

Effect of Ethanol on the Intramuscular Absorption of Water-soluble Drugs in the Rat^{1,2)}

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The effect of ethanol on the intramuscular absorption of water-soluble drugs from the rat thigh muscle was investigated. The presence of ethanol caused a pronounced decrease in the absorption rate of drugs, and the reduction was reflected by the decrease in plasma concentrations. It was also found that the absorption rate of drugs from the site of injection depends mainly on the concentration rather than the absolute amount of ethanol.

A direct relationship between the relative viscosity of the injectable solution and the inhibitory absorption was demonstrated at lower concentrations of ethanol, but not at higher concentration. This shows that the inhibitory absorption effect of ethanol cannot be adequately explained by simple physicochemical factors, such as viscosity, but the existence of other factors must be acting.

A constant intravenous infusion of ethanol showed no effect on the intramuscular absorption of drug. Furthermore, ethanol did not significantly change the permeability of the isolated mesentery to drug molecules. On the other hand, the connective tissue permeability was significantly decreased by the presence of ethanol and had a good correlation to the inhibitory effect of ethanol on the intramuscular absorption of drugs.

From these observations, it can be concluded that the mechanism of the inhibitory effect of ethanol is mainly due to its influence on the extracellular spaces and the connective tissues permeability.

Keywords—intramuscular absorption; effect of ethanol; rat thigh muscle; injection volume; relative viscosity; permeation through rat mesentery; connective tissue permeability

In the field of parenteral preparations, to prepare a safe, stable and efficient injection, several adjuvants are known to be used either as stabilizing agents or to enhance the solubility of certain insoluble drugs. Furthermore, cosolvents are normally used to increase the solubility of drugs specially the non-polar drugs in an aqueous medium. Alcohols are probably the most widely used cosolvents in parenteral preparations.

Physical properties and toxicities of nonaqueous solvents which are used in parenteral preparations had been reviewed by Spiegel and Noseworthy.⁴⁾

J.P. IX states that a digoxin preparation containing 5 to 50% (v/v) alcohol can be used for intramuscular or intravenous administration. Also, USP XIX permits the use of 5 to 50% (v/v) of alcohol in digitoxin injection.

- 1) a) This paper constitutes the 14th report in a series of "Biopharmaceutical Studies on Parenteral Preparations"; b) Preceding paper, Part XIII: H. Kobayashi, T. Peng, R. Kawamura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **25**, 1547 (1977).
- 2) Part of this work was presented at the 96th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1976.
- 3) Location: *Yoshidashimoadachi-cho, Sakyo-ku, Kyoto, Japan.*
- 4) A.J. Spiegel and M.M. Noseworthy, *J. Pharm. Sci.*, **52**, 917 (1963).

Most of the previous work has concentrated on the study of effects of ethanol on the absorption of drugs through the intestine⁵⁾ and gastric mucosa,⁶⁾ drug distribution,⁷⁾ hemolysis,⁸⁾ and drug toxicity.⁹⁾

On the other hand, little is known about effect of ethanol on the intramuscular absorption of drugs. The present investigation was undertaken to see the effects of ethanol on the absorption of drugs after intramuscular injection.

Experimental

Materials—Isonicotinamide, methyl isonicotinate, isonicotinic acid and procainamide hydrochloride were of analytical grade and obtained commercially. All other compounds were of reagent grade or of the highest purity and obtained commercially.

Preparation of Injectable Solutions—Solutions of isonicotinamide (50 and 200 mM), methyl isonicotinate (50 mM), isonicotinic acid (50 mM) and procainamide hydrochloride (50 mM) for intramuscular injection were prepared in saline solution in the presence or absence of ethanol and adjusted by 1 N HCl or 1 N NaOH to pH 7.0. Ethanol (20 and 50% (v/v)) for intravenous administration was prepared in an injectable saline solution.

Absorption Experiments—Male Wistar albino rats weighing 180–250 g were used. Preparation of animals and injection technique have been discussed previously.¹⁰⁾

Measurement of Relative Viscosity—Various concentrations of ethanol in saline solutions were prepared and their relative viscosities were measured at 36° using a B-type Viscometer (Tokyo-keiki Seizojo, Japan).

