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Absorption and Excretion of Drugs. XXXIX. The Absorption of Isonicotinic Acid Derivatives from the Skeletal Muscle of the Rats[1]

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Aqueous solutions of seven isonicotinic acid derivatives were injected to the hind leg of rats and the intramuscular absorption was followed by the local clearance method for a period of 5 min under the conditions in which the constant body temperature and smaller injection volume were maintained. Drug absorption is proportional to the amount remaining in the injection site, and both molecular weight and partition coefficient of drugs affect the intramuscular absorption. The observation presented here supported the conclusion that (1) the absorption of unionized drugs from the injection site was chiefly proceeded by the apparent first order process and (2) the diffusion through the pores of the capillary vessels was predominant compared with the penetration through the capillary endothelial cells. In the case of ionized drugs, there was evidence that the absorption of a drug was influenced by the factor other than the above.

In spite of the fact that parenteral route of drug administration is a useful one for the preclinical screening and the clinical use in human and veterinary medicine, the complete mechanism of drug absorption from various forms of parenteral preparation is still remained unknown.[3] Few studies have been carried out in the rat of parenteral absorption. Schou reported that the experimental method of choice is to record the clearance of injected drug from the local area.[4] Recently, selecting benzyl alcohol as a model drug, Ballard studied subcutaneous drug absorption kinetics in the rat.[5] What appears to be conspicuously absent is an attempt to study basic factors affecting the absorption rates of drugs administered parenterally with well-defined conditions.

The primary purpose of this work was to develop such experimental method and to investigate factors affecting drug transport from the injection site to the capillary vessels and lymphs. The authors have chosen seven isonicotinic acid derivatives for model compounds. Intramuscular absorption process of these compounds was investigated using modified local clearance method. In some cases, time courses of drug absorption were followed varying such factors as initial concentration and volume of injection solutions with special reference to the early stage of drug clearance. Some correlation data on the physical chemical properties of compounds and absorption characteristics are also introduced.

Experimental

Materials——Isonicotinic acid derivatives used in this report are listed in Table IV and Table V. Isoniazid(INH), isonicotinic acid, isonicotinamide, and methyl isonicotinate were obtained from commercially

1) Presented in part to the 17th Annual Meeting of the Kinki Branch, the Pharmaceutical Society of Japan, Nishinomiya, Nov. 1967, and to the 88th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1968.

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

3) *a*) J. Schou, *Pharmacol. Rev.*, **13**, 441 (1961); *b*) J. G. Wagner, *J. Pharm. Sci.*, **50**, 383 (1961); *c*) B. E. Ballard, *J. Pharm. Sci.*, **57**, 357 (1968).

4) R. B. Sund and J. Schou, *Acta Pharmacol. Toxicol.*, **21**, 313 (1964).

5) B. E. Ballard and E. Menczel, *J. Pharm. Sci.*, **56**, 1476 (1967).

available sources, and glucose-INH, lactose-INH were prepared according to the method described by Zinner.[6] Dextran-INH was also prepared from INH and oxidized dextran which was synthesized by the modification of Kobayashi's method.[7] All other materials were of analytical grade.

Procedure of Absorption Experiments——The incision and ligation technique of Schou[4] was adopted. Male Wistar arbino rats weighing 140—180 g were anesthtized with pentobarbital (5 mg per 100 g body weight, intraperitoneal administration) and maintained under anesthesia for entire course of experiment. The temperature in the rectum was measured by the electric themometer, and kept constant ($36 \pm 0.3°$) by laying rats on the hot plate regulated at 36° and by lamping during the absorption experiment. After loose ligation aroundthe *m. extenser quadriseps femoris*, a thin needle (Hamilton N-731) connected to micrometer syringe (Hamilton 702 or 705) was inserted some few mm above the *ligamentum patellae* and the top of the needle was led to the center of the *m. rectus femoris*. Then the sample solution (5—20 μl) was injected. At the end of the absorption period, two ligatures were tied and the muscle was removed. Residual amount of drug in the removed muscle was analyzed for the absorption studies. At least five rats were used for one experiment. The leakof drug solution was demonstrated to be negligible.

