

**Absorption and Excretion of Drugs. XXXVI.¹⁾ Effect of Ca²⁺ on the
Absorption of Tetracycline from the Small Intestine. (I)**KIICHIRO KAKEMI, HITOSHI SEZAKI, HIROYASU OGATA,^{2a)}
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(Received March 23, 1968)

Effect of Ca²⁺ on the absorption of tetracycline was examined *in situ*, *in vitro*, and *in vivo*. It was found that tetracycline disappeared faster from recirculation solution (a) in the presence of small amounts of Ca²⁺ at pH 6.2 and (b) in the presence of Ca²⁺ irrespective of its concentration at pH 8.0. At pH 8.0, partition ratio (isoamyl alcohol/water) of tetracycline increased in the presence of Ca²⁺, exhibiting a similar pattern as that in recirculation experiments. Excretion data of tetracycline in man, however, showed that the antibiotic was less favorably absorbed in the presence of Ca²⁺. These results suggested that tetracycline chelated with metal ions would be bound to intestinal wall and that absorption would be inhibited in the presence of Ca²⁺.

Since tetracycline is widely used for the treatment of many bacterial infections, this series of studies have been initiated in order to elucidate some of the factors which might influence the absorption of this antibiotic.

It has been reported that physiological availability of tetracycline varies from one person to another when it is orally administered³⁾ and that food has a great influence on its absorption.⁴⁾

Although some attempts to increase its availability have been made by administering it with some adjuvants, no one seems to be successful in the attempt. Since it has been observed that the availability of tetracycline decreased to a great extent in the presence of Ca²⁺, it may be considered that both Ca²⁺ in food and endogenous Ca²⁺ play an important role in the absorption of tetracycline. Differences in absorption among individuals may arise from difference in the amounts of Ca²⁺ present in the intestine. The purpose of this series of studies is, thus, to obtain further information on the absorption of tetracycline from the intestine in the presence of Ca²⁺.

Experiments both *in situ* and *in vivo* were carried out at pH 6.2 and pH 8.0, because of minimal change in pH during experiment in the region. Further the pK_a of tetracycline and physiological condition in the intestine were taken into consideration in the choice of these pH values.

Experimental

Absorption Experiments in Rat Small Intestine—Male rats, weighing 130–160 g, were fasted overnight prior to the experiments but allowed free access to water. The animals were anesthetized with urethane (0.5 ml/150 g as a 30% solution) and the small intestine was cannulated for *in situ* recirculation. The intestine was first washed with about 50 ml of a 0.9% NaCl solution maintained at 37°, and then with 25 ml of test solution. After the test solution was forced out, the tubings attached to the inflow and outflow

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3) a) H. Welch, C.N. Lewis, A.W. Saffa, and W.W. Wright, *Antibiot. Med. Clin. Therap.*, **4**, 215 (1957);

b) H. Sibaki, Y. Kato, T. Kaide, S. Kitayama, H. Shibasaki, T. Chiba, M. Tomizawa, I. Nakayama, and R. Saito, *J. Antibiotics*, **13**, 207 (1960).

4) H.A. Hirsch and M. Finland, *Am. J. Med. Sci.*, **237**, 693 (1959).

cannulae were transferred to a flask containing 25 ml of test solution. This volume was then continuously circulated through the small intestine at the rate of 3–5 ml/min. Further experimental detail can be found in previous paper from this laboratory.⁵⁾

Test Solution—In order to eliminate complications which may arise from a buffer solution, a 0.9% NaCl solution rather than a buffer solution was employed to prepare a 400 $\mu\text{g/ml}$ tetracycline hydrochloride solution. Change in pH during recirculation experiments was corrected with either a HCl or NaOH solution. Phenol red was used as an indicator to correct any volume change.

Assay—Sample solutions were appropriately diluted, heated up in a water bath for 3 minutes on acidification with a 5 N HCl solution, and extracted with isoamyl alcohol. This extract was assayed spectrophotometrically at 435 $m\mu$, using a Shimadzu QV-50 spectrophotometer.

