this equation to the experimental curve permits one to obtain K_{sp} and by combining this with the K'_a one can obtain the solubility of the free acid or base. A typical result is shown in Figure 2.

3. Determination of Oil–Water Partition of a Drug. If a compound is added to a two-phase system (i.e., oil and water) in an amount insufficient to saturate either of the phases, the material

Table I. pK_a, Partition Coefficient, and Drug Distribution Coefficients as a Function of Temperature and pH. H_2O Titrations[a]

Compound	Temp, °C	pK_a	$D_{o/w}$	Drug distribution coefficient $P_{7.10}$	$P_{7.35}$	$P_{7.40}$	$P_{7.45}$	$P_{7.50}$	$P_{7.60}$	$P_{7.70}$
Morphine	20	8.02	6.03	0.65	1.06	1.17	1.28	1.40	1.66	1.95
sulfate	37	7.93	6.23	0.80	1.30	1.42	1.54	1.68	1.98	2.30
Nalorphine	20	7.73	57.61	10.94	16.95	18.36	19.83	21.35	24.52	27.80
hydrochloride	37	7.59	71.78	17.55	26.22	28.16	30.16	32.19	36.39	40.41
Codeine	20	8.18	11.88	0.91	1.53	1.69	1.86	2.05	2.47	2.95
phosphate	37	8.10	13.72	1.25	2.07	2.28	2.51	2.75	3.30	3.91
Oxymorphone	20	8.25	3.80	0.25	0.42	0.47	0.52	0.57	0.69	0.83
hydrochloride	37	8.17	6.73	0.53	0.89	0.98	1.08	1.19	1.43	1.71
Naloxone	20	7.94	59.25	7.48	12.12	13.32	14.49	15.78	18.58	21.64
hydrochloride	37	7.82	121.80	19.49	30.82	33.55	36.42	39.43	45.79	52.54
Naltrexone	20	8.38	45.60	2.27	3.89	4.32	4.79	5.31	6.49	7.88
hydrochloride	37	8.13	83.33	7.11	11.86	13.08	14.40	15.82	18.99	22.57
Meperidine	20	8.68	474.00	12.14	21.18	23.64	26.36	29.36	36.40	44.93
hydrochloride (demerol)	37	8.50	527.60	20.20	34.87	38.82	43.17	47.96	58.99	72.17
Levorphanol	20	9.79	1052.90	2.13	3.79	4.24	4.76	5.34	6.72	8.44
tartrate	37	9.37	1305.00	6.89	12.21	13.68	15.33	17.17	21.54	27.00
Levallorphan	20	8.73	2431.00	55.68	97.28	108.62	121.25	135.20	167.77	207.42
tartrate	37	8.43	2590.00	115.73	198.92	220.99	245.50	272.32	333.72	406.60

[a] Since the concentration of the titrated species was significantly less than 0.01 M no activity corrections were used: A. Albert and E. P. Sergeant, "The Determination of Ionization Constants," Chapman and Hall, Ltd., London, 1971.

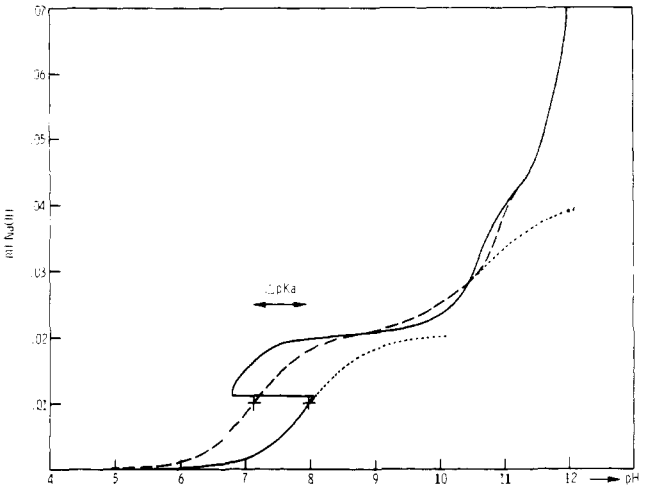

Figure 2. Titration of naloxone hydrochloride in a 10% oil–water mixture. The dashed line represents the titration curve obtained in the presence of 10% octanol. The solid line represents the titration in water solution with the break due to precipitation of the free base in water. The dotted line shows the theoretical curve.

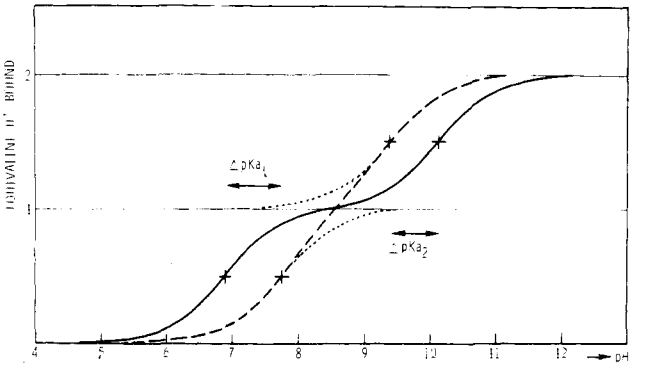

Figure 3. Comparison of the aqueous titration and 10% oil–water titration of nalorphine hydrochloride. The dashed line is the aqueous titration; the solid line is the octanol–water titration. The crosses mark the pK_a values and the ΔpK_a's represent the shifts.

will distribute between the two phases in a definite proportion determined by its distribution coefficient (P).

