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Absorption of Drugs from the Skeletal Muscle of the Rats. (3).[1] Effect of Water-soluble Adjuvants and Vehicles on the Intramuscular Absorption[2]

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The effect of various kinds of adjuvants or vehicles was studied with a view to examine the mechanism of intramuscular drug absorption. From the relationship between *in vivo* absorption studies and various *in vitro* diffusion experiments, it was clarified which step was the rate-limiting one in the intramuscular absorption.

1) The absorption mechanism of a drug with water-soluble adjuvants did not differ from that in aqueous solution without any adjuvant.

2) There was a good correlationship between the parenteral absorption rate and the reciprocal of viscosity of an injectable solution, provided that the molecular weight of an adjuvant was comparatively small such as propylene glycol, glycerin, and PEG 400. Effect of these solvents on the drug absorption was general in nature and not specific to drugs. In the case of an adjuvant having higher molecular weight such as PEG 4000 dextran, and methylcellurose, rate of drug absorption was greater than that expected from the viscosity, suggesting that macromolecules could hardly diffuse through the pores of the capillary wall.

3) From *in vitro* diffusion study using Visking membrane, glass filter, and slice of muscle, it was concluded that the contribution of the diffusion process through the pores of capillary wall was dominant compared with the one through the muscle fiber space.

In the field of aqueous injectable solution, various kinds of adjuvants or vehicles are widely used. Reviews[4,5] are reported on their toxicity or chemical and physical properties, and on their effect on the hemolysis of erythrocytes,[6] but few studies have been performed of their effect on the absorption mechanism.

In the previous papers,[1,7] it was clarified that the intramuscular absorption of aqueous unionized drug solution from the injection site was chiefly proceeded by the apparent first order process and the diffusion through the pores of the capillary vessels was predominant compared with the penetration through the capillary endotherial cells. Therefore physico-chemical properties of injectable solution may serve as a major role in the absorption process provided physiological condition was controlled normally.

The purpose of this work was to examine the effect of the viscosity and the osmotic pressure on the absorption mechanism in the presence of adjuvants. From the relation between *in vivo* absorption study and *in vitro* diffusion rate analysis, it was clarified that the injected solution was absorbed from the injected site through muscle fiber space and then pores of capillary walls, and the latter step might act as the rate-limiting step in the absorptive process.

1) Preceding paper, Part II: Kiichiro Kakemi, Hitoshi Sezaki, Katsuhiko Okumura, and Chiyoko Takada, *Chem. Pharm. Bull.* (Tokyo), **19**, 2058 (1971).
2) Part of this work was presented at 89th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1969.
3) Location: *Yoshidashimoadachi-cho, Sakyo-ku, Kyoto.*
4) A.J. Spiegel and M.M. Noseworthy, *J. Pharm. Sci.*, **52**, 917 (1963).
5) K.S. Lin, J. Anschel, and C.J. Swartz, *Bull. Parenteral Drug Assoc.*, **25**, 40 (1971).
6) D.E. Cadwallader, *J. Pharm. Sci.*, **56**, 351 (1967).
7) K. Kakemi, H. Sezaki, K. Okumura, and S. Ashida, *Chem. Pharm. Bull.* (Tokyo), **17**, 1332 (1969).

Experimental

Materials——All drugs, isonicotinamide and methylisonicotinate, and adjuvants, propylene glycol, glycerin, polyethylene glycol 400 (PEG 400), polyethylene glycol 4000 (PEG 4000), dextran (mol. wt. 70000) and methyl cellulose (4000 cps) were obtained from commercially available sources. All materials, but for PEG 4000 and methyl cellulose, were of analytical grade, and used without further purification. PEG 4000 and methyl cellulose were of extra pure reagent.

Procedure of Absorption Experiments——*The absorption experiments were almost identical with those described in the previous paper from this laboratory.*[1]

Preparation of Solutions for Injection and Diffusion——Isotonic buffer systems and the concentration of drug solution were the same as previously described.[1]

Determination of Possible Complexation of Dextran and PEG 4000 with Isonicotinamide——Ultraviolet spectrometry and equilibrium dialysis method were used.

