

## Absorption of Drugs from the Skeletal Muscle of the Rats. V.<sup>1)</sup> Effect of Potassium Ion on the Absorption of Some Anionic Drugs

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(Received August 2, 1973)

Effect of potassium ion on the intramuscular absorption of some drugs has been examined using the rat thigh muscle clearance method. In the presence of  $K^+$  ( $<200$  mM), a specific and reversible inhibition was noted in the absorption of anionic drugs such as isonicotinic acid, PABA, and sulfa drugs. This inhibition of anionic drug absorption produced by  $K^+$  was reversed by DNP and KCN, but was not abolished by ouabain. This inhibition was minimized by the addition of EDTA. Extracellular  $K^+$  of high concentration produced by anionic drugs may cause the increase of  $Ca^{++}$  entry into the muscle tissue, which inhibits the drug transfer in the muscle by some unknown pharmacological actions.

The intramuscular absorption experiment of Kakemi, Sezaki, Okumura & Ashida<sup>3)</sup> has been used to examine the intramuscular absorption of neutral drugs<sup>3,4)</sup> and protonated drugs<sup>5)</sup> from aqueous solutions. These results demonstrated that neutral drugs were absorbed mainly by simple diffusion through the muscle fiber and capillary wall. In the case of cationic drugs, it was demonstrated that these drugs were absorbed by the complex features involving concentration dependency, vasoactive reaction, and uptake into muscle tissues. However, few studies have been carried out of parenteral absorption of anionic drugs although their absorption mechanism is remained unknown.

In the present study, an attempt has been made to investigate the effect of  $K^+$  on the anionic drug absorption by observing absorption changes produced in various conditions.

### Experimental

**Materials**—All the drugs listed in Table I, sulfisomidine, and the reagents were analytical grade and were obtained commercially.

**Procedure of Absorption Experiments**—Male Wistar albino rats weighing 140 to 180 g were used for animal experiments. Injectable solutions except sulfisomidine solution were prepared isotonicity and adjusted at pH 7.0 with dibasic sodium phosphate-sodium biphosphate buffer. Sulfisomidine was dissolved in citric acid-dibasic sodium phosphate buffers isotonicity for various pH solutions. In the case of  $K^+$  containing buffer, sodium salts were replaced with potassium salts in various ratio. The injection, incision, ligation, and muscle removal techniques were the same as used in the previous report in this series.<sup>3)</sup> The amount of drug remained in the removed muscle was analysed in order to obtain the absorption rate. For the estimation of drug uptake into muscle tissue, previously described method<sup>4)</sup> was used.

**Estimation**—The spectrophotometric or spectrofluorometric determinations were applied to all drugs investigated. Isonicotinic acid, isoniazid, and isonicotinamide were analysed by the method of Nielsch & Giefer.<sup>6)</sup> *p*-Aminobenzoic acid (PABA), procainamide, and sulfisomidine were determined by the regular diazotizing method. For the determination of streptomycin, the spectrofluorometric assay method described by Faure & Blanquet<sup>7)</sup> was used.

- 1) Part IV: K. Okumura, H. Sezaki, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **20**, 1607 (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, and H. Kobayashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 443 (1972).
- 5) K. Okumura, H. Sezaki, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **20**, 1607 (1972).
- 6) W. Nielsch and L. Giefer, *Arzneimittel Forschung*, **9**, 636 (1959).
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## Result and Discussion

In the previous paper,<sup>3)</sup> it was demonstrated that the intramuscular absorption characteristics of isonicotinic acid was very complex and detailed experiments to clarify the specificity of the absorption mechanism of anionic drugs was deemed necessary. Among the specific mechanisms of anionic drug absorption, the effect of  $K^+$  is a very complicated problem. In Table I is shown the effect of  $K^+$  on the intramuscular absorption of various drugs from aqueous pH 7 solutions. The absorption of isonicotinic acid and PABA were diminished in the presence of 100 mM  $K^+$ . Whereas the absorption of neutral or cationic drugs were hardly affected at all, which suggested the possibility that the decreasing tendency in the intramuscular absorption caused by  $K^+$  was specific for anionic drugs.