In the aqueous phase, the compound dissociates into its respective ions to an extent determined by its dissociation constant (K_a) which is obtained from the inflection in the experimentally determined titration curve. When a drug is titrated in the presence of a finely dispersed oil the free acid or base distributes rapidly between water and oil. As the titration proceeds the dissociation equilibria in the water phase continue to follow the relation

$$K'_a = [H^+][A^-]/[HA]$$

for the acid, HA, and a corresponding relation is followed if a base is being titrated. The removal of HA into the oil results in a shift in the apparent pK_a by an amount which depends on the volume of oil and the volume of the water present (the shift occurs to higher pH if an acid is titrated and to lower pH if a base is titrated in the presence of the oil). This apparent shift can be readily related to the distribution coefficient (P) by the relation[12]

$$P = V_w(\text{antilog } \Delta pK'_a - 1)/V_o$$

where V_w = volume of water, V_o = oil volume, and the $\Delta pK'_a$ is the shift of the pK'_a by the addition of the oil.

Experimental determination of the shift in the presence of an oil permits a simple calculation of distribution of the free acid between oil and water phases. This figure applies to the distribution of the undissociated form of the compound between oil and water. One can obtain the partition coefficient of the total drug between oil and water at any pH simply by multiplying the above value of P by the fraction of drug in the undissociated form in the aqueous phase at that pH value. A typical curve illustrating these features is shown in Figure 3.

Results and Discussion

A. Results of This Research. In Table I are presented the measured lowest (corresponding to the deprotonation of the nitrogen) pK_a's, partition coefficients, $D_{o/w}$ (at the pK_a), and the drug distribution coefficients as a function of

Table II. pK_a, Partition Coefficient, and Drug Distribution Coefficients as a Function of Temperature and pH. 50% Ethanol–H_2O Titrations

Compound	Temp, °C	pK_a[a]	$D_{o/w}$	Drug distribution coefficient $P_{7.10}$	$P_{7.35}$	$P_{7.10}$	$P_{7.45}$	$P_{7.50}$	$P_{7.60}$	$P_{7.70}$
Levorphanol tartrate	20	9.78*	1,023.00	2.13	3.79	4.24	4.76	5.34	6.72	8.44
	37	9.36*	1,276.40	6.89	12.21	13.69	15.34	17.18	21.56	27.02
Levallorphan tartrate	20	8.81*	2,929.50	56.02	98.17	109.72	122.52	136.76	170.12	211.06
	37	8.50*	3,046.50	116.63	201.35	224.17	249.30	276.95	340.66	416.76
Methadone hydrochloride	20	9.64*	7,545.00	21.68	38.48	43.16	48.36	54.25	68.21	85.64
	37	9.26*	8,621.00	58.68	103.81	116.33	130.30	145.92	182.92	229.04
α-Acetylmethadol hydrochloride	20	8.98*	19,220.00	250.06	440.22	492.57	551.03	616.02	769.11	958.60
	37	8.61*	20,410.00	611.81	1063.02	1185.25	1321.03	1470.46	1817.45	2235.98
Cyclazocine	20	9.78*	1,852.00	3.86	6.85	7.69	8.62	9.67	12.15	15.28
	37	9.38*	2,079.00	10.86	19.21	21.54	24.14	27.05	33.94	42.55
Pentazocine	20	9.50*	5,000.20	19.83	35.14	39.40	44.17	49.51	62.17	78.01
	37	9.16*	6,484.00	55.99	98.89	110.76	124.00	138.81	173.79	217.29
MR-1256-BS	37	8.42*	1,548.25	70.73	121.43	134.98	149.83	166.16	203.53	247.80
MR-1029-BS	37	7.86*	1,390.00	207.40	330.48	360.29	391.88	425.90	495.90	571.55

[a] pK_{a,H_2O} = pK_a (50% EtOH-H_2O) + 0.5 pH. These pK_a's were determined from titrations in 50% ethanol-H_2O solutions and then applying the relation pK_{a,H_2O} = pK_a (50% EtOH-H_2O) + 0.5 pH. Since the corrective factor of 0.5 pH is not precisely the same for all compounds, these pK_a's should be considered to be approximate.

