Effect of Polysorbates on Absorption Rates of Water-Soluble, Micelle-Free Drugs Administered Intramuscularly in the Rat

HIROSHI KOBAYASHI, TAEKO NISHIMURA, KATSUHIKO OKUMURA, SHOZO MURANISHI, and HITOSHI SEZAKI ˣ

Abstract □ The effects of the polysorbates on the intramuscular absorption of water-soluble, micelle-free drugs were investigated in the rat. The presence of a low concentration of the polysorbates caused a pronounced decrease in the absorption rates of drugs, and the reduction was reflected in their plasma levels. Absorption rates of a drug in the muscle were dependent on the concentration rather than on the absolute amount of the surfactant. The delayed clearance seemed to be operative in various drugs and was caused by the polysorbates *per se*—not by the hydrolysis products.

Keyphrases □ Absorption, intramuscular—effects of surfactants, water-soluble, micelle-free drugs, absorption rates, plasma levels, rat muscle □ Surfactants—effects on intramuscular absorption rate, water-soluble, micelle-free drugs, plasma levels, rat □ Polysorbates—effects on intramuscular absorption rate, water-soluble, micelle-free drugs, plasma levels, rat □ Drugs, water soluble, micelle free—effect of surfactants on intramuscular absorption rate

Studies involving the experimental conditions and biopharmaceutical factors influencing the intramuscular absorption of drugs from parenterally administered aqueous solutions without surfactant have been reported (1–4). Despite the importance in early testing of drugs in animals, in preclinical screening, and in clinical use, little is known about the mechanism of absorption from heterogeneous parenteral preparations such as suspensions and emulsions. When discussing parenteral absorption from such dosage forms, it is important to elucidate the role of surfactants in absorption since they are often formulated in such preparations. Several reports on systemic toxicity and local irritation in general have appeared (5, 6). However, less is known about the effects of surfactants on parenteral absorption than is known about their role in absorption from the GI tract.

In this study, the effects of polysorbates on the absorption of water-soluble, micelle-free drugs were investigated in the rat to elucidate the mechanism of absorption from heterogeneous parenteral preparations.

EXPERIMENTAL

Materials—*Test Substances*—Isonicotinamide[1], methyl isonicotinate[2], isonicotinic acid[1], sulfanilamide[3], and procainamide hydrochloride[3] (JP VIII) were of analytical grade and were obtained commercially. Inulin-(carboxylic acid-^{14}C) was also purchased[4].

Additives—The nonionic surfactants used were polysorbates 20, 40, 60, and 80[5] and a polyoxyethylene derivative of hydrogenated castor oil[6]. Lauric acid (99–100%)[5] was obtained commercially. The hydrolysis products of polysorbates, sorbitan[7], and polyoxyethylene 20 sorbitan[7] also were used.

Procedure of Absorption Experiments—Male Wistar albino rats, 150–180 g, were used in all absorption experiments. The absorption experiments were almost identical to those described previously (1). An injection volume of 10 μl was delivered with a microliter syringe[8]. At various times after injection of drugs into the center of the rat thigh muscle *(musculi rectus femoris)*, the muscle was removed. The muscle was excised and then homogenized in 10 ml of distilled water, and the substance remaining at the injection site and associated area was determined. At least five rats were used at each time interval. Blood samples for plasma level analyses were obtained by cardiac puncture immediately after the muscle was removed.

Preparation of Injection Solutions—In the absorption experiments, the drugs were dissolved in isotonic NaH_2PO_4–Na_2HPO_4 buffer solution of pH 7.0. For the preparation of injection solutions having other pH values, three isotonic buffers were used: *(a)* a citric acid-Na_2HPO_4 system for acidic pH ranges, *(b)* NaH_2PO_4–Na_2HPO_4 for neutral pH ranges, and *(c)* $NaHCO_3$–Na_2CO_3 for alkaline pH ranges. Lauric acid was dissolved with equimolar sodium hydroxide in 0.1% polysorbate 80 or 1.0% polyoxyethylene derivative of hydrogenated castor oil solutions. The final pH values of the solutions injected were determined.

Drug Concentrations—In the absorption experiments, the following concentrations were maintained: 50 m*M*, in usual cases; 25 m*M*, with sulfanilamide; and 2.5 μCi/ml, with inulin-(carboxylic acid-^{14}C). In the plasma level determinations, the concentrations were: 100 m*M*, isonicotinamide; and 2.5 μCi/ml, inulin-(carboxylic acid-^{14}C).