Partition Ratio Determination—Tetracycline hydrochloride solutions (20 $\mu\text{g/ml}$) containing varying amounts of CaCl_2 were prepared and the pH was adjusted with either a HCl or NaOH solution. The test solution were shaken with isoamyl alcohol in a constant temperature bath for 1 hour. An aliquot of the aqueous solution was withdrawn for tetracycline assay as described above. The partition ratio (p.r.) was calculated from the following equation;

$$\text{p.r.} = \frac{\text{Initial concn. in aqueous layer} - \text{Equil. concn. in aqueous layer}}{\text{Equil. concn. in aqueous layer}}$$

In each experiment was recorded the pH of aqueous layer in equilibrium.

Urinary Excretion Studies—Two hundred and fifty milligrams of tetracycline hydrochloride was dissolved in 100–150 ml of water with varying amounts of CaCl_2 and the solution was administered at least 2 hours before breakfast. Urine was collected at appropriate intervals and its tetracycline content was determined according to the procedure described earlier.⁶⁾

Results

Recirculation Experiments in Rat Small Intestine

Disappearance of tetracycline from the recirculation is illustrated in Fig. 1 and 2. Fifteen minutes were allowed for equilibration and 0 time in the figures was shifted to the point 15 min after the perfusion was started.

Relationship between disappearance percent of tetracycline and the ratio of concentrations of Ca^{2+} to those of tetracycline is shown in Fig. 3 and 4.

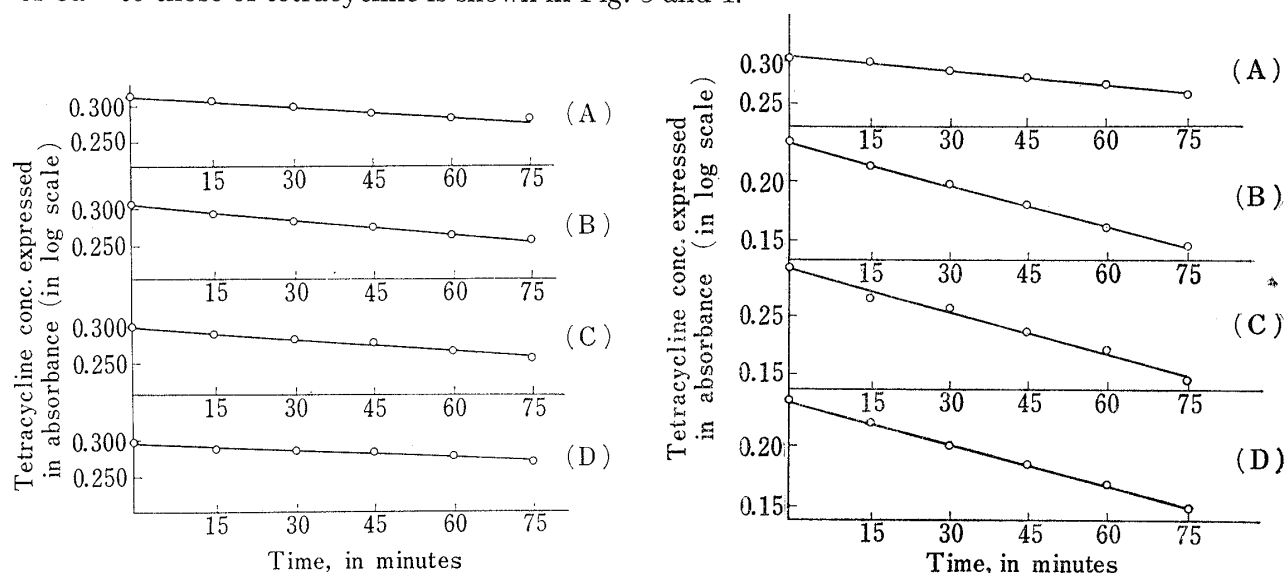


Fig. 1. Plots Showing Disappearance of Tetracycline (TC) from Recirculating Solution in the Presence of Varying Amounts of Ca^{2+} at pH 6.2

