Influence of Hydrophilic–Lipophilic Balance Values of Surfactants on Ephedrine Absorption and Release from Emulsified Systems after Oral Administration to Dogs

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Abstract □ Ephedrine release and availability characteristics *in vivo* were investigated from liquid emulsion systems as they are affected by the hydrophilic-lipophilic nature of the emulsifiers present. Three control and five test groups were used; the latter were all emulsified systems made with surfactants having hydrophilic-lipophilic balance (HLB) values from 10 to 14. All experiments were performed under an acidic urine control, and all urine samples were assayed by GLC to detect the excretion of unchanged drug. The urinary excretion data were best described by the two-compartment model with a zero-order absorption process. Correlations of availabilities between *in vivo* and *in vitro* data were determined, where appropriate. Correlations of ephedrine present in urine *in vivo* with the total amount dialyzed *in vitro* were significant at each HLB value. Poor correlation of absorption rate with dialysis rate was noted at an HLB of 10.

Keyphrases □ Ephedrine—effect of hydrophilic-lipophilic balance values of surfactants on absorption and release from emulsified system, oral administration, dogs, *in vivo-in vitro* correlations □ Surfactants—influence of hydrophilic-lipophilic balance values on ephedrine absorption and release from emulsified systems, oral administration, dogs, *in vivo-in vitro* correlations □ Hydrophilic-lipophilic balance values, surfactants—influence on ephedrine absorption and release from emulsified systems, oral administration, dogs, *in vivo-in vitro* availabilities correlated □ Emulsions—influence of hydrophilic-lipophilic balance values of surfactants on ephedrine absorption and release from emulsified systems

In 1971, Fincher and Waggoner (1) studied the influence of hydrophilic-lipophilic balance (HLB) values of surfactants on ephedrine release rates from emulsified liquid systems. The release rate of ephedrine increased in direct proportion between HLB values of 10 and 14.9. Since this work was conducted *in vitro,* it was desirable to determine if similar results would be observed *in vivo.*

EXPERIMENTAL

The emulsifiers used were polysorbate 60[1] with an HLB value of 14.9 and sorbitan monostearate[2] with an HLB value of 4.7. The amount of the emulsifiers required to obtain the desired HLB values of 10, 11, 12, 13, and 14 was calculated by simple allegation. The drug ephedrine[3] was selected because it was used in previous *in vitro* studies (1). The experimental animals were male beagle dogs, weighing from 9.1 to 13.6 kg (20 to 30 lb), with an average age of 18 months.

Phase I: Preliminary Preparations—To ensure uniformity, all emulsion systems and controls were prepared on a weight basis, with the drug supplying 1%, the surfactant supplying 5%, and the mineral oil[4] and water each supplying 47%, according to the methods previously described (1). An acidic urine was induced and maintained in the dogs by administration of ammonium chloride. One capsule containing 1 g ammonium chloride was given orally every 4 hr on the day before the drug systems were given; one capsule was given 1 hr before administering the drug, and one capsule was given every 4 hr thereafter throughout each experiment to control the urine pH between 4 and 5, thus enhancing drug elimination (2).

Urine samples were collected by catheterization at 1, 2, 4, 8, 12, 24, 36, and 48 hr. The volume and the pH of each sample were

