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Mechanism of the Inhibitory Effect of Polysorbate 80 on Intramuscular Absorption of Drugs. (2)[1]

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The mechanism of absorption inhibitory effect of polysorbate 80 was investigated. The following observations were demonstrated.

1) No significant difference in the uptake of drugs by the muscles either in the presence or absence of polysorbate 80 could be demonstrated.

2) Succinylcholine chloride was used as a muscle relaxant, but no detectable difference in the absorption of isonicotinamide in the presence or absence of polysorbate 80 could be observed. Therefore the effect of polysorbate 80 on the contractility of the muscles was excluded.

3) There was a marked inhibition in the distribution rate of isonicotinamide from blood to muscle, and the extracellular spaces were greatly decreased by pretreatment with polysorbate 80.

4) Permeation of drug through muscle slices and the connective tissue permeability were significantly lowered in the presence of polysorbate 80.

Therefore it can be concluded that the mechanism of the absorption inhibitory effect of polysorbate 80 was mainly due to its influence on the extracellular space and the connective tissue permeability.

Keywords——intramuscular absorption; effect of polysorbate 80; surfactant; rat thigh muscle; uptake of drugs into muscle; extracellular space; connective tissue; permeation of drug through muscle slice

Several works concerned with the intramuscular absorption of drugs have been done. Two main processes for the passage of drug molecules from the injection site to the circulation system have been suggested by Schou.[3] These are the diffusion of drug molecules through the extracellular space of muscle fibers and connective tissues, then the passage of drug molecules through the capillary wall. He states that the first process seems to be the rate limiting one of the intramuscular absorption of drugs from aqueous solutions.

However, less is known about the effect of surfactants on the parenteral absorption of drugs. Consequently, we have investigated the effect of various surfactants on the intramuscular absorption of water soluble drugs[4] and it was found that the capillary permeation process is not the dominant one in the absorption inhibitory effect in the presence of polysorbate 80.[5]

In the present work, researches were proceeded to investigate the effect of polysorbate 80 on the permeation of drug molecules through the extracellular tissue in order to elucidate the mechanism of absorption inhibitory effect caused by the presence of polysorbate 80.

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1) *a*) This paper constitutes the 12th report in a series of "Biopharmaceutical Studies on Parenteral Preparations"; *b*) Preceding paper, Part XI: H. Kobayashi, T. Peng, M. Fujikawa, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **24**, 2383 (1976).

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

3) J. Schou, "Handbook of Experimental Pharmacology," Vol. 28 pt. 1 ed. by B.B. Brodie and J.R. Gillete, Springer Verlag, New York, 1971, pp. 47—63.

4) *a*) H. Kobayashi, T. Nishimura, K. Okumura, S. Muranishi, and H. Sezaki, *J. Pharm. Sci.*, **63**, 580 (1974); *b*) H. Kobayashi, T. Peng, A. Kagayama, K. Okumura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 42 (1975).

5) H. Kobayashi, T. Peng, M. Fujikawa, S. Muranishi, and H. Sezaki *Chem. Pharm. Bull.* (Tokyo),

Experimental

Materials——Isonicotinamide, isonicotinic acid and succinylcholine chloride were of analytical grade and obtained commercially. Polysorbate 80 (Nihon Yushi Co., Japan) and Evans blue (E. Merck A.G., W. Germany) were used without further purification. Inulin-(carboxylic acid-^{14}C) and ^{14}C-sucrose were purchased from Japan Radio Isotope Association (Japan).

Preparation of Injectable Solutions——Solutions of isonicotinamide (50 mM), and succinylcholine chloride (5 mM) for intramuscular injection were prepared in an isotonic pH 7.0 phosphate buffer solution.

Absorption Experiments——Male Wistar albino rats weighing 180—220 g were used. Preparation of animals and injection technique were mentioned previously.[4a)]

Determination of the Uptake of Drugs into a Slice of Muscle——Rats were anesthetized with pentobarbital and the *quadriceps femoris* muscles were separated. Each muscle was cut transversally into two pieces. Tissues were incubated for 10 minutes at 37° in Tyrode solution gassed with 95% oxygen and 5% carbon dioxide, then they were transfered to a Tyrode's solution containing 1 mM drug without or with 1.0% polysorbate 80 and incubated for further 5 or 10 minutes. At the end of the incubation period the tissues were removed, blotted on filter paper, weighed, then homogenized and the amount of drug uptaken was determined.

