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Absorption of Drugs from the Skeletal Muscle of the Rats. (2)[1]

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Biopharmaceutical factors of the formulation of injections such as the effect of buffer components, osmotic pressure, and pH of injection solutions were studied using the rat thigh muscle clearance method and *in vitro* diffusion experiment using muscle slice.

1) It has been shown that the intramuscular absorption of isonicotinamide, a model neutral drug, was hardly affected at all by the buffer components and that of isonicotinic acid, a model anionic drug, was independent for buffer components except potassium ion.

2) Change of the absorption patterns of isonicotinic acid and isonicotinamide due to osmotic pressure of injection solutions was interpreted in terms of a nonspecific, reversible change in the muscle.

3) It was also shown that remarkable reduction of the rate of absorption from acidic solutions was closely related to the irreversible functional change caused by morphological damage.

In our previous paper,[1b] experimental conditions of intramuscular absorption of seven isonicotinic acid derivatives from aqueous solution were studied. From the results, it was found that the rate of drug absorption from the muscle was proportional to the amount remaining in the injection site, and both molecular weight and partition coefficient of drugs affected the absorption. The results also supported the view that the diffusion through the pores of capillary vessels was predominant compared with the penetration through the capillary endothelial cells.

The purpose of this investigation to be described here was to examine additional biopharmaceutical factors of the formulation of injections such as buffer compositions, osmotic pressure, and pH, affecting the absorption of drugs from the rat thigh muscle.

Experimental

Materials——Isonicotinamide, isonicotinic acid, and caffeine were obtained from commercially available sources. Other chemicals used were reagent grade.

Animals——Male Wistar albino rats weighing 140—180 g were used in all the absorption experiments.

Analytical Methods——Isonicotinamide and isonicotinic acid remaining in the muscle were determined as described in the previous report from this laboratory.[1b] Caffeine was analyzed spectrophotometrically after the extraction with chloroform.[3]

Preparation of Injection Solution——In order to adjust the drug solution at certain osmotic pressure, modified Krebs buffer solution (molar ratio= NaCl: KCl: $MgSO_4$: Na_2HPO_4: KH_2PO_4=121:33:0.1:0.3:0.1) and glucose were used while keeping the drug concentration constant. For the preparation of drug solutions of various pH values, isoosmotic buffers, citrate buffer for acidic range, phosphate buffer for neutral range, and carbonate buffer for alkaline range were used.

1) *a*) This paper constitutes the 47 th report in a series of "Absorption and Excretion of Drugs" by Prof. K. Kakemi and his co-workers; *b*) Preceding paper, Part I: K. Kakemi, H. Sezaki, K. Okumura, and S. Ashida, *Chem. Pharm. Bull.* (Tokyo), **17**, 1332 (1969).

2) Location: *Yoshida-Shimoadachi-Cho, Sakyo-ku, Kyoto.*

3) K. Kakemi, T. Arita, R. Hori, R. Konishi, and K. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 248 (1969).

Procedure of Absorption Experiment——The incision, ligation, and injection techniques used are the same as used in the previous report in this series.[1b]

Pretreat Experiment——Incision technique was the same as that described previously.[1b] The hind legs of the rat were fixed on the plate. The micrometer syringe which contained the sample solution for pretreatment and the drug solution separated each other by 0.5 μl of liquid paraffin was connected to micromanipulator. In the beginning, the needle of micrometer syringe was inserted into the muscle and was fixed at this position during the absorption experiment, and then the solution for pretreatment was injected. Five minutes after the pretreatment, drug solution was administered and absorption for the next three minutes was determined as described previously.[1b]

Diffusion Experiment using Muscle Slice——The whole apparatus for the diffusion study using muscle slice is shown in Fig. 1. For the preparation of muscle slice as the diffusion barrier, muscle extensor quadriseps femoris of anesthetized rat weighing 150—200 g was removed. The removed muscle was sliced across the muscle fiber about 0.5 mm thickness by the slicer (Natsume Mfg. Co., type KN-921 slicer). This slice was attached to the end of the tapered glass tube by the instantaneous adhesive (Uron Alpha, TOA Goseikagaku Co.). The slice was tested to insure that there was no leakage before use. The test solution with 50 mM iso nicotinamide was poured into the glass tube with hydrostatic pressure of 40 mm water, which is considered to be equal to the initial injection pressure in the clinical use.[4] The surface level of the test solution in the glass tube was kept almost constant throughout the experiment. When the surface level went down by some reasons, *i.e.* break of slice and others, experimental data were discarded. At the set time intervals, 0.5 ml sample solutions were withdrawn for analysis, and the solutions of both compartments were changed for new ones and the same procedure was repeated. In this way, a series of data can be obtained as the result of a continuous experiment with the same muscular slice.