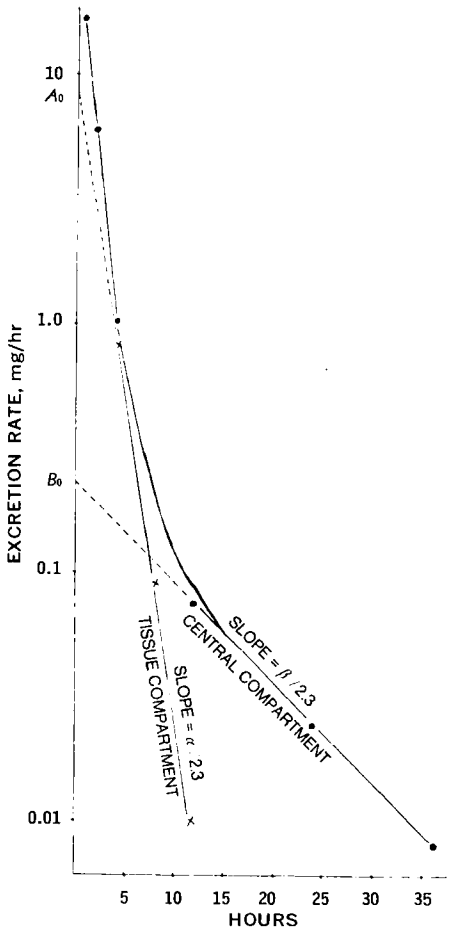

Figure 1—*Log milligrams ephedrine per hour* versus *time after intravenous injection of 20 mg ephedrine aqueous solution to dogs (eight dogs mean data). Curve is fitted by the following equation: excretion rate* $= 9.6e^{-0.58t} + 0.23e^{-0.092t}$.

[1] Tween 60, Atlas Chemical Corp., Wilmington, Del.
[2] Span 60, Atlas Chemical Corp., Wilmington, Del.
[3] Ephedrine NF (hydrous), Merck & Co., Rahway, N.J.
[4] Mineral oil, Sargent-Welch Scientific Co., Chicago, Ill.

Table I—Mean Cumulative Amounts of Ephedrine Excreted in Urine and Total Percent of Recovery from 0 to 48 hr[a]

Hours	Control, mg			Test, mg				
	I (8)	II (6)	III (6)	IV (6)	V (6)	VI (6)	VII (6)	VIII (6)
1	1.60	1.50	0.89	1.68	2.43	1.39	1.58	2.75
2	6.40	3.80	1.03	2.95	4.24	2.96	3.78	4.47
4	14.40	6.20	2.10	4.42	5.57	4.30	5.01	7.04
8	15.60	8.80	5.40	6.06	6.83	6.28	6.95	8.39
12	17.60	10.5	7.30	7.26	6.62	7.62	7.79	10.10
24	17.80	12.90	8.80	8.51	8.20	8.96	8.53	10.66
36	18.20	14.80	9.80	9.56	9.14	9.32	8.74	11.27
48	18.50	15.28	9.96	9.85	9.31	10.03	9.54	11.51
Percent of recovery, 0–48 hr	92.5	76.4	49.8	49.3	46.6	50.2	47.7	57.6

[a] Control: I = intravenous injection of 20 mg ephedrine in aqueous solution, II = oral administration of 20 mg ephedrine in aqueous solution, and III = oral administration of 20 mg ephedrine in oil solution. Test: oral administration of 20 mg ephedrine in an emulsified system at: IV, HLB of 10; V, HLB of 11; VI, HLB of 12; VII, HLB of 13; and VIII, HLB of 14. The number in parenthesis after control or test group indicates the number of dogs utilized in determining the mean.

measured, and the samples were stored at 4° until the time of the assay.

Phase II: Assay Method—The content of ephedrine in urine was determined using a modified GLC method previously reported (3). A gas chromatograph[5] equipped with a flame-ionization detector was used. The chromatographic column was aluminum tubing, 0.635 cm in diameter and 2 m in length. It was packed with 80–100-mesh Anakrom ABS coated with 5% 20M Carbowax. Prior to coating, the support was suspended in 1 *N* NaOH and then was collected by filtration. The column temperature was 180° and the injection temperature was about 200°. The nitrogen carrier flow rate was 36 ml/min.

Urine (10 ml) was added to 5 *N* HCl (0.5 ml) in a 45-ml centrifuge tube fitted with a glass cap. The mixture was extracted with 3 × 5 ml ether, and the ether layers were discarded. To the urine was then added 5 *N* NaOH (1.0 ml) and exactly 1 ml of a 0.1-mg/ml solution of internal standard (α-diethylpropion as the hydrochloride) in water. The mixture was extracted with 3 × 5 ml ether. The extracts were combined and the ether was evaporated under a nitrogen stream to about 1 ml. Approximately 0.5 μl of this solution was injected into the column. The amount of ephedrine was obtained by calculating the ratio of the peak area of ephedrine to that of the internal standard and relating this value to a relative calibration curve.