Measurements of the Distribution of Isonicotinamide, and the Extracellular Space——A 50 μl of 5.0% polysorbate 80 in pH 7.0 phosphate buffer solution was injected into the thigh muscle (one side), then, after 5 minutes a dose of 0.5 ml/100 g body weight of 100 mM isonicotinamide in saline solution was injected from the tail vein. Muscles were separated at a specified period, weighed and the amount of drug distributed into the muscles was determined. Also, at the end of the experiment blood samples were collected from the jugular artery which was cannulated with a polyethylene tube of 0.5 mm i.d. (Dural Plastics and Eng. Pty Ltd.) for estimation of plasma concentration of drug.

In the case of extracellular space measurement, same procedures were followed except that ^{14}C-inulin (5μCi/ml) was used instead of isonicotinamide and the determination was done after 10 minutes of intravenous injection.

Permeation of Drug through Muscle Slice——Permeation studies were done at 37° using the apparatus in Fig. 1 which was essentially the same with the one used in our earlier report.[6)] The *extenser quadriceps femoris* was removed and cut transversely into slices of 0.5 mm thickness. A slice (B) was fixed at the end of the tapered glass tube (A) by an instantaneous adhesive (Alon Alpha, Toa Goseikagaku Co.) and it was tested before carrying the experiment to insure that there is no leakage of solution. The solution containing the drug was introduced into the glass tube (A) under a hydrostatic pressure of 40 mm water, which is considered to be equal to the initial injection pressure in the clinical use.[7)] A polysorbate 80 free solution was introduced in both sides (A, C) with that in compartment A containing 50 mM isonicotinamide. Then, after the lapse of an indicated period solutions in compartment A and C were washed out and replaced with fresh solution containing 1.0% polysorbate 80, and these were changed again at the specified time by polysorbate 80 free solution (50 mM of isonicotinamide was always included in solution of compartment A). A 0.5 ml samples were withdrawn from compartment C at an indicated time to measure the amount of isonicotinamide which diffused through the muscle from compartment A to compartment C.

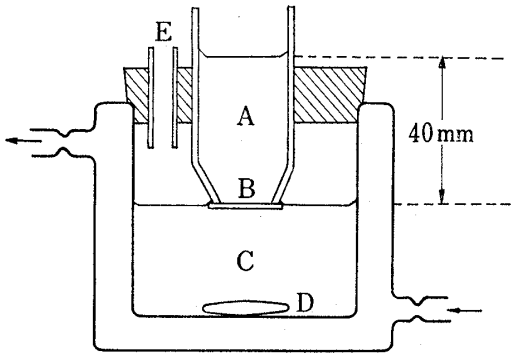

Fig. 1. Schematic Diagram of the Apparatus Used for the Permeation Studies through Muscle Slice

key: A, drug solution (50 mM isonicotinamide); B, muscle slice; C, buffer solution (50 ml); D, magnetic stirrer; E, sampling aperture

Measurement of Connective Tissue Permeability by the Spreading Method Using Evans Blue[8)]——Rats were anesthetized with pentobarbital, fixed on their backs and the ventral regions were shaved with an animal clipper. A depilatory cream was found to be efficient in removing the soft hair without causing any injury or damage to the skin. Solutions of Evans blue in a concentration of 1.6% were prepared in saline, either alone or in the presence of, 0.01% or 1.0% polysorbate 80. Volumes of 50 μl were administered intradermally in the ventral region using a microliter syringe (Terumo MS-N50, Japan). Injections were carried out in symmetrical areas on both sides. The control and test substances were injected alternatively in the right and left side of each animal (3 injections were done in each side), therefore each animal served as

6) K. Kakemi, H. Sezaki, K. Okumura, C. Takada, and S. Furusawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 2058 (1971).
7) J. Schou, *Pharmacol. Rev.*, **13**, 441 (1961).
8) I. Yamagami, Y. Suzuki, M. Ito, and H. Fujimura, *Nippon Yakurigaku Zasshi*, **65**, 61 (1969).

his own control. The areas were measured after 30 minutes of injection. The contour of the blue spots traced on semi-transparent paper, were cut out, weighed and the areas of the spots translated into sq. mm.

