XIII) similar to IX of the classical mechanism except for saturation of the 5,6-bond, but it may be favored since 6-position attack by hydroxide ions on uracils and pyrimidine nucleosides is well documented (4, 6, 8). The resultant active intermediate (IX or XII) may deaminate (XII → XIII → XIV → X or IX → X) to give a uracil derivative, X, or the cytosine ring may open to give a nonchromophoric compound, IX or XII → XI. The subsequent deamination and/or decarboxylation of this acyclic product makes this pathway irreversible. These postulated mechanisms of Scheme IV readily explain the parallel deamination of cytidine to uridine and its direct loss to a nonchromophoric product in agreement with Scheme II.

REFERENCES

(1) E. R. Garrett, *J. Amer. Chem. Soc.*, **82,** 827(1960).

(2) E. R. Garrett and L. J. Hanka, *J. Amer. Pharm. Ass., Sci. Ed.*, **49,** 526(1960).

(3) E. R. Garrett, T. Suzuki, and D. J. Weber, *J. Amer. Chem. Soc.*, **86,** 526(1964).

(4) E. R. Garrett, P. B. Chemburkar, and T. Suzuki, *Chem. Pharm. Bull.*, **13,** 1113(1965).

(5) E. R. Garrett, J. K. Seydel, and A. J. Sharpen, *J. Org. Chem.*, **31,** 2219(1966).

(6) E. R. Garrett, J. H. Nestler, and A. Somodi, *ibid.*, **33,** 3460 (1968).

(7) H. J. Nestler and E. R. Garrett, *J. Pharm. Sci.*, **57,** 1117 (1968).

(8) E. R. Garrett and G. J. Yakatan, *ibid.*, **57,** 1478(1968).

(9) H. Schuster and G. Schramm, *Z. Naturforsch.*, **136,** 697 (1958).

(10) D. O. Jordan, "The Chemistry of Nucleic Acids," Butterworths, Washington, D. C., 1960, p. 65.

(11) R. Shapiro and R. S. Klein, *Biochemistry*, **5,** 2358(1966).

(12) R. E. Notari, *J. Pharm. Sci.*, **56,** 804(1967).

(13) R. E. Notari, M. L. Chin, and A. Cardoni, *Tetrahedron Lett.*, **40,** 3449(1969).

(14) R. E. Notari, M. L. Chin, and A. Cardoni, *J. Pharm. Sci.*, **59,** 28(1970).

(15) H. Hayatsu, *J. Amer. Chem. Soc.*, **91,** 5693(1969).

(16) H. Hayatsu and M. Yano, *Tetrahedron Lett.*, **1969,** 755.

(17) R. Shapiro, R. E. Servis, and M. Welcher, *J. Amer. Chem. Soc.*, **92,** 422(1970).

(18) H. Hayatsu, Y. Wataya, and K. Kai, *ibid.*, **92,** 724(1970).

(19) H. A. Hayatsu, Y. Wataya, K. Kai, and S. Iida, *Biochemistry*, **9,** 2858(1970).

(20) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd ed., Reinhold, New York, N. Y., 1958.

(21) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9,** 199 (1952).

(22) J. J. Fox and D. Shugar, *ibid.*, **9,** 369(1952)

(23) E. R. Garrett, *Arzneim.-Forsch.*, **17,** 795(1967).

(24) E. R. Garrett, *J. Amer. Chem. Soc.*, **80,** 4049(1958).

(25) E. R. Garrett, *Advan. Pharm. Sci.*, **2,** 1(1967).

(26) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," vol. 1, W. A. Benjamin, New York, N. Y., 1966.

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Drug Absorption VI: Water Flux and Drug Absorption in an *In Situ* Rat Gut Preparation

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Abstract □ The influence of net water transfer on drug absorption occurring in an *in situ* rat intestinal preparation was studied using hypotonic, hypertonic, and isotonic intestinal lumenal solutions composed of sodium phosphate and sodium chloride. It was found that the tonicity of the lumenal solution tended to change to a slightly hypertonic value when the initial solutions were isotonic or hypertonic and to isotonicity when the lumenal solution was initially hypotonic. Net water flux occurred from hypotonic solutions and into hypertonic solution. Water loss from isotonic solutions ranged between 7 and 17% in 3.0 hr. The results indicated that the intestinal water loss or gain which occurred with the different tonicity solutions altered the apparent rate constant for sulfaethidole absorption. It was found that the changes could be accounted for by correcting the rate constant for volume and relative available surface area and that when this was done there were no differences between permeability constants for sulfaethidole from the iso- and hypotonic solutions for absorption. However, the hypertonic solution had a reduced permeability constant.

