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Mechanism of the Inhibitory Effect of Polysorbate 80 on Intramuscular Absorption of Drugs. (1)[1]

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The mechanism of the inhibition caused by polysorbate 80 on the intramuscular absorption of water-soluble drugs was studied. It was found that the absorption pattern of isonicotinamide was different from that of polysorbate 80. The inhibition of the absorption requires the existence of polysorbate 80 at the site of injection. Histological studies showed that there is no correlation between the inhibition in absorption and the local effect of polysorbate 80 which was found to cause some inflammation at the site of injection. The influence of polysorbate 80 on the capillary permeability was investigated. This was done by intravenous pre-administration of polysorbate 80, sympatholytic agent or conjugated estrogen. No difference in the intramuscular absorption of the tested drugs in the presence or absence of polysorbate 80, whether the animals were pretreated or not, could be demonstrated. Therefore, the inhibition in the intramuscular absorption of these drugs could not be attributed to a direct nor indirect effect of polysorbate 80 on the capillary walls. It was suggested that the permeation of drug through the intercellular spaces of muscle fibers and connective tissues is the more dominant process.

In the series of our studies that dealing with the investigation of the effect of surface active agents on the intramuscular absorption of drugs we have shown that, the intramuscular absorption of water-soluble drugs was remarkably inhibited in the presence of an extremely low concentrations of polysorbate 80.[3] The reduction in the absorption of these drugs was reflected on their plasma concentrations; and the inhibition caused by the presence of polysorbate 80 was found to be concentration dependent. We also investigated the influence of various kinds of nonionic and ionic surfactants on the intramuscular absorption of drugs and it was found that they possess an inhibitory effect on the absorption process similar to that of polysorbate 80.[4]

Our researches were proceeded to clarify the mechanism of the inhibitory effect of surface active agents on the intramuscular absorption of drugs.

Experimental

Materials——Isonicotinamide was of analytical grade and obtained commercially. Inulin-(carboxylic acid-^{14}C) and ^{14}C-sucrose were purchased from Japan Radio Isotope Association (Tokyo, Japan); Tolazoline·HCl ("Priscol" Takeda Chemical Industries Ltd.,) and conjugated estrogen ("Romeda" Mochida Pharmaceutical Co., Ltd.). All other chemicals were reagent grade. Preparation of ammonium cobalt thiocyanate reagent was done by dissolving 62.0 g of ammonium thiocyanate and 28.0 g of cobalt nitrate hexahydrate in 100 ml of distilled water. Then, the solution was extracted twice with ethylene dichloride.

Preparation of Injectable Solutions——Solutions of isonicotinamide, ^{14}C-sucrose and inulin-(carboxylic acid-^{14}C) for intramuscular injections were dissolved in an isotonic phosphate buffer solution (pH 7.0) in the following concentrations: 50 mM, 5 μCi/ml and 5 μCi/ml respectively. Polysorbate 80, Tolazoline·HCl (1

1) *a*) This paper constitutes the 11th report in a series of "Biopharmaceutical Studies on Parenteral Preparations"; *b*) Preceding paper, Part X: T. Tanaka, K. Taneda, H. Kobayashi, K. Okumura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 3069 (1975).
2) Location: *Yoshidashimoadachi-cho, Sakyo-ku, Kyoto, 606, Japan.*
3) H. Kobayashi, T. Nishimura, K. Okumura, S. Muranishi, and H. Sezaki, *J. Pharm. Sci.*, **63**, 580 (1974).
4) H. Kobayashi, T. Peng, A. Kagayama, K. Okumura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 42 (1975).

mg/ml) and conjugated estrogen (0.2 mg/ml) for intravenous administration were prepared in an injectable saline solution.

Absorption Experiments——Male Wistar albino rats weighing 160—220 g were used. Absorption experiments were carried out as described previously.[5] An injection volume of 10 μl was delivered with a Hamilton microliter syringe. Injection was done into the middle of the rat thigh muscle (*musculi rectus femoris*), then, muscles were removed at the specified periods and the residual amount of drugs was estimated.

