Academy of Pharmaceutical Sciences Symposium, 1971.
(152) M. W. Anders and J. P. Latorre, *Anal. Chem.*, **42,** 1430 (1970).
(153) C. A. Burtis, W. C. Butts, and W. T. Rainey, Jr., *Amer. J. Clin. Pathol.*, **53,** 769(1970).
(154) C. D. Scott, J. E. Attril, and N. G. Anderson, *Proc. Soc. Exp. Biol. Med.*, **1967,** 181.
(155) C. D. Scott, *Clin. Chem.*, **1968,** 521.
(156) C. A. Burtis and K. S. Worren, *ibid.*, **1968,** 290.
(157) C. D. Scott, R. L. Jolley, W. W. Pitt, and W. F. Johnson, *Amer. J. Clin. Pathol.*, **53,** 701(1970).
(158) W. W. Pitt, C. D. Scott, and W. F. Johnson, *Clin. Chem.*, **1970,** 657.
(159) J. E. Mrochek, W. G. Butts, W. T. Rainey, and C. A. Burtis, *ibid.*, **17,** 72(1971).
(160) D. S. Young, *Amer. J. Clin. Pathol.*, **53,** 803(1970).
(161) E. F. Walborg, Jr., and L. E. Kondo, *Anal. Biochem.*, **51,** 320(1970).
(162) A. Floridi, *J. Chromatogr.*, **59,** 61(1971).
(163) M. W. Anders and J. P. Latorre, *Pharmacologist*, **1970,** 180.
(164) M. W. Anders and J. P. Latorre, *J. Chromatogr.*, **55,** 409 (1971).
(165) R. A. Henry, J. A. Schmit, and J. F. Dieckman, *J. Chromatogr. Sci.*, **9,** 513(1971).
(166) J. A. Mollica, C. R. Rehm, J. B. Smith, and R. F. Strusz, APhA Academy of Pharmaceutical Sciences Symposium, 1971.
(167) R. Henry and J. A. Schmit, *Chromatographia*, **1970,** 116.
(168) R. L. Stevenson and C. A. Burtis, *J. Chromatogr.*, **61,** 253 (1971).
(169) J. C. Wolford, J. A. Dean, and G. Goldstein, *ibid.*, **62,** 148 (1971).
(170) G. Ertingshausen, H. J. Alder, and A. S. Reichler, *J. Chromatogr.*, **42,** 355(1969).
(171) C. A. Burtis, *ibid.*, **51,** 183(1970).
(172) F. Murakami, S. Rokushika, and H. Hatano, *ibid.*, **53,** 584(1970).
(173) H. W. Shmukler, *J. Chromatogr. Sci.*, **8,** 581(1970).
(174) *Ibid.*, **8,** 653(1970).
(175) W. P. Kennedy and J. C. Lee, *J. Chromatogr.*, **51,** 203 (1970).
(176) P. R. Brown, *ibid.*, **52,** 257(1970).
(177) T. R. Gabriel and J. Michalewsky, *ibid.*, **67,** 309(1972).
(178) G. Goldstein, *Anal. Biochem.*, **20,** 477(1967).
(179) G. Brooker, *Anal. Chem.*, **42,** 1108(1970).
(180) R. B. Poet and H. H. Pu, APhA Academy of Pharmaceutical Sciences Symposium, 1971.
(181) G. R. Gordon and J. H. Peters, *J. Chromatogr.*, **47,** 269 (1970).
(182) J. Churacek and P. Jondera, *ibid.*, **53,** 69(1970).
(183) H. W. Lange, H. F. K. Mannl, and K. Hempel, *Anal. Biochem.*, **38,** 98(1970).
(184) H. W. Lange and K. Hempel, *J. Chromatogr.*, **59,** 53 (1971).
(185) R. H. Stehl, *Anal. Chem.*, **42,** 1802(1970).
(186) M. Popl, J. Mostecky, and Z. Havel, *J. Chromatogr.*, **53,** 233(1970).
(187) W. F. Beyer, *Anal. Chem.*, **44,** 1312(1972).
(188) L. F. Krzeminski, L. C. Byron, and A. W. Neff, *ibid.*, **44,** 126(1972).