Determination of Viscosities——Viscosities were determined at 36° with B-type viscometer (Tokyo Keiki Seisakusho). In the case of propylene glycol and dextran solution, it was confirmed that the solution was Newtonian fluid by the use of Universal Rheometer UR-1 of Shimazu Manufacturing Co., Ltd.

Determination of Diffusion Coefficients——(A) A Glass Filter and Visking Cellulose Membrane as Diffusion Barrier: The apparatus used for this study is shown in Fig. 1. The apparatus consists of a jacketed glass beaker containing 100 ml of solvent without drug maintained at 37° by circulating water through the jacket. The beaker was closed on the top by a rubber stopper with one hole to keep the temperature constant and to prevent excessive evaporation. For the diffusion barrier, a glass filter (G-3) and a Visking cellulose membrane (Visking Co., Ltd., 24/32, 3 cm diameter) were used, the latter was firmly fixed by rubber band around the diffusion cell. All solutions were kept at 37° before use. In the diffusion cell,[8] the test solution with 50 mM isonicotinamide was filled. The cell was then set to contact with the surface of the solution. Stirring of the solution was achieved by the use of a magnetic stirring bar throughout the whole experiment. Sample solutions of 0.5 ml were withdrawn every one hour in the case of a glass filter and every 30 minutes in the case of the Visking membrane through the hole, and after dilution with 5 ml of water, optical densities were measured at 270 mμ. The logarithmic plots of the residual amounts of isonicotinamide in the diffusion cell versus time were straight line in every case, and the diffusion rate was obtained from the slope.

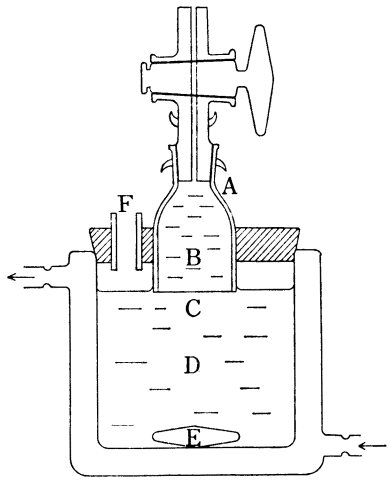

Fig. 1. Schematic Diagram of the Apparatus used to Study the Diffusion Coefficient

A: diffusion cell
B: test solution
C: diffusion barrier (galss filter or Visking cellulose membrane)
D: solvent
E: magnetic stirring bar
F: glass tube for sampling

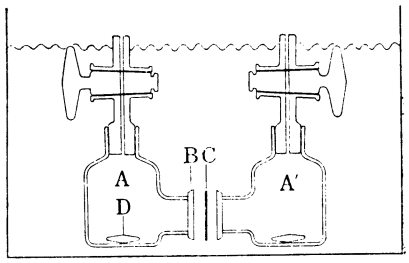

Fig. 2. Schematic Diagram of the Apparatus used to study the Permeability Constant

A,A': cell
B: ground-glass end
C: Visking cellulose membrane
D: magnetic stirring bar

8) Volumes of diffusion cells of Visking membrane and of glass filter were about 4.5 cm^3 and 6.8 cm^3, respectively.

(B) Slice of Muscle as Diffusion Barrier: The experiments were almost identical with those described in the previous paper from this laboratory.[1]

Determination of Apparent Membrane Permeability——The whole apparatus[9] for the permeability study, shown in Fig. 2, consisted of two glass cells, whose volume was about 7 to 8 cm^3. The same Visking membranes as used in Fig. 1 were prepared and attached tightly between the ground-glass end of the glass cell by the rubber bands. The area of the membrane available to permeate was about 5 to 7 cm^2. All solutions were kept at 37° before use. In the cell A, the test solution with 50 mM isonicotinamide was filled and, in the cell A′, the same solution without drug was filled. The entire cell was maintained at 37° by immersing in a constant-temperature water bath. Stirring of the solution was achieved by using magnetic stirring bars and stirring rate was adjusted so as not to affect the permeability. After 2 or 3 hour experiment, the drug concentration of each cell was measured, and apparent membrane permeability was calculated.[10]