Blank urine samples of 10 ml each were spiked with 0.1, 0.25, 0.50, 1.0, 1.5, and 2.0 ml of a 1-mg/ml solution of pure ephedrine hydrochloride to give six sets of ephedrine solution of definite concentration. One milliliter of 1 mg/ml internal standard (α-diethylpropion hydrochloride) was added in each set. Then quantitative analyses of these six sets of samples were performed by the described assay procedure. From the chromatogram, the ratio of peak area of ephedrine to internal standard was measured. Then the relative calibration curve of ephedrine to internal standard was obtained. The area was estimated by multiplying the height times the width at half-height.

Phase III: Group Classification—Control groups were established to aid in the interpretation of the emulsion results.

Control I—This control consisted of the intravenous injection of 20 mg ephedrine in aqueous solution to dogs. The aqueous solution of ephedrine was prepared by dissolving 1 g ephedrine in 99 g water and heating to about 60° with stirring.

Control II—In this control, 20 mg of ephedrine in aqueous solu-

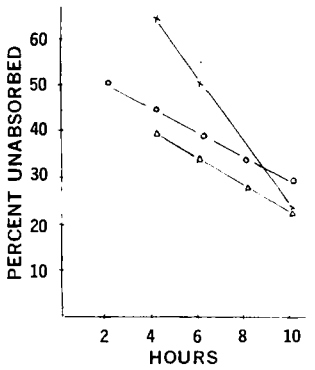

Figure 2—*Oral absorption of ephedrine from emulsion systems made with surfactant having an HLB value of 10. Key: Δ, ephedrine in emulsion system at HLB value of 10; ×, ephedrine in oil preparation; and ○, ephedrine in aqueous preparation.*

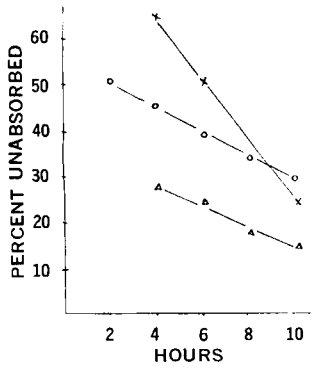

Figure 3—*Oral absorption of ephedrine from emulsion systems made with surfactant having an HLB value of 14. Key: Δ, ephedrine in emulsion system at HLB value of 14; ×, ephedrine in oil preparation; and ○, ephedrine in aqueous preparation.*

[5] Model 900, Perkin-Elmer Corp., Norwalk, Conn.

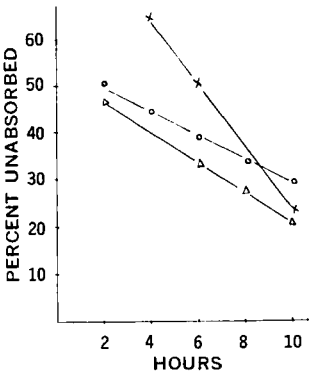

Figure 4—*Oral absorption of ephedrine from emulsion systems made with surfactant having an HLB value of 12. Key: Δ, ephedrine in emulsion system at HLB value of 12; ×, ephedrine in oil preparation; and ○, ephedrine in aqueous preparation.*

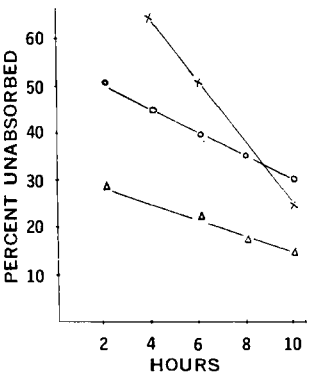

Figure 5—*Oral absorption of ephedrine from emulsion systems made with surfactant having an HLB value of 13. Key: Δ, ephedrine in emulsion system at HLB value of 13; ×, ephedrine in oil preparation; and ○, ephedrine in aqueous preparation.*

Table II—Estimated Parameters Fitting Two-Compartment Open Model at Different Doses to Dogs

Parameters[a]	Intravenous Ephedrine Injection		
	10 mg	20 mg	40 mg
K_d, hr^{-1}	0.4465	0.5159	0.4522
$K_{1,2}$, hr^{-1},	0.0450	0.0526	0.0453
$K_{2,1}$, hr^{-1}	0.1485	0.1034	0.1725
α	0.51	0.58	0.52
β	0.13	0.092	0.15
$T_{1/2}$, hr	5.3	7.5	4.6