Analytical Methods——Isonicotinamide was determined spectrophotometrically as mentioned previously.[5]

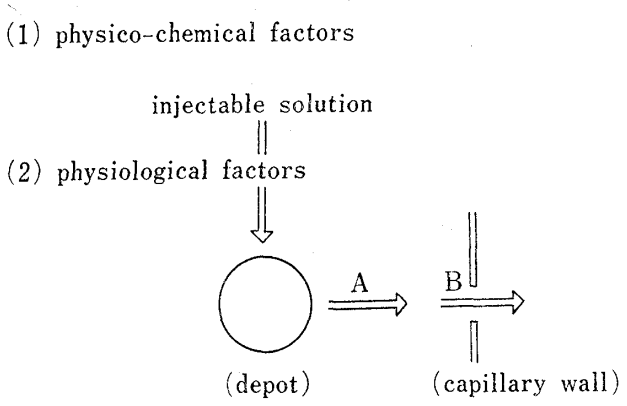
Polysorbate 80: This was estimated by the modified method of Griff.[6] A 10 ml of muscle homogenate was extracted with 15 ml of ethylene dichloride for 3 hours then, 8.0 ml of ethylene dichloride extract was shaken with 5.0 ml of ammonium cobalt thiocyanate, 5.0 ml distilled water and 3.0 g NaCl for 40 min. The extinction of polysorbate 80 in the organic layer was measured at 322 nm.

Histology——Muscles after separation were immediately fixed in 10% neutral buffered formalin solution. Then, they were embedded in liquid paraffin and sections of 4—6 μm thickness were prepared and stained with hematoxylin and eosin-phloxine.

Measurement of Relative Viscosity——Various concentrations of polysorbate 80 in pH 7.0 phosphate buffer solution (ionic strength 0.1) were prepared and their relative viscosities were measured at 25° using Ostwald viscometer (5 ml).

Results and Discussion

Figure 1 is an illustration which shows the route of drug passage from the site of injection until it reaches the blood circulatory system. This can be divided into two stages, stage A demonstrates diffusion of drug through the intercellular spaces of muscle fibers or connective tissues, and stage B represents the diffusion through the pores of the capillary wall. Also it



A: Diffusion through the intercellular spaces of muscle fibers or connective tissues
B: Diffusion through the pores of the capillary wall

Fig. 1. Absorption Model

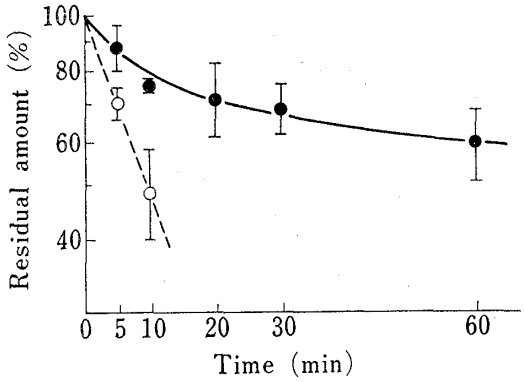


Fig. 2. Semilogarithmic Plots of the Disappearance of Polysorbate 80 and Isonicotinamide from the Rat Thigh Muscle

A 50 μl of 50%polysorbate 80 containing 50 mM isonicotinamide was injected intramuscularly. Vertical bars indicate standard deviation.
key: —●—: polysorbate 80
---○---: isonicotinamide

TABLE I. Effect of Polysorbate 80 on the Relative Viscosity[a]

Concentration of polysorbate 80 (% w/v)	0	0.05	0.1	0.5	1.0	5.0	10.0
Relative viscosity (η/η_0)	1.00	1.00	1.01	1.02	1.04	1.10	1.78

a) A 0.05 M phosphate buffer (pH 7.0, ionic strength 0.1) was used.

5) K. Kakemi, H. Sezaki, K. Okumura, and S. Ashida, *Chem. Pharm. Bull.* (Tokyo), **17**, 1332 (1969).
6) R.A. Greff, E.A. Setzkorn, and W.D. Leslie, *J. Am. Oil Chem. Soc.*, **42**, 180 (1965).

shows that the intramuscular absorption process can be influenced by other factors such as the physico-chemical and physiological factors, which have already been reported in previous studies[5,7,8] and other reviews.[9–11]

As it was previously demonstrated that the viscosity of the solvent could affect the intramuscular absorption process,[8] in the present study we have examined the influence of polysorbate 80 on the relative viscosity and, it was found that, the change in the relative viscosity due to the presence of polysorbate 80 within the range of 0.05—5.0% was very small (Table I), consequently its influence on the intramuscular absorption of isonicotinamide was negligible.