Preparation of Injectable Solutions——In order to adjust the drug solutions at pH 7.0, phosphate buffer solution consisted of NaH_2PO_4 and Na_2HPO_4 was used but for the case of isonicotinic acid. In the case of isonicotinic acid, equimolar NaOH and phosphate buffer soltuion were used. The osmotic pressures of the solutions were regulated at isotonic range by adding NaCl. In the case of ionized drug, the osmotic pressure was checked with Mechrolab vapor pressure osmometer model 301A. As the drug concentration, 50 mM was employed for usual case. All derivatives except dextran-INH did not reveal any appreciable decomposition under the experimental conditions. Stability of dextran-INH in the phosphate buffer solution was measured by the method of Fujiwara.[8]

Analytical Methods——The removed muscle was minced with scissors and after adding some portion of water, homogenized with Potter–Elvehjem glass homogenizer. Centrifuging at 3000 ***g*** for 30 min, isonicotinamide or isonicotinic acid in the supernatant was determined by the method of Nielsch.[9] In the case of INH derivatives, drugs in the supernatant were oxidized to isonicotinic acid by adding bromine water, then sufficient $NaNO_2$ was added and determined as described above. As dextran-INH decomposed to INH in water considerably, the experimental data of dextran-INH ($6.4 \pm 2.8\%$) was corrected by subtracting the decomposed INH absorption. The effects of viscosity and physiological property of dextran were also corrected using the absorption data of INH in the presence of dextran. For the determination of methyl isonicotinate, each whole homogenate was heated in boiling water with 1 ml of 1N NaOH for 1 hr. At the end of the hydrolysis, these samples were neutralized with HCl solutions and centrifuged, then resulting isonicotinic acid was determined similarly. In these methods the recoveries of drugs from muscles were more than 95%.

Results and Discussions

Effect of Body Temperature

In contrast with the case of unanesthetized rat, body temperature of anesthetized rat varies with the enviromental temperature and is one of the factors which can significantly alter the circulation of the exposed muscle. So it was assumed to be indispensable to demonstrate the effect of body temperature on muscular absorption for the establishment of animal experiments. Figure 1 shows the intramuscular absorption of isonicotinamide and isonicotinic acid within 3 min at different rectal temperatures. Increasing tendency of absorption with ascending rectal temperature was observed in both cases. It was previously reported that local cooling was used in order to prolong the action of the drug which was parenterally administered,[10] and the clearance rate of intramuscularly administered $Na^{24}Cl$ from the humstring muscles of dogs decreased compared to controls when an ice–salt mixture was applied over the injection site.[11] Recently, Hyman reported the effects of the local temperature on the clearance rate of water blue from micro–injected sites in living rat muscle and established a definite quantitative relationship between clearance rate and local temperature from the

6) H. Zinner and W. Bock, *Chem. Ber.*, **89**, 1124 (1956).
7) T. Kobayashi and Y. Tsukano, *Nippon Nogei-Kagaku Kaishi*, **27**, 33 (1953).
8) H. Fujiwara, *Yakugaku Zasshi*, **78**, 1034 (1958).
9) W. Nielsch and L. Giefer, *Arzneimittel Forsch.*, **9**, 636 (1959).
10) M. Trumper and A. M. Hutter, *Science*, **100**, 432 (1944); *idem*, *J. Am. Med. Assoc.*, **130**, 627 (1946).
11) F. R. Franke, J. B. Boatman, and R. George, *Proc. Sci. Exptl. Biol. Med.*, **74**, 417 (1950).

