

Absorption of Drugs from the Skeletal Muscle of the Rats. (4)¹⁾ Absorption of Cationic Drugs from the Muscle

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Absorption profiles of intramuscularly administered drugs, protonated at the physiological pH, and the fundamental problems concerning the specificity of their absorption were studied, using the rat thigh muscle clearance method and *in vitro* uptake experiments.

1) The intramuscular absorption rates of cationic drugs were lower than that of neutral or anionic drugs.

2) The absorption of cationic drug from the skeletal muscle was diminished as its concentration increased, and the absorption of isonicotinamide was depressed by the presence of cationic drugs. These results indicated that the vascular effect of cationic drug might influence the cationic drug absorption.

3) It was noted that cationic drugs were incorporated more rapidly than the other drugs into the muscle pieces suggesting the storage or affinity of these drugs to the muscle tissue. This was proposed as one of the factors influencing the low-absorptive nature of cationic drugs.

In our previous papers, it was demonstrated that intramuscularly administered neutral drugs were mainly transported into blood vessels by diffusing through the muscle fibers and then through the pores of the capillary wall,³⁾ in which the latter process appeared to be the late limiting one. Concomitantly, the effects of pH and osmotic pressure of injectable solution⁴⁾ and addition of water-soluble adjuvants¹⁾ were established, supporting above hypothesis. However, it was also suggested that anionic or cationic drugs might show the different pattern in the intramuscular absorption compared with that of neutral drugs.

The present investigations were undertaken to examine systematically the specificity of the mechanism of the cationic drug absorption.

Experimental

Materials—All drugs listed in Table I, isonicotinamide, and the reagents were obtained from the commercially available sources of analytical grade, and used without further purification.

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

Procedure of Absorption Experiment—The incision, ligation, and injection techniques used are the same as in the previous report in this series.³⁾

Preparation of Injectable Solution—All injectable solutions used in this study were prepared isototically and adjusted at pH 7.0 using phosphate buffer.

Determination of the Uptake of Drugs to the Muscle—The muscle extensor quadriceps femoris of the rat weighing 180—200 g was removed after the anesthesia with pentobarbital, and was cut into two pieces by transverse section. Then the muscle was preincubated for 10 min in the Tyrode solution of the following composition (mM); NaCl, 136.8; KCl, 2.7; CaCl₂, 2.5; MgCl₂, 1.0; NaH₂PO₄, 0.4; NaHCO₃, 11.9, and glucose, 5.5, which was gassed with a mixture of 95% oxygen and 5% carbon dioxide (v/v) at 37°. After preincubation, muscle pieces were incubated in the Tyrode solution which contained 1 mM drug for 10 or 20 min. At the end of the incubation period the muscle was removed from the bath and blotted on the

1) Preceding paper, Part III: K. Kakemi, H. Sezaki, K. Okumura, H. Kobayashi, and S. Furusawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 443 (1972).

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

3) K. Kakemi, H. Sezaki, K. Okumura, and S. Ashida, *Chem. Pharm. Bull.* (Tokyo), **17**, 1332 (1969).

4) K. Kakemi, H. Sezaki, K. Okumura, C. Takada, and S. Furusawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 2058 (1971).

filter paper. Then the muscle was weighed and homogenized with 6% trichloroacetic acid solution. After centrifugation, drug in the supernatant was determined spectrophotometrically.

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

A: Isonicotinamide: The drug was analyzed by the method described previously.³⁾

B: Quinine and 2-Allyloxy-4-chloro-N-(2-diethylaminoethyl) benzamide (A.C.D.B.): These drugs were determined by the methods reported from this laboratory.^{5,6)}

C: N,N'-Anhydro-bis- β -hydroxyethyl Biguanide (A.B.O.B.): The muscle homogenate containing drug was shaken with 13 ml isoamylalcohol for 30 min and centrifuged. A 10 ml of aliquot of isoamylalcohol layer was shaken with 5 ml of pH 7 phosphate buffer for 20 min. The separated aqueous layer was determined at 240 m μ .

D: Procainamide, Metoclopramide, and *p*-Aminobenzoic Acid (P.A.B.A.): These drugs were determined by the regular diazotizing method.⁷⁾

E: Thiamine and N-Methylnicotinamide: The spectrophotofluorometric assay described by Matsui⁸⁾ and Carpenter⁹⁾ were used respectively.