Keyphrases □ Drug absorption—effect of tonicity and net water transfer, *in situ* rat intestine □ Intestinal absorption, *in situ*, rat—effect of tonicity and net water transfer

In situ rat gut techniques, based on a simple surgical operation and measurement of drug disappearance from the gut perfusion solution, are often used in studies of drug absorption. A single perfusion method (1), a recirculation method (2), and the method of Doluisio *et al.* (3) are examples of *in situ* gut techniques that have been used by various workers to study the dynamics of the drug absorption process. One problem common to all these methods is the loss of water that occurs during experimentation. In our laboratories, we attempted to minimize this factor by limiting experimentation to less than 40 min.; this time is adequate for drugs that are absorbed rapidly, but it is not adequate for drugs that are absorbed slowly. Hayton (4) conducted *in situ* experiments for longer periods and compensated for water loss by periodically adding solvent (isotonic

Table I—Preparation of Phosphate Buffer Solutions

	$NaH_2PO_4 \cdot H_2O$, g./l.	NaCl, g./l.
Hypertonic Solution A	11.4	13.0
Hypertonic Solution B	11.4	7.0
Isotonic Solution C	11.4	4.0
Hypotonic Solution D	11.4	2.0
Hypotonic Solution E	11.4	—

saline); other investigators (1, 2) monitored water loss using an unabsorbable marker and corrected for volume changes. It has been shown that intestinal water loss is often secondary to the absorption of ions such as sodium (5). In studies of drug absorption that are conducted for long periods of time, the absorption of ions greatly influences the transport of water and, hence, estimation of drug absorption.

This paper reports upon changes in tonicity and in net water transfer that occur in an *in situ* rat intestinal preparation (3) when hypotonic, hypertonic, and isotonic intestinal lumenal solutions are employed and the influence of these changes on the drug absorption process. Methods are also reported that make possible the calculation of absorption rate constants when volume change is appreciable.

EXPERIMENTAL

Reagents and Equipment—All of the chemicals employed in this study were reagent grade. Sulfaethidole[1] and polyethylene-1,2-^{14}C glycol[2], 0.2 μc./mg., were obtained from commercial sources. The perfusion solution consisted of 1.45×10^{-1} *M* NaCl, 4.56×10^{-3} *M* KCl, 1.25×10^{-3} *M* $CaCl_2$, and 5.0×10^{-3} *M* NaH_2PO_4 dissolved in distilled water. A pH meter[3], a spectrophotometer[4], an osmometer[5], and a scintillation counter[6] were utilized.

Preparation of Sample Solutions—In the tonicity experiments, phosphate buffer solutions (pH 6.0) were adjusted to appropriate tonicity by the addition of sodium chloride (Table I). To determine net water transport, sample solutions were prepared by adding 0.5 or 0.05 μc. of polyethylene-1,2-^{14}C glycol to 10 ml. of each phosphate buffer solution.

Sulfaethidole solutions (70 mg.%) were prepared using pH 6.8 phosphate buffer.

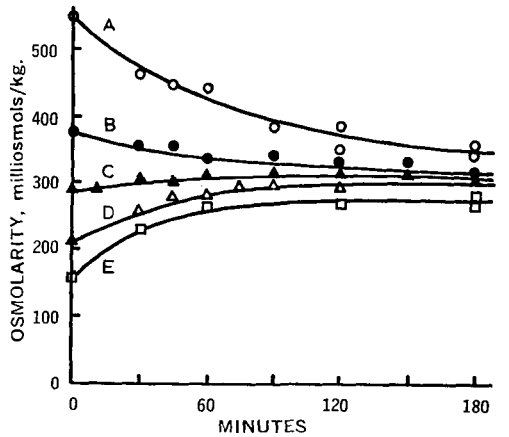

Figure 1—*Changes in osmolarity of phosphate buffer solution (pH 6.0) in the* in situ *rat intestinal lumen. (See Table I.)*

[1] Smith Kline and French Laboratories.
[2] New England Nuclear.
[3] Beckman Zeromatic II.
[4] Gilford 240.
[5] Fiske, model G.
[6] Packard Tri-Carb model 3375.