[a] $K_d = \frac{\alpha\beta(A + B)}{A\beta + B\alpha}$

$K_{1,2} = \frac{AB(\beta-\alpha)^2}{(A + B)(A\beta + B\alpha)}$

$K_{2,1} = \frac{A\beta + B\alpha}{A + B}$

$t^1/_2 = 0.693/\beta$

$\alpha/2.3$ = slope of tissue compartment line

$\beta/2.3$ = slope of central compartment line

tion was administered orally to dogs. This aqueous solution of ephedrine was prepared by the same way as Control I.

Control III—Oral administration of 20 mg of ephedrine in oil solution to dogs was utilized. This oil solution of ephedrine was prepared by dissolving 1 g ephedrine in 99 g mineral oil and heating to about 60° with stirring.

Test Groups—There were five test groups; each dog separately and orally received 20 mg ephedrine in an emulsion made from the surfactants having HLB values of 10, 11, 12, 13, and 14, respectively. The emulsion was gently shaken by hand before it was administered.

Phase IV: Pharmacokinetic Study—Separately, single doses of 10, 20, and 40 mg ephedrine in aqueous solution were administered by intravenous injection to dogs. Acidic urine control, collection of urine, and assay of urine samples were described previously. This study was necessary to determine if the kinetic parameters (*e.g.*, volumes of distribution and rate constants) are changed with dose (4).

Phase V: Treatment of Data—The average urinary excretion rates with respect to time were plotted on semilogarithmic graph paper and then the best fit biexponential equation:

$$\text{excretion rate} = A_0e^{-\alpha t} + B_0e^{-\beta t} \qquad \text{(Eq.1)}$$

was obtained (Fig. 1). The value of α was calculated from the slope of the tissue compartment equilibration line obtained by subtracting the extrapolated portion of the terminal linear portion of the curve from the beginning nonlinear portion; $\alpha/2.3$ is equal to the slope. The value of β was calculated from the slope of the terminal linear portion of the central compartment line; $\beta/2.3$ is equal to the slope. The values of A_0 and B_0 were estimated from the Y intercepts of the tissue and central compartment lines, respectively.

RESULTS AND DISCUSSION

Kinetic Studies—Cumulative amounts of unchanged ephedrine excreted in urine and the total percentages of ephedrine recovery

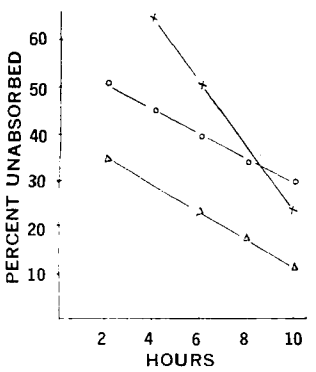

Figure 6—*Oral absorption of ephedrine from emulsion systems made with surfactant having an HLB value of 11. Key: △, ephedrine in emulsion system at HLB value of 11; ×, ephedrine in oil preparation; and ○, ephedrine in aqueous preparation.*

Table III—Ephedrine Absorption Rate Constants (in Dogs) and Dialysis Rate Constants

System[a]	Absorption Rate Constant ($-K_a$), hr^{-1}	Dialysis Rate Constant ($-m$), min^{-1}
1	2.60	0.279
2	6.80	0.101
3	2.90	0.055
4	2.30	0.077
5	3.20	0.089
6	1.90	0.061
7	2.90	0.116

[a] 1 = aqueous preparation system, and 2 = oil preparation system. The emulsified systems were: 3, HLB 10; 4, HLB 11; 5, HLB 12; 6, HLB 13; and 7, HLB 14.