Analytical Methods——The spectrophotometric determination was applied to all the drugs investigated.

i) Isonicotinamide: In absorption experiments, same spectrophotometric methods were used as described previously.[1] For the determination of the samples other than the absorption experiments, 5 ml of distilled water was added to 0.5 ml sample solution and its optical density was measured at 270 mμ.

ii) Methylisonicotinate: In absorption experiments, same spectrophotometric methods were used as previously described.[7]

iii) Propylene Glycol: In absorption experiments, removed muscle was homogenized and centrifugated in the manner as described previously.[7] Supernatant, deproteinized by trichloroacetic acid, was used as 0.5 ml sample solution and 0.5 ml of distilled water, 0.5 ml of 0.1N hydrochloric acid, and 5 ml of 1.6% w/v sodium metabisulfite were added. After centrifugation, 1 ml of supernatant was separated. Then 2.5 ml of chromotropic acid solution, prepared by solubilizing 200 mg of chromotropic acid in 2 ml of distilled water and sufficient sulfuric acid to make 50 ml under cooling in ice-bath, was added, and the aliquot was boiled for 30 minutes. After cooling, 10 ml of sulfuric acid was added and the optical density was determined at 565 mμ.

Result and Discussion

Contrary to such pharmaceutical dosage form as suspensions,[11] oily solutions,[12] and emulsions,[13] very little has been understood about the effect of vehicles on the absorption of aqueous injection solutions.

(1) Effect of Adjuvants on the Time Course of Drug Clearance

For the purpose of examining the possibility of change in the absorption mechanism caused by these adjuvants, the time course of drug clearance in the rat muscle was investigated. According to our previous paper,[1] it was proved that the time course of the drug absorption from aqueous solution followed in most cases apparent first-order kinetics. Figure 3 shows the logarithmic plots of the amounts of isonicotinamide remaining in the muscle versus time after injection of 10 μl of 40% propylene glycol solution and 10% dextran solution. As is evident from the figure, straight lines were obtained in both cases, which shows that the absorption was proceeded by the apparent first-order kinetics. Accodingly, it was suggested that the absorption mechanism of the drugs with water-soluble adjuvants did not essentially differ from that in aqueous solution without any adjuvant.

(2) Effect of Injection Volume in the Presence of Adjuvants

As reported in the previous papers,[1,7] it was suggested that the parenteral absorption from aqueous solution was not affected by the variation of the injection volume. So the effect of injection volume was also examined in the presence of adjuvants. Absorption of isonicotinamide within 3 min when 40% propylene glycol or 10% dextran was added to injectable solution is shown in Fig. 4. No remarkable difference on absorption was observed between

9) This apparatus was originally designed by Prof. Masayuki Nakagaki of Kyoto University and Mr. Masakatu Yonese of Nagoya City University.
10) M. Nakagaki and M. Koga, *Yakugaku Zasshi*, **82**, 1134 (1962).
11) F.H. Buckwalter and H.L. Dickson, *J. Pharm. Sci.*, **47**, 661 (1958).
12) J.C. Bauernfeind and H.L. Newmark, *Bull. Parenteral Drug Assoc.*, **24**, 169 (1970).
13) J.J. Windheuser, M.L. Best, and J.H. Perrin, *Bull. Parenteral Drug Assoc.*, **24**, 286 (1970).

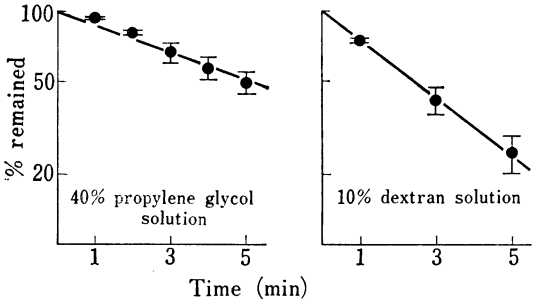

Fig. 3. Clearance Curves for Isonicotinamide

Each point represents the mean value of at least five experiments. Vertical bars indicate S.D.