Determination of Possible Interaction of Drugs with Polysorbate 80—Molecular sieve technique employing a cross-linked dextran gel[9] (9) was used. To prepare the swollen gel, 4 g of the dry gel was added to pH 7.0 isotonic phosphate buffer (15 ml) and the gel was allowed to swell (2 hr). The solutions containing the constant drug concentration (0.1 m*M*) in polysorbate 80 solutions of known but varying concentrations were then added along with additional phosphate buffer so that the total volume of liquid added to the gel was always 25 ml. The system was then equilibrated by shaking (1 hr) at 37°. After equilibration, the concentration of drug in the supernatant liquid was determined colorimetrically. By plotting the ratio of total (free and micellar) drug to free drug in solutions containing varying concentrations of polysorbate 80 against the polysorbate 80 concentration, the apparent distribution constants were determined.

Assay—*Isonicotinic Acid Derivatives*—Isonicotinic acid derivatives were determined by the same spectrophotometric method as described previously (1). In the case of blood samples, 2.0 ml of plasma was deproteinized by the addition of 1.5 ml of 30% trichloroacetic acid and centrifuged. After neutralization, 3.0 ml of the supernate was used for the estimation.

Procainamide Hydrochloride and Sulfanilamide—These drugs were determined spectrophotometrically for the diazotizable amines by a previously reported method (10).

Inulin-(carboxylic acid-^{14}C)—The muscle, swollen overnight in

[1] Fluka Co., Switzerland.
[2] Tokyo Kasei Kogyo Co., Japan.
[3] Dai-ichi Seiyaku Co., Japan.
[4] Japan Radio Isotope Association, Tokyo, Japan.
[5] Nihon Yushi Co., Japan.
[6] HCO-100, Nikko Chemicals Co., Japan.
[7] Nikko Chemicals Co., Japan.
[8] Hamilton.
[9] Sephadex G-25 fine grade.

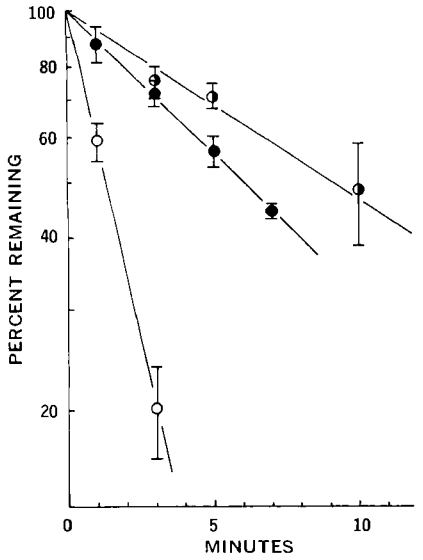

Figure 1—*Effect of polysorbate 80 on the clearance of isonicotinamide from the rat thigh muscle. Each point represents the mean value of at least five animals. Vertical bars indicate standard deviation, and straight lines are the result of least-squares regression analysis. The isonicotinamide concentration was 50 mM. Key (polysorbate 80 concentration, w/v): ○, 0.0%; ●, 1.0%; and ◑, 5.0%.*

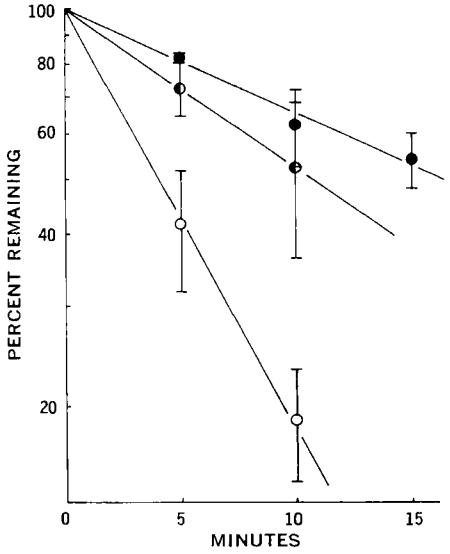

Figure 2—*Effect of polysorbate 80 on the clearance of ^{14}C-inulin from the rat thigh muscle. Each point represents the mean value of at least five animals. Vertical bars indicate standard deviation, and straight lines are the result of least-squares regression analysis. The ^{14}C-inulin concentration was 2.5 μCi/ml. Key (polysorbate 80 concentration, w/v): ○, 0.0%; ◐, 0.5%; and ●, 1.0%.*