Isolated Mesentery Permeation Study—Permeation studies were done at 37° using the apparatus shown in Fig. 1 which is a modification of a previously reported one.¹¹⁾ Male albino rats weighing 180 g were anesthetized by ethyl ether and the blood was shed out through an incision in the neck. A portion of intact mesentery, free of visible fat and blood vessels was excised, rinsed with physiological saline solution and fixed at the end of the tapered glass tube (A) by an instantaneous adhesive (Alon Alpha, Toa Goseikagaku Co., Japan) and it was tested before carrying the experiment to insure that there is no leakage of the solution. A 0.5 ml portion of a saline solution containing isonicotinamide (20 mM) or isonicotinamide (20 mM) and ethanol (20% (v/v)) was introduced into the glass tube (A). A 100 ml of saline or 20% (v/v) ethanol saline solution free from drug were introduced into the beaker (C). Solutions in both compartments (A and C) were kept at the same level. A 1.0 ml samples were withdrawn from compartment C at the indicated time and immediately replaced with an equal volume of either saline or 20% (v/v) ethanol saline solution to maintain the volume of the solution in compartment C constant, then the amount of isonicotinamide permeated through the mesentery from compartment A to C was determined.

Measurement of Connective Tissue Permeability by the Spreading Method Using Evans Blue—Experimental procedures were the same as mentioned previously.¹¹⁾ A solution of Evans Blue at a concentration of 1.6% (w/v) was prepared in either saline alone, or in the presence of 20 or 50% (v/v) ethanol. Volumes of 50 μ l were administered intradermally in the ventral region using a microliter syringe (Terumo MS-N50, Japan). The area of the dye spots was measured 30 minutes after injection. The contour of the blue spots was traced on semi-transparent paper, cut out, weighed and the areas of the spots translated into sq. mm.

Analytical Methods—Isonicotinic Acid Derivatives: These were determined by the method described previously.¹⁰⁾ In the case of blood samples, 2.0 ml of plasma were deproteinized by the addition of 1.5 ml of 30% (w/v) trichloroacetic acid and centrifuged. The supernatant was neutralized with 1 N NaOH, and 3.0 ml of this solution were used for the estimation.

Procainamide Hydrochloride: This compound was determined spectrophotometrically by a previously reported method.¹⁰⁾

Ethanol: Muscles were separated and homogenized as described previously.¹⁰⁾ The ethanol was determined by gas chromatography. A gas chromatograph (Shimadzu GC-5A) with a flame-ionization

- 5) M.P. Magnussen, *Acta Pharmacol. Toxicol.*, **26**, 130 (1968); R. Koysooko and G. Levy, *J. Pharm. Sci.*, **63**, 829 (1974); R. Koysooko, E.F. Ellis, and G. Levy, *ibid.*, **64**, 299 (1975).
- 6) V.P. Dinoso, S. Ming, and J. McNiff, *Am. J. Dig. Dis.*, **21**, 626 (1976); E. Baraona, R.C. Pirola, and S. Lieber, *Gastroenterology*, **66**, 226 (1974); E. Rubin, B.J. Rybak, J. Lindenbaum, C.D. Gerson, G. Walker, and C.S. Lieber, *ibid.*, **63**, 801 (1972); W.Y. Chey, *Digestion*, **7**, 239 (1972).
- 7) L.W. Whitehouse, C.J. Paul, B.B. Coldwell, and B.H. Thomas, *Res. Commun. Chem. Pathol. Pharmacol.*, **12**, 221 (1975).
- 8) S. Ku and D.E. Cadwallander, *J. Pharm. Sci.*, **63**, 60 (1974).
- 9) I. Vuorinen, J. Heinonen, and P. Rosenberg, *Acta Pharmacol. Toxicol.*, **38**, 31 (1976).
- 10) H. Kobayashi, T. Nishimura, K. Okumura, S. Muranishi, and H. Sezaki, *J. Pharm. Sci.*, **63**, 580 (1974).
- 11) H. Kobayashi, T. Peng, R. Kawamura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **25**, 569 (1977).