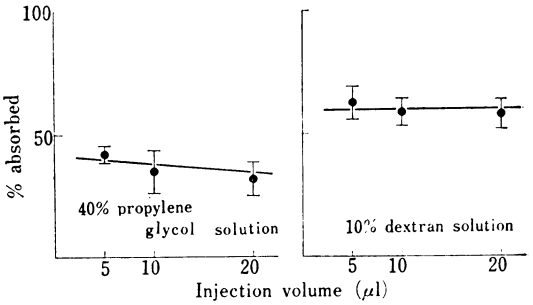

Fig. 4. Effect of Injection Volume on Parenteral Absorption

these two vehicles in the range of injection volume investigated. This suggests that there is little possibility of physiological alteration caused by adjuvant.

As the further study for confirming whether the decrease of absorption accompany with the increase of the amount of propylene glycol, influence of pre-treatment by adjuvant on parenteral absorption was examined. In this experiment, 10 μl of 40% propylene glycol without drug was injected initially and 5 min after the first injection, when propylene glycol was expected to be mostly absorbed, the absorption of 50 mM isonicotinamide within 3 min was measured by injection of aqueous drug solution into the same injection site. No significant difference caused by pre-treatment was observed. It is conceivable, therefore, that there is little possibility of change in the absorption mechanism caused by the local effect of the adjuvants.

(3) Effect of Osmotic Pressure

Hydrophilic pharmaceutical solvents are usually incorporated in very high concentrations and, in this experiment too, fairly high concentrated solutions were used. Therefore, the osmotic pressure of the injected solutions with the solvents of small molecular weight is naturally hypertonic. Thus the effect of osmotic pressure on the absorption was examined, particularly on the hypertonic range.

Effect of osmotic pressure on the absorption from muscle is shown in Fig. 5. In this case N,N-dimethylacetamide, a non-aqueous solvent of which the contribution of viscosity is almost negligible, was added to 50 mM isonicotinamide solution to make the osmotic pressure of the final injection solution in the range of 50 mosM to 3 osM which exceeds the physiological osmotic pressure. As is evident from the figure, no significant difference of the absorption was observed either in isotonic or in hypertonic range, which rule out the possible effect of osmotic pressure of the solvents on the drug absorption.

(4) Effect of Adjuvant on Drug Absorption

(A) Contribution of Viscosity——In Fig. 6 is shown the result of the examination of the effect of propylene glycol on absorption in which the left vertical axis is for the absorption rate constant, the right vertical one for the reciprocal viscosity. The horizontal one for the concentration of propylene glycol in pH 7.0 phosphate buffer. The solid line indicates the absorption rate constant calculated from the percentage-absorbed within 3 min and the dotted line denotes the reciprocal of viscosity. Good correlationship was obtained between these two parameters. Absorption rate of propylene glycol itself is indicated by the mark (□). As shown in this figure, absorption rate constant of isonicotinamide and that of propylene glycol are very close. The observed results rationalize the view that both of the components are absorbed by the same route. In other words this suggests that in the case of unionized drug such as isonicotinamide, drug is not separated from the solvent but is transported together with the solvent into blood thus the viscosity of the solvent affects the

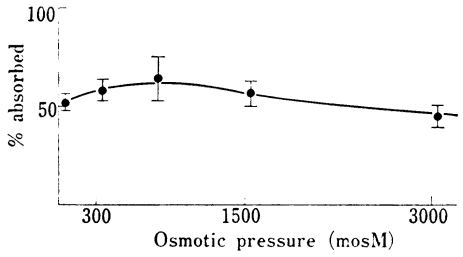

Fig. 5. Effect of Osmotic Pressure on Parenteral Absorption

vehicle: N,N-dimethylacetamide-water
drug: isonicotinamide

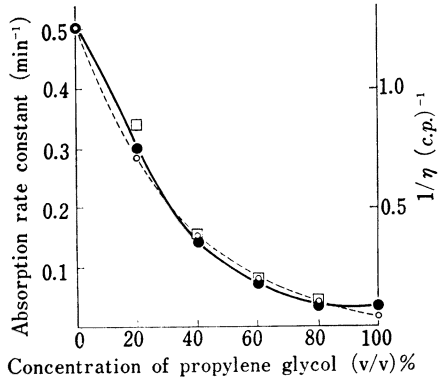

Fig. 6. Effect of Propylene Glycol on the Absorption Rate Constant of Isonicotinamide and Propylene Glycol