3 ml of 0.5 *N* NaOH, was dissolved by heating to 90–95° with intermittent vigorous shaking in a glass-stoppered tube for about 2 hr. When cooled, the sample solutions were diluted to 5.0 ml with distilled water, and 2.5 ml of a 10% solution of zinc sulfate was added to precipitate the protein. After centrifugation, 0.5 ml of the supernate was taken and mixed well with 10 ml of scintillation medium[10] and 0.5 ml of 1 *N* HCl. In the case of blood samples, 1 ml plasma underwent zinc hydroxide precipitation to remove protein. After spinning, 0.5 ml clear supernate from a sample was added to 10 ml of the scintillation medium and 0.5 ml of 1 *N* HCl. The radioactivity of each sample was measured in a liquid scintillation system[11].

RESULTS AND DISCUSSION

To determine the possible effects of the polysorbates upon the kinetics of drug absorption, it was first necessary to study the time course of drug clearance from the site of injection. In earlier studies (1–4), it was found that the clearance of drugs from muscle could be described by a pseudo-first-order process. As shown in Figs. 1 and 2, clearance of isonicotinamide and ^{14}C-inulin was monoexponential, although there was a pronounced decrease in the rate of absorption. It appears that there was no change in the basic mechanism of absorption in the presence of the surfactant, and a reduced absorption might be ascribed to the effect of polysorbate 80 on some factors governing the passive diffusion of the drugs.

The results of the studies of isonicotinamide and ^{14}C-inulin plasma concentrations in rats are shown in Figs. 3 and 4, respectively. The decrease in the clearance from the site of injection is well reflected by the plasma level of the two drugs having a considerable molecular weight difference from one another (isonicotinamide, mol. wt. 122.13; inulin, mol. wt. approximately 5000). There is a pronounced decrease in the plasma concentration of isonicotinamide and ^{14}C-inulin in the presence of polysorbate 80. From this observation, together with the results shown in Figs. 1 and 2, it is evident that surfactants exert an absorption inhibitory effect through a similar mechanism not only for drugs of low molecular weight but for those of comparatively high molecular weight such as inulin.

Several formulation factors, such as the pH and the volume of injection solution, can influence the rate of intramuscular absorption from aqueous solutions. It has been shown that absorption of parenterally administered drugs can be influenced by the pH of the injected solution. Marked decreases in absorption in the acidic pH range contrary to a slight or negligible decrease in the alkaline pH range were reported (2). Figure 5 shows the effect of the pH of the injection solution on the 3-min absorption of isonicotinamide in the presence and absence of polysorbate 80. The apparent pattern of the pH–absorption profile in the presence of polysorbate 80 is somewhat similar to the one seen with the control experiment, although a general decrease in absorption is noticed

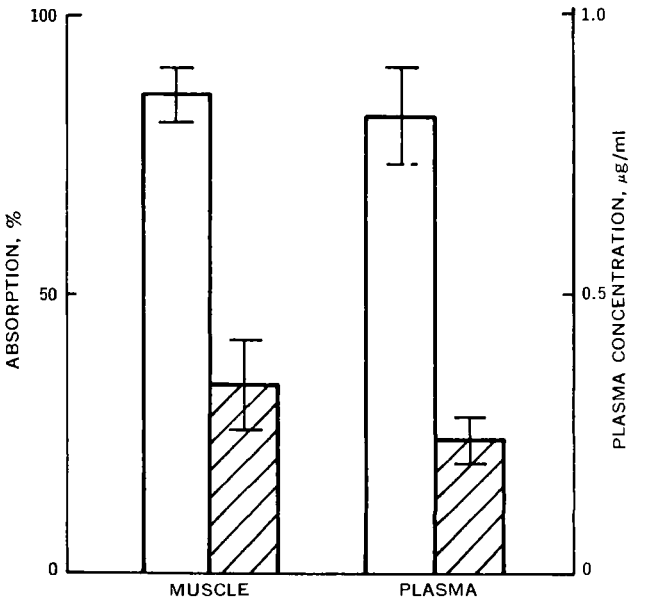

Figure 3—*Relationship between absorption and plasma concentration of isonicotinamide, and effect of polysorbate 80 at end of 3 min. Each column represents the mean of results from experiments with six animals with standard deviation. The isonicotinamide concentration was 100 mM. Key (polysorbate 80 concentration, w/v): open columns, 0.0%; and hatched columns, 5.0%.*

[10] NT-scintillation medium: a mixture of toluene (700 ml), nonylphenol-polyethoxyethanol (300 ml), and 2,5-diphenyloxazole (4 g).
[11] Beckman LS-232.