Analytical Methods——Isonicotinamide and isonicotinic acid were determined spectrophotometrically, and the total content of ^{14}C-inulin in tissues was estimated by liquid scintillation method as described previously.[4a)]

Results and Discussion

Effect of Polysorbate 80 on Muscle Uptake of Drugs

Interaction of drug molecules with muscle tissue plays a role in the absorption of drugs from the intramuscular injection site.[9)] Doluisio, *et al.*[10)] found a lower rate of intramuscular absorption of dicloxacillin solution compared with that of ampicillin, which referred to a difference in tissue binding of the two antibiotics. Okumura, *et al.*[11)] indicated that the intramuscular absorption rates of cationic drugs were lower than that of neutral or anionic drugs. They ascribed it to a rapid incorporation of the cationic drugs into the muscle tissues than the other drugs due to an increased affinity of the former to the muscle tissues.

Hence, it was of importance to demonstrate whether polysorbate 80 increases the binding of drugs to proteinaceous muscle tissue or to the proteins in interstitial fluids and consequently, decreases the availability of drug molecules for absorption from the injection site. So, the uptake of isonicotinamide and isonicotinic acid into muscle pieces were studied. As shown in Fig. 2, no significant difference of the uptake of both drugs either in the presence or absence of polysorbate 80 could be demonstrated.

Therefore, the inhibitory effect of polysorbate 80 on the intramuscular absorption of drugs could not be attributed to an increase in tissue binding and consequently a decrease of availability of drug molecules for absorption from the injection site.

Effect of Succinylcholine Chloride

Since, it has been reported that the absorption rate of the intramuscularly injected drugs is greatly affected by muscle contraction or muscle movement.[6)] Therefore, we have studied

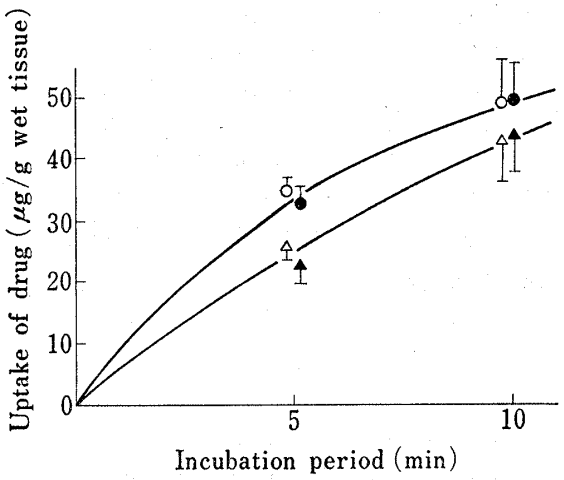

Fig. 2. Effect of Polysorbate 80 on Drug Uptake by Muscles

Each point represents the mean value of 4 or more experiments. Vertical bars indicate standard deviation.
key: ○ ●, isonicotinamide; △ ▲, isonicotinic acid; open symbols, in the absence of polysorbate 80; solid symbols, in the presence of 0.5% polysorbate 80

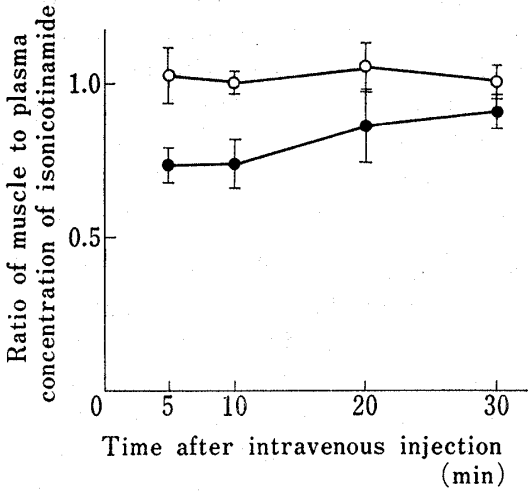

Fig. 3. Effect of Polysorbate 80 on the Distribution of Isonicotinamide into Muscles