—●—: absorption rate constant of isonicotinamide
□ : absorption rate constant of propylene glycol
---○---: reciprocal of viscosity

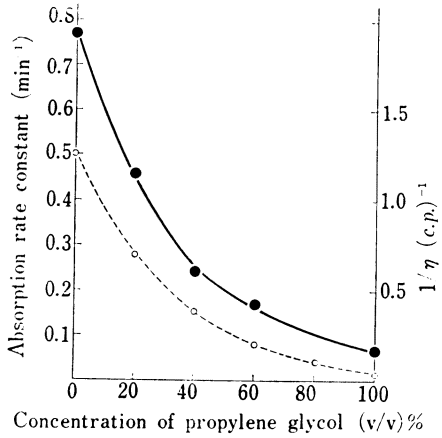

Fig. 7. Effect of Propylene Glycol on the Absoption Rate Constant of Methyl Isonicotinate

—●—: absorption rate constann of methyl isonicotinate
—○—: reciprocal of viscosity

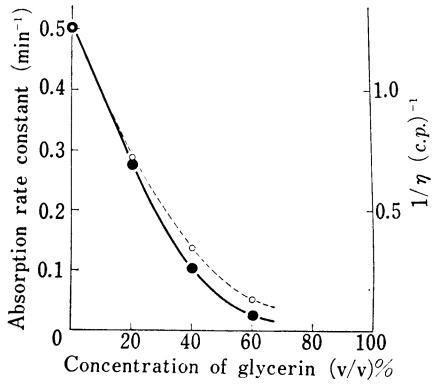

Fig. 8. Effect of Glycerin on the Absorption Rate Constant of Isonicotinamide

—●—: absorption rate constant of isonicotinamide
---○---: reciprocal of viscosity

absorption remarkably. This relationship was also reported by Coles, *et al.* in the absorption experiment of various vaccine formulation administered subcutaneously.[14]

Further examination was made about methylisonicotinate, a drug having high lipid solubility. Similar tendency with the case of isonicotinamide, shown in Fig. 7, suggests that the nature of the effect of these solvents on drug absorption is not of specific to drugs. The absorption rate of methylisonicotinate is larger than that of isonicotinamide by some definite value, which is independent of propylene glycol concentration. This difference can be attributed to the greater lipid-solubility of the former. In general, the diffusion process through pores of the capillary wall and the partition process through the lipid component

14) C.L.J. Coles, K.R. Heath, M.L. Hilton, K.A. Lees, P.W. Muggleton, and C.A. Walton, *J. Pharm. Pharmacol.*, **17** (*Suppl.*), 87s (1965).

of the vascular endothelial cells have been reported for the mechanism of capillary permeability. In the latter process which is considered to be more dependent on lipid solubility, effect of the water-soluble adjuvants is comparatively small. In the limited sense, parenteral drug absorption with such adjuvant seems to be proceeded dominantly by the former process. Similar examination was made for other vehicles such as glycerin, and PEG 400. The results are shown in Fig. 8 and. Fig. 9, respectively. As is shown in the figures, good correlationship between parenteral absroption rate and the reciprocal of viscosity was obtained.

On the basis of these experiments, it may be proposed that the prediction of the parenteral absorption rate of a drug from the injectable solution could be possible by the viscosity of the solvents, provided that the solvents are of comparatively small molecular weight and exert little local effect. On the other hand, in the case of adjuvants of macro molecules such as PEG 4000, dextran, and methyl cellulose, size of molecules outweighs the viscosity. As

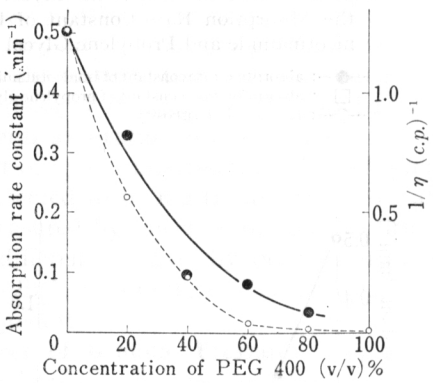

Fig. 9. Effect of PEG 400 on the Absorption Rate Constant of Isonicotinamide

—●—: absorption rate constant of isonicotinamide
---○---: reciprocal of viscosity