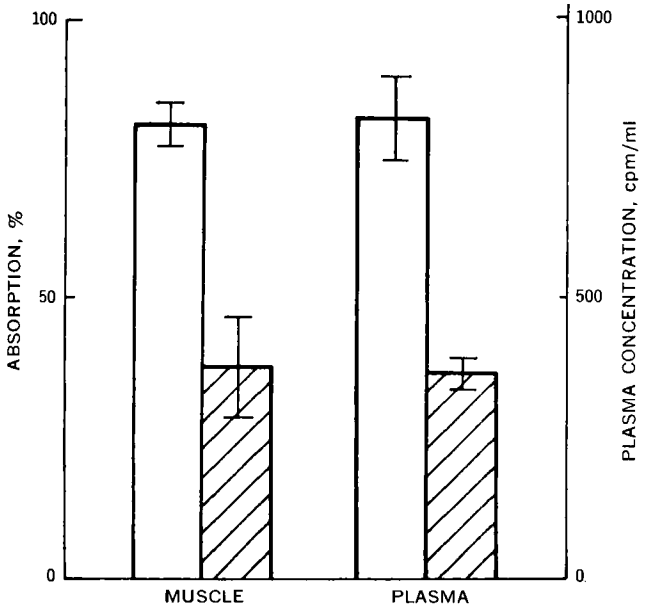

Figure 4—*Relationship between absorption and plasma concentration of ^{14}C-inulin, and effect of polysorbate 80 at end of 10 min. Each column represents the mean of results from experiments with five animals with standard deviation. The ^{14}C-inulin concentration was 2.5 μCi/ml. Key (polysorbate 80 concentration, w/v): open columns, 0.0%; and hatched columns, 1.0%.*

in the former. This supports the view that the basic kinetics of absorption in the presence of polysorbate 80 is somewhat similar to that in the absence of surfactant.

The effect on the rate of intramuscular absorption of isonicotinamide by varying the injection volume was examined in the presence of 0.1 and 1.0% polysorbate 80. For isonicotinamide (50 m*M*), the percentages of the injected dose remaining were examined for injection volumes of 5, 10, and 20 μl. The results of the 3-min absorption of isonicotinamide are shown in Table I. For comparison, the control values obtained in the absence of the surfactant are listed. Within the range of volumes investigated, the injection volumes do not particularly influence absorption rate of isonicotinamide. In contrast, the absorption rate depends markedly upon the concentration of the surfactant.

The possibility of the delayed clearance of drug in the presence of polysorbate 80 regardless of the physicochemical characteristics

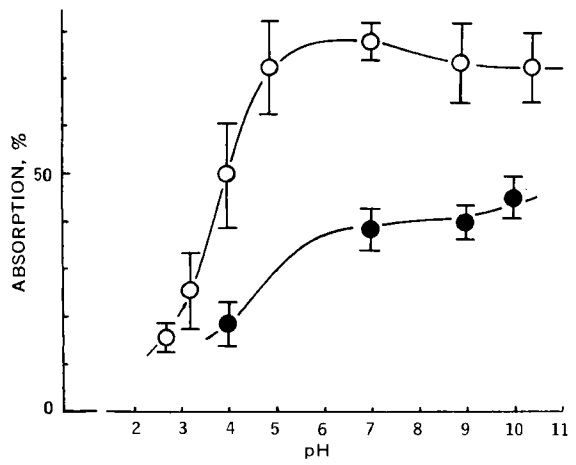

Figure 5—*Effect of pH and polysorbate 80 on the absorption of isonicotinamide at end of 3 min. Each point represents the mean values of at least five animals. Vertical bars indicate standard deviation. The isonicotinamide concentration was 50 mM. Key (polysorbate 80 concentration, w/v): ○, 0.0%; and ●, 1.0%.*

Table I—Effect of Injection Volume on the Absorption of Isonicotinamide in the Presence of Polysorbate 80[a]

Concentration of Polysorbate 80, % w/v	Absorption[b], % Injection Volume, μl		
	5	10	20
0	74.0 ± 7.8 (5)	78.0 ± 4.1 (5)	80.2 ± 6.4 (5)
0.1	52.9 ± 6.8 (4)	51.1 ± 4.9 (5)	50.8 ± 5.8 (3)
1.0	30.8 ± 12.5 (8)	28.3 ± 3.8 (9)	31.0 ± 5.9 (5)