Absorption of Polysorbate 80 and Isonicotinamide from the Rat Thigh Muscle

The disappearance of polysorbate 80 from the site of injection and its influence on the absorption of isonicotinamide were studied. From Fig. 2 it can be seen that the absorption pattern of polysorbate 80 is composed of two phases, an initial phase of rapid absorption followed by a second phase of slow absorption. While, that of isonicotinamide is entirely different as it was proceeded following a first order kinetics which is composed of only one phase.

Effect of Pre-treatment with Polysorbate 80

Pre-treatment was done by the injection of 50 μl of 1.0 or 5.0% polysorbate 80 into the thigh muscle, then the animals were divided into three groups. Isonicotinamide was injected after 6 hours in the first, 24 hours in the second, and 72 hours in the third group. Disappearance of the drug from the site of injection was estimated. Table II demonstrates that after 6 hours the inhibition in the absorption of isonicotinamide was about 17 or 20% and, after 24 hours it was about 30 or 40% in the case of pre-treatment with either 1.0 or 5.0% polysorbate 80 respectively. While, after the elapse of 72 hours, no inhibition in the absorption of isonicotinamide could be demonstrated.

At the same time the residual amount of polysorbate 80 at the injection site was measured, and it is clear in Table III that no polysorbate 80 could be detected after 72 hours where it was

Table II. Effect of Pre-treatment with Polysorbate 80 on the Absorption of Isonicotinamide

Time after pre-treatment (hr)	Absorption,[a] %	
	Control	Pre-treated
	1.0% polysorbate 80 pre-treatment[b]	
6	78.96±2.85 (4)	65.70±6.37[c] (4)
24	80.37±3.39 (4)	55.02±9.09[c] (4)
72	74.66±5.65 (4)	78.57±6.68 (4)
	5.0% polysorbate 80 pre-treatment[b]	
6	81.44±3.46 (9)	60.05±10.63[d] (9)
24	78.78±3.29 (7)	45.96± 5.62[d] (7)
72	72.74±3.90 (11)	76.16± 7.16 (11)

a) A 10 μl of 50 mM isonicotinamide was injected intramuscularly and the amount absorbed was determined after 3 minutes. Values represent the mean±SD. Figures in parentheses are the number of animals.
b) The injection volume of polysorbate 80 was 50 μl.
Significant difference was observed statistically *c*) $p<0.05$, *d*) $p<0.01$ in comparison with the control values.

7) K. Kakemi, H. Sezaki, K. Okumura, C. Takada, and S. Furusawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 2058 (1971).
8) K. Kakemi, H. Sezaki, K. Okumura, H. Kobayashi, and S. Furusawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 443 (1972).
9) J. Schou, *Pharmacol. Rev.*, **13**, 441 (1961).
10) J. Schou, "Handbook of Experimental Pharmacology," 28 (part I), Springer-Verlag, Berlin, 1971, p. 48.
11) B.E. Ballard, *J. Pharm. Sci.*, **57**, 357 (1968).

Table III. Disappearance of Polysorbate 80 from the Rat Thigh Muscle

Time after injection (hr)	Percent remaining
6	10.51±0.79 (3)
24	4.85±0.10 (3)
72	0 (5)

Values represent the mean ± SD. Figures in parentheses are the number of animals. Injection volume of 5.0% polysorbate 80 was 50 μl.

almost completely disappeared. These results (Table II and III) demonstrate that the inhibition in the absorption of isonicotinamide requires the existence of polysorbate 80 at the site of injection and its effect is reversible.

Histological Changes Following Injection of Polysorbate 80

Although it has been reported that injection of surfactants whether subcutaneously[12] or intramuscularly[13] causes a local inflammation at the site of injection, little has been known of its relationship to drug absorption. Consequently, our researches were conducted to examine whether a remarkable histological changes which could influence the intramuscular absorption of drugs was occurred. Therefore, a 10 μl of 1.0% polysorbate 80 was injected into the thigh muscle, after the elapse of the specified time, sections were prepared and examined microscopically.