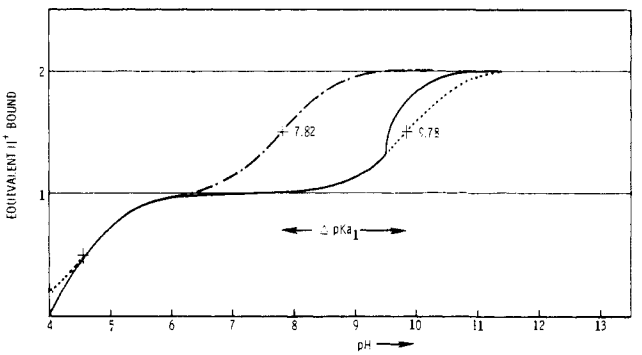

Figure 4. Comparison of the aqueous titration and the 10% oil–water titration of levorphanol tartrate. The solid line is the aqueous titration, the short dashed (- - -) line is the extrapolation of the aqueous titration curve, and the · · · · line is the octanol–water titration. The crosses mark the pK_a values and the ΔpK_{a_1} represents the shift.

temperature and pH for a variety of water-soluble narcotics and where possible their congener–narcotic antagonists. The partition coefficients and drug distribution coefficients are reported as absolute values and not as the much less sensitive log P values. This enables the clinically significant differences to be ascertained accurately. The drug distribution coefficients have been measured smoothly from pH values of ca. 3–12. In Table I are reported only the values in the physiologically significant pH range from 7.10 to 7.70.

In cases where precipitation did not occur the same pK_a values were obtained if one dissolved the material in NaOH solution and then titrated with HCl. In cases where precipitation occurs by titration with base the difficulties encountered are not remedied by reversing the situation and titrating with the acid since again precipitation occurs before the inflection point is reached.

Table II gives a tabulation of similar results obtained with compounds where the pK_a's were determined from titration in ethanol–water mixtures. Levorphanol tartrate and levallorphan tartrate are common to both tables since these compounds are sufficiently soluble in H_2O to give a sufficient portion of a titration curve so that a determination of their aqueous pK_a could be made. The ethanol–H_2O solvent titrations for the pK_a's were made using the procedure of Casy and Wright[10] in which the aqueous pK_a is obtained by adding 0.5 to the pK_a obtained in the 50% ethanol–water solvent. In this present article any pK_a's that were determined by us in 50% ethanol–H_2O solutions and then corrected by adding 0.5 pH are denoted with an asterisk. (Most of the drugs investigated are well-known ones. MR-1256-BS is a "pure antagonist"; MR-1029-BS is a "mixed agonist–antagonist".[13])

B. Comparison with Previous Results. 1. pK_a's. In Table III available literature values of the pK_a's are compared to those in this study. It is clear that in all cases where a comparison could be made in aqueous titration good agreement is realized. In two cases, i.e., levorphanol tartrate and levallorphan tartrate, discrepancies occur between the only available literature values and ours. The reported literature values[14,18] were obtained by a method using ethanol–water mixtures as solvents.[19] The literature values[14,18] were said to have been measured in various alcohol–water mixtures (50% ethanol–H_2O,[14,18] 75% propanol–H_2O[18]) but with no specifications in each case as to which solvent was used. These reported values are apparently uncorrected back to aqueous pK_a values. The measured pK_a in 75% propanol–water solution will be even lower than the pK_a measured in 50% ethanol–water solution. We consider the case of levorphanol tartrate first. This compound has sufficient solubility in water so that a theoretical extension of the titration curve permits us to realize an aqueous pK_a. This gives us a pK_a of 9.78 and our 50% ethanol–water titration curve (corrected back to the value in H_2O) gives 9.79*, both of which are considerably higher than the reported literature value of 8.18 obtained from ethanol–water titration.[14] Two points need comment. One is the discrepancy between our values and the reported literature values and the other is the magnitude of our pK_a since it is higher than the other first pK_a's listed in Tables I and II. One might suspect that this is the pK_a corresponding to the phenolic hydrogen. These points can best be discussed by referring to Figure 4 where our levorphanol tartrate–NaOH titration curves are presented for oil–water, ethanol–water, and water. It is clear that precipitation takes place suffi-

Table III. Comparison of pK_a's (This Research and Literature Values)

Compound	Temp, °C	This research: pK_{a_1} (proton on N)	This research: pK_{a_2} (phenolic H)	Literature: pK_{a_1}	Literature: pK_{a_2}	Ref
Morphine sulfate	20	8.02	9.76	8.02		14
	37	7.93	9.63			
Morphine hydrochloride	20			8.05		14
Morphine (free base)	20				9.85	14
Morphine	15			8.07	9.85	3a (K47)
Morphine hydrochloride	20			8.03		3a (P16)
Morphine	25			8.21		3a (O1)
Morphine				8.31	9.51	3b (S12)
Nalorphine hydrochloride	20	7.73	9.36	7.83		14
	37	7.59	9.28			
Codeine phosphate	20	8.18		8.22		14
	37	8.10		8.2		18
Codeine	15			8.15		3a (K4)
Codeine hydrochloride	20			8.25		3a (P16)
Codeine hydrochloride				8.04		3a (R6)
Codeine				8.17		3a (B7)
Codeine	25			8.21		3a (O1)
Oxymorphone hydrochloride	20	8.25	9.71			
	37	8.17	9.54			
Naloxone hydrochloride	20	7.94	9.44			
	37	7.82	9.25			
Naltrexone hydrochloride	20	8.38	9.93			
	37	8.13	9.51			
Meperidine hydrochloride	20	8.68		8.72		14
	37	8.50		8.72		15
Levorphanol tartrate	20	9.79	*a*	8.18		14
	37	9.36	*a*			
Levallorphan tartrate	20	8.81*	*a*	8.3		18
	37	8.50*	*a*			
Methadone hydrochloride	20	9.64*		8.25		14
	37	9.26*		10.12		16
	23			8.62	(100% MeOH unextrapolated)	17
Methadone	20			10.12		3a (M14)
	25			8.94		3a (B41)
α-Acetylmethadol hydrochloride	20	8.98*				
	37	8.59*				
Cyclazocine	20	9.78*	*a*			
	37	9.38*	*a*			
Pentazocine	20	9.50*	*a*			
	37	9.16*	*a*			