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RESEARCH ARTICLES

Absorption and Distribution of Naloxone in Rats after Oral and Intravenous Administration

S. H. WEINSTEIN▲, M. PFEFFER, J. M. SCHOR, L. FRANKLIN, M. MINTZ, and E. R. TUTKO

Abstract □ The effect of route of administration on the absorption and distribution of naloxone, a narcotic antagonist, was investigated in rats. Plasma levels were determined by GLC. Five minutes after intravenous administration of 1 mg./kg., the plasma concentration was 258 ng./ml. Plasma levels after low oral doses were undetectable; but after 100 mg./kg. orally, the peak level of unchanged drug was almost 5000 ng./ml. In terms of percent of administered dose, the maximum amount of naloxone in the calculated plasma volume is 1.04% of the intravenous dose *versus* 0.19% of the oral dose. Pharmacokinetic parameters were generated with a computer program; the models constructed are of a rapidly absorbed and rapidly excreted and/or metabolized drug. These results, together with results from absorption studies with ligated intestinal loops, indicate that poor absorption of naloxone is not the cause of its relatively low oral potency. *In vitro* metabolic studies with rat liver slices confirmed rapid naloxone metabolism, suggesting that the lower potency of oral naloxone compared to parenteral naloxone is due to rapid first-pass liver metabolism.

Keyphrases □ Naloxone hydrochloride—absorption and distribution after oral and intravenous administration, rats □ Absorption and distribution, naloxone hydrochloride—after oral and intravenous administration, plasma levels, liver metabolism, rats

Naloxone [(−)-*N*-allyl-14-hydroxynordihydromorphinone] is a potent narcotic antagonist upon parenteral administration to laboratory animals (1, 2) or man (3), but it is approximately one-fiftieth as po-

tent when administered orally in human subjects (3). A similar relationship was reported for rats and mice (4).

The reduced oral effectiveness of a drug may be due to poor or incomplete absorption from the GI tract or to rapid first-pass metabolism in the liver since orally administered drugs enter the systemic circulation *via* the hepatic portal system. To determine the extent of absorption of naloxone from the intestine, *in vivo* intestinal loop experiments were performed in rats. This technique has been used to study the absorption of many compounds including quaternary ammonium compounds (5) and digitalis glycosides (6).

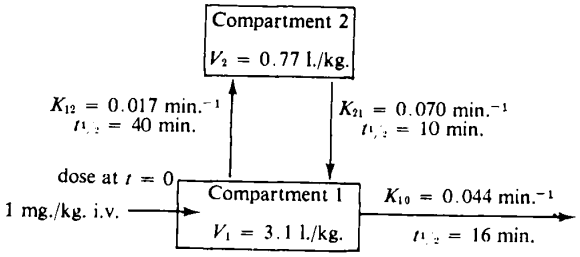
In vitro rat liver slice experiments were performed to determine the extent of naloxone metabolism in the liver. This method was previously applied to the study of morphine metabolism (7). Naloxone has been reported to undergo glucuronidation, *N*-dealkylation, and reduction of the 6-oxo group (8, 9).

Plasma levels of naloxone in rats were determined following oral and intravenous administration. Absorption and elimination rate constants, as well as other pharmacokinetic parameters, were calculated to gain insight into the fate of naloxone following administration by the two routes.

EXPERIMENTAL

Analytical Methods—Extraction of naloxone from rat plasma was performed as described by Mulé (10), except that chloroform containing 1% isopropanol was used as the solvent and was back-extracted with 1.3 ml. of 0.1 *N* HCl. A 1-ml. portion of the final hydrochloric acid extract was evaporated to dryness[1]. An internal standard, 500 ng. of tetraphenylethylene (25 μl. of a 20 mcg./ml. methanol solution), was then added. The samples were again evaporated to dryness, and 25 μl. of bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane[2] was added. Wilkinson and Way (11) demonstrated the efficacy of bis(trimethylsilyl)trifluoroacetamide as a silylating agent for morphine, and it was equally useful for naloxone. The tubes were flushed with dry nitrogen and closed with ground-glass stoppers, and the silylation reaction was carried out for 30 min. at 60–65° in a dry heating block[3]. One-microliter aliquots of the silylated samples were injected into a gas chromatograph[4], with carbon tetrachloride as a solvent flush. Naloxone was measured by comparing the peak height ratio of naloxone–tetraphenylethylene in the experimental samples to the peak height ratio obtained from standards extracted from plasma.