TABLE I. Parenteral Absorption of Drugs in the Presence of  $K^+$  a)

Drug	Absorption within 3 min (%)	
	Control	In the presence of $K^+$
Isonicotinic acid	73.6 ± 9.7	28.4 ± 9.6
PABA	66.5 ± 5.1	28.5 ± 13.2
Isonicotinamide	78.0 ± 4.1	74.3 ± 5.6
Isoniazid	66.9 ± 4.8	69.3 ± 7.8
Procainamide	35.5 ± 14.5	29.5 ± 10.7
Streptomycin	35.2 ± 15.1	43.4 ± 8.9

drug concentration 50 mM, injection volume 10  $\mu$ l

a)  $K^+$  concentration 100 mM

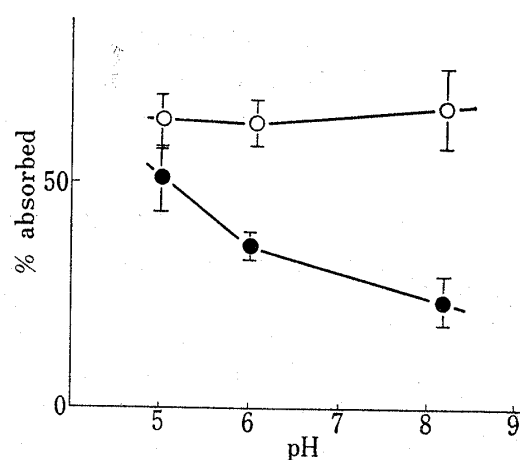


Fig. 1. Intramuscular Absorption vs. pH Profile of Sulfisomidine

—○—: absorption in three minutes from  $K^+$  deficient solutions; —●—: absorption in three minutes from 100 mM  $K^+$  containing solutions; Ten  $\mu$ l of 50 mM drug solution was injected. Each point represents the mean value of at least five experiments. Vertical bars indicate S.D.

In order to reinforce the impression given by the data presented in Table I, the absorption of sulfisomidine was examined at various pH's. Sulfisomidine is mostly anionic above pH 8, as its  $pK_a$  value is 7.3.<sup>8)</sup> As shown in Fig. 1, in the absence of  $K^+$ , there is no difference in the intramuscular absorption of sulfisomidine from solutions of various pH's ranging from pH 5 to pH 8.2. On the other hand, the decrease of absorption at pH 8.2 in the presence of  $K^+$  was noted which can better be explained by the anionic form of the drug. These findings suggest that low absorption rate of anionic drug in the presence of  $K^+$  could be attributed to inhibition of anion absorption. Mayersohn & others,<sup>9,10)</sup> reported that  $K^+$  produced a marked reduction of passive solute transfer across the everted rat intestine. In their experiments, however, transfer inhibition produced by  $K^+$  correlated with molecular weights of solute molecules and unrelated with their charge. It is therefore reasonable that the transfer inhibition process in the intestine is different from that in the muscle.

In order to confirm the  $K^+$  effect on absorption, time courses of anionic or neutral drug clearance in the presence of  $K^+$  was investigated. The results are shown in Fig. 2.

8) E. Krüger-Thiemer and P. Bünger, *Proceedings of the European Society for the Study of Drug Toxicity Meeting*, 6, 185 (1965).

9) H. Karaki, S.S. Ganeshanandan, M. Ikeda, and N. Urakawa, *Jap. J. Pharmacol.*, 19, 569 (1969).

10) N. Urakawa, H. Karaki, and M. Ikeda, *Jap. J. Pharmacol.*, 20, 360 (1970).