[a] Cannot be determined.

ciently high on the aqueous titration curve so that a meaningful theoretical extension can be made of the titration curve giving the 9.78 value. The second point that is obvious from these titration curves is that the shift of the pK_a as the amount of sodium hydroxide equal to the equivalent of the first proton bound to the levorphanol moiety is added in the presence of oil is in the downward direction characteristic of a nitrogen pK_a. If we were dealing with a pK_a associated with a phenolic hydrogen, the shift would be toward higher values as illustrated in Figure 3. We also prepared levorphanol free base and the pK_a value obtained by titration agrees with our above values for levorphanol tartrate. A pK_a of the order of 9.79* for the nitrogen proton in levorphanol is not unexpectedly high if one compares this to the pK_a of piperidine which is reported to be 11.280 (20°), 11.123 (25°), and 10.818 (30°).[20]

A similar situation appears to exist in the case of levallorphan tartrate. We report a value of 8.81* whereas the literature value is 8.3.[18] The corresponding titration curves are shown in Figure 5. This compound is less soluble in water than the levorphanol tartrate; consequently, we have a shorter section of the titration curve before precipitation to use for fitting a theoretical extension curve and, thus, the extrapolation may be less accurate. However, we feel that the data still permit meaningful extrapolation to be made. This gives us a pK_a of 8.73 in water and 8.81* from the alcohol–water titration. Again the shift in apparent pK_a on addition of oil is downward corresponding to the re-

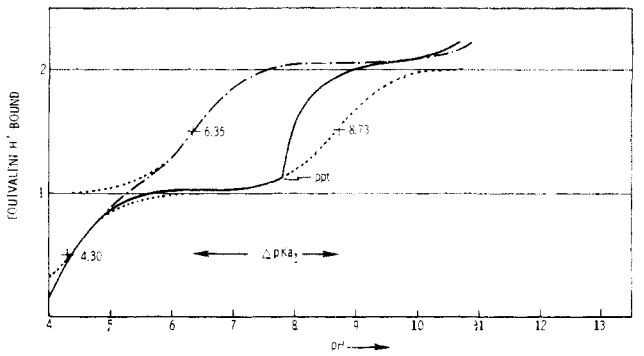

Figure 5. Comparison of the aqueous titration and the 10% oil–water titration of levallorphan tartrate. The solid line is the aqueous titration, the short dashed (- - -) line is the extrapolation of the aqueous titration, and the - · - · line is the octanol–water titration. The crosses mark the pK_a values and the ΔpK_{a1} represents the shift.

moval of a proton from nitrogen. Lowering of the measured pK_{a1} of levallorphan relative to levorphanol, 0.98, is commensurate with what one would expect for substitution of an allyl group for the methyl group in a compound of this kind. One would not expect the pK_a for levallorphan, an allyl-substituted amine, to be higher than the pK_a for levorphanol, the comparable methyl-substituted amine (although this is what the previous literature data for the pK_a's of levorphanol, 8.18,[14] and levallorphan, 8.3,[18] would imply). The substitution of an allyl group on piperidine, for example, lowers the pK_a by 1.47.[21]

Another question which now must be addressed is that we were not able to determine a second pK_a for these two compounds in aqueous solution. There are several possible contributions to this behavior: (1) interference of the precipitation; (2) the pK_{a2} might be raised so high that it is out of the measured range; (3) also plausible is the known tendency toward zwitterion formation in amphoteric substances when the pK_a of the basic group (in our case, the nitrogen) is comparable with, or greater than, the pK_a of the acid group (in our case, the phenolic group).[22]

The same comments apply to cyclazocine for which we found a measured pK_{a1}, 9.38* at 37°, and for which we could not determine a second higher pK_a.

There is no agreement on the literature values for the pK_a of methadone. They range from 8.25[14] to 10.12.[3a,16] Our value of 9.64*, compared to the literature value of 8.25[14] (measured by the same Canadian laboratory which performed the pK_a measurements of levorphanol and levallorphan), is commensurate with our measured pK_a values in 50% EtOH–H_2O being in the order of 1.5 units higher than their reported values. The most recent literature value, 8.62[17] at 23° (from titration in 100% MeOH), is quite reasonable compared to our values of 9.64* (20°) and 9.26* (37°) since the correction factors for converting the measured pK_a from 100% MeOH to its value in aqueous solution would be much greater than adding 0.5 pH and would be closer to adding almost 1 pH unit to the 100% alcohol value.