Naloxone was extracted from the liver and intestine by homogenizing the tissue in a total of 4 ml. of water (including the volume of water used to rinse the homogenizer), and the protein was precipitated by addition of 0.5 ml. of 10% $ZnSO_4$ and 0.5 ml. of 0.5 *N* NaOH. This was centrifuged 15 min. at 3000×*g*, and 3 ml. of the protein-free supernate was diluted to 15 ml. with 0.5 *M* phosphate buffer, pH 7.4. The diluted supernate was then sequentially extracted with three 15-ml. portions of chloroform. The pooled chloroform extracts were evaporated to dryness in a rotary evaporator[5], and the residue was quantitatively washed out of the evaporating flask with 5 ml. of chloroform into a conical centrifuge tube. This chloroform was removed by evaporation to dryness[1]. The residue was redissolved in 0.1 ml. chloroform containing 300 ng. of *N*-cyclobutylmethyl-7,8-dihydro-14-hydroxynormorphine (nalbuphine, 3 mcg./ml.) as the internal standard. A portion of this chloroform solution was injected into the gas chromatograph, and the naloxone was measured by comparing the peak height ratio of naloxone–nalbuphine to the peak height ratio obtained for standards also extracted from intestinal loops or rat liver slices. Metabolites such as noroxymorphone and naloxone glucuronide are not extracted under these conditions.



Scheme I—*Two-compartment open pharmacokinetic model for intravenous naloxone*

The gas chromatograph was equipped with a flame-ionization detector. The column was 3.8% UC-W98 on high performance Chromosorb W, 80–100 mesh. Conditions used were: oven, 245°; detector, 310°; flash heater, 300°; hydrogen flow rate, 37 ml./min.; helium, 75 ml./min.; and air, 350 ml./min.

Animal Studies—Three to five fasted, male, CFN rats (average weight 397 g.) were used for each time interval of the intravenous study. Naloxone hydrochloride (1 mg./ml., aqueous solution) was administered *via* the tail vein at a dose of 1 mg./kg. while the rats were under light ether anesthesia. At 5, 10, 15, 22, 30, 38, 45, 50, and 60 min. after dosing, blood was obtained by cardiac puncture with heparinized syringes. The plasma was separated by centrifugation at 3000×*g* for 20 min.

For the oral studies, naloxone hydrochloride was administered to fasted rats (average weight 375 g.) in aqueous solution (100 mg./ml.) *via* stomach tube at a dose of 100 mg./kg. At 2, 3.5, 5, 15, 22, 30, 35, 45, 50, and 60 min. after dosing, blood was obtained as already described. Four to 11 rats were used for each time interval.

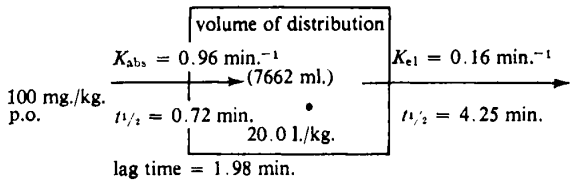
Intestinal loops were prepared, while fasted rats were under ether anesthesia, by exposing the intestine and locating a section supplied by three blood vessels, approximately 20–22 cm. from the pyloric sphincter. One end of a segment approximately 2.54 cm. (1 in.) in length was securely ligated with thread. The other end of the segment was ligated with a 22-gauge needle entering the end through the center of the tightened loop of thread. Five hundred micrograms of the drug in 0.5 ml. of saline solution was injected and, as the needle was removed, the ligature was completed. The midline incision made to expose the intestine was then closed. At 30, 60, or 90 min. after treatment, the animals were again anesthetized and the previously tied section of gut was removed for analysis as already described. Controls for these intestinal loop experiments consisted of the incubation of 500 mcg. of naloxone in gut sections *in vitro* at 37° in normal saline for a time interval equal to that of the *in vivo* incubation. Loss of drug by absorption was determined by comparing the naloxone recovered from *in vivo* intestinal loops to the naloxone recovered from *in vitro* loops. Loss of drug due to metabolism by intestinal tissue itself was determined by comparing the naloxone recovered after incubation *in vitro* in intestinal loops to the naloxone recovered from *in vitro* loops that were not incubated but were homogenized and extracted immediately after the drug was placed within the loop.

For liver slice experiments, ether-anesthetized rats were killed and the livers were immediately removed. The livers were washed with ice-cold normal saline solution and sliced with a hand microtome[6]. Three hundred milligrams of liver slices was placed in 25-ml. conical flasks containing 1.5 ml. of Krebs–Henseleit solution. Then 0.5 mg. of naloxone hydrochloride in 0.5 ml. of Krebs–Henseleit solution was added, giving a total incubation volume of 2.0 ml. Prior to use, the Krebs solution was saturated with 95% O_2–5% CO_2 (7).