The following observations could be seen:

A) Control——Figure 3 is a control showing a normal muscle in which the muscle fibers are combined together to form muscle bundles, which are fixed together by connective tissues.

B) Injection of pH 7.0 Phosphate Buffer (10 and 60 min after the Injection)——No detectable histological change could be demonstrated except a slight inflammation of some neutrocyte which might be due to the disruption caused by the needle during the injection (not shown here).

C) Injection of 1.0% Polysorbate 80——1) 10 min after the Injection: Swelling, vacuolation and disruption of muscle cell membrane accompanied by oedema, hyperdemia and slight haemorrhagia were observed (Fig. 4).

2) 60 min after the Injection: No significant difference from the previous one (not shown here).

3) One Day after the Injection: Detectable inflammation of round cells (lymphocytes, neutrocytes and monocytes) was demonstrated. Swelling and degeneration of muscle cells were increased, and partial degeneration in nervous fibers occasionally accompanied by necrosis in muscle fibers was observed (Fig. 5).

4) Two Days after the Injection: Changes which suggest that regeneration process was working could be seen (Fig. 6).

5) Three Days after the Injection: Swelling and degeneration of muscle cells at the periphery subsided and fibroblasts appeared (not shown here).

6) Five Days after the Injection: Cellular inflammation subsided, also immatured muscle fibers and cross striation had been recognized (not shown here).

7) Seven Days after the Injection: Cellular inflammation subsided further and the regenerative muscle fibers became matured. Also a distinctive appearance of cross striation was observed (not shown here).

8) Ten Days after the Injection: Regeneration of muscle fibers was almost completed and cellular inflammation almost disappeared (Fig. 7).

12) H. Mima, T. Yashiki, H. Nakatani, S. Shintani, and T. Usui, *Yakugaku Zasshi*, **82**, 1171 (1962).
13) M. Suzuki, *Hyomen*, **6**, 392 (1968).

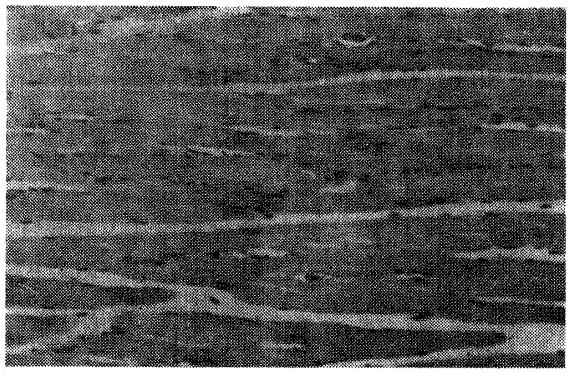


Fig. 3. Control (15×10)

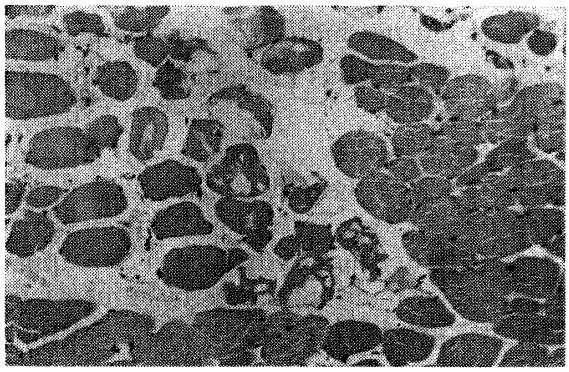


Fig. 4. 1.0% Polysorbate 80 (10 min) (15×10)

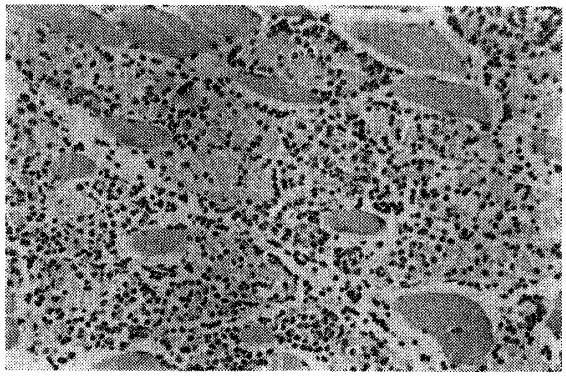


Fig. 5. 1.0% Polysorbate 80 (1 day) (15×10)