Each point represents the mean value of 6 or more animals. Vertical bars indicate standard deviation.
key: ○, control; ●, 5.0% polysorbate 80 pretreated

9) S. Feldman, *Bull. Parenteral Drug Ass.*, **28**, 53 (1974).
10) J.T. Doluisio, J.C. LaPiana, and L.W. Dittert, *J. Pharm. Sci.*, **60**, 715 (1971).
11) K. Okumura, H. Sezaki, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **20**, 1607 (1972).

Table I. Effect of Succinylcholine Chloride on the Absorption of Isonicotinamide

Concentration of polysorbate 80 (% w/v)	Absorption (%)		Statistical difference
	Control	Succinylcholine chloride	
0.0	70.35± 8.90(6)	66.01±7.74(6)	$P>0.2$
0.1	49.49± 7.24(6)	49.76±5.85(6)	$P>0.5$
1.0	25.79±10.67(6)	22.18±8.90(6)	$P>0.2$

A 50 μl of 5 mM succinylcholine chloride was injected into the thigh muscle (one side), then, after 5 minutes a 10 μl of 50 mM isonicotinamide was injected intramuscularly and the amount absorbed after 3 minutes was determined. Values represent the mean±SD. Figures in parentheses are the number of animals.

whether polysorbate 80 has an influence on muscle contraction and thereby affects the absorption process. A 50 μl of 5 mM succinylcholine chloride, as a depolarizing muscle relaxant, was injected into the thigh muscle (one side) 5 minutes prior to the intramuscular injection of 10 μl of isonicotinamide in the absence or presence of either 0.1% or 1.0% polysorbate 80. Table I demonstrates no significant difference of the absorption percentage of isonicotinamide between the control and pretreated animals in the absence or presence and irrespective of the concentration of polysorbate 80. Consequently, it was clarified that the inhibitory effect of polysorbate 80 was not mainly due to the influence of polysorbate 80 on muscle contraction.

Effect of Polysorbate 80 on the Distribution of Isonicotinamide into Muscle

In this investigation preliminary experiments showed that intramuscular injection of the buffer system has no effect on the distribution of drugs from the blood into the muscle, therefore in the case of control animals no intramuscular injection was done.

As it is shown in Fig. 3 the distribution of isonicotinamide from blood into muscle reached equilibrium within the first 5 minutes, where the ratio of muscle to plasma concentration of isonicotinamide approached unity. In contrast, on intramuscular pretreatment with polysorbate 80, the ratio was smaller than that of the control and it approached unity after a comparatively longer period, which demonstrates a significant reduction in the distribution rate of isonicotinamide by pretreatment with 5.0% polysorbate 80. But, although the intramuscular absorption of drugs was inhibited in the presence of polysorbate 80 and also their distribution from the blood to the muscle was reduced, it remained uncertain whether the absorption and distribution processes proceed through the same pathway. This needs further investigations.

Effect of Polysorbate 80 on the Extracellular Space of Muscles

Various studies have been made to determine the extracellular space of muscles,[12] and it was shown that inulin is a proper marker for measuring it.[13] Hence, in this study, ^{14}C-inulin was used to determine the extracellular space.

As is evident in Table II, the ratio of muscle to plasma concentration of ^{14}C-inulin in the polysorbate 80 pretreated animals is lower by approximately 20% than that of the untreated animals, which illustrates a decrease in the distribution of inulin into the extracellular space due to the influence of polysorbate 80.