The incubations were carried out in duplicate on a shaking incubator[7] at 37° under a 95% O_2 5% CO_2 atmosphere delivered at a rate of 5 ft.^{3}/hr.

After 30, 60, and 120 min., duplicate incubation mixtures were homogenized. Extraction of naloxone from the homogenized in-

[1] Evaporation performed in 5-ml. conical centrifuge tubes with a Buchler Evapo-mix.
[2] Regisil, Regis Chemical Co.
[3] TEMP-BLOK Heater, Lab-Line Instruments.
[4] Hewlett-Packard, F&M 402.
[5] Rinco rotary evaporator.
[6] Stadie-Riggs hand microtome.
[7] Dubnoff.



Scheme II—One-compartment open model for orally administered naloxone

cubation mixture was carried out as previously described. The extracts were analyzed for unmetabolized naloxone by GLC. The peak height ratio (naloxone–nalbuphine) of naloxone obtained from extracts of incubation mixtures was compared with peak height ratios obtained from extracts of unincubated liver–naloxone mixtures. Duplicate determinations were performed for each sample.

As controls, to determine whether loss of naloxone was an assay artifact or due to liver metabolism, incubations were also carried out with boiled liver slices.

Pharmacokinetic Calculations—A computer program (COMPT) for optimizing the solution of integral nonlinear compartmental models of drug distribution, written in extended BASIC for use in time-sharing computer systems, was used to generate pharmacokinetic parameters (12).

The mathematical formulations for compartmental models (Schemes I and II) reduce, in their most general form, to sums of exponential terms:

$$f(T) = \sum_{i=1}^{M} N_i \exp(-a_i T) \qquad \text{(Eq. 1)}$$

The formulations for the one- and two-compartment models are, respectively:

$$C = \left(\frac{D}{V}\right)\left(\frac{K_{abs}}{K_{abs} - K_{el}}\right)[\exp(-K_{el}T) - \exp(-K_{abs}T)] \qquad \text{(Eq. 2)}$$

$$C_1 = \frac{D}{V_1(a_1 - a_2)}[(K_{21} - a_2)\exp(-a_2T) - (K_{21} - a_1)\exp(-a_1T)] \qquad \text{(Eq. 3)}$$

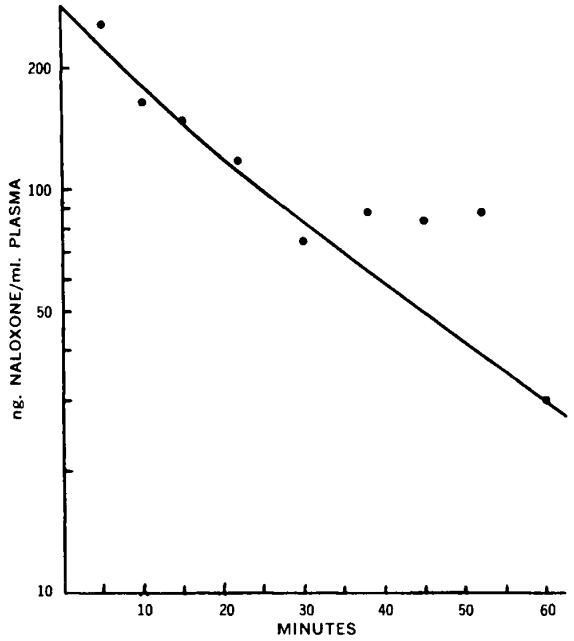


Figure 1—*Semilog plot of naloxone plasma concentrations in rats following 1 mg./kg. i.v.*

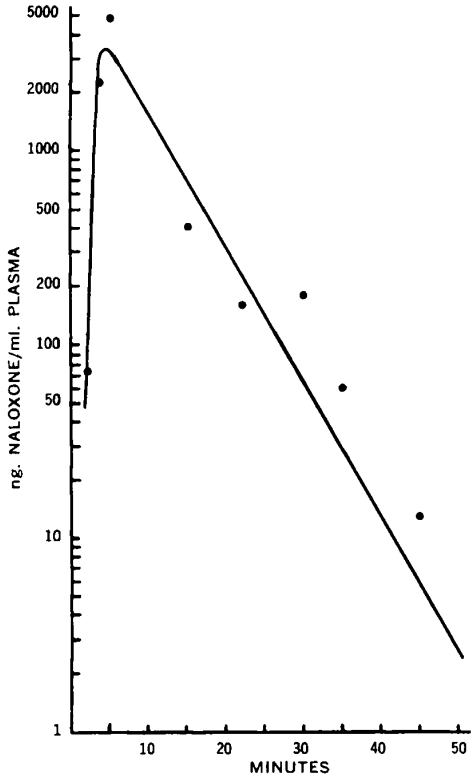


Figure 2—*Semilog plot of naloxone plasma concentrations in rats following 100 mg./kg. p.o.*

N_i and a_i are obtained from the line projected from the terminal portion of the log C *versus* T curve. Calculation of pharmacokinetic parameters for these models was extensively discussed by Wagner (13).