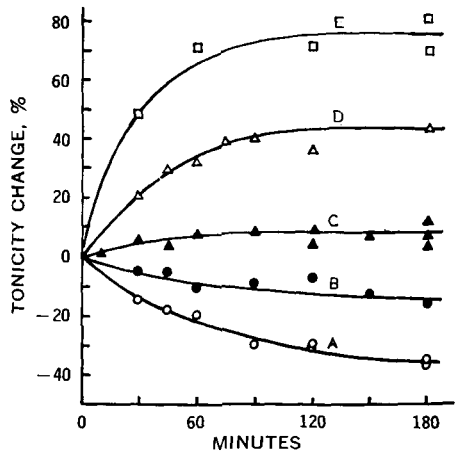

Figure 2—*Percent changes in osmolarity of phosphate buffer solution (pH 6.0) in the* in situ *rat intestinal lumen. (See Table I.)*

Preparation of Scintillation Solution—The scintillation solution used was prepared as follows: 4 g. of 2,5-diphenyloxazole, 100 mg. of 1,4-di-[2-(5-phenyloxazolyl)]benzene, 360 g. of octylphenoxypolyethoxyethanol[7], and distilled toluene to make 1 l.

Test Animals—Male Sprague-Dawley albino rats weighing 250–480 g. were fasted about 16 hr. prior to surgery; however, drinking water was allowed *ad libitum*. The rats were kept in cages having wide mesh floors to minimize coprophagy.

Experimental Procedures—The procedure for studying drug disappearance from the *in situ* rat gut lumen was described previously (3, 6), except that the threeway stopcock arrangement was eliminated by drilling a hole above the 10-ml. line of the plastic syringes. Separate experiments had to be conducted for tonicity changes and for water loss and drug absorption due to the volume of sample required for analysis and the desire to prevent radioactive contamination of the osmometer. In the experiment measuring change in tonicity, 15 ml. of sample solution was introduced into the intestine. A 3-ml. aliquot was removed immediately, and the remaining solution was returned to the intestine. In the experiments using polyethylene-1,2-^{14}C glycol and sulfaethidole, 10 ml. of the sample solution was introduced into the intestine by means of the same method and a 0.1-ml. aliquot was removed at periodic intervals.

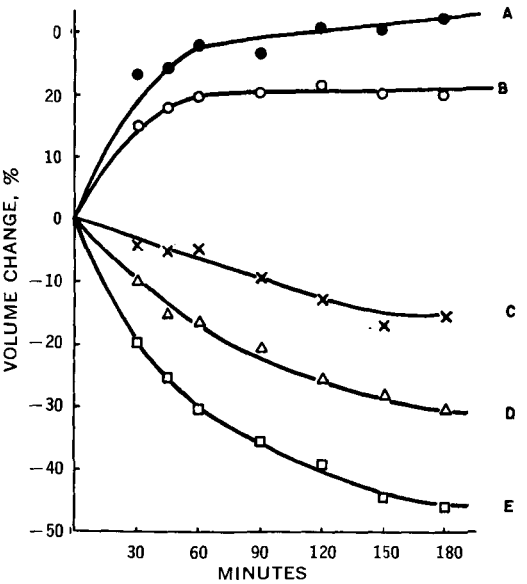

Figure 3—*Percent changes in lumenal water volume with time in the* in situ *rat intestinal preparation. (See Table III.)*

[7] Triton X-100.

Table II—Net Water Flow in the *In Situ* Rat Intestinal Lumen Expressed as Percent Volume Change[a]

Solution	Mean Percent Change of Volume in Minutes ± *SD*						
	30	45	60	90	120	150	180
Hypertonic Solution A	+23.0 ± 6.0	+24.1 ± 8.7	+28.0 ± 8.1	+26.5 ± 10.9	+30.2 ± 5.8	+30.3 ± 6.1	+32.2 ± 7.6
Hypertonic Solution B	+14.9 ± 3.8	+17.8 ± 4.7	+19.5 ± 4.8	+20.3 ± 4.3	+21.4 ± 2.9	+20.2 ± 4.0	+20.0 ± 5.7
Isotonic Solution C	−4.2 ± 3.9	−5.2 ± 3.8	−5.0 ± 5.9	−9.6 ± 5.2	−13.2 ± 5.3	−17.2 ± 6.6	−15.5 ± 6.0
Hypotonic Solution D	−10.2 ± 3.2	−15.3 ± 4.9	−16.2 ± 2.9	−20.9 ± 6.7	−25.6 ± 7.4	−28.0 + 7.2	−30.5 ± 7.3
Hypotonic Solution E	−10.9 ± 4.4	−25.5 ± 6.8	−30.2 ± 9.2	−35.7 ± 11.7	−30.6 ± 10.0	−44.9 ± 11.8	−46.7 ± 12.8

[a] Four rats for each determination.