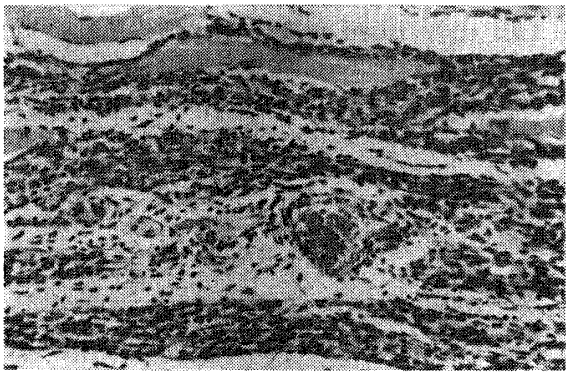


Fig. 6. 1.0% Polysorbate 80 (2 days) (15×10)

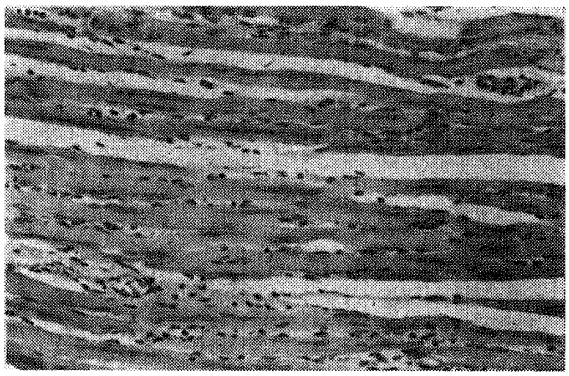


Fig. 7. 1.0% Polysorbate 80 (10 days) (15×10)

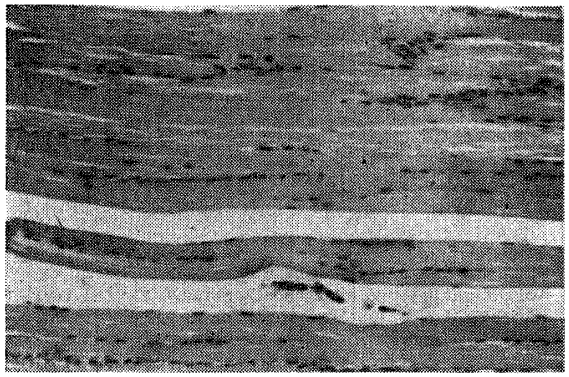


Fig. 8. 1.0% Polysorbate 80 (15 days) (15×10)

9) Fifteen Days after the Injection: Cellular inflammation entirely disappeared and the muscle structure returned back to its normal condition (Fig. 8).

We had tried other concentrations of polysorbate 80 and it was found that the higher the concentration the more acute the inflammation it causes although there was no shift in the time course of the previously indicated local changes.

Since, the appearance of the inhibitory effect caused by polysorbate 80 was rapid[3] and it requires the presence of surfactant at the site of injection (Table II and III), while, the appearance of the local effect of polysorbate 80 was rather late and varied with time, moreover the inflammation was detected even after the disappearance of the surfactant, therefore, the relationship between the occurrence of the inflammation and the influence on the absorption of

isonicotinamide was ruled out. Furthermore it was reported by Ito[14] that inflammation enhances but not inhibits the tissue permeability.