from 0 to 48 hr are given in Table I. In the control groups, the aqueous intravenous injection (I) yielded the highest percentage of drug recovery, followed by the aqueous oral solution (II), and the oil oral solution (III) in order of decreasing yields. In five test groups, it was found that there was a stairwise fluctuation of percent of ephedrine recovery from 0 to 48 hr in the emulsified systems which had HLB values from 10 to 14. Higher percentages were obtained at HLB values of 10, 12, and 14, with the highest at 14. This indicated that the release of ephedrine from the emulsified systems was greatest when the emulsified system had an HLB value of 14. In no case did the percent drug recovery from the emulsified systems exceed that of Controls I and II which were aqueous solutions administered intravenously and orally. However, when the five test groups were compared to Control III (the oil solution administered orally), the emulsified systems having surfactant with HLB values of 12 and 14 yielded a higher percent recovery than that of Control III by 0.8 and 15.7%, respectively. The emulsified systems having emulsifiers with HLB values of 10, 11, and 13 were lower than the Control III by 1.0, 6.4, and 4.2%, respectively. These results are partially in agreement with the *in vitro* data (1) where the emulsions containing a surfactant with the high HLB values released the ephedrine faster than those of lower HLB values.

By utilizing the two-compartment model and excretion data after single intravenous doses, some kinetic parameters were estimated (Table II). The kinetic parameters were not dependent on dose, and the $t_{1/2}$ based on $0.693/\beta$ was in the range of 4.6–7.5 hr, which was near the value of 5.99 hr (5.01–7.46 hr) found using the two-compartment model and biexponential fitting (5).

By utilizing the two-compartment model and measuring unchanged drug excreted in the urine, the absorption rate constant, K_a, was estimated by a digital computer using Wagner's (4) equations:

$$f(A)_{tn} = 1/K_d(dAe/dt)_{tn} + (Ae)_{tn} + T_n \qquad \text{(Eq.2)}$$

where:

$$T_n = 1/K_d[(dAe/dt)_{tn-1}(K_{1,2}/K_{2,1})(1 - e^{-K_{2,1}\Delta t})] + 1/K_d[(dAe/dt)_{tn} - (dAe/dt)_{tn-1}]K_{1,2}\,\Delta_{t/2} + T_{n-1}e^{-K_{2,1}\Delta t} \qquad \text{(Eq.3)}$$

and K_d is the overall elimination rate constant, $K_{1,2}$ is the transfer rate constant going from the central to the tissue compartment, $K_{2,1}$ is the transfer rate constant going from the tissue to the central compartment, Ae is the cumulative amount of drug excreted in the urine at time t, f is the fraction of drug absorbed, A is the amount of drug absorbed, t is the time, Δt is the time interval, and n is a positive integer, *i.e.*, $n = 0, 1, 2 \ldots$. Then:

$$\text{percent absorbed} = \frac{(f)(A)_{tn}}{(f)(A)_{\max}} \times 100 \qquad \text{(Eq.4)}$$

The value of Eq. 2 progressively increases until it reaches a maximum or asymptotic value. When the values are expressed as percentage of this asymptotic value, the desired percents absorbed are obtained; *i.e.*, percent unabsorbed = 100% − percent absorbed.

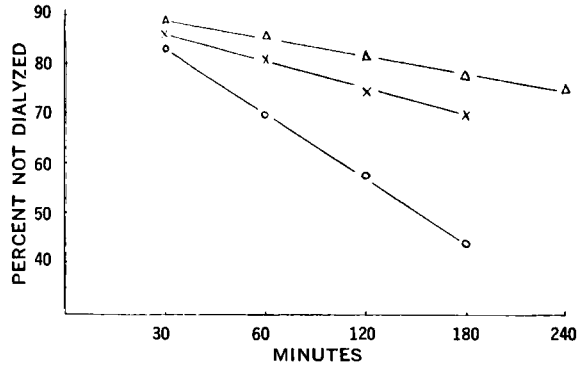

Figure 7—*Dialysis of ephedrine from emulsion systems made with surfactant having an HLB value of 12. Key: △, emulsion system at HLB 12; ×, oil preparation; and ○, aqueous preparation.*

By plotting the percent unabsorbed *versus* time on linear graph paper, a linear relationship (Figs. 2–6) was obtained and the zero-order absorption rate constant (K_a) was determined from the slope of the line. In a similar manner, to correlate these data with *in vitro* work (1), the dialysis rate constant, m, was determined by calculating the slope of the percent not dialyzed *versus* time plots (Figs. 7–11). Data for K_a and m are given in Table III.