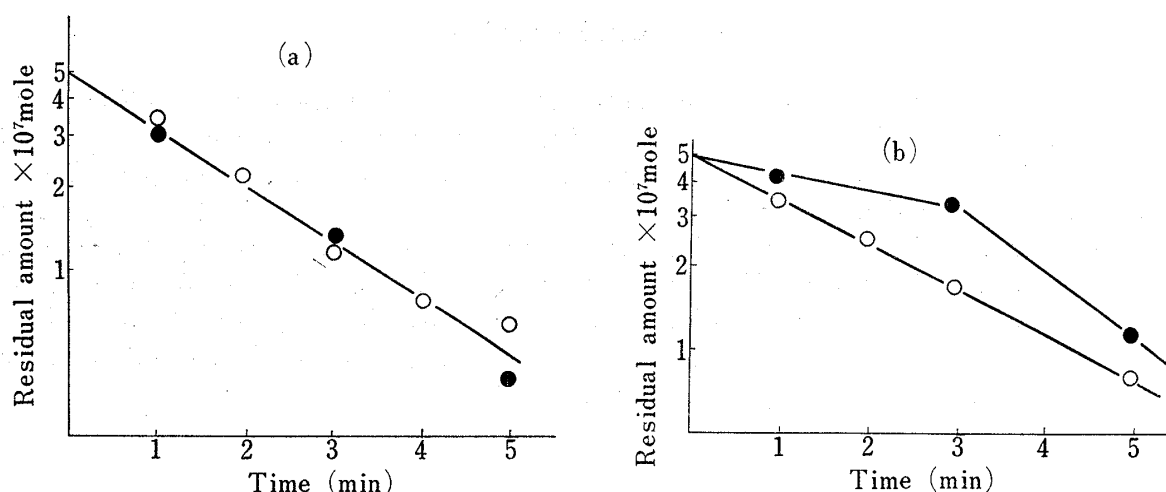


Fig. 2. Clearance Curves for Isonicotinamide (a) and Isonicotinic Acid (b)

—○—:  $K^+$  deficient solution; —●—: 100 mM  $K^+$  containing solution; Ten  $\mu$ l of 50 mM drug solution was injected.

The clearance curve of isonicotinic acid from the rat muscle in the presence of  $K^+$  is different from that in the absence of  $K^+$ . Comparatively slow absorption rate and curved line indicate that absorption inhibition is reversible in nature. Concomitantly, in the case of isonicotinamide there is no difference in absorption between  $K^+$  containing buffer and  $K^+$  deficient buffer. These results may be explained by the rapid exchange of  $K^+$  through the muscle membrane. The rapid exchange or absorption of  $K^+$  into muscle cells was further confirmed by an experiment in which the preinjection of isotonic KCl solution caused no inhibition of isonicotinic acid absorption. These results support the view that extracellular  $K^+$  may act a major role in the inhibition process. Karaki, Ganeshanandan & others demonstrated<sup>9)</sup> that high  $K^+$  in the incubation medium caused an increase in muscle tension and in  $Ca^{++}$  uptake into muscle tissue of guinea pig *taenia coli*. In this limited sense, it was expected that extracellular  $K^+$  of high concentrations may cause some pharmacological response which causes the inhibition of the drug absorption.

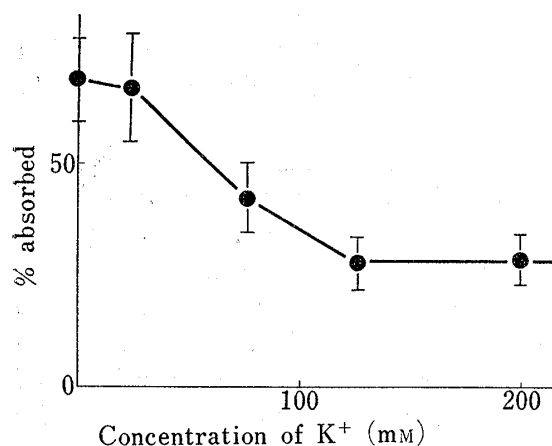


Fig. 3. Effect of  $K^+$  Level on Isonicotinic Acid Absorption at the End of Three Minutes

Ten  $\mu$ l of 50 mM drug solution was injected. Each point represents the mean value of at least five experiments. Vertical bars indicate S.D.

The effect of  $K^+$  concentration on the inhibition of isonicotinic acid absorption is shown in Fig. 3. The percentage absorption of isonicotinic acid decreased as  $K^+$  concentration increased and reached a constant value over 100 mM. In the following experiments, therefore, 100 mM was chosen as  $K^+$  concentration of injectable solutions since maximal inhibition of absorption was achieved at this concentration.