TC conc. = 400 $\mu\text{g/ml}$, Ca^{2+}/TC molar ratio = (A) 0, (B) 2, (C) 10, and (D) 20

Fig. 2. Plots Showing Disappearance of Tetracycline (TC) from Recirculating Solution in the Presence of Varying Amounts of Ca^{2+} at pH 8.0

TC conc. = 400 $\mu\text{g/ml}$, Ca^{2+}/TC molar ratio = (A) 0, (B) 2, (C) 10, and (D) 20

5) K. Kakemi, T. Arita, and S. Ohashi, *Yakugaku Zasshi*, **82**, 348 (1962).

6) K. Kakemi, T. Arita, H. Sezaki, and T. Nadai, *Yakugaku Zasshi*, **83**, 264 (1963).

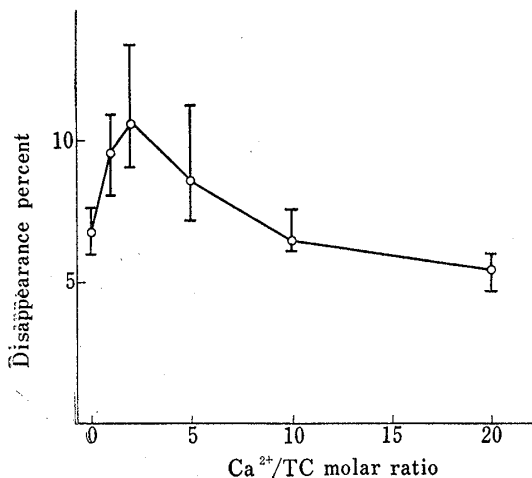


Fig. 3. Effect of Ca^{2+} on the Disappearance Percent of Tetracycline from Rat Small Intestine in 75 min at pH 6.2, Conc. of Recirculating Tetracycline Solution = 400 $\mu\text{g/ml}$

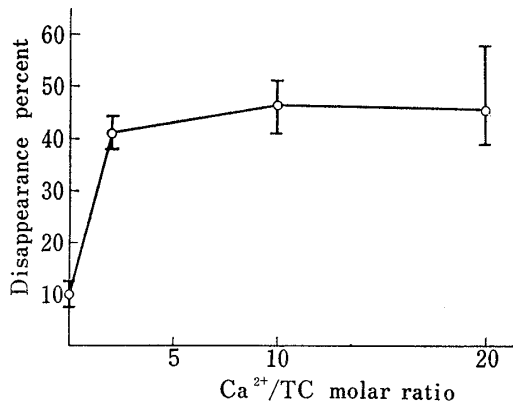


Fig. 4. Effect of Ca^{2+} on the Disappearance Percent of Tetracycline from Rat Small Intestine in 75 min at pH 8.0, Conc. of Recirculating Tetracycline Solution = 400 $\mu\text{g/ml}$

From these experiments at pH 6.2 it may be seen that the disappearance of tetracycline follows the apparent first order kinetics (Fig. 1), and that disappearance percent reaches its maximum at $\text{Ca}^{2+}/\text{tetracycline} \approx 2$ and it decreased gradually with further addition of Ca^{2+} (Fig. 3). Additional experiments carried out at pH 6.2 and with $\text{Ca}^{2+}/\text{tetracycline} = 2$ led to the observation that disappearance percent decreased to that without Ca^{2+} when twice as much EDTA as Ca^{2+} was added to the recirculating solution and that disappearance percent was not modified in the presence of 2,4-dinitrophenol ($2 \times 10^{-4}\text{M}$), which is believed to inhibit an active transport process.

Recirculation experiments at pH 8.0, on the other hand, indicated that disappearance percent increased steadily in the presence of Ca^{2+} , approaching a certain value on further addition of Ca^{2+} (Fig. 4).