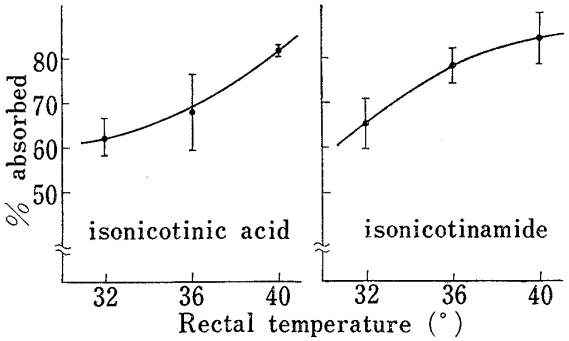

Fig. 1. Effect of Rectal Temperature on Parenteral Absorption

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

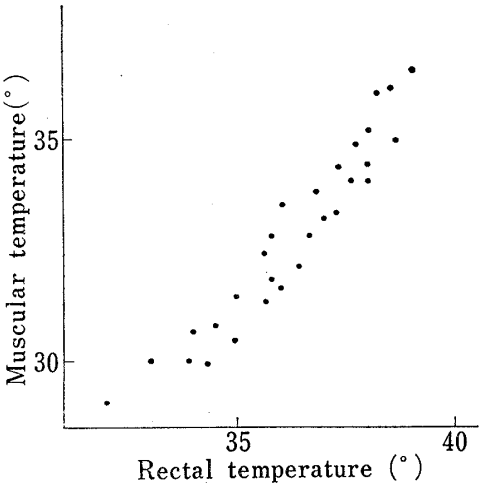

Fig. 2. Correlation between Muscular Temperature and Rectal Temperature

These points indicate the results of three rats.

Arrhenius plot.[12] It is obvious that microcirculation in the true capillary is a tremendously difficult problem for the studies of drug clearance from the muscle or the connective tissue and is affected by the local temperature. From this result it is considered that the severe control of the local temperature is necessary for the animal experiment. As good correlation between muscular temperature which was measured by needle type thermistor thermometer and rectal temperature was observed as shown in Fig. 2, the rectal temperature instead of the muscular temperature of the animal was regulated at 36±0.3° in the following experiments.

Effect of Injection Volume

The effect of injection volume on the parenteral absorption is a weighty problem to establish the experimental condition and to solve the absorption mechanism. Figure 3 shows the time courses of isonicotinamide clearance from the muscle under the condition in which two different injection volumes were used. That two straight lines with a same slope were obtained within 3 min indicates the similarity of absorption processes. In Table I the relationship between absorption and injection volume of isonicotinamide and isonicotinic acid is shown. No significant difference is observed in this range of injection volume. According to the papers of Warner[13] and Schou,[4] an increase in the rate of clearance corresponding to a decrease in volume was noted. But these authors used larger volumes compared with our experiments. Our results suggest that the drug diffusion from the middle of the depot to the outer layer is not a limiting factor and the physiological alteration caused with volume change is negligible. It is also suggested that relative absorbing area may not vary in the case of small injection volume ranging from 5 to 20 μl. So it is proper to inject the drug solution within 20 μl for this animal experiment.

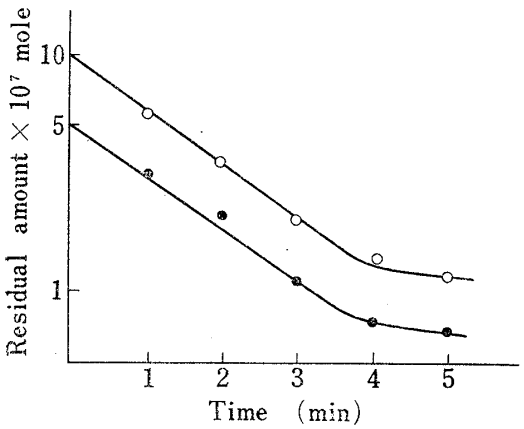

Fig. 3. Effect of Injection Volume on Clearance Rates of Isonicotinamide

—○—: 20 μl —●—: 10 μl

12) C. Hyman and R. Paldino, *Circulation Research*, **10**, 89 (1962).
13) G. F. Warner, E. L. Dobson, N. Pace, M. E. Johnston, and C. R. Finney, *Circulation*, **8**, 732 (1953).

Table I. Effect of Injection Volume on Parenteral Absorption

Drug	Injection volume (μl)	Number of experiment	% absorbed[a] ±S.D.
Isonicotinic acid[b]	5	6	60.2±1.4
	10	6	64.9±6.2
	20	5	64.2±7.1
Isonicotinamide[c]	5	5	74.0±7.8
	10	5	78.0±4.1
	20	5	80.2±6.4

a) experimental period: 3 min *b*) drug concentration: 34 mM *c*) drug concentration: 50 mM

Absorption of One Drug in the Presence of Another

In order to determine whether there is some local vascular effect exerted by the drug, experiments were performed in which two drugs were studied simultaneously. The data of Table II indicate that the presence of one drug had no significant effect on the absorption of the other, each being absorbed to its usual extent.