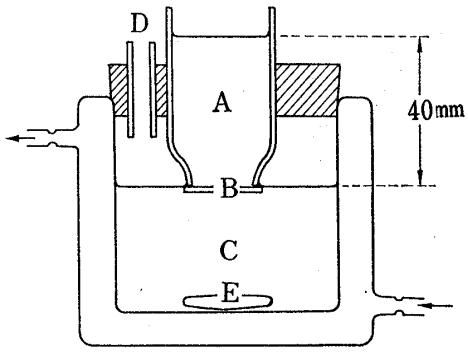

Fig. 1. Diffusion Apparatus using Muscle Slice

A: drug solution
B: muscle slice
C: isotonic buffer solution
D: hole for sampling
E: stirrer

Result and Discussion

Effect of Buffer Component

In preparing the injections, solutions are adjusted to a definite pH or to within a pH range by using many kinds of buffers and adjuvants to increase the stability of the preparation and to minimize pain, irritation, and necrosis on injection. Contrary to the absorption from the gastrointestinal tract, this aspect of the importance of pH as well as buffer components in the injections has been very little understood.

Drug solutions, made isotonic at pH 7 by adding various buffer components or adjuvants, were injected into rat thigh muscle and drug absorption was investigated by the local clearance

Table I. Effect of Buffer Component on Parenteral Absorption of Isonicotinamide

Buffer component		% absorbed[a] ±S.D.
NaH_2PO_4–Na_2HPO_4	250 mosM	76.5±5.6
NaH_2PO_4–Na_2HPO_4 NaCl	210 mosM 40 mosM	78.0±4.0
NaH_2PO_4–Na_2HPO_4 NaCl	50 mosM 200 mosM	77.3±6.0
NaCl	250 mosM	79.9±5.1

a) experimental period: 3 min
concentration of isonicotinamide: 50 mM

4) J. Schou, *Pharmacol. Rev.*, 13, 441 (1961).

Table II. Effects of Buffer Component and Adjuvant on Parenteral Absorption of Isonicotinamide

Adjuvant[a]	% absorbed ±S.D.[b]
KH_2PO_4–K_2HPO_4	74.3±5.6
Krebs buffer	78.0±8.1
Glucose	80.1±8.2
Propylene glycol	76.2±6.8

a) concentration of Adjuvant: 250 mosM
b) experimental period: 3 min

method. Tables I and II show the effect of buffers and adjuvants on the muscular absorption of isonicotinamide, a neutral drug. No difference was observed when phosphate anion was relpaced with chloride anion in any ratio in sodium contaning buffers. Also, any difference in absorption was noted when sodium ion was replaced with potassium ion. Glucose and propylene glycol, commonly used organic adjuvants in the formulation of injections, do not seem to exert any effect on the absorption of isonicotinamide in this isotonic range. It may be concluded, therefore, that intramuscular absorption of a neutral drug is hardly affected at all by the buffer components or adjuvants in isotonic range unless viscosity, pH, osmotic pressure, and other physicochemical properties are changed. However, there is a strong evidence that the absorption of anionic or cationic drugs in the presence of potassium ion is very complex. This aspect of the effect of buffer components is now being studied and will be discussed in the future report from this laboratory.