Figures 5 and 6 illustrate the effect of concentration of tetracycline on absorption behaviors of tetracycline when $\text{Ca}^{2+}/\text{tetracycline}$ molar ratio was kept constant at 10. It can be seen that tetracycline disappears at a constant rate irrespective of its initial concentration so long as $\text{Ca}^{2+}/\text{tetracycline}$ molar ratio is constant with the exception of the initial parts of the experiments at pH 8.0.

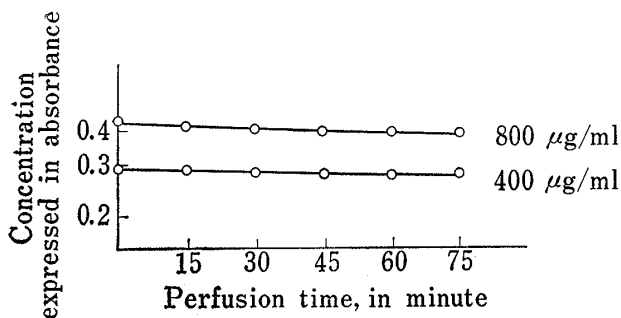


Fig. 5. Effect of Concentration on Disappearance of Tetracycline from Rat Small Intestine at pH 6.2

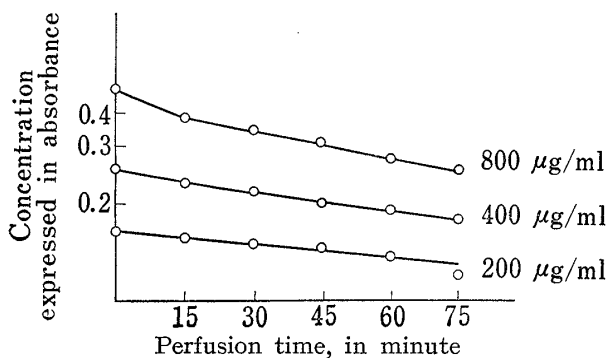


Fig. 6. Effect of Concentration on Disappearance of Tetracycline from Rat Small Intestine at pH 8.0

Figure 7 illustrates the results of the 2 repeated recirculation experiments in the same rat with 75 min perfusion in each experiment. There was no difference in disappearance rate between the two.

Effect of Ca²⁺ on the Partition Ratio of Tetracycline

Partition ratios of tetracycline between isoamyl alcohol and water in the presence of varying amounts of Ca²⁺ over the pH range between 2 and 10 are recorded in Fig. 8. It may be seen that tetracycline becomes fat soluble in the presence of Ca²⁺ in the alkaline region. The effect of Ca²⁺/tetracycline molar ratio on the partition ratio was studied at pH 8.0 (0.01 M Tris buffer) and is shown in Fig. 9. It is interesting to note that this curve is similar to that in Fig. 4. There seems to be some correlation between the partition ratio and the disappearance percent.

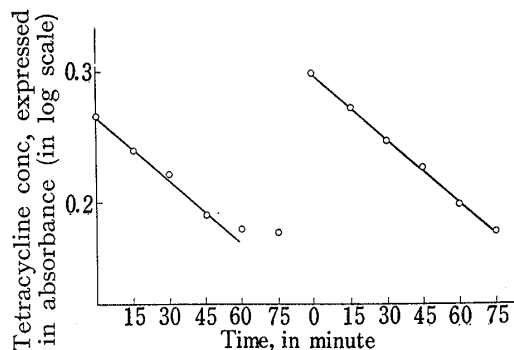


Fig. 7. Plots Showing the Tetracycline Disappearance from Rat Small Intestine in Recirculation Experiments in the Same Rat, TC conc. =400 µg/ml

