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Effect of Some Ionic and Nonionic Surfactants on the Intramuscular Absorption of Isonicotinamide[1,2]

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The effects of various surfactants on the intramuscular absorption of water-soluble, micelle-free drug was studied using isonicotinamide as a model compound.

1) Lineality of the clearance curves of the drug was kept even in the presence of either nonionic or ionic surfactants, which indicates that the fundamental mechanism of absorption was unaltered.

2) Absorption inhibitory effects were not specific to nonionic surfactants but were also found in ionic ones in almost equal degrees, and concentration dependency as well.

3) In regard to the homologous series of polyoxyethylene derivatives of hydrogenated castor oil (HCOs), it has been observed that the smaller the molecular weight and the hydrophlie lipophlie balance are, the greater is the inhibitory effect.

Surface active agents are one of the most important groups of adjuvants in pharmaceutical preparations. The wide-spread use of surfactants as well as their unique physical-chemical properties continues to be of great interest in the effect of these agents upon the biological availability and pharmacological activity of drugs.

Enhancement or inhibition of the absorption and pharmacological activity of drugs have been observed in the presence of surfactants. A number of investigations on the surfactants' effects have been reported in the field of gastrointestinal absorption,[4] skin permeability,[5] hemolysis,[6] and biological membrane permeability.[7–10] However, there appears to be much less known about the effects of surfactants on parenteral absorption.

It was reported in a previous paper[11] that polysorbates, nonionic surfactants, exert remarkable inhibitory effect on the absorption of water-soluble, micelle-free drugs from the rat thigh muscle.

In this paper such a pronounced decrease in the absorption rates of drugs observed by the presence of a low concentration of the polysorbates was examined in various types of surfactants with the view to clarify the mechanism of inhibitory effects of surfactants.

Experimental

Materials——Isonicotinamide was of analytical grade and was obtained commercially. The following surfactants were used——*viz.*, nonionic surfactants: polyoxyethylene 40 stearate, polyoxyethylene 55 stea-

1) *a*) This paper constitutes the 8th report in a series of "Biopharmaceutical Studies on the Parenteral Preparations"; *b*) Preceding paper Part VII: T. Nishimura, H. Kobayashi, K. Okumura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **22**, 1275 (1974).

2) Part of this work was presented at the 93th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1973.

3) Location: *Yoshidashimoadachi-cho, Sakyo-ku, Kyoto.*

4) C.J. Kreutler and W.W. Davis, *J. Pharm. Sci.*, **60**, 1835 (1971).

5) M. Mezei and K.J. Ryan, *J. Pharm. Sci.*, **61**, 1329 (1972).

6) M. Kondo, M. Yoshimura, and T. Okumura, *Seikagaku*, **44**, 849 (1972).

7) J.M.N. Gillan and A.T. Frorence, *J. Pharm. Pharmac.*, **25**, *Suppl.*, 137P (1973).

8) W. Saski, M. Mannelli, M.F. Saettone, and F. Bottari, *J. Pharm. Sci.*, **60**, 854 (1971).

9) C.W. Whitworth and H.W. Jun, *J. Pharm. Sci.*, **62**, 1890 (1973).

10) J.A. Anello and G. Levy, *J. Pharm. Sci.*, **58**, 721 (1969).

11) H. Kobayashi, T. Nishimura, K. Okumura, S. Muranishi, and H. Sezaki, *J. Pharm. Sci.*, **63**, 580 (1974).

rate,[12] triton X-100, sucrose fatty acid ester,[13] and polyoxyethylene derivatives of hydrogenated castor oil (HCO-20, 40, 50, 60, 100, and 120); anionic surfactants: sodium lauryl sulfate, sodium deoxycholate, and sodium cholate; cationic surfactant: cetyl trimethyl ammonium bromide. All compounds were commercially available materials, and no attempt at further purification was made except sodium lauryl sulfate.

Procedure of Absorption Experiments——Male Wistar albino rats weighing between 150 and 180 g were used in all absorption experiments. The absorption experiments were almost identical with those described in a previous paper from this laboratory.[14] The customary injection volume of 10 μl was delivered with a Hamilton microliter syringe. At various times after injection of drugs into the center of the *m. rectus femoris*, the muscle was removed. The muscle was excised, 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 in each experiment.

Preparation of Injectable Solutions——All injection solutions used in this study were prepared isotonically and adjusted at pH 7.0 using NaH_2PO_4-Na_2HPO_4 buffer solution. The concentration of isonicotinamide was 50 mM.