RESULTS

Gas Chromatography and Extractions—Under the described chromatographic conditions, tetraphenylethylene had a retention time of 2.2 min. and the trimethylsilyl derivative of naloxone had a retention time of 4.5 min. Underivatized naloxone and nalbuphine had retention times of 3.6 and 7.8 min., respectively.

The use of parallel extraction standards as controls was necessary because the recovery of naloxone from plasma was 50–60%. There was a linear, reproducible relationship between GLC response and plasma concentration of naloxone.

Preliminary experiments showed that naloxone was recovered from intestinal loops and liver slices in quantities sufficient for GLC analysis without silylation and that there was a linear relationship between naloxone concentration and GLC response.

Experiments in which naloxone was incubated *in vitro* in sections of gut showed that the gut itself did not metabolize naloxone. Therefore, disappearance from *in vivo* loops cannot be attributed to destruction within the loop but must be ascribed to absorption from the loop. When naloxone was incubated with boiled liver slices, the naloxone was recovered intact, indicating that the disappearance found with unboiled slices was due to metabolism. Metabolites such as naloxone glucuronide or noroxymorphone are not extracted under the conditions used.

Plasma Levels of Naloxone—Figure 1 is a plot of log of rat plasma levels of naloxone *versus* time after intravenous administration. The increases in drug levels between 38 and 52 min. may be due to biliary recycling. The curve shown is the best fit computed with the COMPT program, neglecting those points attributable to biliary recycling.

Initially, an oral dose of 10 mg./kg. was administered, but naloxone was not detectable in plasma at this level. At a dose of

Table I—Absorption of Naloxone from *In Vivo* Intestinal Loop in Rat[a]

Minutes	Number of Animals	Naloxone Remaining in Loop, % ± *SD*
30	5	55.5 ± 17.9
60	8	18.8 ± 12.5
90	4	4.73 ± 3.6

[a] Five hundred micrograms injected into ligated intestinal loop *in vivo* and assayed by GLC after indicated time. See text for details.

Table II—Percent Naloxone Remaining after Incubation with Rat Liver Slices

Minutes	Naloxone Remaining, %	
	Experiment 1[a]	Experiment 2
30	48.5	73.5
60	23.6	42.2
120	13.5	13.4

[a] Experiments 1 and 2 represent two separate experiments, each performed in duplicate.

100 mg./kg., detectable levels were obtained. Figure 2 is a plot of log of rat plasma levels of naloxone *versus* time after oral administration. The curve shown is the best fit computed with the COMPT program.

Absorption from Ligated Intestinal Loop—The percent of administered naloxone absorbed from *in situ* ligated intestinal loops at various time intervals is presented in Table I. Naloxone is well absorbed; 95.3% was absorbed at 90 min.

Metabolism by Rat Liver Slices—Table II presents the results of the incubation of naloxone with rat liver slices. After 2 hr., almost 90% of the added naloxone was metabolized. Earlier time intervals showed some variations; but in 30 min., 25–50% of the naloxone was metabolized.

Pharmacokinetics—The plasma level data obtained for intravenously administered naloxone (Fig. 1) fit a two-compartment open model (Scheme I). The data for orally administered naloxone (Fig. 2) fit a one-compartment model with first-order absorption (Scheme II).

DISCUSSION

The pharmacokinetic model (Scheme I) for intravenous naloxone yields a very rapid elimination rate ($t_{1/2}$ = 16 min.). The relatively large volume of distribution in Compartment 1 could indicate either extensive tissue binding or rapid metabolism, because the model is simply attempting to account for the absence of a large fraction of the administered dose as unchanged drug in the plasma. The small values of K_{12} and V_2 and the larger K_{21} value indicate slow entry into, little binding in, and rapid removal from a peripheral compartment, respectively.