All emulsified systems were unabsorbed at lesser percentages (more drug was absorbed) than that of the controls, both aqueous and oil systems. Similar treatment of the *in vitro* data (1) is given in Figs. 7–11. All emulsified systems were not dialyzed to the same extent as were the aqueous and oil controls, except the emulsified system having the surfactant with an HLB value of 14.

Correlations of *In Vivo* and *In Vitro* Studies—Correlation of the rate of availability for *in vivo* and *in vitro* studies at each HLB value of the emulsified system was obtained. There was poor correlation at HLB values of 11, 12, 13, and 14 (Fig. 12). The correlation coefficient was $r = 0.45$ and the regression coefficient was $b = 0.021$ for all HLB values. There was no correlation at an HLB value of 10.

Correlations of the amount of drug recovered between *in vivo* and *in vitro* studies are shown in Fig. 13. *In vivo*, the percent of total amount recovered was calculated from 0 to 48 hr using the intravenous injection control as a standard; *in vitro*, the percent of total amount dialyzed was determined from 0 to 240 min. The correlation coefficient was $r = 0.92$ and the regression coefficient was $b = 1.14$. It can be concluded from these correlations that there was poor correlation between the rate of dialysis and the rate of absorption at four HLB values studied, and no correlation was obtained at an HLB value of 10; there was good correlation between the extent of drug dialyzed and the extent of ephedrine recovered in the urine of dogs after oral administration at each HLB value.

In vitro work demonstrated that the ephedrine was dialyzed in two phases. There was an initial fast release pattern followed by a slower secondary release pattern. The slope of each percent not dialyzed *versus* time line was based on the secondary release phase, and this did not correlate well with the overall *in vivo* absorption rates obtained. The initial phase release of ephedrine from the emulsion systems was probably not observed *in vivo* because ephedrine is not absorbed from the stomach in the protonated form. The delay in absorption time, due to a mixing of the emulsion with stomach contents and stomach emptying rate, probably obliterated the potential effects of the fast initial release observed *in vitro* but not *in vivo*.

The extent of drug dialyzed did correlate well with the extent of drug recovered from the urine and at each HLB value. When consideration is given to the physiological system, the latter is a more valid correlation and demonstrates that *in vitro* work will

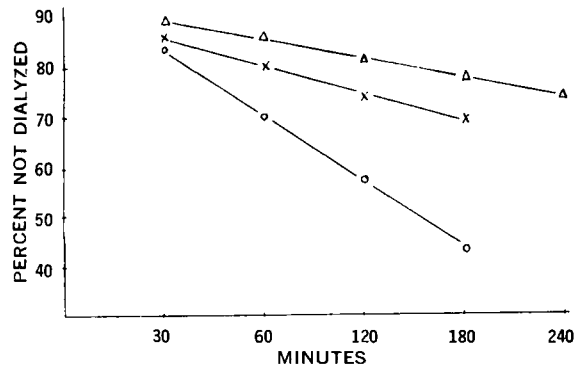

Figure 8—*Dialysis of ephedrine from emulsion systems made with surfactant having an HLB value of 10. Key: △, emulsion system at HLB 10; ×, oil preparation; and ○, aqueous preparation.*

Figure 9—*Dialysis of ephedrine from emulsion systems made with surfactant having an HLB value of 11. Key: △, emulsion system at HLB 11; ×, oil preparation; and ○, aqueous preparation.*

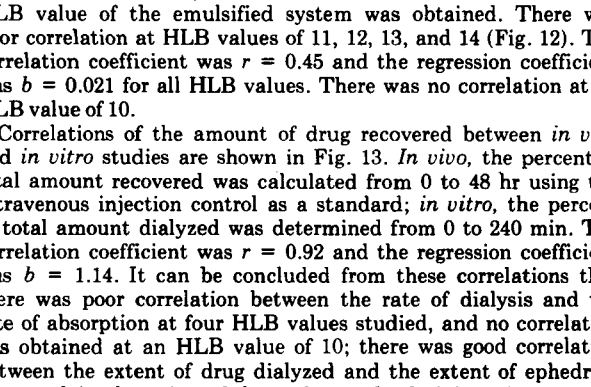

Figure 10—*Dialysis of ephedrine from emulsion systems made with surfactant having an HLB value of 13. Key: △, emulsion system at HLB 13; ×, oil preparation; and ○, aqueous preparation.*