Analytical Method——Isonicotinamide was determined by the same spectrophotometric method as described in a previous report from this laboratory.[14]

Results and Discussion

The presence of a low concentration of the polysorbates exerts remarkable inhibitory effect on the intramuscular absorption of drugs. It is of great interest to examine whether other surfactants also have the same effect.

Since it was made clear in the previous paper[11] that no selectivity of drugs was observed in the inhibitory effect of polysorbates on the absorption of water-soluble drugs with very little micellar interaction, experiments in this study were conducted with isonicotinamide as a model drug.

(1) Effect of Nonionic Surfactants

For nonionic surfactants, POE(44)[15] stearate and POE(55)[15] stearate; HCO-20, 40, 50, 60, 100, and 120, a polyoxyethylene derivative of hydroganated castor oil; triton X-100, octyl phenol ethyleneoxide; and sucrose fatty acid ester were used.

Effect of Nonionic Surfactants on the Time Course of Isonicotinamide Clearance——Figure 1 shows the time course of the clearance of isonicotinamide from the site of injection after the intramuscular administration of the drug with POE(55) stearate (0.0, 1.0, and 5.0%). As is evident from the Figure, the lineality of the clearance curves is maintained even in the presence of the surfactant. Same results were obtained with the other nonionic surfactants used in this study. It has become clear that even in the presence of nonionic surfactants, drug clearance from the site of injection is apparently described by a pseudo first-order process as in the case of polysorbates reported in the previous paper.[11]

Effect of Concentration of Nonionic Surfactants on Drug Absorption——Investigation was made on the influence of the initial concentration of nonionic surfactants such as POE(40) stearate, POE(55) stearate, HCOs, triton X-100, and sucrose fatty acid ester, on the absorption of isonicotinamide from the muscle 3 minutes after injection. As shown in Figure 2, it is apparent that in the presence of nonionic surfactants, absorption inhibitory effect is more or less universally observed and that surfactants' concentration dependency is involved. About HCOs, their clearance curves are shifted toward the side of higher concentration compared with those of the other nonionic surfactants. Also, great difference is observed in the inhibitory effect among HCOs of the same concentration with different polymerization grades. The figure shows the order of the strength of inhibitory effect of HCOs,— HCO-40, 20, 50, 60, 100,

12) Marketed as MYS 40 and MYS 55 by Nikko Chemicals Co., Ltd., Japan.
13) Marketed as Nittoester (Japanese Standards of Food Additives) by Dainihonseito Co., Ltd., Japan.
14) K. Kakemi, H. Sezaki, K. Okumura, and S. Ashida, *Chem. Pharm. Bull* (Tokyo), **17**, 1332 (1969).
15) POE (40): polyoxyethylene 40, POE(55): polyoxyethylene 55.

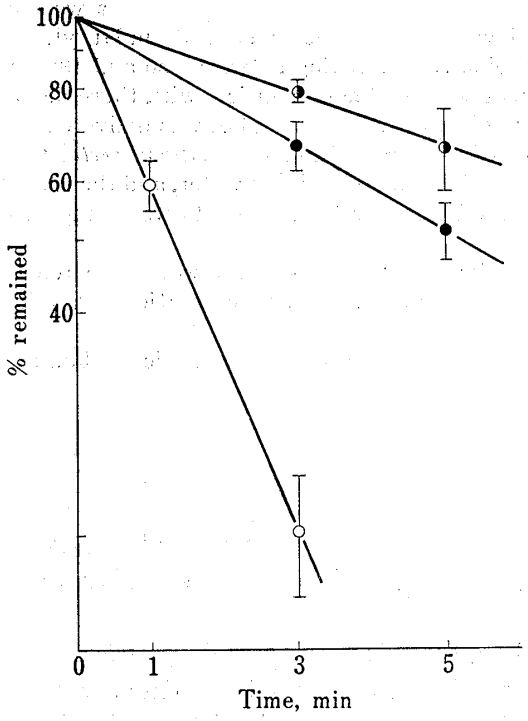

Fig. 1. Effect of POE (55) Stearate on the Clearance of Isonicotinamide from Rat Thigh Muscle

Each point represents the mean values of at least five animals. Vertical bars indicate S.D. and straight lines are the result of least squares regression analysis.
isonicotinamide: 50 mM; POE (55) stearate, w/v: ○, 0.0%; ●, 1.0%; ◑, 5.0%