detector and a column 1.0 m long and 3.0 mm i.d., packed with Chromosorb W, 60–80 mesh, coated with 20% PEG 600 was employed. Temperature of the column was 90°, and the injection port and detector were at 120°. Ethyl acetate was added to the samples as an internal standard. Nitrogen gas was used as a carrier at a rate of 60 ml/min; the flow rate of hydrogen and air were 50 and 900 ml/min respectively. A 0.1 ml portion of a 0.1% (v/v) ethyl acetate solution was added to 1.0 ml of the supernatant of the muscle tissue homogenate, and 7 μ l aliquot was injected onto the column. A plot of the area of the ethanol peak divided by the area of the internal standard peak as a function of ethanol concentration was linear and passed through the origin.

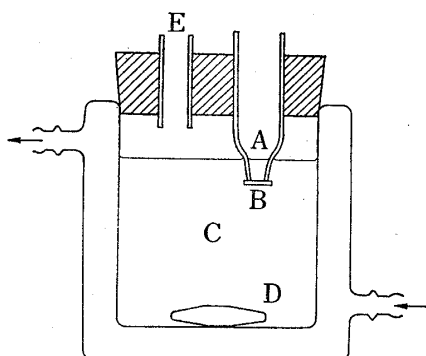


Fig. 1. Schematic Diagram of the Apparatus Used for the Permeation Studies through Isolated Mesentery

Key: A, drug solution (0.5 ml of 20 mM isonicotinamide); B, isolated mesentery; C, buffer solution (100 ml); D, magnetic stirrer; and E, sampling hole.

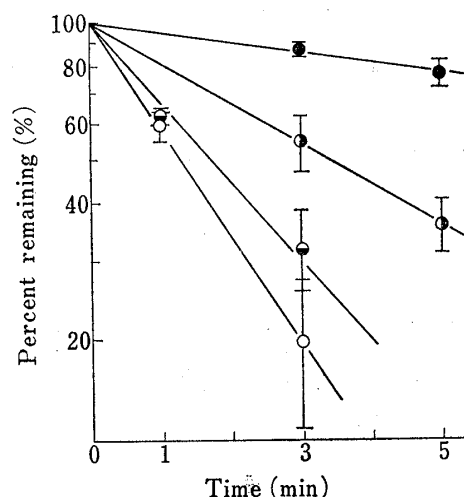


Fig. 2. Effect of Ethanol on the Disappearance of Isonicotinamide from the Rat Thigh Muscle

Each point represents the mean value of at least five animals. Vertical bars indicate standard deviation, and straight lines are the result of least-square regression analysis. Isonicotinamide concentration was 50 mM.

Key (ethanol concentration, v/v): ○, 0.0%; ◐, 10.0%; ◑, 20.0%; and ●, 50.0%.

Results and Discussion

Effect of Ethanol on the Absorption of Isonicotinamide

In order to determine the possible effect of ethanol upon the kinetics of drug absorption after intramuscular injection, the rate of disappearance of isonicotinamide from the site of injection was measured as a function of time. Figure 2 shows the percentages of the residual amount of isonicotinamide at the site of injection in the absence, or presence of different concentrations of ethanol. As is evident from the Figure, the linearity of the disappearance curves is maintained in both cases. However, the presence of ethanol causes a pronounced decrease in the absorption rate of isonicotinamide.

Since the disappearance of drug molecules from the injection site is always accompanied by an increase of the drug concentration in the blood, the concentrations of isonicotinamide in plasma after the intramuscular injection with or without ethanol were measured. As can be seen from Fig. 3, the plasma concentration of isonicotinamide injected with ethanol appeared lower than in the case of injection without ethanol. These results are in a good agreement with the observations shown in Fig. 2. Based on the above fact, it is clear that ethanol inhibits drug absorption from the site of injection.