In our previous paper,<sup>3)</sup> it was noticed that the percentage absorption in three minutes of isonicotinic acid increased as its initial concentration increased. This concentration dependency of anionic drug absorption was examined in the presence or absence of  $K^+$ . However, as is evident from Fig. 4, increased absorption of isonicotinic acid and PABA at high drug concentration in the presence of  $K^+$  is similar to that in the absence of  $K^+$ . This

suggests that the inhibition process produced by  $K^+$  and self-acceleration or concentration-

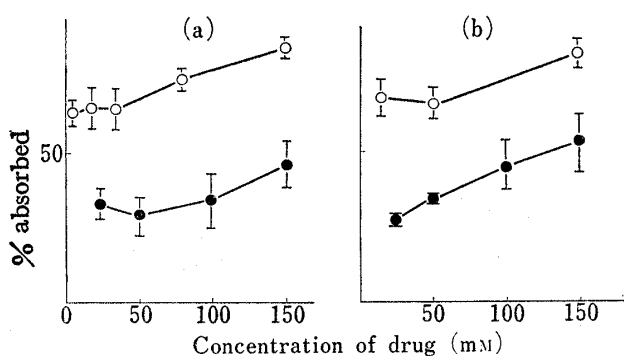


Fig. 4. Effect of Initial Concentration on the Absorption of Isonicotinic Acid (a) and PABA (b)

For key see Fig. 1.

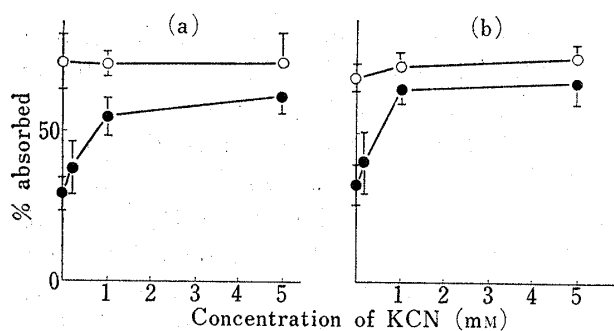


Fig. 6. Effect of KCN on the Absorption of Isonicotinic Acid (a) and PABA (b)

For key see Fig. 1.

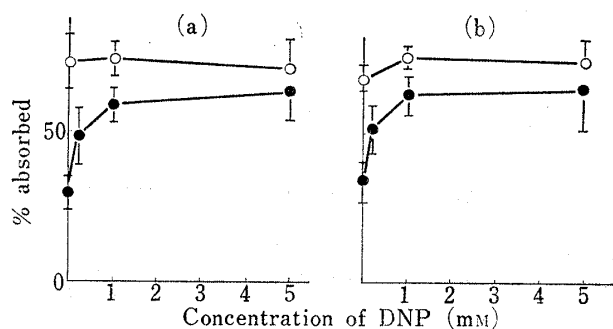


Fig. 5. Effect of DNP on the Absorption of Isonicotinic Acid (a) and PABA (b)

For key see Fig. 1.

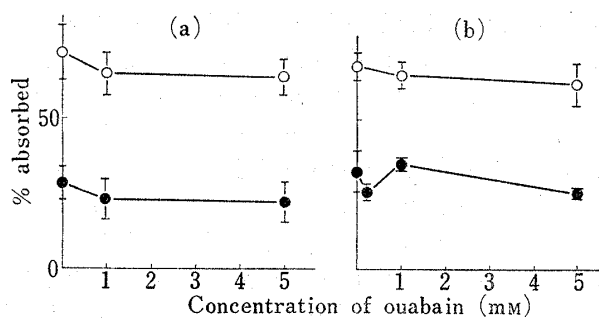


Fig. 7. Effect of Ouabain on the Absorption of Isonicotinic Acid (a) and PABA (b)

For key see Fig. 1.

dependent process of anionic drug absorption are independent. Furthermore, uptake nature of anionic drugs into muscle in  $K^+$  containing buffer was investigated using isonicotinic acid and PABA. As  $K^+$  did not alter the uptake of these drugs, possibility that inhibition of uptake results in the transport inhibition in the presence of  $K^+$  was ruled out.

To elucidate the possibility that an active metabolic process was mediated in the inhibition of anionic drug absorption, effect of metabolic inhibitors on anionic drug absorption was investigated as shown in Figs. 5, 6, and 7. As shown in the figures, it was found that 2,4-dinitrophenol (DNP) and KCN could reverse the reduced absorption rate of isonicotinic acid and PABA caused by  $K^+$ , although they did not affect the absorption of anionic drugs from the  $K^+$  deficient buffer. It is worthy to note that ouabain failed to reverse the inhibited anionic drug absorption. It is well-known that DNP and KCN are the inhibitors of oxidative phosphorylation and ouabain is the inhibitor of ATPase. From the facts described above, we may conclude that the inhibition of anionic drug absorption caused by  $K^+$  could possibly be related to energy-rich phosphate compounds. It was previously reported that the increase in both  $Ca^{++}$  influx and tissue  $Ca^{++}$  of the muscle in high  $K^+$  solution were abolished by DNP, but were not affected by ouabain.<sup>10)</sup> Therefore, the possibility exists that some of the stages of inhibitory process in the anionic drug absorption caused by  $K^+$  are limited by  $Ca^{++}$  influx or other active process.