[a] Values represent the mean ± *SD*. Figures in parentheses are the number of animals. Concentration of isonicotinamide was 50 m*M*. [b] Percent absorption was determined for 3 min.

of the drugs was tested. For that purpose, isonicotinic acid and procainamide hydrochloride were chosen as charge-bearing compounds at the pH of the absorption experiment. For nonionic drugs, three types were selected according to their relative magnitude of lipid solubility, namely isonicotinamide and ^{14}C-inulin as the least soluble, sulfanilamide as intermediate, and methyl isonicotinate as the most soluble. Figure 6 shows the results obtained through the molecular sieve technique. As can be seen, the ratios of total to free isonicotinamide, isonicotinic acid, and procainamide hydrochloride are approximately unity within the concentration range of surfactant, which implies that these drugs also are free from micellar interaction. In the case of methyl isonicotinate and sulfanilamide, however, some interaction was observed. Semilogarithmic plots of the residual amounts of drugs *versus* time yielded straight lines for solutions containing up to 10.0% of polysorbate 80. The absorption rate constants obtained are summarized in Table II. As is evident from these results, absorption of this rather diverse group of drugs is decreased as the concentration of polysorbate 80 is increased. To compare the magnitude of the delayed clearance effect on a variety of drugs, a plot of the absorption rate constant ratio *versus* the log of surfactant concentration was constructed. Absorption rate constants are expressed as a ratio relative to the rate constant observed with injection solutions containing only drug and buffer. As illustrated in Fig. 7, absorption of a variety of drugs was reduced to an almost similar extent independent of their ionogenic nature, lipophilicity, molecular size, and pharmacological class. In the case of muscular absorption of drugs, it has been reported that diffusion through pores of the capillary wall is dominant compared with the penetration process through the vascular endothelial cells, unlike the GI absorption (8, 11, 12). Also it has been pointed out that in contrast to the GI tract, absorption from muscle could not be related to the pKa's and lipid solubilities of the substances (1). Nonselectivity in the absorption-decreasing action of the polysorbate may support the foregoing view. In the following experiments, isonicotinamide was selected as a model drug because of its negligible micellar interaction as well as its well-documented absorption characteristics (1–4).

Further experiments were conducted in which the absorption inhibitory effect of other polysorbates was investigated. As in the case of polysorbate 80, first-order kinetics were observed in the absorption of isonicotinamide in the presence of polysorbates. The results of the study with polysorbate 20, 40, and 60 (Table III) are in good agreement with the results obtained with polysorbate 80. This finding may be explained by the fact that these polysorbates share the common polyol moiety and only the fatty acid portions

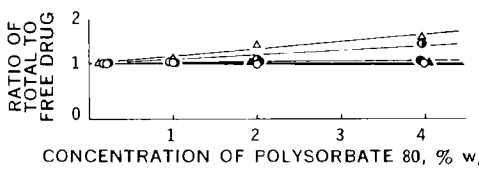

Figure 6—*Ratio of total to free drug containing various concentrations of polysorbate 80 as determined by a molecular sieve technique. Key: ●, isonicotinamide; ◑, methyl isonicotinate; ○, isonicotinic acid; ▲, procainamide hydrochloride; and △, sulfanilamide.*

Table II—Effect of Polysorbate 80 on the Absorption Rate Constants[a] of Drugs

Drug	Absorption Rate Constant, min^{-1} Concentration of Polysorbate 80, % w/v							
	0	0.01	0.05	0.1	0.5	1.0	5.0	10.0
Isonicotinamide	0.537	0.483	0.252	0.240	0.147	0.116	0.074	0.054
Methyl isonicotinate	0.613	—	—	0.256	—	0.103	—	—
Isonicotinic acid	0.463	0.430	0.220	0.140	0.100	0.073	0.038	—
Sulfanilamide	0.230	—	0.097	0.040	—	0.030	0.026	—
Procainamide hydrochloride	0.170	—	0.093	0.073	0.024	—	—	—
^{14}C-Inulin	0.171	—	—	0.065	—	0.042	—	—

[a] Absorption rate constants were calculated from clearance curves.