As a comparison, the disappearance of ethanol from the site of injection was studied. From this study, it was found that the rate of ethanol disappearance is different from that of isonicotinamide as shown in Fig. 4. Since ethanol is a small molecule like water, it was

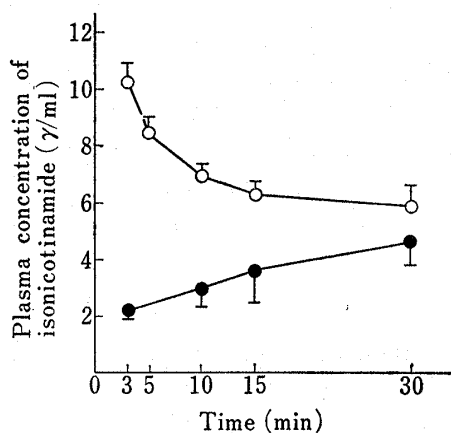


Fig. 3. Plasma Concentration of Isonicotinamide after Its Intramuscular Administration

Each point represents the mean value of at least four animals. Vertical bars indicate standard deviation. A 25 μ l of 200 mM isonicotinamide in saline or 50% (v/v) ethanol in saline solution was injected into both thigh muscles.

Key: \circ , saline solution; and \bullet , 50% (v/v) ethanol in saline solution.

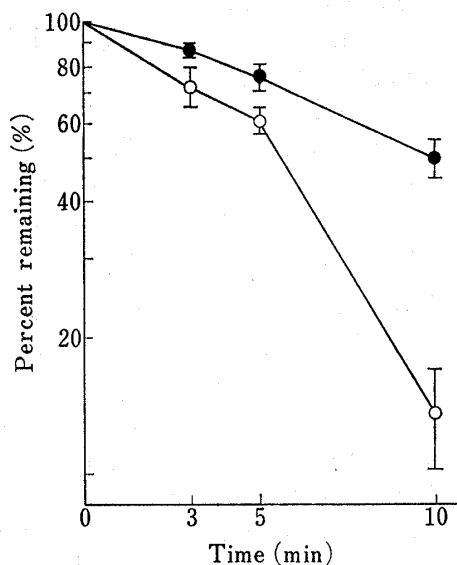


Fig. 4. Semilogarithmic Plots of the Disappearance of Ethanol and Isonicotinamide from the Rat Thigh Muscle

A 50 μ l of 50% (v/v) ethanol containing 50 mM isonicotinamide was injected intramuscularly. Vertical bars indicate standard deviation.

Key: \circ , ethanol; and \bullet , isonicotinamide.

expected based on the results obtained by Sund and Schou¹²⁾ that the rate of absorption is much fast. But the experimental results show a little delayed absorption rate of ethanol. This indicate that there must be self inhibitory effect on its own absorption.

Also, Fig. 4 shows that isonicotinamide disappeared in a linear fashion in the earlier phase after injection. In the later, however, the rate of disappearance increased slightly. This increased rate of disappearance arises from the less inhibitory effect of ethanol due to the rapid ethanol disappearance during the same time interval.

Effect of Injection Volume

The effect of injection volume on the intramuscular absorption of drugs was examined in the presence of 0, 10, 20 and 40% (v/v) ethanol. The absorption experiments were carried

TABLE I. Effect of Injection Volume on the Absorption of Isonicotinamide in the Presence of Ethanol^{a)}

Concentration of ethanol (%(v/v))	% absorption ^{b)}		
	5 μ l	10 μ l	20 μ l
0	74.0 \pm 1.4 (5)	78.0 \pm 4.1 (5)	80.2 \pm 6.4 (5)
10	70.2 \pm 3.6 (6)	68.3 \pm 6.3 (14)	66.3 \pm 5.9 (5)
20	47.7 \pm 4.2 (5)	45.8 \pm 7.6 (8)	43.9 \pm 8.6 (5)
40	16.2 \pm 4.2 (6)	17.2 \pm 3.1 (4)	6.4 \pm 2.3 (5)

a) Values represent the mean \pm S.D. Figures in parentheses are the number of animals. Concentration of isonicotinamide was 50 mM.

b) Percent absorbed was determined after 3 minutes.

out for 3 minutes and the injection volume was varied from 5 to 20 μl . Table I shows that the injection volume of solutions containing the same concentration of ethanol has no significant effect on the absorption of isonicotinamide, except in the case of 40% (v/v) ethanol level where 20 μl was injected. However, it is interesting to see that, although the absolute amount of ethanol in 5 μl of 40% (v/v) is equal to a 10 μl portion of 20% (v/v) and a 20 μl portion of 10% (v/v), the absorption percentage of isonicotinamide decreases with an increase in the concentration of ethanol. This result shows that the absorption rate of isonicotinamide is mainly affected by the concentration rather than the absolute amount of ethanol.