The attempt was made to examine the effect of  $Ca^{++}$  on the intramuscular absorption of anionic drug in order to account for the above possibility. In Fig. 8 are shown the effects of  $Ca^{++}$  and ethylenediaminetetraacetic acid (EDTA) on the intramuscular absorption of PABA. Addition of  $Ca^{++}$  to the injectable solution caused a remarkable decrease in the absorption of

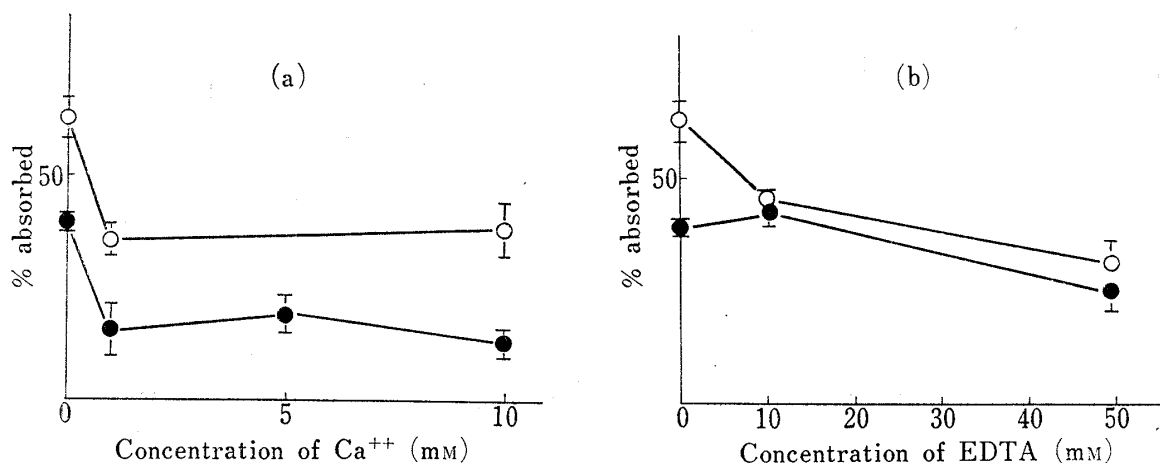


Fig. 8. Effect of Ca<sup>++</sup> (a) and EDTA (b) on the Absorption of PABA

For key see Fig. 1.

PABA from both K<sup>+</sup> containing and K<sup>+</sup> deficient buffer solutions simultaneously. The same result was obtained in the absorption of isonicotinic acid. Therefore, Ca<sup>++</sup> in the extracellular space is considered to be an important factor in the inhibition of the drug absorption because of the contraction of muscle or other responses. Further investigation was made for the effect of endogenous Ca<sup>++</sup> in the extracellular space of the muscle as shown in Fig. 8b.

The absorption of PABA in K<sup>+</sup> containing buffer solution was not significantly different from that in K<sup>+</sup> deficient buffer solution in the presence of EDTA. It could be anticipated that the decrease of endogenous Ca<sup>++</sup> due to the addition of EDTA reduces the difference of absorption rate of PABA from the two buffer systems. Same results were observed in the case of isonicotinic acid absorption. From these experiments, it can be postulated that the inhibition of the absorption of these drugs due to K<sup>+</sup> may be the results of increasing Ca<sup>++</sup> influx caused by K<sup>+</sup>.

The reason why the absorption is inhibited by K<sup>+</sup> only in the case of anionic drug is not certain yet. As reported by Ginzburg,<sup>11)</sup> K<sup>+</sup> loss from the cells produced by anionic compounds resulted in high K<sup>+</sup> concentration in the extracellular space, which might inhibited the drug transfer in the muscle. This problem should be made clear in the future work.

11) M. Ginzburg, B.Z. Ginzburg, and D.C. Tosteson, *J. Membrane Biol.*, **6**, 259 (1971).