Analytical Procedures—For the measurement of tonicity, each sample was filtered and the osmotic pressure of the filtrate was determined by freezing-point depression using the osmometer. For determination of radioactivity, each sample solution was pipeted into a scintillation vial containing 15 ml. of the scintillation solution, and the radioactivity was counted using a scintillation counter. The counting efficiency was approximately 90%, with a background of 27–30 c.p.m. Sulfaethidole was analyzed by the method of Bratton and Marshall (7).

RESULTS AND DISCUSSION

To understand the influence of tonicity on the intestinal absorption of sulfaethidole, the changes occurring in different tonicity solutions were examined in the *in situ* rat intestine preparation. These changes were measured using hypertonic (A and B), isotonic (C), and hypotonic (D and E) phosphate buffer solutions which possessed the osmotic pressures of 555, 380, 285, 207, and 149 milliosmols/kg. water[8], respectively. Results are summarized in Fig. 1. The tonicity of hypertonic Solutions A and B decreased gradually and reached values of about 350 and 325 milliosmols/kg. water, respectively, after 3 hr. The tonicity of hypotonic Solutions D and E increased gradually and reached values of about 275 and 300 milliosmols/kg. water, respectively, after 3 hr. The tonicity of isotonic Solution C also showed a slight increase, from 285 to approximately 310 milliosmols/kg. water (freezing-point depression −0.58°), after 3 hr. A plot of percent change of tonicity in each phosphate buffer solution *versus* time is shown in Fig. 2.

From these results, it is apparent that the tonicity of a sample solution in the *in situ* rat small intestine preparation has the tendency to change to a slightly hypertonic value (about 310 milliosmols/kg. water) when initial solutions are isotonic or hypertonic owing to the transport of water and/or salts through the gut membrane. Solutions that are initially hypotonic seem to change to isotonicity (288 milliosmols/kg. water). The rates of the change in tonicity of the hypertonic solutions were apparently slower than either the isotonic or hypotonic solutions.

Studies were conducted to determine the flux of water in the *in situ* rat small intestines using polyethylene-1,2-^{14}C glycol as an unabsorbable marker. The changes in volume in the intestinal fluid using phosphate solutions, A, B, C, D, and E, are summarized in Table III and Fig. 3. A decrease in volume is due to net water loss from the intestine (absorption), and an increase in volume is due to net water flux into the intestine. Accordingly, it was found that water was absorbed *from* the hypotonic solutions and absorbed *into* the hypertonic solutions. Water loss from Solution C ranged between 7 and 17% in 3.0 hr. As expected, these results illustrate that the absorption and secretion of water are greatly influenced by the concentration of salts in the sample solution.

The influence of volume changes on the intestinal absorption of sulfaethidole is illustrated in Fig. 4. For the hypertonic (A) and hypotonic (E) solutions, semilogarithmic plots of sulfaethidole intestinal concentrations *versus* time were nonlinear; a similar plot for the isotonic (C) solution appeared linear for 2 hr. Using the percent volume change (Fig. 3) for each solution, the amount of drug unabsorbed at each time was calculated and plotted semilogarithmically *versus* time in Fig. 5. Since the volume changes with time for the hypertonic (A) and hypotonic (E) solutions, one would expect these plots to be nonlinear during the period of appreciable volume change, about 1 hr. As shown in Fig. 2, the plots do exhibit nonlinearity. For the isotonic solution, the volume change is small and the plot appears linear.