Table III—Effect of Polysorbates on Absorption of Isonicotinamide[a]

Concentration of Polysorbate, % w/v	Absorption, %			
	Polysorbate 80	Polysorbate 20	Polysorbate 40	Polysorbate 60
0.01	76.5 ± 7.8 (6)	80.9 ± 5.8 (5) N.S.	80.7 ± 6.8 (9) N.S.	85.1 ± 2.6 (5) $p < 0.05$
0.05	52.9 ± 4.8 (6)	51.1 ± 4.1 (5) N.S.	52.0 ± 2.2 (4) N.S.	60.1 ± 4.7 (7) $p < 0.05$
0.1	51.1 ± 4.9 (5)	34.5 ± 4.8 (7) $p < 0.01$	48.8 ± 6.2 (8) N.S.	57.8 ± 6.6 (12) N.S.
0.5	35.6 ± 3.2 (4)	17.5 ± 2.6 (5) $p < 0.001$	35.3 ± 5.7 (6) N.S.	39.7 ± 7.6 (3) N.S.
1.0	43.4 ± 3.5* (4)	12.3 ± 1.4* (4) $p < 0.001$	40.3 ± 8.9* (5) N.S.	38.8 ± 3.6* (4) N.S.
5.0	29.1 ± 3.7* (7)	12.2 ± 8.0* (3) $p < 0.01$	30.8 ± 10.1* (3) N.S.	31.8 ± 8.6* (5) N.S.
10.0	23.9 ± 4.5* (5)			

[a] Percent absorption was determined for 3 min except for items marked by asterisk which indicates 5 min. Values represent the mean ± *SD*. Figures in parentheses are the number of animals. A Student *t* test was performed among the same concentration against the value of polysorbate 80. N.S. = not significant ($p > 0.1$).

differ from each other rather than the molecular weight, hydrophilic–lipophilic balance, and other physicochemical properties. The more pronounced effect exerted by polysorbate 20 in concentrations above 0.1% could be attributed to its tendency to release the endogenous biogenic compounds or to the self-depression of absorption caused by histamine (13). In the case of polysorbate 60, at concentrations of 0.05% and below, the absorption inhibitory effect was somewhat lessened but the cause was not clear.

The polysorbates are readily hydrolyzed in the GI tract to fatty acid and polyol (polyoxyethylene 20 sorbitan) (14). Levy and Anello (15) noted the permeability-modifying effect of polysorbate hydrolysis products on secobarbital absorption in goldfish. Little is known about the biotransformation of these compounds in the muscle. But it is of interest to determine the effect of such hydrolysis products of polysorbates on the intramuscular absorption, since the accidental presence of any of the hydrolysis products as contaminants may be expected. The results of studies with lauric acid and with sorbitan and polyoxyethylene 20 sorbitan are shown in Figs. 8 and 9, respectively. Various concentrations of lauric acid, the fatty acid component of polysorbate 20, the surfactant that showed the strongest absorption-inhibiting tendency, were solubilized with 0.1% polysorbate 80 and 1.0% polyoxyethylene derivative of hydrogenated castor oil. As is evident from Fig. 8, the inhibitory effect of lauric acid on the 3-min absorption of isonicotinamide was almost negligible. In the case of sorbitan, no delayed absorption of isonicotinamide was observed even at a concentration as high as 5.0%.

The polyol moiety, at a concentration higher than 2.0%, decreased significantly the absorption of isonicotinamide, but the pattern of the curve was somewhat different from that of polysorbate 80 which exerted a much more pronounced absorption-inhibiting effect at lower concentrations. The contribution due to the possible presence of hydrolysis products in polysorbates is negligible because they are considered small in quantity. These observa-