Table II. Parenteral Absorption of One Drug in the Presence of a Second Drug

Drug 1	Drug 2	% absorbed within 3 min±S.D. Drug 1		Drug 2	
		Alone	In presence of drug 2	Alone	In presence of drug 1
Isonicotinamide	Isonicotinic acid	78.0±4.1	74.0±7.4	67.3±9.1	69.0±5.1
Methylisonicotinate	Lactose-INH	89.9±2.1	86.1±4.8	59.5±9.6	61.4±5.9

Time Courses of Drug Clearance

For the purpose of clarifying the mechanism of the parenteral absorption, time courses of drug clearance from the rat muscle were investigated. Figures 4 and 5 show the plots of logarithm of the amounts of isonicotinamide and isoniazid remaining in the muscle versus time after administration. Although slightly curved tendency was observed for 3—5 min

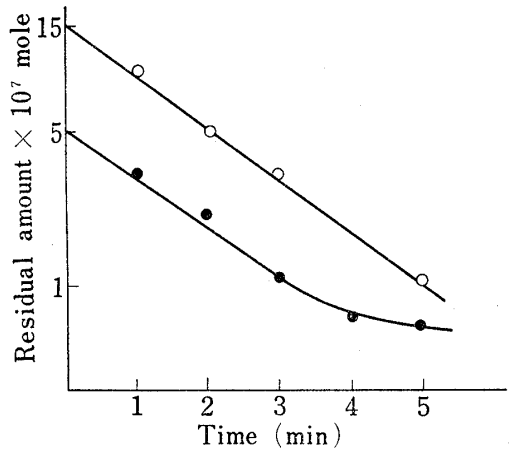

Fig. 4. Effect of Drug Concentration on Clearance Rates of Isonicotinamide

—○—: 150 mM —●—: 50 mM

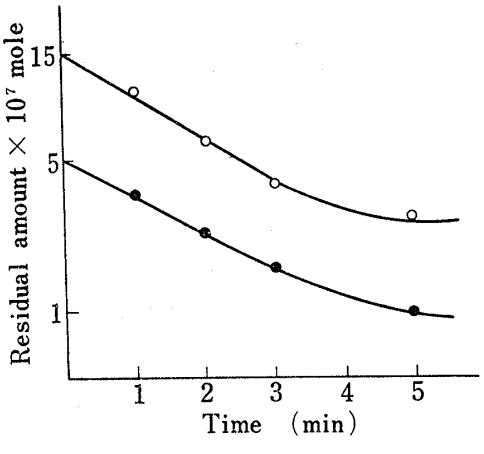

Fig. 5. Effect of Drug Concentration on Clearance Rates of Isoniazid

—○—: 150 mM —●—: 50 mM

after the administration, straight lines were obtained at least within 3 min. As a reason of slow absorption rates during 3—5 min after the administration, the effects of protein binding, local damage and other physiological factors were suggested. However, in our experiments,

this slow absorption process was neglected because of its small contribution to whole absorption process. Since more than 65% of administered dose was absorbed within 3 min in most of drugs, it is more appropriate to discuss the initial absorptive phase for the kinetical study, where first order process is predominant in the muscular absorption.

Effect of Initial Concentration

As shown in Fig. 4 and Fig. 5 the effect of initial concentration on parenteral absorption process was also examined. In both concentrations, 50 mM and 150 mM, straight lines with a same slope were obtained denying the concentration dependence in the muscular absorption. For more exact demonstration on concentration independence, absorption of isonicotinamide and isoniazid within 3 min at various concentration was examined as shown in Table III.

Table III. Effect of Drug Concentration on Parenteral Absorption

Drug	Concentration (mM)	Number of experiment	% absorbed ± S.D.[a]
Isonicotinamide	17	5	72.2 ± 4.6
	50	6	78.0 ± 4.1
	250	6	74.1 ± 6.4
Isoniazid	17	5	61.7 ± 3.3
	50	6	66.9 ± 4.8
	150	6	74.2 ± 5.6
	250	5	71.7 ± 8.2

a) experimental period: 3 min

No significant difference was observed in this concentration range. Therefore, in the case of unionized drugs, such as isonicotinamide and isoniazid, there is no concentration dependence on the permeation of drugs from the injection site to the capillary vessels. From this viewpoint, it was suggested that the type of absorption of unionized drug is of passive nature and a specialized active mechanism is not mediating. In the previous paper of Teorell, it was assumed that the drug administered by extravascular route would disappear from the injection site according to the first order kinetics,[14] *i.e.*, the rate of release from the injection site is proportional to the amount remaining in the injection site. Some authors who produced data for the proof of this hypothesis, failed to make a straight line in the semi-logarithmic plot.[15] This is mainly due to the difficulty of measuring initial absorption rates correctly because of its rapid absorption. In the recent paper, Ballard demonstrated that subcutaneous absorption of benzyl alcohol is proceeded by first order process.[5] He could slow down the absorption rate by an unique technique, and could estimate exactly the initial absorption process, which is in good agreement with our result.