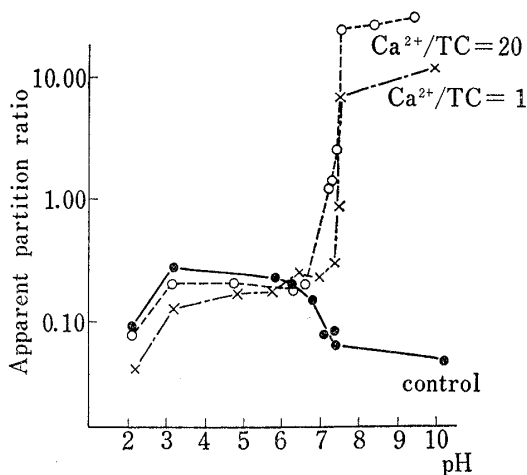


Fig. 8. Effect of Ca²⁺ on the Apparent Partition Ratio (Isoamyl Alcohol/Water) of Tetracycline in Various pH's at 37° conc. of tetracycline=20 µg/ml

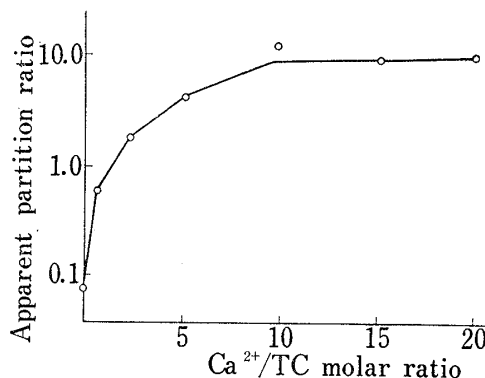


Fig. 9. Effect of Ca²⁺ on the Apparent Partition Ratio of Tetracycline at pH 8.0 and 37° conc. of tetracycline solution=100 µg/ml

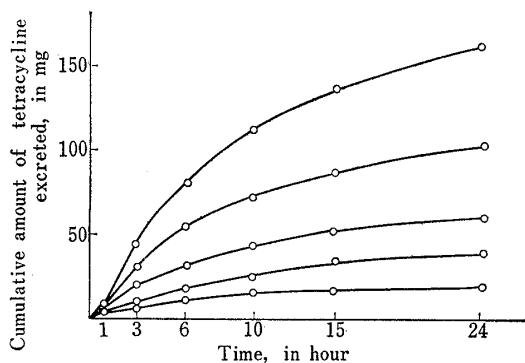


Fig. 10. Effect of Ca²⁺ on the Cumulative Excretion Curve of Tetracycline in Human dose=250 mg TC-HCl; Ca²⁺/TC molar ratio= from the top, 0,1,2,5, and 10

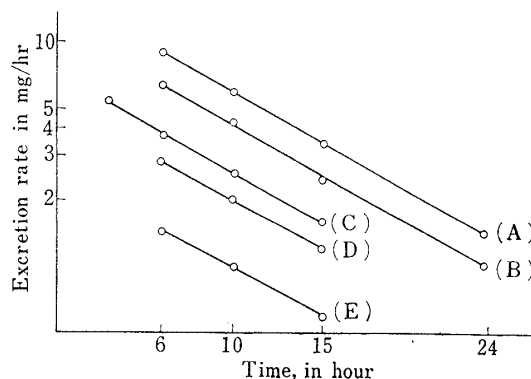


Fig. 11. Relationship between Excretion Rate of Tetracycline in Humans and Time in the Presence of Varying Amounts of CaCl₂ dose=250 mg TC-HCl; CaCl₂/TC molar ratio= (A) 0, (B) 1, (C) 2, (D) 5, and (E) 10

Partition studies at pH 6.2, on the other hand, did not exhibit any marked change in partition ratio with increasing amount of Ca^{2+} .