2. Partition and Drug Distribution Coefficients. In Table IV are presented for comparison the available literature values for the partition coefficients (true partition coefficients) and the drug distribution coefficients (apparent partition coefficients). While this distinction has been emphasized by many previous investigators (as in ref 10) and is nicely defined in Hansch's review,[4] it is often not stated precisely in some of the pharmacological literature which of the coefficients is really being reported. The advantage of the drug distribution coefficients is that they are the coefficients most significant for pharmacological implications.

Our tabulated partition coefficients, Tables I and II, are the average of at least four determinations in the range of 2–20% oil in water. As expected, the precision was poorest in the cases that the shift in pK_a was smallest. In such cases as morphine sulfate or oxymorphone hydrochloride the maximum scatter was about 15% of the value listed. In other cases it was much smaller. In cases where it was necessary to estimate the pK_a from alcohol–water solvent measurements, all of the comments made under pK_a determinations are applicable here. Again, the results are tabulated separately and users should take the limitations under which the results were obtained into consideration.

Our value for the true partition coefficient of morphine, 6.03 at 20° (octanol–H_2O), agrees quite well with that of Hansch and Anderson,[23] 5.75 at room temperature (octanol–H_2O). Their value was reported as $\log P = 0.76 \pm 0.02$. Thus their value for P is 5.75 (−0.25 to +0.28). We consider the agreement between the established classical methods of obtaining the morphine octanol–H_2O partition coefficient with the values obtained in this study as a verification of our procedure. Our values for the partition and drug distribution coefficients of codeine were measured directly in octanol–water. The various values for the partition coefficients of codeine reported in Hansch's tables[4] were mostly calculated by regression from different other solvents. Although there is a considerable spread in values of P(true) obtained for codeine by various classical methods (Table IV) it is clear that our value of 11.88 is a reasonable one which falls about in the middle of the range of the previously reported measured values. The values for the partition coefficients of levorphanol calculated using either the aqueous pK_a's or the corrected pK_a*'s from ethanol–water titration (which were only 0.01 unit different) agree to within 2.5%. The drug distribution coefficients, which are less affected by a slight shift in the measured reference pK_a's, agree even more closely, to within ca. 0.1%. The slightly greater difference between the aqueous pK_a of levallorphan and the corrected ethanol–water value led to about a 15% difference in the calculated partition coefficients but to only an average 4% or less difference in the pharmacologically significant drug distribution coefficients.

Our value for the true partition coefficient of methadone is considerably higher than that reported in Hansch's tables.[4] However, from examination of the original reference,[26] while it is not completely clear, the value of 33.88 seems to be those authors' measured value[26] for the apparent partition coefficient in ethyl oleate–H_2O. (In that article[26] there is no mention of the experimental details nor of the pH or the temperature at which this partition coefficient was measured.) It does not appear to be the true partition coefficient in octanol–H_2O although it is quoted as such in ref 4. Also, a value of 33.88 is not incompatible with the values we obtain for the methadone drug distribution coefficient at 20°. Moreover, to obtain the true partition coefficient from the measured apparent partition coefficient involves using the measured pK_a to get the amount of free base present. As data in Table III indicate, there have been previous wide discrepancies in the reported pK_a values of methadone from 8.25 to 10.12. A quite recent value of the apparent partition coefficient of methadone, 55.5, obtained by shaking radiolabeled drug with an octanol–H_2O mixture at pH 7.4 and ~25°, separating and counting the drug in the two fractions,[40] is not as compatible with our results. [However, in an even more recent reference from the same laboratory[41] (appearing after the original submission of this present article), the value for

Table IV. Literature Values for Partition Coefficients (Apparent and True)

	P' (apparent)	P (true)	Temp, °C	Solvent	Ref
Morphine	0.15	0.8	37?	Cyclohexane–H_2O	10
		5.75	RT	Octanol–H_2O	23
		5.24	RT	Octanol–H_2O	4
	1.0 (pH 7.4)		~25	Octanol–H_2O	40
	1.4 ± 0.2 (pH 7.4)		?	Octanol–H_2O	41
	<0.00001 (pH 7.4)		?	Heptane–H_2O	24, 25
	<0.0504 (pH 7.4)		?	Ethylene chloride–H_2O	24
	0.02 (pH 7.0)		?	Ethylene dichloride–H_2O	42
Nalorphine	3.9 (pH 7.4)		~25	Octanol–H_2O	40
	0.028 (pH 7.4)		?	Heptane–H_2O	24
	1.7 (pH 7.4)		?	Ethylene chloride–H_2O	24
	3.9 (pH 7.4)		?	Ethylene dichloride–H_2O	42
Codeine		15.49			4
		10.23			4
		6.02	RT	Octanol–H_2O	
		7.58			
		26.30		(Calcd in ref 4 by regression from different other solvents)	
		42.20			
Naloxone	5.4 (pH 7.4)		~25	Octanol–H_2O	40
	3.1 ± 0.1 (pH 7.4)		?	Octanol–H_2O	41
	4.0 (pH 7.0)		?	Ethylene dichloride–H_2O	42
Meperidine	11.5 (pH 7.0)		?		42
Levorphanol	8.7 (pH 7.4)		~25	Octanol–H_2O	40
	<0.01 (pH 7.4)		?	Heptane–H_2O	43
	0.12 (pH 7.4)		?	Ethylene dichloride–H_2O	42
Levallorphan	0.35 (pH 7.4)		?	Heptane–H_2O	24
Methadone	33.88[a]		RT	Ethyl oleate–H_2O	26
l-Methadone	57.3 (pH 7.4)		?	Octanol–H_2O	44
	55.5 (pH 7.4)		~25	Octanol–H_2O	40
	37.0 ± 2.8 (pH 7.4)		?	Octanol–H_2O	41
	24.0 (pH 7.0)		?	Ethylene dichloride–H_2O	42
d-Methadone	28.3 (pH 7.4)		?	Octanol–H_2O	41

[a] See text for discussion.