Effect of Osmotic Pressure

Solutions intended for parenteral injection should be adjusted to approximately the same osmotic pressure as the body fluids with which they come in contact. However, for the purpose of maintaining enough stability of drugs or keeping the adequate injection volume, paratonic solutions are marketed widely for clinical and veterinary use. The effects of osmotic pressure of injection solutions on the red blood cells[5] and the pain and irritation at the injection site[6] have been examined by various authors. However, the past work on the effect of osmotic pressure on the parenteral absorption of drugs has been relatively sparse despite the apparent potential of the problem in biopharmaceutical dosage form design. Thus, comparisons have been made with the paratonic solutions in the muscular absorption of isonicotinic acid and isonicotinamide. Figure 2 shows the three minutes absorption of the drug at pH 7 at different osmotic pressure regulated by modified Krebs buffer. Decreasing tendency of absorption at lower osmotic pressure and slightly increasing tendency at higher osmotic pressure were observed in both drugs. Similar tendency was observed in the experiments in which three-minute absorption of isonicotinamide was investigated as a function of osmolarity. Osmolarity was varied

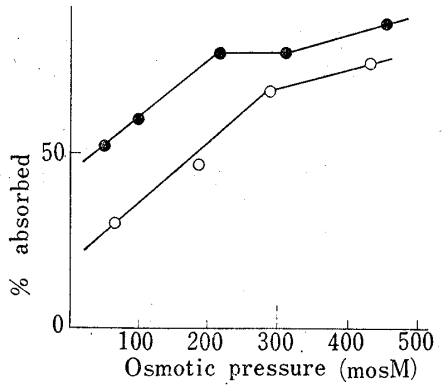

Fig. 2. Effect of Osmotic Pressure on Parenteral Absorption using Buffer Solution

—●—: isonicotinamide
—○—: isonicotinic acid

5) I. Setnikar and O. Temelcou, *J. Am. Pharm. Assoc.*, **48**, 628 (1959).
6) I. Setnikar and M.R. Paterlini, *J. Pharm. Sci.*, **49**, 5 (1960).

over a three-fold range by the addition of glucose and the result is shown in Fig. 3. It is conceivable, therefore, that such effect of osmotic pressure is a nonspecific one, independent on drug and medium component, and could be attributed to a reversible change in the muscle caused by simple physico-chemical properties. In order to get further insight into the nature of physiological change caused by paratonic solutions, several investigations have been undertaken. In the first, effect of injection volume of paratonic solutions was studied. As shown in Fig. 4, absorption decreased slightly in hypotonic range, which was less conspicuous in the injection of 10 μl solution than 20 μl. This suggests the rapid change and recovery of absorptive phase.

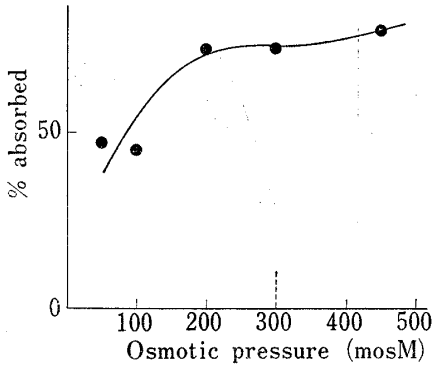

Fig. 3. Effect of Osmotic Pressure on Parenteral Absorption of Isonicotinamide using Glucose Solution

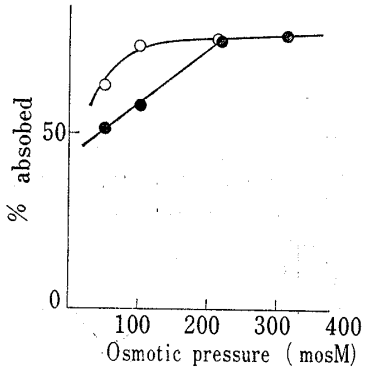

Fig. 4. Effect of Injection Volume on Parenteral Absorption at Low Osmotic Pressure

—●—: 20 μl injected
—○—: 10 μl injected

In the second, effect of osmotic pressure was interpreted from the stand-point of blood flow. Marshall, *et al.*[7] demonstrated the increase of blood flow in intraarterial injection of hypertonic saline solution. In our experiment, however, absorbed drugs are transported into capillary vessels and then carried to vein, not into artery or pre-capillary sphincter which usually regulates blood flow. Also increasing tendency of absorption in the hypertonic range is not notable as shown in Fig. 2. These considerations rule out the possibility that the change of muscular absorption by osmotic pressure is attributable to the action of vasoactive nature.

In the third, in order to prove that the change in absorption by osmotic pressure was caused by a reversible physiological change and not by the histological damage, effect of pretreatment with distilled water was investigated. The rate of absorption of isonicotinamide from isotonic solution five minutes after the pretreatment with 10 μl of distilled water is shown

Table III. Parenteral Absorption of Isonicotinamide in the Usual Condition from the Rat Muscle Pretreated with Distilled Water and pH 3.2 Buffer

Treatment	% absorbed ±S.D.[a)]
Control	78.0± 4.0
Pretreated with dist. water	73.5± 7.3
Pretreated with pH 3.2 buffer	52.0±14.5

a) Absorption of isonicotinamide from isotonic solution was measured 5 minutes after the pretreatment. For details see text.