Effect of Ethanol on the Absorption of Drugs

The effect of ethanol on the intramuscular absorption of some drugs possessing different physicochemical characteristics was investigated. Isonicotinic acid and procainamide hydrochloride were chosen as charge-bearing compounds at the pH of the absorption experiments. Methyl isonicotinate and isonicotinamide are nonionic compounds with a high and low lipophilicity respectively. Figure 5 shows the absorption percentage of isonicotinic acid derivatives within 3 minutes and of procainamide hydrochloride within 5 minutes. As is evident from the Figure, in spite of the difference in their ionogenic nature, lipophilicity or pharmacological class, the inhibitory effect of ethanol on the intramuscular absorption of drugs is not selective.

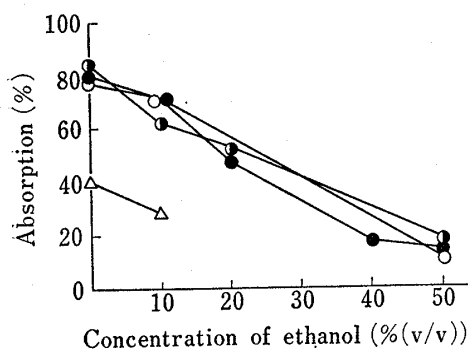


Fig. 5. Effect of Ethanol on the Absorption of Drugs

Each point represents the mean value of at least five animals. Percent absorbed was determined after 3 minutes, except for procainamide hydrochloride which was for 5 minutes.

Key: ●, isonicotinamide; ●, methylisonicotinate; ○, isonicotinic acid; and △, procainamide hydrochloride.

TABLE II. Relative Viscosity of Ethanol Saline Solution

Concentration of ethanol (%(v/v))	Relative viscosity (η/η_0) ^{a)}
1	1.00
10.0	1.213
20.0	1.503
30.0	1.675
40.0	1.761
50.0	1.836

a) Relative viscosity was measured at 36° using B-type Viscometer.

Relative-Viscosity of Ethanol Saline Injectable Solution and Its Relationship to the Absorption Inhibitory Effect

As is clear from Fig. 2, the absorption of drug molecules from the site of injection occurs by a passive diffusion process. Hence the disappearance rate from the injection site can be described by Fick's law:¹³⁾

$$dN/dt = -\bar{D}A(dc/dx)$$

where, dN/dt is the disappearance rate, and t is the time. In a well-stirred system, the rate is proportional to the mean diffusion coefficient of the drug in the membrane, D , the area, A , of the absorbing membrane exposed to the solution and the concentration gradient, dc/dx , of the drug across the membrane. Furthermore, from the Stokes's Law D is inversely proportional to the viscosity. In this study the relative viscosities of the injectable solutions,

13) B.E. Ballard, *J. Pharm. Sci.*, **57**, 357 (1968).

containing various concentrations of ethanol, were measured and the relationship to the reciprocal of the relative viscosity was examined. It was found that the relative viscosities of injectable solutions were increased by increasing the concentration of ethanol (Table II).

Moreover, Fig. 6 shows that at lower concentration of ethanol there was a direct relationship between the relative viscosity and the absorption inhibitory effect, and it shows that the viscosity of the injectable solution contributed much to the absorption inhibitory effect. On the other hand, at higher concentrations of ethanol the inhibition in the absorption of isonicotinamide becomes more pronounced. This can not be explained on the basis of viscosity alone, but the involvement of other factors must be considered.

In the case of intramuscular absorption of water-soluble drugs, it has been reported¹⁴⁾ that the main process for the passage of drug molecules from the injection site to the circulatory system is the diffusion through the intercellular spaces of muscle fibers or connective tissues and the capillary walls. To clarify the inhibitory mechanism of ethanol on the intramuscular absorption, the influence of ethanol on the above-mentioned passage route of absorption of the drug molecules was investigated.