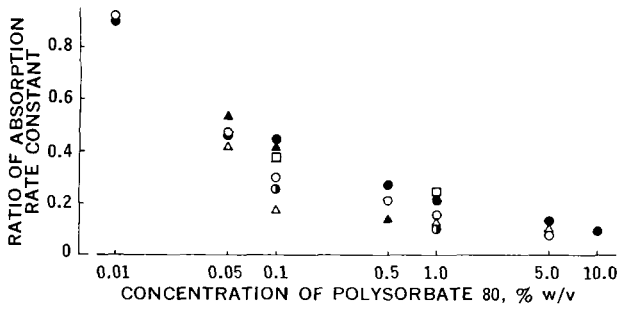

Figure 7—*Relationship between ratio of absorption rate constant and concentration of polysorbate 80. Ratios of absorption rate constants are expressed as a ratio relative to that observed with injection solutions containing only drug and buffer. There are no significant differences by the variance analysis in the ratio of absorption rate constant among the drugs at the polysorbate 80 concentrations of 0.01, 0.05, and 5.0% [0.01%, $F_{calc} = 1.85 < F(1,7; 0.05) = 5.59$; 0.05%, $F_{calc} = 0.68 < F(3,19; 0.05) = 3.13$; 5.0%, $F_{calc} = 2.06 < F(2,15; 0.05) = 3.68$]. Key: ●, isonicotinamide; ◑, methyl isonicotinate; ○, isonicotinic acid; △, sulfanilamide; ▲, procaine amide hydrochloride; and □, ^{14}C-inulin.*

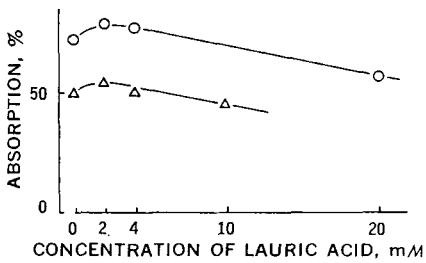

Figure 8—*Effect of lauric acid on the absorption of isonicotinamide at the end of 3 min. Each point represents the mean value of at least five animals. Key: ○, with 1.0% (w/v) polyoxyethylene derivative of hydrogenated castor oil; and △, with 0.1% (w/v) polysorbate 80.*

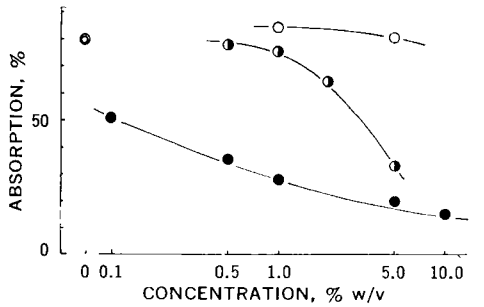

Figure 9—*Effect of sorbitan, polyoxyethylene 20 sorbitan, and polysorbate 80 on the absorption of isonicotinamide at the end of 3 min. Each point represents the mean value of at least five animals. Key: ⓪, control; ○, sorbitan; ◑, polyoxyethylene 20 sorbitan; and ●, polysorbate 80.*

tions suggest the possibility that the delayed absorption of a drug is caused by the surfactant *per se* and not by its hydrolysis products.

The results in this study indicate that: *(a)* a low concentration of the polysorbates dramatically reduces the absorption of water-soluble, micelle-free drugs and such reduction is reflected on the plasma levels of drugs; *(b)* the relative absorption rate of a drug solution in the muscle is dependent on the surfactant concentration rather than on the absolute amount; and *(c)* the delayed clearance seems to be operative in a variety of drugs and is caused by the polysorbates *per se*. The exact cause of the phenomenon is uncertain at present. Several mechanisms could operate alone or in combination to bring about the decrease of absorption observed in the presence of the polysorbates. Previous investigations concerning the absorption and viscosity of aqueous injection solutions (3) and on the membrane transport phenomena (16) in the presence of a surfactant under negligible micellar interaction ruled out the possibility that the reduction of the rate of absorption is caused mainly by the decreased diffusion rate of drugs in the injected solution containing surfactant. There is a possibility that the biological events that depend on other complex and interrelated events, which cannot be adequately explained by a simple physicochemical concept, influence the absorption.

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PHARMACEUTICAL ANALYSIS

GLC Analysis of Ergonovine Maleate

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Abstract □ A method is described for the quantitative determination of ergonovine maleate by GLC. Data are presented to show that this method provides the quantitative and semiquantitative results of the USP colorimetric assay and TLC in a single determination. Favorable quantitation is achieved by using brucine as internal standard. The sample is dried in the dark, derivatized with *N*-trimethylsilyldiethylamine and *N*-trimethylsilylimidazole in pyridine, and chromatographed on a column containing a nonpolar methyl silicone liquid phase. Degradation of the sample by preanalytical preparation (pH changes, air, heat, and light) are thereby minimized.

Keyphrases □ Ergonovine maleate—GLC analysis □ GLC—analysis, ergonovine maleate

It is generally recognized that the chemical purity of ergonovine (I) maleate is affected by oxygen, light, temperature, and extremes in pH (1–7). The chemical nature of the degradation products (6, 7) may cause falsely high values when assays are performed by the colorimetric method described in USP XVIII