Effect of Intravenous Pre-administration of Polysorbate 80 on the Intramuscular Absorption of Isonicotinamide

Several studies concerned with the influence of surface active agents on the capillary permeability has been done. Suzuki[15] reported that the capillary permeability was increased by intracutaneous injection of surfactants. Most of the studies were concerned with the distribution of drugs or dyes from the capillary system into the muscle or subcutaneous tissues. On the other hand, little has been mentioned of the effect of surfactants on drug permeation from the muscle into the capillary system. Hence, our studies were directed to investigate the influence of polysorbate 80 on the capillary permeability and subsequently on drug absorption. Therefore, different concentrations of polysorbate 80 were injected intravenously, and the absorption percentage of isonicotinamide after its intramuscular injection was determined. Since there is not enough informations concerned with the distribution volume of polysorbate 80 and its rate of metabolism, a higher dose of polysorbate 80 (2.5 and 10 mg/100 g body weight) was injected intravenously. As it is clear in Fig. 9, the influence of pre-administration of polysorbate 80 on isonicotinamide absorption was negligible. Accordingly, the inhibition caused by polysorbate 80 could not be attributed to its direct effect on the capillary walls.

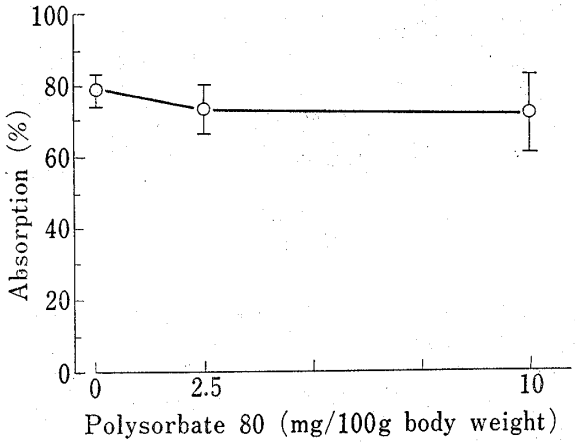


Fig. 9. Effect of Intravenous Pre-administration of Polysorbate 80 on the Absorption of Isonicotinamide

2.5 or 10 mg polysorbate 80 in saline solution/100 g body weight was injected intravenously through the tail vein then, 10 μl of 50 mM isonicotinamide was injected intramuscularly and its absorption after 3 minutes was determined.

Table IV. Effect of Pre-administration of Tolazoline·HCl on the Absorption of Isonicotinamide

Concentration of polysorbate 80 (% w/v)	Absorption (%)[a]	
	Control	Tolazoline·HCl[b]
0.0	74.02± 8.92(4)	73.54± 4.85(6)
0.1	66.21± 6.45(4)	65.56± 7.59(6)
1.0	25.38± 12.87(4)	20.55± 10.67(5)

a) A 10 μl of 50 mM isonicotinamide was injected intramuscularly and the amount absorbed was determined after 3 minutes. Values represent the mean±SD. Figures in parentheses are the number of animals.

b) Tolazoline-HCl (0.05 mg/100 g body weight) was pre-administered from the tail vein 5 minutes before the intramuscular injection of isonicotinamide.

Effect of Tolazoline·HCl Pre-administration on the Intramuscular Absorption of Isonicotinamide

It has been previously mentioned that the intramuscular absorption of drugs from aqueous solutions is greatly affected by blood flow at the injection site. Bederka, *et al.*[16] found that the absorption of benzylpenicillin in the presence of epinephrine was decreased due to the local vasoconstricting effect of the latter and thereby reduction in blood flow. In contrast, the absorption was enhanced by prostaglandin E_2 which was found to increase the muscle blood flow. Evans, *et al.*[17] indicated a significant differences in human muscle blood flow

14) M. Ito, *Nippon Yakurigaku Zasshi*, **66**, 618 (1970).
15) M. Suzuki, *Nippon Yakurigaku Zasshi*, **60**, 62 (1964).
16) J. Bedreke, Jr., A.E. Takemori, and J.W. Miller, *Europ. J. Pharmacol.*, **15**, 132 (1971).
17) E.F. Evans, J.D. Proctor, M. Fraktin, J. Velandia, and A.J. Wasserman, *Clin. Pharmacol. Therap.*, **17**, 44 (1975).

which affect the rate of absorption and peak of serum level. Schou, *et al.*[18] showed that the co-administration of adrenaline or nor-adrenaline inhibits the intramuscular absorption due to their vasoconstricting effect. Accordingly, it was of importance to demonstrate whether polysorbate 80 has a similar inhibitory effect on the intramuscular absorption process. Therefore, tolazoline·HCl, a sympatholytic compound known by its vasodilating effect on the capillaries, was injected intravenously 5 minutes prior to the intramuscular injection of drug in the absence or presence of polysorbate 80. Table IV demonstrates no significant difference of the absorption percentage of isonicotinamide between the pre-treated and control animals in the presence or absence of polysorbate 80. Consequently, the influence of polysorbate 80 on blood flow by inducing a direct vasoconstricting effect or an indirect effect by liberating intragenous amines is not acceptable.