To determine the relationship between volume changes and absorption, studies were conducted *in situ* with isotonic sulfaethidole solutions of different initial volumes. Since isotonic solutions have small volume changes over the 1st hr., these studies give a comparison of absorption for several different volumes that are essentially constant. The results of these studies (Table III) show that as the volume of the intestinal solution increased the apparent rate constant for absorption decreased. Since it is known that the available surface area of the intestines changes with the volume, *i.e.*, distension of the intestines, two factors probably are involved

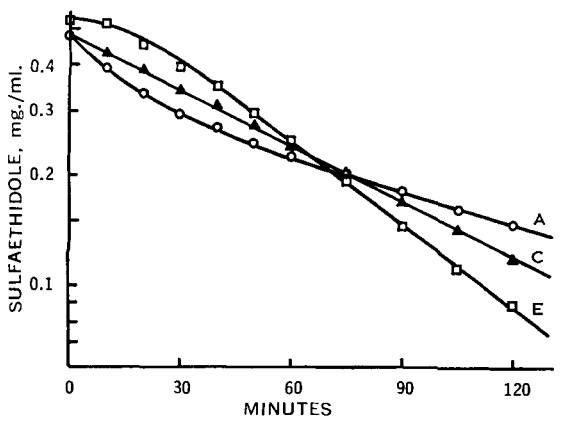

Figure 4—*Semilogarithmic plot of sulfaethidole unabsorbed* versus *time, uncorrected for water loss. Key:* ○, *hypertonic A;* ▲, *isotonic C; and* □, *hypotonic E.*

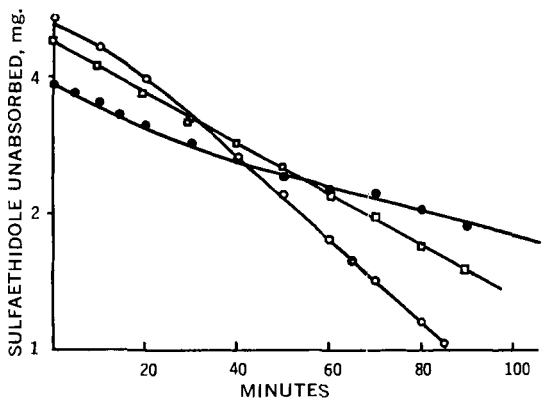

Figure 5—*Semilogarithmic plot of amount of sulfaethidole unabsorbed* versus *time. Key:* ●, *hypertonic A;* ○, *hypotonic E; and* □, *isotonic C.*

[8] Osmolality in milliosmols/kg. water = 1000 ΔT/1.858, where ΔT is the actual freezing-point depression, and 1.858 is the cryoscopic constant equal to the molal freezing-point depression of water.

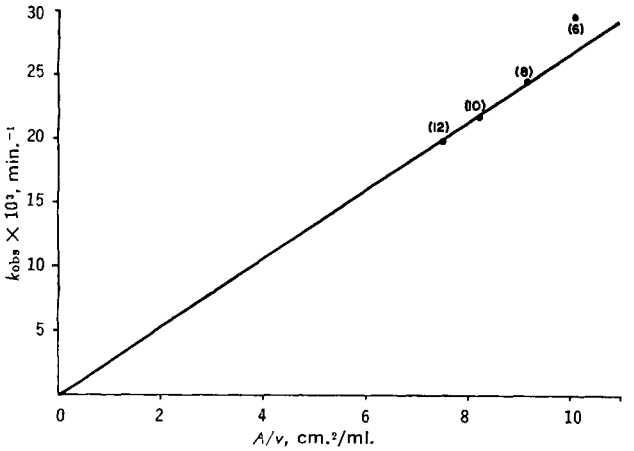

Figure 6—*Relation between* k_{obs} *and* A/v *for isotonic sulfaethidole solutions of different initial volumes, pH 6.8.*

in this change in apparent absorption rate constant: surface area and volume.

It would be difficult to determine the actual surface area of the intestines. However, if a relationship is assumed to exist between area and volume over a small range of volumes, 6–12 ml. for this *in situ* method, a *relative* surface area may be accounted for by using a simple model. Since a constant length of intestine was employed in this experimental technique, the surface area changes were approximated by assuming the intestine to be a cylinder of constant length and varying radius as a function of volume. The area was calculated in this manner for each volume used (Table III).

From Fick's law the observed absorption rate constant is described by:

$$k_{obs} = \frac{D \times A}{v \times dx} \qquad \text{(Eq. 1)}$$

where D is the diffusion coefficient, A is the surface area, v is the volume, and dx is the membrane thickness. By assuming that D and dx are constant, the following linear relationship should exist between the observed rate constant and A/v:

$$k_{obs} = k' \times \frac{A}{v} \qquad \text{(Eq. 2)}$$

The A/v values are shown in Table III, and the plot of k_{obs} *versus* A/v is shown in Fig. 5. The relationship was linear and gave the expected y-intercept of 0.0. It is important to note that the slope of this line gives an "intrinsic" constant (or permeability constant), k', which is independent of volume or surface area and as such would allow a comparison of the effects of tonicity on drug absorption other than volume and surface area.