The one-compartment model for naloxone plasma levels after oral administration (Scheme II) satisfies the available data. If a more sensitive analytical method were available, a leveling off of plasma concentrations probably would have been observed after 45 min., and the use of a two-compartment model would be indicated. The observed terminal straight-line portion of the curve in Fig. 2 is probably really the distributive phase. The computer-fitted curve does, however, show a very rapid absorption rate ($t_{1/2}$ = 0.72 min.) for naloxone. The large volume of distribution may indicate either extensive tissue binding or rapid metabolism. The pharmacokinetic models of naloxone are, therefore, those of a rapidly absorbed and rapidly excreted and/or metabolized drug.

Figures 1 and 2 show the plasma concentrations of naloxone after intravenous and oral administration. The peak concentration after oral administration, 4856 ng./ml., occurs at 5 min. In terms of percent of the administered dose, calculated from the average plasma volume of rats (40.4 ml./kg.) and the average weight of the rats used, the maximum amount of naloxone found in plasma is 0.19% of the oral dose. Five minutes after intravenous administration, the plasma concentration is 258 ng./ml. or 1.04% of the dose. This could indicate that a large portion of the oral dose is either not absorbed or is metabolized before reaching the systemic circulation.

The rapid absorption rate observed for orally administered naloxone is in agreement with the results of the ligated intestinal loop experiments. At 30 min., 45% of the drug is absorbed and after 90 min. absorption is virtually complete (Table I). In these experiments the entire intestinal loop was analyzed for naloxone, eliminating the possibility of naloxone loss through binding to some component of intestinal tissue, as reported by Levine *et al.* (5) for quaternary ammonium compounds.

These results lead to the conclusion that the lower therapeutic effectiveness of orally administered naloxone is not the result of poor absorption from the intestine. The remaining factor, rapid first-pass liver metabolism, therefore, appears to be the mechanism by which efficacy of orally administered naloxone is diminished. This conclusion is strongly supported by the rapid metabolism of naloxone by rat liver slices (Table II). To be sure, plasma concentrations of naloxone in rats are erratic after oral administration (coefficient of variation 1.10 compared to 0.25 for intravenous administration), but this may be due to the well-known biological variation in drug-metabolizing enzymes rather than to erratic absorption from the GI tract.

The data presented show that low oral effectiveness of naloxone cannot be attributed to poor absorption. Pentazocine's low oral effectiveness (compared to parenteral dosing) has been attributed to poor absorption (14), but this is now questionable since Berkowitz *et al.* (15) reported finding an additional metabolite in plasma following oral administration which has not been found after intramuscular administration. This indicates route-dependent metabolism of pentazocine.

REFERENCES

(1) H. Blumberg, H. B. Dayton, and P. S. Wolf, *Fed. Proc.*, **24**, 676(1968).

(2) H. Blumberg, P. S. Wolf, and H. B. Dayton, *Proc. Soc. Exp. Biol. Med.*, **118**, 763(1965).

(3) M. Fink, A. Zaks, R. Sharoff, A. Mora, A. Bruner, S. Levit, and A. M. Freedman, *Clin. Pharmacol. Ther.*, **9**, 568(1968).

(4) H. B. Dayton and H. Blumberg, *Fed. Proc.*, **29**, 686(1970).

(5) R. M. Levine, M. R. Blair, and B. B. Clark, *J. Pharmacol. Exp. Ther.*, **114**, 78(1955).

(6) M. J. Greenberger, R. P. MacDermott, J. F. Martin, and S. Dutta, *ibid.*, **167**, 265(1969).

(7) F. Bernheim and M. L. C. Bernheim, *ibid.*, **82**, 85(1945).

(8) S. H. Weinstein, M. Pfeffer, J. M. Schor, L. Indindoli, and M. Mintz, *J. Pharm. Sci.*, **60**, 1567(1971).

(9) J. M. Fujimoto, *Proc. Soc. Exp. Biol. Med.*, **133**, 317(1970).

(10) S. J. Mulé, *Anal. Chem.*, **36**, 1907(1964).

(11) G. R. Wilkinson and E. L. Way, *Biochem. Pharmacol.*, **18**, 1439(1969).

(12) M. Pfeffer, *J. Pharmacokin. Biopharm.*, **1**, 138(1973).

(13) J. G. Wagner, "Biopharmaceutics and Relevant Pharmacokinetics," 1st ed., Drug Intelligence Publications, Hamilton, Ill., 1971.

(14) A. H. Beckett, J. F. Taylor, and P. Kourounakis, *J. Pharm. Pharmacol.*, **22**, 123(1970).

(15) B. A. Berkowitz, J. H. Asling, S. M. Snider, and E. L. Way, *Clin. Pharmacol. Ther.*, **10**, 320(1969).

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