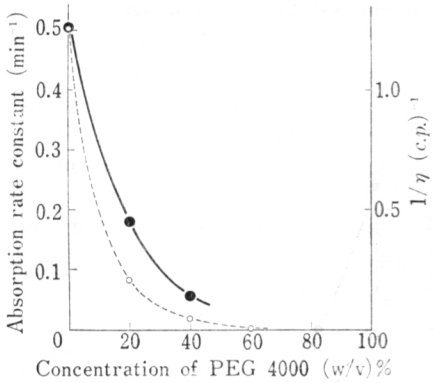

Fig. 10. Effect of PEG 4000 on the Absorption Rate Constant of Isonicotinamide

—●—: absorption rate constant of isonicotinamide
—○—: reciprocal of viscosity

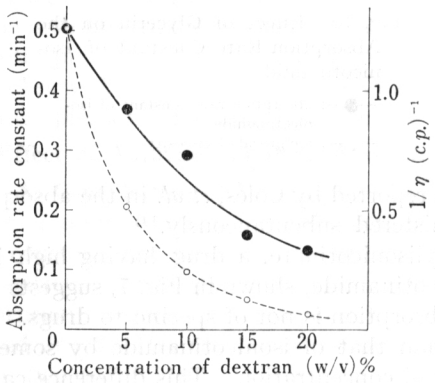

Fig. 11. Effect of Dextran on the Absorption Rate Constant of Isonicotinamide

—●—: absorption rate constant of isonicotinamide
---○---: reciprocal of viscosity

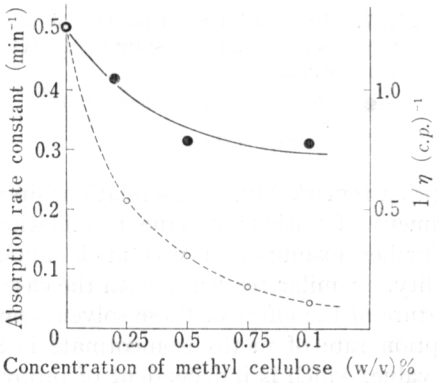

Fig. 12. Effect of Methyl Cellulose on the Absorption Rate Constant of Isonicotinamide

—●—: absorption rate constant of isonicotinamide
---○---: reciprocal of viscosity

shown in Fig. 10, 11, and 12, absorption rate is greater than that expected from the viscosity. It is proper to consider that the water-soluble solution injected into muscle is transported into blood from the injection site through two steps; as the first step, it diffuses through the intercellular space of muscle fiber or connective tissue, and as the second step, it diffuses through the pores of the capillary wall. According to Pappenheimer,[15] the pore radius of capillary wall is 30—40 Å, while the intercellular space is much larger than that.

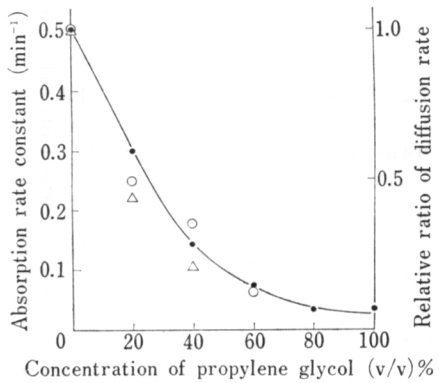

Fig. 13. Relationship between the Absorption Rate Constant and the Diffusion of Isonicotinamide with Propylene Glycol added as an Adjuvant

—●—: absorption rate constant of isonicotinamide
○ : diffusion of isonicotinamide through the Visking membrane
△ : diffusion of isonicotinamide through the glass filter