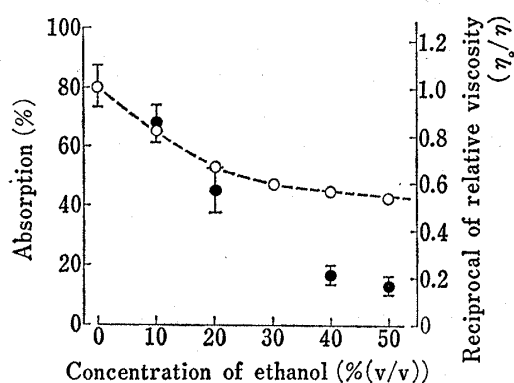


Fig. 6. Relationship between the Absorption of Isonicotinamide and Reciprocal of the Relative Viscosity of Injectable Solutions

Key: ●, absorption percent; and ---○---, reciprocal of relative viscosity.
Percent absorbed was determined after 3 minutes.
Vertical bars indicate standard deviation.

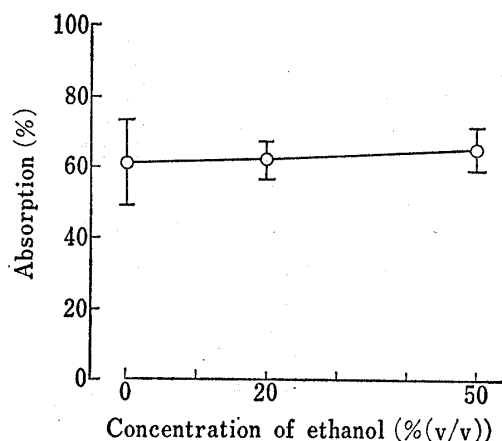


Fig. 7. Effect of Intravenous Constant Infusion of Ethanol on the Absorption of Isonicotinamide

20 or 50% (v/v) ethanol in saline solution was injected intravenously through the tail vein at a rate of 0.05 ml/min, then, 10 μ l of 50 mM isonicotinamide was injected intramuscularly and its absorption after 3 minutes was determined. Vertical bars indicate standard deviation.

Effect of the Intravenous Infusion of Ethanol on the Intramuscular Absorption of Isonicotinamide

Several studies concerned with the influence of ethanol on the capillary permeability and blood circulations had been done. Suzuki, *et al.*¹⁵⁾ reported that the capillary permeability was increased by intracutaneous injection of alcohols. Also it has been known that ethanol alters the vascular physiology by increasing blood flow¹⁶⁾ and inducing contraction of vascular smooth muscles.¹⁷⁾

The effect of ethanol on intramuscular absorption was investigated by a constant intravenous infusion of saline solution or 20 and 50% (v/v) ethanol in saline solution at a rate of 0.05 ml/min. As is evident from Fig. 7, irrespective of the presence of ethanol the absorption

- 14) J. Schou, "Handbook of Experimental Pharmacology," Vol. 28, ed. by B.B. Brodie and J.R. Gillete, Springer-Verlag, Berlin, Heidelberg, New York, 1971, pp. 47-66.
15) M. Suzuki and H. Arai, *Nippon Yakurigaku Zasshi*, **62**, 241 (1966).
16) G. Blanquat, Y. Lamboeuf, and R. Derache, *Eur. J. Pharmacol.*, **34**, 219 (1975).
17) H.F. Dalske, *Arch. Int. Pharmacodyn.*, **218**, 54 (1975).

of isonicotinamide was not affected. This result illustrates that the inhibitory absorption effect of ethanol is attributed neither to a direct effect on the capillary wall nor to a systemic mechanism.