Effects of Partition Coefficient and Molecular Size

There appears to be much less known about absorption from various parenteral sites of administration than is known about absorption from the gastrointestinal tract. Partition properties and molecular weight, generally known to be influencing factors in the drug absorption process in the gastrointestinal tract, may also be affecting in this route of absorption. For this experiment, seven isonicotinic acid derivatives were selected and absorption for 3 min after injection was measured. These drugs have been selected because of their water solubility and a wide range of physical properties, as a group, including partition coefficient, diffusibility, and pK_a useful for this study. Since the initial process of the parenteral absorption was proved to be of passive nature, the effects of partition coefficient and molecular weight

14) T. Teorell, *Arch. Intern. Pharmacodyn.*, **57**, 205 (1937).
15) H. Shriftman and A. A. Kondritzer, *Am. J. Physiol.*, **191**, 591 (1957).

of drugs were primarily investigated to estimate the main route of drug absorption. The results of these animal experiments are summarized in Table IV and Table V. Table IV

Table IV. Effect of Partition Coefficient on Parenteral Absorption

Compound	Mol. wt.	pK_a[a]	Partition[b] coefficient	% absorbed ± S.D.
Isonicotinic acid	123.11	1.82 4.78	0	67.3 ± 9.7
Isonicotinamide	122.13	3.61	0.06	78.0 ± 4.1
Methylisonicotinate	137.15	1.72	102	89.9 ± 2.1

a) These values were obtained from Landoldt-Börnstein: "Zahlenwerte und Funktionen aus Physik, Chemie, Astronomie, Geophysik, Technik," Springer Verlag, II Band, 7 Teil (1960).
b) Partition Coefficient was measured between water and chloroform at pH 7.0.

Table V. Effect of Molecular Weight on Parenteral Absorption

Compound	Mol. wt.	pK_a[a]	Partition coefficient	% absorbed ± S.D.
INH	137.15	2.13 3.81 11.03	0	66.9 ± 4.8
Glucose-INH	299.3		0	68.1 ± 2.6
Lactose-INH	461.4		0	59.5 ± 9.6
Dextran-INH	55000		0	<3

a) These values were obtained from *Chem. Pharm. Bull.* (Tokyo), **11**, 797 (1963).

shows the effects of partition coefficient of drugs on muscular absorption. Although three drugs have similar molecular weight, the absorption rate rises with the increase of partition coefficient. This tendency, however, was not so conspicuous compared with other passive transfer of drugs such as into brain,[16] in the mammary gland,[17] and in the alimentary tract.[18] Table V shows the effects of molecular weight of drugs. In spite of the fact that these four INH derivatives have almost negligible partition coefficient to organic solvent, these drugs, except dextran-INH, were readily absorbed from the muscle. It is also clear that absorption tends to decrease with the increase of molecular weight of drugs, *i.e.*, lactose-INH is absorbed slightly slower than INH or glucose-INH and dextran-INH is hardly absorbed at all. This tendency agrees with the results which were reported by some authors.[19] In our result, the correlation between absorption rate and free diffusion coefficient in which molecular size is a major parameter is not demonstrated well in the range of these small molecular sizes. These results indicate that both diffusion constant and partition coefficient to organic solvent contribute to the parenteral absorption, and the former may act as a major role than the latter. The diffusion process through pores of the capillary wall and the partition process through the lipid component of the vascular endothelial cells were reported for the mechanism of capil-