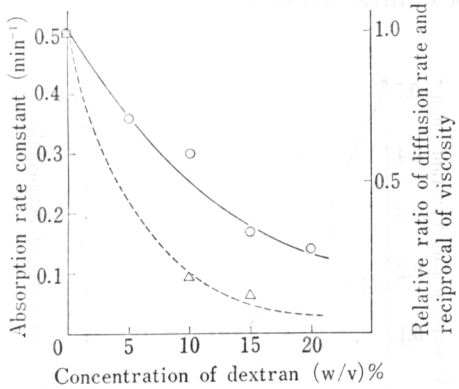

Fig. 14. Relationship between the Absorption Rate Constant and the Diffusion of Isonicotinamide with Dextran added as an Adjuvant

——: absorption rate constant of isonicotinamide
------: reciprocal of viscosity
○ : diffusion of isonicotinamide through the Visking membrane
△ : diffusion of isonicotinamide through the glass filter

(B) Contribution of Diffusion Rate——In order to gain further insight into the nature of intramuscular absorption of drugs from aqueous vehicles, various diffusion experiments have been undertaken. In these experiments, cellulose Visking membrane with similar pore size with capillary pores (average pore size of about 24 Å) and the glass filter (No. 3) (average pore size of about 40—20 μ) were used for diffusion barrier. Fig. 13 is the result of diffusion of isonicotinamide with propylene glycol added as an adjuvant of low molecular weight. The left vertical axis is for absorption rate constant and the right vertical one for the value of the relative ratio of the diffusion rate without adjuvant to that with the adjuvant of respective concentration. As is evident from the figure, all these three plots have shown good correlationship.

As for dextran which belong to the adjuvants of macro molecular group, results of the experiments are shown in Fig. 14. In this adjuvant, good correlation was obtained with the absorption curve when the Visking membrane was used. However in the case of glass filter the plots appeared almost on the curve of the reciprocal of viscosity. These results again indicate that, in the case of the solvent of small molecular weight such as propylene glycol, the solvent molecule itself permeates into the blood through pores of capillary wall, the drug and the solvent are transported together, so that the rate of transportation is rather limited by the solvent viscosity. When PEG 4000, dextran or methyl cellulose, which does not easily permeate the capillary wall by themselves, was added as an adjuvant, the transportation through the capillary wall is now becomes a rate-limiting step. The drug and the adjuvant

15) J.R. Pappenheimer, *Physiol. Rev.*, **33**, 387 (1953).

are presumably separated and only the former diffuses through pores of capillary wall as the simple aqueous solution. This may be considered to be the reason why the absorption rate *in vivo* is greater than the one anticipated from the solvent viscosity. It is conceivable also that the pharmacological effect of dextran itself, *i.e.* enhancement of capillary permeability caused by histamine release,[16] should be taken into consideration. Schou, however, demonstrated that the self-depression of drug absorption was very pronounced when the injected drug is a chemical histamine liberator,[17] and in our experiments such effect is considered to be of little account.

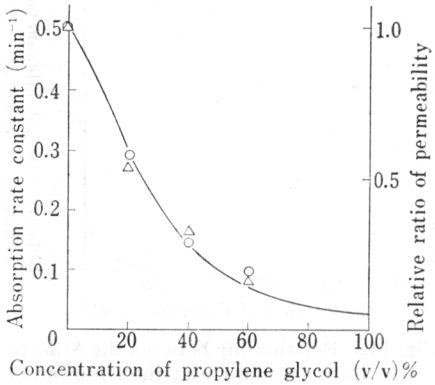

Fig. 15. Relationship between the Absorption Rate Constant and the Apparent Membrane Permeability through the Visking Membrane, Permeability through the Slice of Muscle

——: absorption rate constant of isonicotinamide
△ : permeability of isonicotinamide through the Visking membrane
○ : permeability of isonicotinamide through the slice of muscle

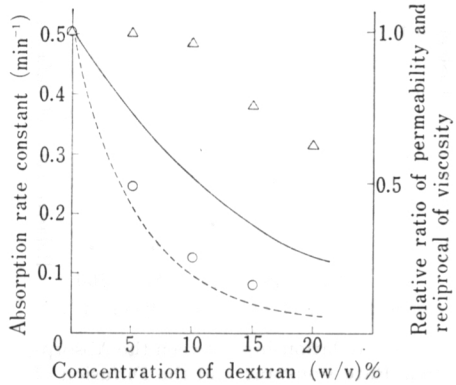

Fig. 16. Relationship between the Absorption Rate Constant and the Apparent Membrane Permeability through the Visking Membrane, Permeability through the Slice of Muscle