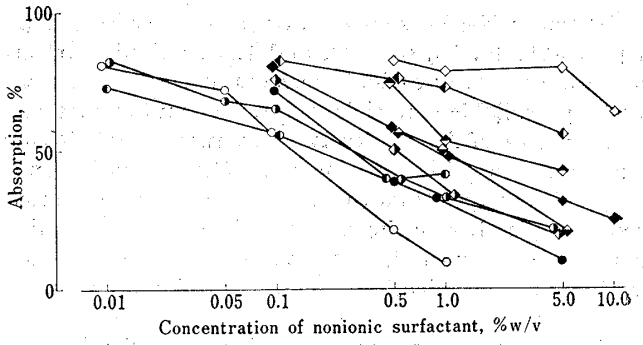

Fig. 2. Effect of Nonionic Surfactants on the Absorption of Isonicotinamide

Absorption period is 3 minutes.
Each point represents the mean values of at least five animals.
isonicotinamide: 50 mM
○: triton X-100
◐: POE (40) stearate
◑: POE (55) stearate
●: sucrose fatty acid ester
⬙: HCO-20
◑: HCO-40
◆: HCO-50
⬘: HCO-60
◐: HCO-100
◇: HCO-120

Table I. Molecular Weight and HLB of HCOs

Surfactant	mol.wt.[a]	HLB[b]
HCO-20	1818	9.3
HCO-40	2698	11.8
HCO-50	3138	13.4
HCO-60	3578	14.1
HCO-100	5338	16.5
HCO-120	6218	

a) Molecular weight was calculated from the chemical structure.
b) data from Nikko Chemicals Co., Ltd., Japan

120; that is, the smaller the molecular weight is, the greater is the inhibitory effect. This difference of inhibitory effect might be attributed to the difference of molecular weights and HLB values, as shown in Table I. Wide difference is seen between HCO-20 and HCO-120. The molecular weight of the former being 1818 and that of the latter 6218, calculated from their chemical structures; and the hydrophylic lipophylic balances (HLB) are 9.3 and 16.5 respectively. However, HCO-20 seems exceptional because of its smaller inhibitory effect than might be expected from its molecular weight and HLB, but this may be due to its lower solubility in water. Unlike the other HCOs, HCO-20 yields a little cloudy injectable solution, which might have depressed its inhibitory effect.

Thus it has been demonstrated that there is a concentration dependency in the inhibitory effect of the nonionic surfactants used here and that, with the same series of surfactants, the smaller the molecular weight is, the greater is the inhibitory effect, as was observed with a series of HCOs.

Relationship between HLB Values of Nonionic Surfactants and Decrease in Absorption—— Figure 3 shows the relationship between HLB and percentage absorption of isonicotinamide in 3 minutes after injection of the drug solution containing 0.5, 1.0, and 5.0% HCOs. As the degree of polymerization of ethylene oxide chain, hydrophilic portion of HCOs, increases in the order of HCO-20, 40, 50, 60, 100, 120, their HLB values also increase in this order. The figure shows that plots of the percentage absorption of isonicotinamide in the presence of the same concentration (w/v%) of HCO-40, 50, 60, and 100 against their HLB values become linear except less water-soluble HCO-20. However, in the uppermost curve, which corres-

ponds to the values of 0.5%, the point representing the absorption rate in the presence of HCO-100 takes no higher value than that of HCO-60, unlike the cases of 1.0% or 5.0%. This may be attributed to the fact that the percentage absorption within 3 minutes was almost identical to the one of the control value, as shown in Figure 1. Furthermore, as HLB has additivity, 1% solution was prepared by putting the equivalent volume of HCO-40 and 100 together. The percentage absorption of isonicotinamide from this solution was measured and was plotted on the HLB value of 14.15, which is the avarage value of each HLB. As is indicated by the closed circle, this point almost overlaps the middle point between HCO-40 and 100 of 1.0%. Thus it is suggested that, with the same series of surfactants, there exists some correlationship between HLB and absorption inhibitory effect; that is, the greater the hydrophilicity of the surfactant is, the less is the inhibitory effect. However, when plotted in the same way polysorbates, whose HLB values of about 15—17,[16] gave 3-minutes absorption of 12.3—40.3%,[11] which are widely off the curves obtained with HCOs. Also SLS, whose HLB is around 40,[16] has stronger inhibitory effect than can be expected from these data, as is mentioned elsewhere.

Suzuki, *et al.*[17] reported that with nonionic surfactants of the homologous series there is a significant correlationship between the effect of surfactants on the extravasal dye leakage and their HLB. In the case of intramuscular absorption shown in Figure 3, however, this relationship is not common to all cases, but is confined to some limited range of HLB and to the surfactants of the homologous series.