16) L. C. Mark, J. J. Burns, L. Brand, C. I. Campomanes, N. Trousof, E. M. Papper, and B. B. Brodie, *J. Pharmacol. Exptl. Therap.*, **123**, 70 (1958).
17) G. E. Miller, N. C. Banerjee, and C. N. Stowe, *J. Pharmacol. Exptl. Therap.*, **157**, 245 (1967).
18) L. S. Schanker, P. A. Shore, B. B. Brodie, and A. M. Hogben, *J. Pharmacol. Exptl. Therap.*, **120**, 528 (1957); L. S. Schanker, D. T. Tocco, B. B. Brodie, and A. M. Hogben, *ibid.*, **123**, 81 (1958); K. Kakemi, T. Arita, and S. Muranishi, *Chem. Pharm. Bull.* (Tokyo), **13**, 861 (1965).
19) P. Kruhoffer, *Acta Physiol. Scand.*, **11**, 37 (1946); P. Malek, J. Kolc, M. Herold, and J. Hoffman, *Antibiot. Ann.*, **1957-1958**, 546; C, R. Beresford, L. Golberg, and J. P. Smith, *Brit. J. Pharmacol.*, **12**, 107 (1957); R. L. Paldino, and C. Hyman, *Am. J. Physiol.*, **210**, 576 (1966).

lary permeability,[20] though the former process was proved morphologically by only few authors.[21] But there is few work connected with the correlation of both processes, using a series of derivatives. In this limited sense, the diffusion process through pores of the capillary wall is dominant compared with the penetration process through the vascular endothelial cells in the case of muscular absorption of drugs having usual molecular weight. This tendency is slightly different from the gastrointestinal absorption.

Absorption of Isonicotinic Acid

In the case of gastro-intestinal absorption, organic anions permeated into the blood stream much faster than is supposed by their low lipid solubility.[22] Contrary to pH partition hypothesis, even some highly ionized cationic drugs are absorbed from the rat intestine according to the unpublished data of our laboratory. In the case of muscular absorption, entire picture of the absorption of ionized drugs is still not well understood. Absorption process of the anionic drug was demonstrated in Fig. 6, which shows the time courses of isonicotinic acid clearance from the rat muscle at different initial concentrations. Straight lines were obtained within 3 min in all cases, but the slope in the case of 150 mM is different from the others.

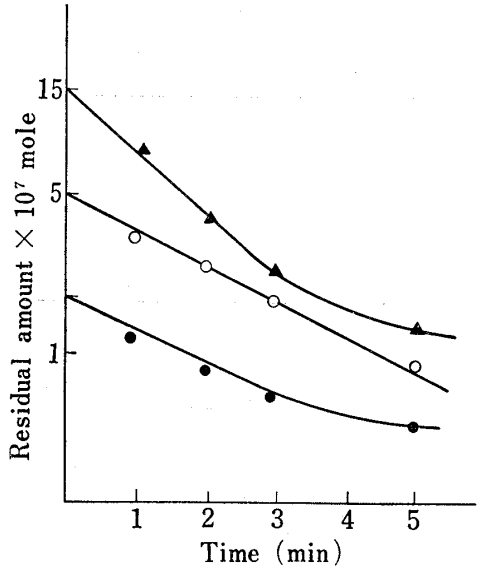

Fig. 6. Effect of Drug Concentration on Clearance Rates of Isonicotinic Acid

—▲—: 150 mM —○—: 50 mM
—●—: 17 mM

Table VI. Effect of Drug Concentration on Parenteral Absorption of Isonicotinic Acid

Concentration (mM)	Number of experiment	% absorbed ± S.D.
4	5	62.4±4.6
9	6	62.7±3.8
17	5	64.2±7.0
21	5	67.0±13.1
34	5	64.2±7.1
50	6	67.3±9.7
80	5	75.2±3.2
150	6	85.0±2.6

Table VI shows the absorption of isonicotinic acid within 3 min at various concentrations. No significant difference in absorption was observed under the concentration below 50 mM, but in the high concentration over a range of 80 mM to 150 mM increased absorption was observed. This result suggests that some specific absorption beside processes associated with unionized drugs may exist in the case of isonicotinic acid which was almost completely ionized at pH 7. This problem was not analyzed in this experiment and will be discussed in the following report.

20) J. R. Pappenheimer, E. M. Renkin and L. M. Borrero, *Am. J. Physiol.*, **167**, 13 (1951).
21) K. Mori and R. Ito, *Nagoya Med. J.*, **4**, 115 (1958).
22) J. H. Weatherby and R. W. Depuy, *Arch. Intern. Pharmacodyn.*, **135**, 127 (1962).