7) R.J. Marshall and J.T. Shepherd, *Am. J. Physiol.*, **197**, 951 (1959).

in Table III. No significant difference was observed between control and distilled water pretreated runs.

Finally, drug diffusion through slice was examined *in vitro* and the results are shown in Fig. 5. As seen in Fig. 5, drug diffusion through the muscle fiber is reversible and correlated with the muscle volume which is sensitive to the osmotic pressure of the medium. Also it has been reported that the volume change of muscle or kidney tissue by bathing in hyper or hypotonic solution was reversible and proceeded rapidly.[8] On the bass of these experiments, a mechanism of action for osmotic pressure to muscular absorption can be proposed. Hypo- or hypertonic solution does not cause any irreversible damage in the injection site and reversible physiological volume change of muscle fiber or capillary endothelial cell inhibits or accelarates the diffusion of the injected drug.

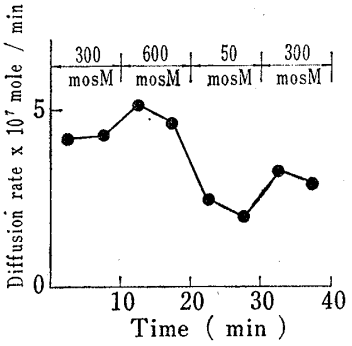

Fig. 5. Effect of Osmotic Pressure on the Drug Diffusion through the Muscle Slice

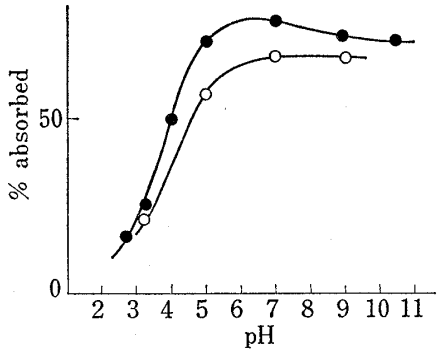

Fig. 6. Parenteral Absorption *v.s.* pH Profiles of Isonicotinamide and Isonicotinic Acid

—●—: isonicotinamide
—○—: isonicotinic acid

Effect of pH

In the clinical field, it is desirable to use injections of neutral pH to avoid any histological damage or local pain at the injection site. But there are many drugs that are unstable at neutral pH region and can be made much more stable by correct choice of pH. In our previous report, pH of injection solution was maintained to physiological neutral pH range, however, it was expected that acidic or alkaline solution may cause morphological damage.

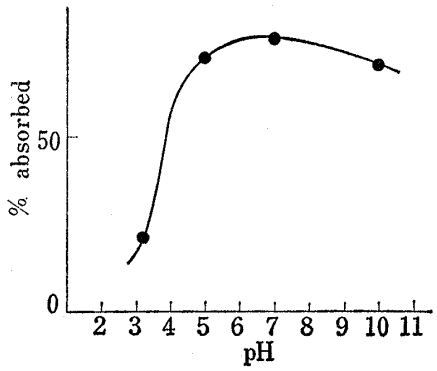

Fig. 7. Parenteral Absorption *v.s.* pH Profile of Caffeine

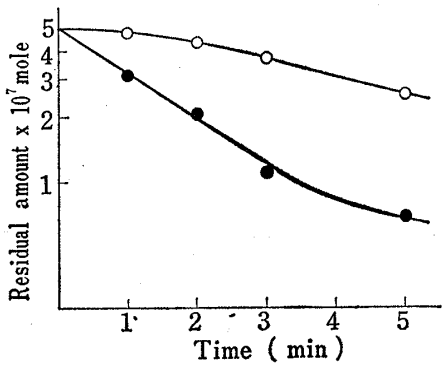

Fig. 8. Clearance Curves for Isonicotinamide at pH 3.2 and pH 7.0

—○—: pH 3.2 —●—: pH 7.0

8) A.F. Brading and J. Setekleiv, *J. Physiol.*, **195**, 107 (1968).

which would affect the parenteral absorption. For this reason, three minute absorption of isonicotinamide, isonicotinic acid, and caffeine from isotonic solutions of various pHs were investigated. As is evident from Fig. 6 and 7, remarkable decrease of absorption in the acidic pH range and no or slight decrease of absorption in the alkaline pH range were observed. Since any significant difference due to buffer components was not observed except potassium ion in the parenteral absorption of these drugs in the preceding experiment, differences of buffer component could not be a contributory cause of the decrease of absorption in the acidic pH range.