Figure 11—*Dialysis of ephedrine from emulsion systems made with surfactant having an HLB value of 14. Key: △, emulsion system at HLB 14; ×, oil preparation; and ○, aqueous preparation.*

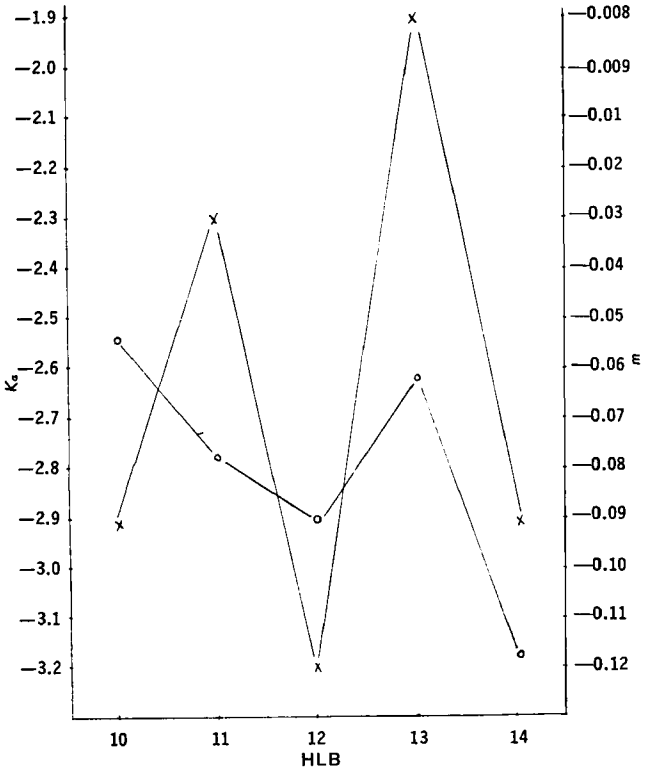

Figure 12—*Correlation of* in vivo *absorption rate constant* (K_a) *and* in vitro *dialysis rate constant* (m) *from the emulsion systems having HLB values from 10 to 14. Key:* ×, in vivo; *and* ○, in vitro.

correlate well with *in vivo* work if an appropriate method of correlation can be found.

CONCLUSIONS

1. The urinary excretion data obtained with ephedrine were adequately described by the two-compartment model with a zero-order absorption process.

2. The transfer rate constants, K_d, $K_{1,2}$, and $K_{2,1}$, for ephedrine in dogs were not dose dependent in the range tested.

3. The HLB value of the surfactant affected the absorption rate and the extent of ephedrine recovery from the orally administered mineral oil-water emulsified system.

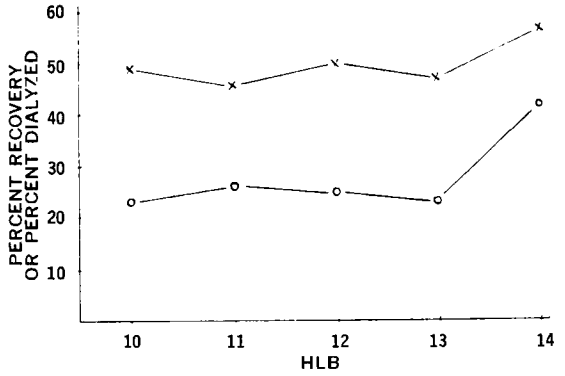

Figure 13—*Correlation of ephedrine recovery from urine and quantity dialyzed from 0 to 240 min. Key:* ×, *ephedrine recovery from urine; and* ○, *ephedrine quantity dialyzed from 0 to 240 min.*

4. *In vivo* correlations of the amount of ephedrine recovered from the urine (0–48 hr) with the total dialyzed *in vitro* (0–240 min) were significant at each HLB value.

5. Poor correlations of rates of availability with the rates of dialysis at all HLB values were noted. The correlations mentioned in Conclusion 4 are more valid considering the nature of the systems involved.

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