Effect of Ethanol on the Permeability of the Mesentery

The peritoneal mesothelium has been reported to be a suitable model for studying the permeation through the vascular endothelium, and the mesentery has been used to predict the capillary behavior.¹⁸⁾

TABLE III. Effect of Ethanol on the Permeation through Isolated Mesentery

Time (min)	Percent remained Experimental No.					
	1	2	3	4	5	5
	Control					
15	88.7	86.7	91.5	91.7	91.2	89.3
30	78.1	76.4	83.6	83.7	82.5	79.1
45	67.4	66.4	76.1	75.9	74.3	68.7
60	57.3	58.0	68.8	67.9	65.9	58.0
<i>a</i>	-0.00958	-0.00898	-0.00633	-0.00662	-0.00720	-0.00958
<i>r</i>	0.9985	0.9998	0.9997	0.9988	0.9991	0.9971
	$\bar{a} = -0.00807 \pm 0.00153$					
	20% (v/v) Ethanol					
15	84.4	91.0	89.1	86.0	86.7	87.9
30	71.0	81.1	79.0	72.4	73.5	76.1
45	59.4	71.7	69.6	63.0	62.2	65.9
60	49.3	64.1	61.1	55.1	52.5	56.7
<i>a</i>	-0.01194	-0.00784	-0.00839	-0.00983	-0.01114	-0.00972
<i>r</i>	0.9999	0.9998	0.9998	0.9981	0.9999	0.9999
	$\bar{a} = -0.00981 \pm 0.00156 (0.10 > p > 0.05)$					

r = correlation coefficient, *a* = slope.

Table III shows the effect of ethanol on the permeability of the mesentery to isonicotinamide. As is clear from the Table III, the permeation of the mesentery to isonicotinamide, although slightly increased, was not significantly affected by the presence of ethanol.

From these studies it can be concluded that the inhibitory effect of ethanol on the intramuscular absorption of drugs can not be attributed to an effect on the capillary permeation process nor the vasoconstrictive or vasoactive effects.

TABLE IV. Effect of Ethanol on Dermal Tissue Permeability and Relationship to Intramuscular Absorption

Concentration of ethanol (% (v/v))	Area of dye spot ^{a)} in dermal tissue (mm ²)	Intramuscular absorption ^{b)} of Isonicotinamide (%)
0	92.71 ± 7.13 (7)	79.81 ± 3.79 (8)
10	83.00 ± 7.66 (6)	68.30 ± 6.34 (14)
50	23.83 ± 5.34 (6)	13.72 ± 2.96 (6)

a) 0.05 ml of 1.6% Evans Blue saline solution was injected intradermally in the ventral region and the areas of the spots were measured after 30 minutes of injection.

b) Percent absorbed was determined after 3 minutes.

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

18) E.A. Rasio, *Am. J. Physiol.*, 226, 962 (1974).

Effect of Ethanol on Connective Tissue Permeability

As is shown in Table IV, the area of dye spots measured 30 minutes after the intradermal injection was diminished when the 10% (v/v) ethanol solution was included in the injection medium, and was remarkably reduced when the concentration of ethanol was increased to 50% (v/v). This demonstrates that ethanol significantly decreased the connective tissue permeability.

Furthermore, there is a good correlation between the effect of ethanol on the intramuscular absorption of isonicotinamide and dermal connective tissue permeability as it is obvious from Table IV and Fig. 8. These results suggest that the mechanism of the inhibitory effect of ethanol on absorption is not due to the inhibition of the passage of drug molecules through the capillary wall, but mainly to a decrease in permeability of the connective tissues and extracellular spaces.

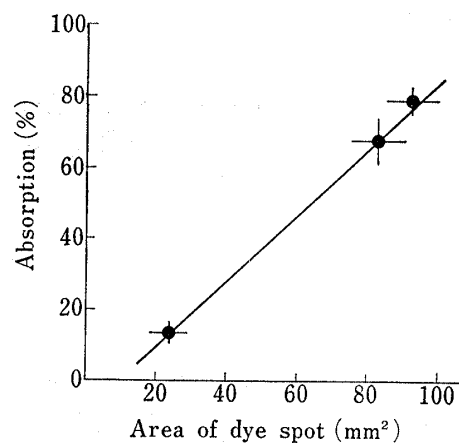


Fig. 8. Relationship between the Absorption of Isonicotinamide and the Area of Dye Spots

Bars indicate standard deviation. Straight line is the result of least-square regression analysis ($y=0.947x-9.011$, $r=0.9995$).