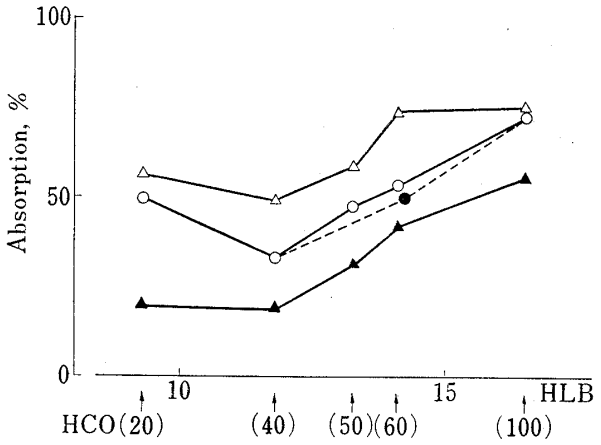

Fig. 3. Relationship between the Effect of HCOs on the Absorption of Isonicotinamide and their HLB Values

Absorption period is 3 minutes.
Each point represents the mean values of at least five animals.
isonicotinamide: 50 mM
HCOs, w/v: △: 0.5%; ○: 1.0%; ▲: 5.0%; ●: 1.0% HCO-40+1.0% HCO-100

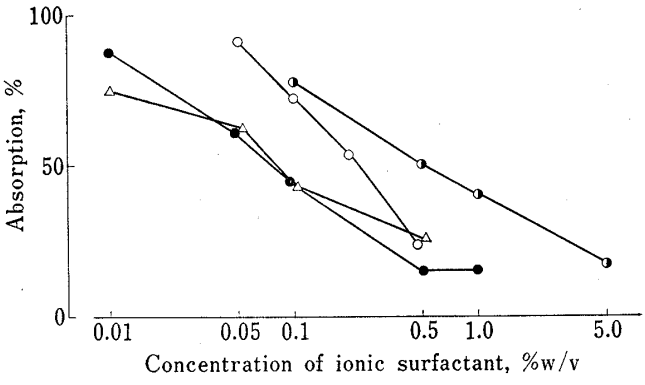

Fig. 4. Effect of Ionic Surfactants on the Absorption of Isonicotinamide

Absorption period is 3 minutes.
Each point represents the mean values of at least five animals.
isonicotinamide: 50 mM
○: sodium deoxycholate; ●: SLS;
◑: sodium cholate; △: CTAB

(2) Effect of Ionic Surfactants

So far various nonionic surfactants were taken up and their absorption inhibitory effect was investigated. It would be of great significance in clarifying the influence of surfactants on the absorption of drugs from muscle to examine whether these effects are specific to nonionic surfactants, or whether nonionic surfactants are different in their effects from ionic ones as was already reported about the rate of peritoneal dialysis of urea and creatinine in the rabbits,[18]

16) Y. Ishii, "Hiion-kaimenkasseizai," Seibun-do Shinko-sha Ltd., Japan, 1962, p. 142.
17) M. Suzuki, K. Motoyoshi, H. Arai, and H. Horikawa, *Jap. J. Pharmacol.*, 17, 525 (1967).
18) S.C. Penzotti and A.M. Mattocks, *J. Pharm. Sci.*, 57, 1192 (1968).

histological effect on the mouse gastrointestinal tract,[19] absorption of phenol red from rat colon,[20] and hemolysis.[6] From this point of view, sodium lauryl sulfate and two bile salts, sodium deoxycholate and sodium cholate, were chosen among anionic surfactants, and cetyl trimethyl ammonium bromide was chosen among cationic surfactants. A similar investigation was made on their relation to absorption of drugs. In these cases also lineality was kept in the clearance curves of isonicotinamide administered into muscle in the presence of ionic surfactants. Absorption is apparently a pseudo first-order process as in the case of POE(55) stearate shown in Figure 1. Therefore, it is clear that the fundamental mechanism of absorption is unchanged even in the presence of either nonionic or ionic surfactants. Figure 4 shows the absorption curves of isonicotinamide in the presence of ionic surfactants, such as sodium lauryl sulfate, sodium deoxycholate, sodium cholate, and cetyl trimethyl ammonium bromide, plotted in the same way as in Figure 2. As is clearly shown in this figure, ionic surfactants also have remarkable inhibitory effects and concentration dependency as well. Moreover, about these absorption inhibitory effects, similar results were obtained to those seen in the cases of nonionic surfactants, *viz.* polysorbates, triton X-100, POE(40) stearate, POE(55) stearate, and sucrose fatty acid ester. Therefore, it has been demonstrated that, unlike the cases of gastrointestinal absorption or hemolysis, there is hardly any fundamental difference between nonionic and ionic surfactants in the absorption inhibitory effect.