Law, *et al.*[14] reported that the vascular space in rat *gastrocnemius* is approximately 2.74% of total muscle volume. Accordingly, the decrease of inulin space due to polysorbate 80 pre-

12) G. Esposito, A. Faelli, and V. Capraro, *Pflügers Arch.*, **331**, 70 (1972); F.H. Osman, J.L. Munson, and D.M. Paton, *Comp. Biochem. Physiol.*, **45A**, 1047 (1973); D.J.L. McIver and A.D.C. Macknight, *J. Physiol.*, **239**, 31 (1974).
13) F.H. Osman, J.L. Munson, and D.M. Paton, *Comp. Biochem. Physiol.*, **40A**, 45 (1971).
14) R.O. Law and C.F. Phelps, *J. Physiol.*, **186**, 535 (1966).

Table II. Effect of Polysorbate 80 on the Extracellular Space of Muscles

Ratio of muscle to plasma concentration of ^{14}C-inulin		Polysorbate 80/Control
Control	5.0% polysorbate 80	
0.245	0.205	0.836
0.297	0.240	0.808
0.312	0.260	0.833
0.334	0.276	0.826
0.256	0.175	0.823
		mean±SD.=0.797±0.065

Extracellular space was measured using ^{14}C-inulin (5 μCi/ml) as a marker and its distribution was determined after 10 minutes of intravenous injection.

Table III. Effect of Polysorbate 80 on Dermal Tissue Permeability

Concentration of polysorbate 80 (% w/v)	Area of dye spot (mm^2)
0	95.07±6.07(11)
0.01	73.39±9.45 (6)
1.0	25.00±1.87 (5)

Values represent the mean±SD. Figures in parentheses are the number of experiments.

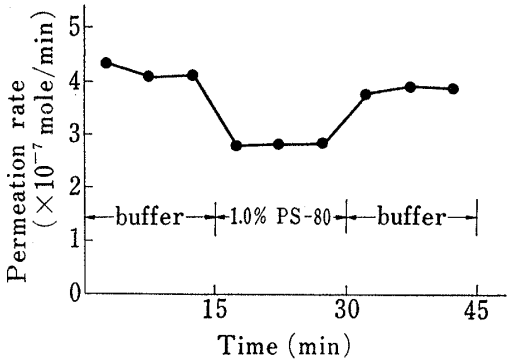

Fig. 4. Effect of Polysorbate 80 on the Permeation of Isonicotinamide through Muscle Slice

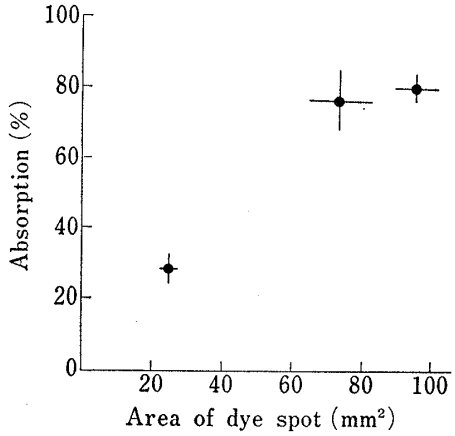

Fig. 5. Correlation between the Absorption of Isonicotinamide[4a] and the Area of Dye Spot

Bars indicate standard deviation.

treatment can be attributed not to a decrease of blood volume in muscle, but to a decrease of inulin space, which means decrease of the distribution volume of inulin into extracellular space or connective tissues.

Effect of Polysorbate 80 on the Permeation of Drug through Muscle Slice

This was examined *in vitro* and the results are shown in Fig. 4. As it is clear in the Figure the permeation of drug is decreased by the presence of polysorbate 80 and its effect was reversible where the permeation through the muscle returned back to its normal state when polysorbate 80 solution was replaced by buffer system.

Effect of Polysorbate 80 on Connective Tissue Permeability

As is shown in Table III, the areas of dye spots measured at 30 minutes after intradermal injection were diminished in the presence of polysorbate 80, which demonstrates that polysorbate 80 significantly decreased the connective tissue permeability.

Furthermore, there is a good correlation between the effect of polysorbate 80 on the intramuscular absorption of isonicotinamide and dermal connective tissue permeability (Fig. 5). From these results and our previous observations,[4,5] it can be concluded that the mechanism of absorption inhibitory effect of surfactants are not due to the inhibition of passage of drug molecules through the capillary wall, but mainly to a decrease of permeability in the connective tissues and extracellular space.