The determination of this relationship allows one to compare the absorption of sulfaethidole from solutions of different tonicity where volume changes are rapid. As seen in Fig. 5, a plot of log amount remaining *versus* time is linear when the volume is constant; under this condition, k_{obs} can be calculated from the slope of the line. When the volume change was appreciable during drug absorption, it was necessary to determine rate constants over small time intervals, where the volume change was negligible, using Eq. 3:

$$\frac{2.303 \log (M_1/M_2)}{t_2 - t_1} = k_{obs} \qquad \text{(Eq. 3)}$$

where M is the amount at time t. As would be expected from the previous results for the isotonic solutions in Table III and from Fick's law, k_{obs} was inversely proportional to the volume in the intestine. The area corresponding to each volume was then calculated, and the intrinsic (or permeability) constant was determined from the slope of the plot, k_{obs} *versus* A/v, for the hypotonic (E), isotonic (C), and hypertonic (A) solutions. The intrinsic constants thus calculated are given in Table III. When the means were compared by Student's t test, it was found that the hypotonic and isotonic means were not significantly different ($p > 0.05$) but that the hypertonic mean was significantly different ($p < 0.01$).

Table III—Changes in Intestinal Fluid Volume

Solution	k_{obs}, min.$^{-1}$	A/v, cm.^{2} ml.$^{-1}$	v, ml.	A, cm.^{2}	k' (intrinsic), cm. min.$^{-1}$
Isotonic C	0.0297	10.56	6	63.36	0.00281
Isotonic C	0.0247	9.13	8	73.04	0.00271
Isotonic C	0.0216	8.18	10	81.80	0.00264
Isotonic C	0.0198	7.48	12	89.76	0.00265

Solution	$k' \times 10^3 \pm SD$[a], cm. min.$^{-1}$
Isotonic C, pH 6.8	2.70 ± 0.08
Isotonic C, pH 6.0	1.71 ± 0.2
Hypotonic E, pH 6.0	2.08 ± 0.2
Hypertonic A, pH 6.0	1.20 ± 0.08

[a] Three rats for each solution.

These results indicate there was apparently no difference in the permeability of the membrane to the hypotonic and isotonic sulfaethidole solutions when the data were corrected for volume and surface area changes. It was found that the apparent permeability of the membrane to sulfaethidole was significantly decreased when the hypertonic solution was used. Thus, changes in absorption of sulfaethidole from a hypertonic solution are *not* due simply to changes in volume.

These studies showed that drug absorption profiles vary due to volume changes that occur in the *in situ* absorption technique. It was demonstrated that for the passively absorbed drug sulfaethidole, these changes in volume may be corrected for in the calculation of a permeability constant that is independent of volume and surface area. The model employed for these corrections is very elementary and would only apply without further considerations for drugs that are passively absorbed and studied using this *in situ* technique.

Other investigators (8) reported a much greater loss of water than was found in these studies involving isotonic solutions. It is possible that this discrepancy may be due to excessive positive pressure being exerted by these investigators when intestinal solutions were being expelled for sampling. At any rate, if appreciable water change occurs during experimentation, absorption plots may be nonlinear and may have to be calculated as stated here.

REFERENCES

(1) L. S. Schanker, P. A. Shore, B. B. Brodie, and C. A. M. Hogben, *J. Pharmacol. Exp. Ther.*, **120**, 528(1957).

(2) K. Kakemi, T. Arita, R. Hari, and R. Konishi, *Chem. Pharm. Bull.*, **15**, 1534(1967).

(3) J. T. Doluisio, N. F. Billups, L. W. Dittert, E. T. Sugita, and J. V. Swintosky, *J. Pharm. Sci.*, **58**, 1196(1969).

(4) S. Hayton, *Ann. Agr. Coll. Swed.*, **22**, 139(1955).

(5) S. G. Schultz and P. F. Curran, "Handbook of Physiology," section 6, vol. III, American Physiological Society, Washington, D. C., 1968.

(6) J. T. Doluisio, G. H. Tan, N. F. Billups, and L. Diamond, *J. Pharm. Sci.*, **58**, 1200(1969).

(7) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537(1939).

(8) G. Levy and A. Perala, *J. Pharm. Sci.*, **59**, 874(1970).

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