Result and Discussion

Absorption of Cationic Drugs

It was reported that the absorption of ²⁴NaCl¹⁰⁾ or atropine¹¹⁾ from the intramuscularly injected site was very complex. However there appears to be much less known about the absorption study in which series of organic cations were examined or cationic drug absorption was compared with that of neutral or anionic drugs. In order to clarify these points, seven cationic drugs were selected and intramuscular absorption at pH 7 for 3 min after injection was measured. These drugs are mostly protonated at the physiological pH range. The results of these animal experiments are shown in Table I indicating that cationic drugs are absorbed more slowly than neutral or anionic drugs which are absorbed more than 65% within 3 min as described previously.³⁾ This is inferred to be generally applicable to wide variety of cationic drugs which differ in chemical structure. To confirm above trend that the absorption rate of protonated drugs is slower than that of nonprotonated drugs, absorption of thiamine was examined at various pHs in which the difference of the ionic nature of the compound should allow closer and more meaningful study of the factors influencing it. As shown

TABLE I. Parenteral Absorption of Basic Drugs

Basic drug	pK _a	% absorbed ^{a)} \pm S.D.
Quinine	8.4 ^{b)}	12.9 \pm 9.2
A.C.D.B.	7.9 ^{c)}	16.4 \pm 5.7
Procainamide	9.2 ^{d)}	32.8 \pm 12.8
A.B.O.B.	strong	33.9 \pm 8.9
N-methylnicotinamide	strong	31.4 \pm 5.8
Metochlopramide	9.0 ^{e)}	37.2 \pm 5.1
Thiamine	9.1 ^{f)}	42.2 \pm 6.9

a) Each result represents the mean value of at least five experiments.

b) This value was obtained from *J. Pharmacol. Exptl. Therap.*, **119**, 361 (1957).

c) This value was obtained from *Yakuzaigaku*, **27**, 229 (1967).

d) This value was determined in our laboratory.

e) This value was obtained from *Chem. Pharm. Bull.* (Tokyo), **17**, 1641 (1969).

f) This value was obtained from reference (12).

- 5) K. Kakemi, H. Sezaki, S. Muranishi, and Y. Tsujimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 1641 (1969).
- 6) K. Kakemi, H. Sezaki, and S. Horiuchi, *Yakuzaigaku*, **27**, 229 (1967).
- 7) K. Kakemi, T. Arita, and S. Muranishi, *Chem. Pharm. Bull.* (Tokyo), **13**, 861 (1965).
- 8) K. Matsui, *Vitamine*, **6**, 459 (1953).
- 9) K.J. Carpenter and E. Kodicek, *Biochem. J.*, **46**, 421 (1950).
- 10) F.R. Franke, J.B. Boatman, and R.S. George, *Proc. Soc. Exptl. Biol. Med.*, **74**, 417 (1950).
- 11) H. Schriftman and A.A. Kondritzer, *Am. J. Physiol.*, **191**, 591 (1957).

in Fig. 1, the decrease of absorption in acidic range and increasing tendency of absorption in the alkaline pH range were obtained. The decrease of absorption in the acidic pH range is agreed with the previously described report,⁴⁾ in which the reduction of absorption from acidic solution was concluded to be related closely to the irreversible functional change caused by morphological damage. On the other hand, the increase of absorption in alkaline pH range can better be explained by the reduction of protonated form of thiamine whose pK_a value is 9.1.¹²⁾ This result suggests that the low absorption rate of cationic drugs may be caused by the specific inhibition processes of the protonated form, including vasoactive property and uptake nature into the muscle tissue and other factors.

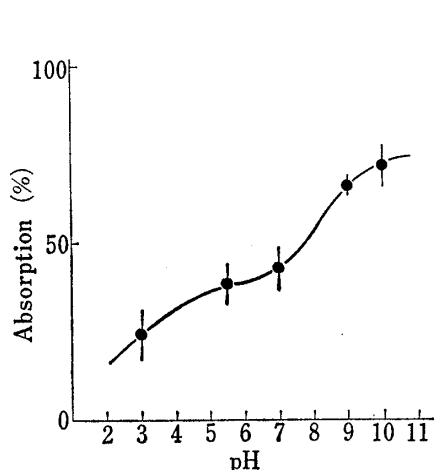


Fig. 1. Absorption vs. pH Profile of Thiamine

Vertical bars indicate S.D.