Table V. Free Base Solubilities

Compound	Temp, °C	pK_a	Solubility of free base, g/l.
Morphine	20	8.02	0.149
	37	7.93	0.184
Levorphanol and dextrorphan	20	9.49	0.494
	37	9.36	0.567
Naloxone	20	7.94	0.134
	37	7.82	0.140

Table VI. Literature Values for Solubility of Morphine Free Base

mol/l.	g/l.	Temp, °C	Ref[a]
4.7×10^{-4}	0.143	18	27
	0.149	20	28
5.0×10^{-4}	0.147	20	29
6.1×10^{-4}	0.181	18–20	30
	0.25	18–20	31
	0.288	15	32
	0.283	18–20	33

[a] These references came from F. K. Beilstein, "Handbuch der organische Chemie," Vol. 27, 2nd ed, Springer-Verlag, Berlin, 1955, p 123.

the apparent partition coefficient of methadone in octanol–H_2O of 37.0 ± 2.8 measured by the same radiolabeled method (presumably at room temperature) is more compatible with our results.]

3. Aqueous Solubility. It was possible with the technique that was used in this study to determine the aqueous solubilities of the free bases of several of the compounds under investigation (Table V). As far as we are aware there are very few reliable values for the solubilities of these compounds in the literature. Even in the case of morphine where a number of determinations have been reported there is a surprisingly large spread in reported results. In the case of morphine Maus[27] reported a value of 0.143 g/l. at 18°, Baggesgaard-Rasmussen et al.[28] give a value of 0.149 at 20°, and Kolthoff[29] reported a value of 0.147 at 20°. These values agree well with our value of 0.149 g/l. On the other hand, values have been reported in the literature in the 15–20° range which have a spread ranging from 0.181 to 0.288[30–33] g/l. (Table VI). We consider the close agreement of our value with the prior three values which appear to be more reliable as a verification of this technique of aqueous solubility measurement.

Pharmacological Significance. There are several important points to be noted which have pharmacological significance. Firstly, the drug distribution coefficients are extremely sensitive to pH at values which span the range of attainable human physiological blood pH values. While a pH of 7.40 is considered as the norm, a patient who is very ill and in acidosis can have a blood pH as low as 7.1, while a patient hyperventilated under anesthesia during an opera-

tion can have a pH as high as 7.7. There is an approximate 300–400% increase in drug distribution coefficient between the low and high pH values. Even for "normal individuals", between the narrow pH ranges of 7.35 and 7.45, which span closely the so-called "normal" physiological blood pH, there is still approximately a 20% increase in drug distribution coefficients. This strong pH dependence has significant implications for proper scaling of drug dosage under various clinical situations.[34] There is clinical evidence which indicates that a patient who is under a narcotic analgesic and hyperventilates under concomitant administration of a general anesthetic (i.e., the blood pH rises and the patient is then in alkalosis) goes into a deeper plane of analgesia as if he had been given a larger dose of the narcotic.[35] This strong pH dependence is also extremely important in the type and dosage of narcotic and narcotic antagonist which should be given to obstetrical patients in labor. Because the pH of the fetus, 7.2, is lower than that of the mother, the same amounts of narcotics (or narcotic antagonists) do not traverse the placental barrier in both directions and this can lead to an undesirable buildup in narcotic concentration in the fetus.[34] There are several factors which have been cited as of the most importance in governing the transfer of drugs from mother to fetus.[36] First, the placental membrane is mainly lipoprotein in nature; therefore, lipophilic drugs can pass more easily. Second, the membrane carries a charge so that ionized drugs tend not to cross easily. (However, those authors do not state whether the membrane carries a positive or a negative charge.) The third (and what those authors consider as perhaps the most important) factor in controlling transfer is the concentration gradient across the placental membrane. Once the free base form of the drug has traversed the placental barrier from the mother to the fetus (the propensity of which is proportional to the lipid–water drug distribution coefficient), then the lower the pK_a of the narcotic (or narcotic antagonist), the more apt it is to become concentrated in the fetus, since only the free base can retraverse the placental barrier.[34] While there has been a great deal of prior research in perinatal pharmacology, even in a recent excellent review of that field,[37] while a number of factors are cited as affecting transplacental drug movement, there is no specific mention of the effect that the lower fetal pH will have on altering the ability of the drug to retraverse the placental barrier. This is an important consideration and should be taken into account in prescribing any kind of drugs (especially CNS drug) to pregnant women or administering them to women in labor.