——: absorption rate constant of isonicotinamide
------: reciprocal of viscosity
△ : permeability of isonicotinamide through the Visking membrane
○ : permeability of isonicotinamide through the slice of muslce

(C) Contribution of Apparent Membrane Permeability——For the purpose of further confirmation, apparent membrane permeability of isonicotinamide through the same cellulose Visking membrane as that used in the previous diffusion experiment was examined. Results of propylene glycol and dextran are shown in Fig. 15 and Fig. 16, respectively. The right vertical axis denoted the value of the relative ratio of the apparent membrane permeability without adjuvant to that when the adjuvant of respective concentration is added. In the case of propylene glycol, a good correlation was observed between the absorption rate *in vivo* and the membrane permeability. However, in the case of dextran, relative ratio of the apparent membrane permeability is greater than the ratio of diffusion rate. Particularly, in the range of low concentration of dextran, such as 5% w/v, the ratio of apparent membrane permeability is almost unity.

It may be concluded that with the solvent of small moleculer weight such as propylene glycol, the pore of the cellulose membrane is filled with propylene glycol solution so that the membrane permeability of isonicotinamide is rather limited by the solvent viscosity. On the other hand, dextran cannot easily permeate the pores of the cellulose membrane and isonicotinamide molecules have to diffuse through the pores filled with water. At the range

16) R.H. Poyser and G.B. West, *Brit. J. Pharmacol.*, **25**, 602 (1965).
17) J. Schou, *Nature*, **182**, 324 (1958).

of high concentration of dextran, however, the pore masking effect of dextran becomes predominant thus reduces the apparent membrane permeability.

In the present report two apparatus with Visking membrane as the barrier have been used to investigate the mechanism of intramuscular absorption of drug with water-soluble adjuvant. The concentration gradients in each apparatus and in the case of practical intramuscular injection are shown in Fig. 17. Considering the correlationship between *in vivo* absorption data and *in vitro* diffusion data, it may be concluded that, in our experimental condition, the diffusion apparatus shown in Fig. 1 is the most promising one for the investigation of the mechanism of intramuscular absorption of drug. It is also suggested that in the case of macromolecules, the absorption process through the intracellular space or its masking effect could not be neglected in determining the rate of drug absorption.

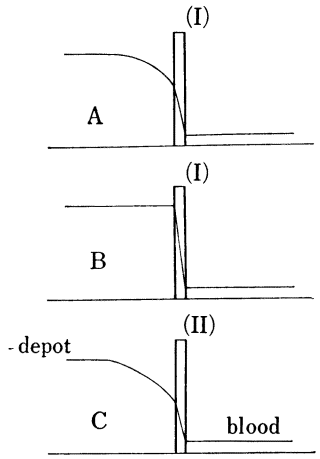

Fig. 17. Model of Drug Concentration Gradient

A: apparatus shown in Fig. 1
B: apparatus shown in Fig. 2
C: practical intramuscular injection
(I) Visking membrane
(II) capillary wall

(D) Contribution of Permeability through Isolated Muscular Slices——Finally to confirm the foregoing considerations, we have examined the permeability of the drug through isolated muscular slices. The result is shown in Fig. 15 and Fig. 16. As can be seen cleary from Fig. 15 the ratio of diffusion rate to the control declines with the increase of the concentration of propylene glycol. Both isonicotinamide and prolylene glycol permeated the slice of muscle almost at the same rate, which agrees with the data obtained in the *in vivo* absorption experiment. On the other hand, as shown in Fig. 16, diffusion rate decreases with the increase of the concentration of dextran, which can be explained by the increase of its viscosity. This data differs significantly from the absorption data *in vivo*. Consequently results of muscle slice diffusion experiment on dextran could be interpreted that the contribution of the diffusion process through the pores of capillary wall to the drug absorption is dominant compared with the process through the muslce fiber space. This again supports our conclusion.