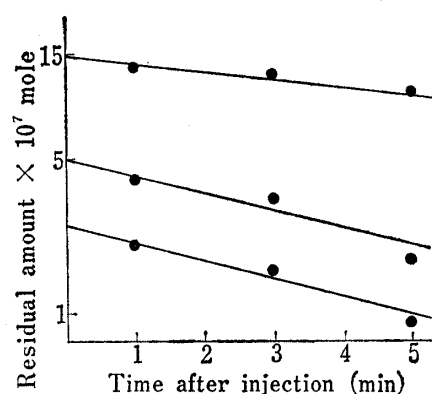


Fig. 2. Clearance Curves for A.B.O.B.

injection volume: 10 μ l
initial concentration: upper line-150 mM
middle line-50 mM
lower line-25 mM

Effects of Initial Concentration and Injection Volume

With the aim of illustrating the absorption mechanism of cationic drugs, the effect of initial concentration of drugs on drug absorption was studied. Figure 2 reveals the time courses of A.B.O.B. clearance at various drug concentrations. Although straight lines were obtained in all cases, the slope in the case of 150 mM is slightly smaller than the others. Similarly the time courses of thiamine clearance were examined as shown in Fig. 3, which also represents the concentration dependency of drug absorption. In order to get further insight into the depression of absorption caused by the increase of drug concentration, absorption for 3 min at various concentrations were investigated and the results are listed in

TABLE II. Effect of Concentration on Parenteral Absorption of Basic Drugs^{a)}

Compound	10 mM	25 mM	50 mM	100 mM	150 mM
Procainamide	42.5 \pm 6.4		32.8 \pm 12.8	24.1 \pm 11.8	
Thiamine	36.0 \pm 9.5	36.1 \pm 8.7	42.2 \pm 6.9	25.5 \pm 6.9	
A.B.O.B.		37.8 \pm 8.3	33.9 \pm 8.9	25.8 \pm 9.6	18.2 \pm 5.6

a) Each result represents % absorbed \pm S.D.

Table II. It is demonstrated that the self-depression of drug absorption in the high concentration over the range of 100 mM to 150 mM is obvious. It is conceivable, therefore, that such effect of drug concentration may reflect the slow absorption, and can be attributed to

12) J.J. Windheuser and T. Higuchi, *J. Pharm. Sci.*, **51**, 354 (1962).

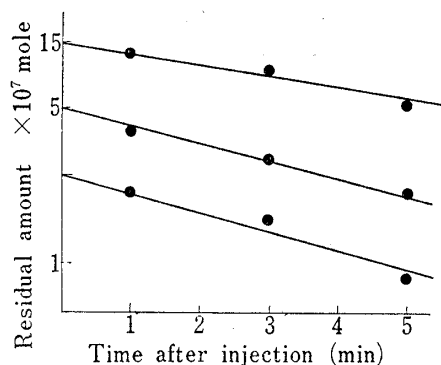


Fig. 3. Clearance Curves for Thiamine
injection volume: 10 μ l

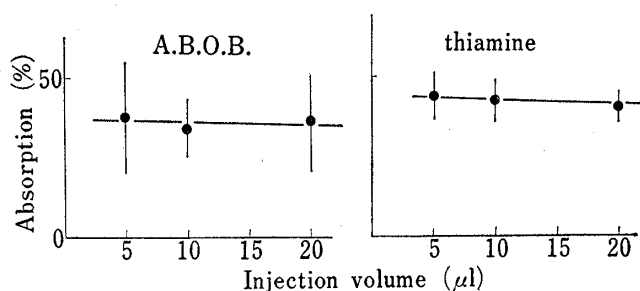


Fig. 4. Effect of Injection Volume on the Parenteral Absorption of Basic drugs

drug concentration: 50 mm
Vertical bars indicate S.D.

the vascular effect caused by the drugs as mentioned for anticholinergic and adrenergic agents by Schou.¹³⁾ Influence of injection volume on absorption was also examined in more detail with particular emphasis on the nature of concentration dependency. As shown in Fig. 4, any significant change on the absorption of thiamine and A.B.O.B. in the injection volume ranging 5 to 20 μ l was not observed. Therefore, self-depression of absorption observed at high concentration was considered to be independent of injection volume or total amount of drug. This result suggests that diffusing process of drugs from the injection site to absorbing area is unchanged, and diffusing through the capillary wall was affected by cationic drugs because of decrease of true capillary for absorption.