Secondly, it has apparently been customary for pharmacologists to measure pK_a's and partition coefficients at 20° (sometimes at 25° or at room temperature—often the temperatures are not even reported in the articles) and to use the numerical results obtained at these temperatures in discussing pharmacological implications. However, physiological body temperature is 37°. As a check, the pK_a's, partition coefficients, and drug distribution coefficients were also measured at 37°. It is apparent from the values in Tables I and II that raising the temperature from 20 to 37° results in significant increases in drug distribution coefficient ranging from 21% for morphine to 200% for naltrexone. The nonregularity of the increases with temperature emphasizes the necessity for careful attention to the temperature dependence of these properties. It appears not to be valid merely to extrapolate by any constant factor to body temperature the measurements made at lower temperatures. Even the true partition coefficients increase markedly with temperatures from 20 to 37°.

Thirdly, there is more of a difference in the drug distribution coefficients for naloxone and naltrexone than might have been expected from the similarities in their structures, with naltrexone being significantly less lipophilic than naloxone. This would imply that this would lead to naloxone having a more rapid onset for antagonist activity and likewise a shorter duration of action than naltrexone.[7] Clinical studies confirmed this prediction.[38]

It was suggested by Martin[39] that opiate antagonists enter the brain more rapidly than opiate agonists. Examination of the data in Tables I and II indicates that while this is true for congener agonist–antagonist pairs, it is not true in general. Compounds such as meperidine, methadone, and α-acetylmethadol (all narcotic agonists) have higher drug distribution coefficients than nalorphine, naloxone, or naltrexone (the last two of which are almost pure narcotic antagonists).

The onset and duration of narcotic agonist and antagonist activity are related to lipid solubility. The effective potency of the drugs is governed both by their lipid solubilities and by their specific chemical structures and absolute configurations.[1] This latter propensity appears to be reflected in the quantitative differences in their binding to the "opiate receptor".[2]

Conclusions

The procedures outlined in this article permit one to deconvolute overlapping pK_a's and have enabled us to ascertain the two different pK_a's in most of the molecules investigated. Reliable "true" partition coefficients depend on knowing the correct pK_a quite precisely since the "true" partition coefficient refers to the partitioning of the same species—the free base—between lipid and water.

We have also measured sensitively the partition coefficients and total drug distribution coefficients of these compounds as a function of pH and temperature. These quantities exhibited a more pronounced nonuniform temperature dependence than pharmacologists customarily appear to take into consideration when using such data in predicting or correlating pharmacological behavior. The drug distribution coefficients, which are vital for understanding drug transport, are shown also to have a much more pronounced pH dependence, even in a small range around normal physiological pH, than apparently has been customarily taken into consideration in pharmacological correlations.

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References and Notes

(1) E. Kutter, A. Herz, H.-J. Teschemacher, and R. Hess, *J. Med. Chem.*, 13, 801 (1970).
(2) M. J. Kuhar, C. B. Pert, and S. H. Snyder, *Nature (London)*, 245, 447 (1973).
(3) (a) D. D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solution", Butterworths, London, 1965; (b) "Dissociation Constants of Organic Bases in Aqueous Solution: Supplement 1972", Butterworths, London, 1972.

(4) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).
(5) (a) T. V. Parke and W. W. Davis, *Anal. Chem.*, **26**, 642 (1954); (b) W. W. Davis and C. J. Kreutler, papers presented before the Division on Analytical Chemistry, 164th National Meeting of the American Chemical Society, Washington, D.C., Sept 1971, Abstracts ANAL 8 and ANAL 9.
(6) J. J. Kaufman and W. S. Koski in "Drug Design", Vol. V, E. J. Ariens, Ed., Academic Press, New York, N.Y., 1975, Chapter 6, p 251.
(7) (a) J. J. Kaufman and W. S. Koski, presented at the International Symposium on Quantum Biology and Pharmacology, Sanibel Island, Jan 1974; *Int. J. Quantum Chem., Biol. Symp.*, 1, 289 (1974); (b) J. J. Kaufman, presented at the Committee on Problems of Drug Dependence, Satellite Session, Cocoyoc, Mexico, March 1974.
(8) W. S. Koski, paper presented at the 167th National Meeting of the American Chemical Society, Los Angeles, Calif., April 1974, PHYS 4.
(9) G. Schill and K. Gustavii, *Acta Pharm. Suec.*, 1, 24 (1964).
(10) A. F. Casy and J. Wright, *J. Pharm. Pharmacol.*, **18**, 677 (1966).
(11) This expression may be derived in the following manner

$$K_{sp} = [H^+][A^-]$$

if C is the molar concentration of the salt and α is the fraction of 1 equiv of hydrogen ion bound by the sample at any given pH

$$[A^-] = C(1 - \alpha)$$

$$K_{sp} = [H^+]C(1 - \alpha)$$

$$\log K_{sp}/C = \log [H^+] + \log (1 - \alpha)$$

$$pH = \log (1 - \alpha) - \log K_{sp}/C$$

(12) This expression may be obtained in the following manner. The pH of a solution of a weak acid being titrated by a strong base is given by