Contribution of ionization of the drugs to the remarkable decrease of their parenteral absorption in acidic pH region can be ruled out by the similar absorption pattern of caffeine which bears no net charge at the pH range investigated. However, as shown in Table III, pretreatment of the muscle with the injection of 5 μl of pH 3.2 citrate buffer significantly alter the following muscular absorption of isonicotinamide from pH 7 phosphate buffer solution. Moreover the time course of isonicotinamide clearance from pH 3.2 citrate buffer, shown in Fig. 8, did not obey apparent first order kinetics contrary to the one obtained in neutral pH. Moreover, when the solution of pH 3.2 having higher buffer capacity was injected, absorption of isonicotinamide became constant and the rate of absorption was almost independent on injection volume as shown in Fig. 9 and 10.

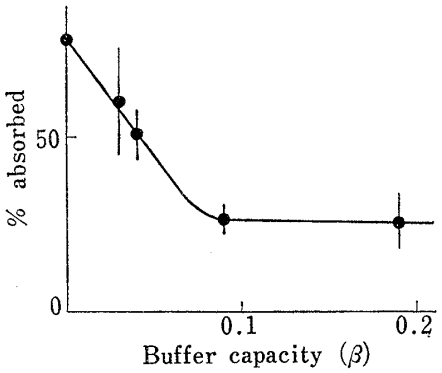

Fig. 9. Effect of Buffer Capacity on Parenteral Absor ption at pH 3.2

Vertical bars indicate S.D.

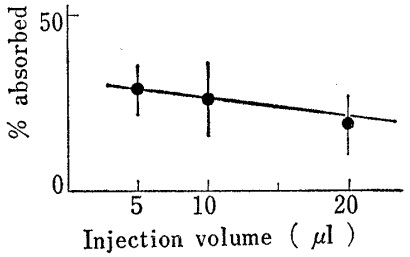

Fig. 10. Effect of Injection Volume on Parenteral Absorption at pH 3.2

Drug diffusion analysis by the muscle slice failed to demonstrate such effect since irreversible leakage from the slice occured when acidic medium was used, which suggests the morphological change of the muscle tissue. These findings may be rationalized on the basis of morphological damage caused by the injection of acidic solutions.

Madison and Christian[9] studied the heart blood level of $^{22}NaCl$ or $^{23}NaCl$ administered subcutaneously with various buffer solutions. In their results, from pH 2.5 to 10 inclusive, there was little effect on the normal absorption rate of sodium ion, and at pHs of 1.0 and 2.0, a decrease in sodium ion absorption was observed, while at pH of 11 and 12 an increase in rate occurred. However, in their experiment, blood sample was removed 45 minutes after the injection when the absorption seemed almost completely ceased. Besides, the result obtained from blood samples contains many factors including absorption, distribution, and excretion. Such factors apparently obscure the pH profiles obtained. On the other hand, Cutts and Walker[10] studied the LD_{50} of nitrogen mustard intraperitoneally administered with pH 2 and pH 8 buffer, and they interpreted the slow absorption of drug in acidic pH range in terms of the ionization of the drug.

9) W.L. Madison and J.E. Christian, *J. Am. Pharm. Assoc.*, **39**, 689 (1950).
10) J.H. Cutts and I.G. Walker, *Cancer Res.*, **26**, 1386 (1966).

From our result, however, their observation could better be explained by the functional change in the injection site caused by acidic solution. Shintani, *et al.*[11] studied the morphological change of rabbit thigh muscle caused by solutions of various pH and demonstrated that morphological damage caused by acidic solution was dominant than by alkaline solution. These results support our conclusion.

11) S. Shintani, M. Yamazaki, M. Nakamura, and I. Nakayama, *Toxicol. Appl. Pharmacol.*, **11**, 293 (1967).