Absorption of Isonicotinamide in the Presence of Cationic Drugs

Because of the variation among the intramuscular absorption rates of cationic drugs and their concentration dependency, it was expected that local vascular effect exerted by the cationic drugs might influence the absorption. Table III shows the intramuscular absorption

TABLE III. Parenteral Absorption of Isonicotinamide in the Presence of Basic Drugs^{a, b)}

Basic drug	% absorbed of isonicotinamide ^{c)}
A.C.D.B.	22.6 \pm 3.8
Procainamide	53.7 \pm 15.6
A.B.O.B.	71.2 \pm 9.1
N-methylnicotinamide	32.0 \pm 11.2
N-methylisonicotinamide	42.1 \pm 7.7
Metoclopramide	51.9 \pm 11.6
Thiamine	50.8 \pm 7.9
None	78.0 \pm 4.0

a) initial concentration of isonicotinamide: 50 mm

b) initial concentration of basic drug: 50 mm

c) mean value \pm S.D.

rate of isonicotinamide, a neutral drug, for 3 min in the presence of some cationic drugs. In most cases, inhibiting tendency of isonicotinamide absorption was observed by the presence of cationic drugs, indicating that vasoactive property of cationic drugs was one of the factors for their low absorption rate. However, the inhibition property of cationic drugs varied from drug to drug. Although A.C.D.B., N-methylnicotinamide, and N-methylisonicotinamide markedly inhibited the isonicotinamide absorption, the inhibition by thiamine, procainamide, and metoclopramide was comparably mild, furthermore A.B.O.B. scarcely inhibited the

13) R.B. Sund and J. Schou, *Acta Pharmacol. Toxicol.*, **21**, 339 (1964); J. Schou, *ibid.*, **26**, 253 (1968).

absorption of the neutral drug. These results seem to favor the view that the vasoactive property is not the only factor for the inhibition of absorption of cationic drugs.

To reinforce the impression given by this data, the time courses of both isonicotinamide and A.B.O.B. clearance from the muscle under the condition in which two drugs presented together in the same injection solution were investigated. Figure 5 indicates that the presence of A.B.O.B. at the concentration of 50 mM has no significant effect on the absorption of isonicotinamide, each being absorbed by the apparent first-order process and to its usual extent. Then it would be proved that the slow absorption of cationic drugs was due not only to the vasoactive property, but also to the uptake nature of drugs into muscle tissue or other factors.

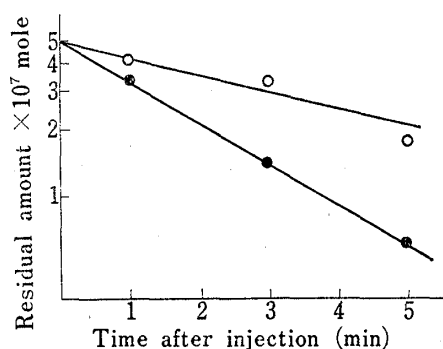


Fig. 5. Clearance Curves for A.B.O.B. and Isonicotinamide from the Same Solution

—○—: A.B.O.B.
—●—: isonicotinamide

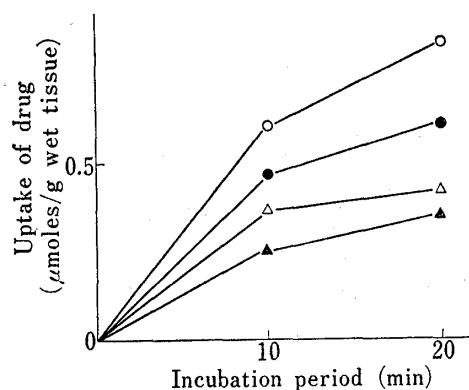


Fig. 6. Uptake of Drugs into the Muscle Tissue

—○—: metoclopramide
—●—: procainamide
—△—: isonicotinamide
—▲—: p-aminobenzoic acid

Uptake of Drugs into Muscle Tissue

In order to clarify the possible inhibiting factors other than vasoactive property, the uptake of cationic, anionic and neutral drugs were compared. In Figure 6 the uptake patterns of procainamide, metoclopramide, isonicotinamide and P.A.B.A. are demonstrated. Both the cationic drugs, procainamide and metoclopramide, were highly incorporated into muscle tissue compared with neutral or acidic drugs. In the case of monovalent cation, its permeability has been investigated in detail, suggesting that inorganic cations permeate more easily than inorganic anions through muscle fiber membrane by some electrically neutral mechanism.¹⁴⁾ This report indicates the possibility of high permeability of organic cation through the muscle fiber membrane. From this standpoint, it can be considered that the uptake of cationic drugs into muscle tissue may inhibit the transport into the capillary.

It seems reasonable to conclude from these results that the comparatively slow intramuscular absorption of cationic drugs is mainly due to the vasoactive property and uptake nature into muscle tissue of the cationic drugs.

14) S. Hagiwara, K. Toyama, and H. Hayashi, *J. General Physiol.*, **57**, 408 (1971).