$$pH = pK_a + \log [salt]/[acid]$$

where K_a is the dissociation constant which in our case is

$$K_a = \frac{[H^+][A^-]}{[HA]} = \frac{[B][H^+]}{[BH^+]}$$

In the absence of any oil phase the $pH = pK_a$ when the concentration of acid and salt is equal. In the presence of the oil when 50% of the acid is neutralized the amount of acid present in the aqueous phase equals the sum of the free base present in the aqueous and the oil phases so

$$V_w[BH^+] = V_w[B_w] + V_o[B_o]$$

where V_w and V_o are the volumes of water and oil, respectively, and B_w and B_o are the concentrations of the free base in the water and oil, respectively. Substituting this equality in the first equation gives

$$pH = pK_a + \log \frac{(V_w[B_w] + V_o[B_o])}{V_w[B_w]}$$

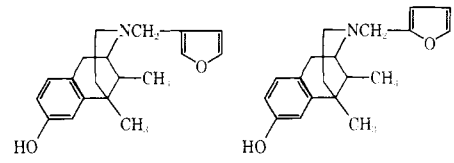
In the presence of the oil when 50% of the acid is consumed the pH will be equal to an apparent pK_a which is shifted by an amount ΔpK_a from the pK_a obtained in the aqueous solution; then

$$\text{antilog } \Delta pK_a = 1 + V_o/V_w$$

where P = distribution of the free base between oil and water = $[B_o]/[B_w]$. Solving for P we have

$$P = V_w/V_o \text{ (antilog } \Delta pK_a^{-1})$$

(13) H. Merz, private communication.



MR-1255-BS MR-1029-BS

(14) C. G. Farmilo, P. M. Oestricher, and L. Levi, *Bull. Narc.*, **6**, 7 (1954).
(15) A. H. Beckett, *J. Pharm. Pharmacol.*, **8**, 848 (1956).
(16) P. B. Marshall, *Br. J. Pharmacol.*, **10**, 270 (1955).
(17) P. S. Portoghese and D. A. Williams, *J. Med. Chem.*, **12**, 839 (1969).
(18) L. Martin, K. Genest, J. A. R. Cloutier, and C. G. Farmilo, *Bull. Narc.*, **15**, 17 (1963).
(19) L. Saunders and R. S. Srivastava, *J. Pharm. Pharmacol.*, **3**, 78 (1951).
(20) R. G. Bates and V. E. Bower, *J. Res. Natl. Bur. Std.*, **57**, 153 (1956).
(21) H. K. Hall, *J. Phys. Chem.*, **60**, 63 (1956).
(22) J. Clark and D. D. Perrin, *Q. Rev., Chem. Soc.*, **13**, 295 (1964).
(23) C. Hansch and S. Anderson, *J. Org. Chem.*, **32**, 2583 (1967).
(24) A. Herz, *Psychopharmacologica*, **26**, 219 (1972).
(25) A. Herz, *Neuropharmacology*, **12**, 111 (1973).
(26) G. Valette and J. Cariou, *C. R. Seances Soc. Biol. Ses Fil.*, **152**, 317 (1958).
(27) P. Maus, Dissertation, Tübingen, 1904.
(28) H. Baggesgaard-Rasmussen and F. Reimers, *Arch. Pharm. (Weinheim, Ger.)*, 129 (1935).
(29) J. M. Kolthoff, *Biochem. Z.*, **162**, 339 (1925).
(30) A. Heiduschka and M. Faul, *Arch. Pharm. (Weinheim, Ger.)*, **255**, 452 (1917).
(31) E. Winterstein, *Ar. Pht.*, **62**, 141 (1910).
(32) G. Guerin, *J. Pharm. Chim.*, **7**, 438 (1913).
(33) Muller, *Apoth. Ztg.*, 18, 258 (1903).
(34) D. W. Benson, J. J. Kaufman, and W. S. Koski, *Curr. Res. Anesth. Analg.*, submitted for publication.
(35) D. W. Benson (Anesthesiologist-in-Chief, The Johns Hopkins Hospital, and Chairman, Department of Anesthesiology, The Johns Hopkins University School of Medicine), private communication.
(36) I. Speirs and A. W. Sim, *Br. J. Anesth.*, **44**, 370 (1972).
(37) S. J. Yaffe and M. R. Juchau, *Annu. Rev. Pharmacol.*, **14**, 219 (1974).
(38) A. Kurland, private communication, March 1974.
(39) W. R. Martin, *Pharmacol. Rev.*, **19**, 464 (1967).
(40) A. L. Misra, R. B. Pontani, and S. J. Mule, *Xenobiotica*, **4**, 17 (1974).
(41) S. J. Mule, G. Casella, and D. H. Clouet, *Res. Commun. Chem. Pathol. Pharmacol.*, **9**, 55 (Sept 1974). (This reference added in proof since it appeared after original submission of our article for publication. We thank Dr. Mule for bringing this article to our attention.)
(42) D. H. Clouet and N. Williams, *Biochem. Pharmacol.*, **22**, 1283 (1973).
(43) A. Herz and H.-J. Teschemacher, *Adv. Drug. Res.*, **6**, 79 (1971).
(44) A. L. Misra and S. J. Mule, *Nature (London)*, **241**, 281 (1973).