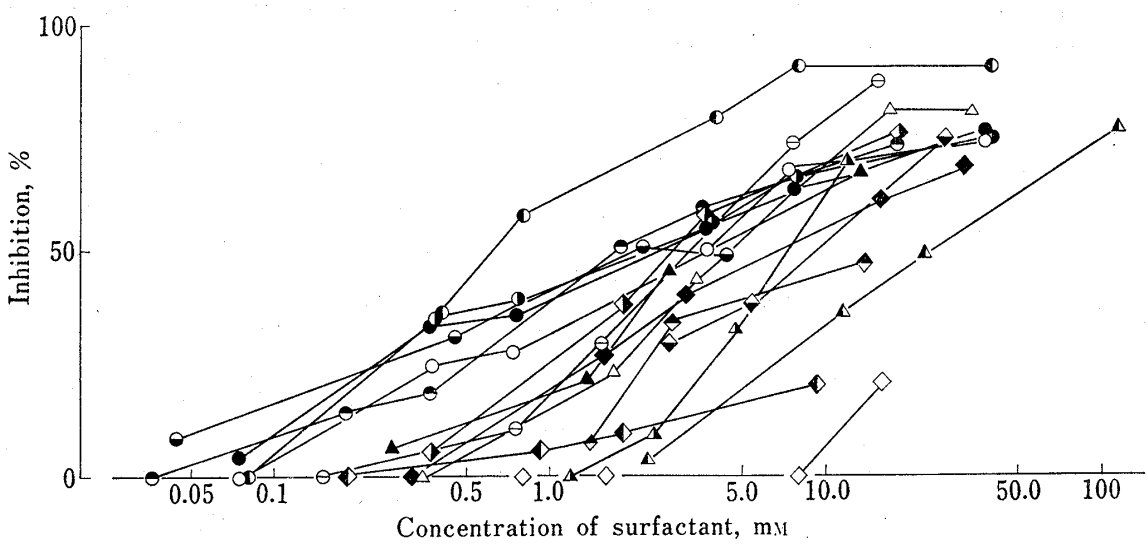

Fig. 5. Relationship between Inhibitory Effect on the Absorption and Concentration of Surfactants

◐: polysorbate 20[a] (1226); ◑: polysorbate 40[a] (1282); ○: polysorbate 60[a] (1310); ●: polysorbate 80[a] (1308); ⊖: triton X-100 (649); ◓: POE (55) stearate (2705); ◒: POE (40) stearate (2245); ◈: HCO-20 (1818); ◈: HCO-40 (2698); ◆: HCO-50 (3138); ◈: HCO-60 (3578); ◈: HCO-100 (5338); ◇: HCO-120 (6218); △: SLS (288.38); ▲: sodium deoxycholate (414.57); ▲: sodium cholate (430.57); ▲: CTAB (364.46);

Figures in parentheses are the molecular weight of surfactant calculated from chemical structure.
a) The curves of polysorbates are drawn on the basis of the data in the previous paper.[11]

(3) Relationship between Inhibitory Effect and Concentration of Surfactants

So far, comparative observation of various surfactants has been conducted with their concentrations of percentages of weight *versus* volume basis. Figure 5 indicates the comparison of their inhibitory effects with molar concentrations basis. Percentage inhibition is obtained by comparing 3-minutes absorption after injection with those where no surfactant is present. Molar concentrations were calculated from their molecular weight shown in the parentheses

19) J.A. Nissim, *Nature*, **187**, 305 (1960).
20) P.M. Lish and J.H. Weikel, Jr., *Toxicol. Appl. Pharmacol.*, **1**, 501 (1959).

in the figure. A more significant correlationship might be obtained, if the actual avarage molecular weights are measured and the figures are replaced by corrected ones. It is evident that inhibitory effect of the surfactants is not specific to nonionic ones but are also found in ionic surfactants in almost equal degrees.

The present study is confined to the phenomenal aspects of absorption inhibition only. This observation, however, would be of great help in clarifying the mechanism of influence of surfactants on absorption from the muscle. In the intramuscular absorption of water-soluble drugs, as was previously reported, either of the following two is considered to be a rate-limiting step in the absorption of drugs,— (1) the diffusion through the intercellular space of muscle fiber or connective tissue, (2) the diffusion through the pores of the capillary wall. Besides, as is clarified in this and the last reports[11] the absorption of drugs follows apparently 1st order kinetics regardless of the nature of the surfactant incorporated in the injectable solutions. Work designed to elucidate the mechanism of the inhibitory action of surfactants on the intramuscular absorption of drugs is continuing in this laboratory.