Effect of Intravenous Estrogen Pre-administration on the Intramuscular Absorption of Isonicotinamide

From the previous works, it was clear that the intravenous injection of conjugated estrogens have been widely used clinically to control bleeding. Several theories were tried to interprete their mechanism of action. The histological observations of Schiff, *et al.*[19] showed that the administration of a preparation of water-soluble conjugated estrogenic substances affects the ground substance in and around the capillary walls by increasing the amount of acid mucopolysaccharides and lengthening their polymers which shifts the sol-gel equilibrium toward the gel side and thus increases the solidity of the ground substance. Thus, our attention was directed to investigate the influence of intravenous pre-administration of estrogens on the intramuscular absorption of drugs in the absence and presence of polysorbate 80. Consequently, the conjugated estrogen was injected intravenously 30 minutes prior to the intramuscular injection of ^{14}C-sucrose or ^{14}C-inulin solutions prepared in either free or polysorbate 80 containing medium. Then, their disappearance from the site of injection was determined. The results shown in Table V and VI could not demonstrate any significant effect on the absorption of both drugs in the presence or absence of polysorbate 80 whether the animals were pre-treated or not.

From this study it can be concluded that the inhibitory effect of polysorbate 80 on the intramuscular absorption of drugs can not be attributed to the capillary permeation process nor to the vasoconstrictive or vasoactive effect. There is contradiction between our observa-

Table V. Effect of Pre-administration of Estrogen on the Absorption of ^{14}C-sucrose

Concentration of polysorbate 80 (% w/v)	Time (min)	Absorption (%)[a]	
		Control	Estrogen[b]
0	2	54.45±10.69 (8)	56.82± 9.55 (7)
	5	81.90± 4.80(12)	83.88± 4.18 (6)
0.1	5	39.53± 8.25 (5)	43.17±10.16 (5)
	10	69.26± 5.34 (6)	66.56± 8.58 (6)
1.0	5	24.23± 5.23 (8)	24.52± 6.72(12)
	10	46.70± 3.88 (8)	49.52± 6.50 (7)
	15	58.90± 6.59 (8)	63.29± 8.68(11)

a) Values represent the mean±SD. Figures in parentheses are the number of animals.
b) Estrogen (0.4 mg/100 g body weight) was pre-administered from the tail vein 30 minutes before the intramuscular injection of isonicotinamide.

18) J. Schou, *Acta Pharmacol. Toxicol.*, **26**, 253 (1968).
19) M. Schiff and H.F. Burn, *Arch. Otolaryngol.*, **73**, 63 (1961).

Table VI. Effect of Pre-administration of Estrogen on the Absorption of ^{14}C-inulin

Concentration of polysorbate 80 (% w/v)	Time (min)	Absorption (%)[a]	
		Control	Estrogen[b]
0	5	35.77±4.13 (9)	33.46±5.26 (9)
	10	54.71±4.29 (4)	49.58±5.75 (7)
0.1	5	20.81±3.05 (5)	18.86±6.27 (7)
	10	37.24±3.60 (4)	34.36±6.02 (7)
1.0	5	11.99±7.80 (7)	8.19±5.88 (4)
	10	18.53±7.71 (10)	19.17±4.53 (6)

a) Values represent the mean±SD. Figures in parentheses are the number of animals.
b) Estrogen (0.4 mg/100 g body weight) was pre-administered from the tail vein 30 minutes before the intramuscular injection of isonicotinamide.

tions and those reported by Suzuki[15] in which he stated that, surfactants increase the capillary permeability. Therefore, it was postulated that the capillary permeability is not the rate-limiting one in the case of intramuscular absorption of drugs in the presence of polysorbate 80 but, permeation through intercellular spaces of muscle fibers and connective tissues is the more dominant process. Further studies are proceeding for more clarification.