Effect of CaCl_2 on the Urinary Excretion of Tetracycline

The urinary excretion of tetracycline in man in the presence of varying amounts of CaCl_2 is shown in Fig. 10. Amounts excreted in the urine decreased with increasing amounts of CaCl_2 and it seems to approach a certain value on further addition of the salt. Data in

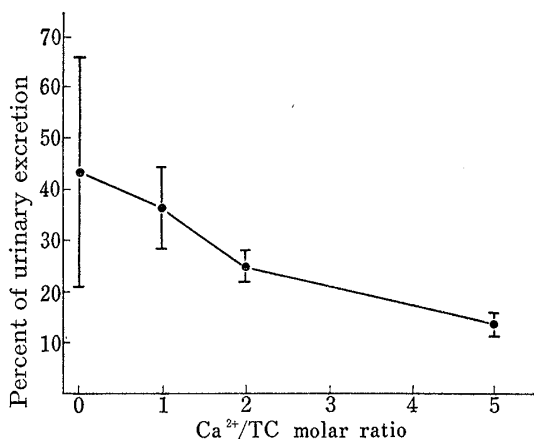


Fig. 12. Effect of Ca^{2+} on the Excretion of Tetracycline in Humans
dose=250 mg TC-HCl

Fig. 10 are pharmacokinetically treated⁷⁾ and are shown in Fig. 11. Parallel straight line relationships are obtained between the logarithms of excretion rate and time irrespective of Ca^{2+} /tetracycline molar ratio, suggesting that the biological half-life of tetracycline is constant.

The same type of experiments as shown in Fig. 10 were repeated on a large number of subjects and the results are summarized in Fig. 12, where vertical lines indicate deviation among individuals. As it is apparent from the figure, the urinary excretion of tetracycline decreased with increases in Ca^{2+} , variation among individuals diminishing at the same time.

Discussion

It is evident that Ca^{2+} plays an important role in modifying the disappearance percent of tetracycline from the intestine in the recirculation experiments. Chelate formation of tetracycline with divalent ions has been known⁸⁾. Physical properties of tetracycline may be expected to change greatly on chelation. Some of the results in recirculation experiments may best be interpreted on this basis. Results of partition studies suggest that tetracycline chelate is much more fat soluble than the parent antibiotic. Increase in fat solubility may parallel increase in disappearance percent.

Remarkable increase in the partition ratio between pH 7 and 8 (Fig. 8) may illustrate irregularity in the disappearance rate of tetracycline observed in the initial stage of experiments as depicted in Fig. 6. Because no buffer was employed in these experiments, there might have been relatively large change in pH during the initial stage of the recirculation. Schachter and Rosen⁹⁾ and others¹⁰⁾ suggested that Ca^{2+} would be absorbed by either active transport or facilitated diffusion mechanism as well as by passive transport process. Possibility that tetracycline may be actively transported along with Ca^{2+} may be neglected since dinitrophenol, which is known to inhibit active transport or facilitated diffusion, did not have any influence on the absorption behaviors of tetracycline in the presence of Ca^{2+} .

Thus the increase in disappearance percent with small amount of Ca^{2+} at pH 6.2 should be attributed to other origin. With either *in situ* or *in vitro* experiments alone one might erroneously conclude that administration of Ca^{2+} with tetracycline would increase the absorption of tetracycline from the intestine if its pH is around 8. Tonelli, *et al.*,¹¹⁾ however, reported

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- 8) J.T. Doluisio and A.N. Martin, *J. Med. Chem.*, **6**, 16 (1963).
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- 11) G. Tonelli, L. Alfano, and E. Takesue, *J. Pharmacol.*, **145**, 386 (1964).

that serum tetracycline concentration decreased in the presence of Ca^{2+} in their experiments in rat. Urinary excretion experiments in man also demonstrated that absorption of tetracycline decreased in the presence of Ca^{2+} . As it is shown in Fig. 11, the biological half-life of tetracycline was not affected in the presence of Ca^{2+} . Thus it is assumed that Ca^{2+} modify the absorption of tetracycline in the transport process across the gastrointestinal membrane rather than it changes the fate of tetracycline after the drug enters into the body fluids. Doluisio and Martin¹²⁾, and Kohn¹³⁾ have shown that tetracycline is bound to protein or barbital in the presence of divalent metal ions. The observation in the present work may be rationalized on this basis. Namely tetracycline may be bound to protein molecules present in the intestinal wall when CaCl_2 is given with it. The binding to intestinal wall seems to be rather strong, thus tetracycline will not permeate as it would otherwise do.

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