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# A Comparison of Bioavailability of Free Bases and Hydrochloride Salts of Chlortetracycline, Demethylchlortetracycline, and Methacycline[1]

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Gastrointestinal absorption of the free base and the hydrochloride of chlortetracycline (CTC) was studied in rats and human subjects. Following oral administration, the free base produced higher CTC levels in the body fluids than the hydrochloride. The difference between the bioavailability of the free base and that of the hydrochloride was due to the difference in the solubility characteristics at the gastric pH. Similar investigations were carried out with demethylchlortetracycline (DMCT) and methacycline (MOTC). The results indicated that plasma level after oral administration of MOTC base to rats was slightly higher than that of the hydrochloride and plasma levels of DMCT base and the hydrochloride were essentially identical. In the study comparing the cumulative amounts of MOTC base and the hydrochloride after oral administration to human subjects, the free base gave greater cumulative amounts than the hydrochloride in two of the three subjects.

In the previous paper,[3] it was confirmed that the free base of chlortetracycline (CTC), demethylchlortetracycline (DMCT), and methacycline (MOTC) was more soluble than the corresponding hydrochloride salt in the pH range of stomach (pH 1.2—1.4 for CTC and DMCT and pH 1.2—2.1 for MOCT). It was expected that such a change might affect the solubility characteristics of these antibiotics in the stomach, and as a consequence the bioavailability would be influenced.

Welch, *et al.*[4] reported that the chlortetracycline hydrochloride (CTC-HCl) produced somewhat lower serum concentration than did CTC base. However, systematic investigations on the solubility characteristics of the base and the hydrochloride and their influences on the absorption characteristics have not been made. Furthermore, the effect of solubility differences between the base and the hydrochloride of DMCT and MOTC on the bioavailability following their administration has never been reported.

It is the purpose of this report to examine the effect of solubility differences in the hydrochloric acid solutions between the base and the hydrochloride of these three tetracycline antibiotics on their bioavailability.

## Experimental

**Materials**——The sample of tetracycline antibiotics employed in this study was identical to that used in the previous work.[3] The MOTC (anhydrate) and DMCT base (contained 3/2 mole of water) were commercial products. The CTC-HCl (anhydrate), CTC base (contained 3/2 mole of water), and DMCT-HCl (anhydrate) were prepared by the methods described previously.[3] The DMCT-HCl (monohydrate) was obtained by the method of Origoni and Winterbottom.[5] The size of the crystal samples was not controlled in the absorption studies.

**Procedures for Absorption Studies**——a) Plasma Levels in Rats: Male Wistar rats, weighing 180—220 g, were fasted for 20—24 hr before the experiment. At the time of dosing, the fasted rats were lightly anes-

1) A part of this work was presented at 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April 1974.
2) Location: *Kita-12, Nishi-6, Kita-ku, Sapporo.*
3) S. Miyazaki, M. Nakano, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **23**, 1197 (1975).
4) H. Welch, W.W. Wright, and C.N. Lewis, *Antibiotics Medicine and Clinical Therapy*, **4**, 414 (1957).
5) V.E. Origoni and R. Winterbottom, U.S. Patent 3023239, Feb. 27, 1962, Appl. Dec. 9 (1959) [through *C.A.*, **56**, 14407*i* (1962)].

thetized with ether. The samples were administered orally to the rats by stomach tube as a suspension in 0.5 ml of aqueous solution of hydrochloric acid. The doses were 35 mg of CTC, 40 mg of DMCT, and 60 mg of MOTC; the pH values of suspension medium were 1.4 for CTC and DMCT and 1.6 for MOTC. In order to obtain uniform dispersion of crystal powder of MOTC, 0.1% carboxymethylcellulose was added to the suspension. The time between the addition of samples to the aqueous solutions and the administration was 2 min. The animals were sacrificed by decapitation and the plasma was separated immediately by centrifugation (2000 rpm).

b) Urinary Excretion in Human Subjects: Three healthy human subjects were used in this study. After an overnight fast, each subject was given orally CTC hydrochloride, 300 mg, in oblates with 20 ml of water. No food was permitted until 2 hr after drug administration. The urine was collected periodically 2, 4, 6, 8, and 10 hr following administration and the cumulative amount of CTC excreted in urine at each collection period was estimated. The cross-over test was carried out after one week; the 300 mg of the free base was administered to the same subject. A comparison of the urinary excretion of MOTC base and the hydrochloride was also carried out under the same conditions as mentioned above.

Both plasma and urine samples were determined following Kohn's method.[6]

## Results

In order to compare the bioavailability of CTC base and the hydrochloride, plasma concentrations of CTC were determined after oral administration of the base and the hydrochloride to groups of five rats. As indicated in Fig. 1, the administration of the CTC base gave higher concentrations in plasma than those after the administration of the hydrochloride. Especially, mean plasma concentration (±S.E.) at 2 hr after the administration of the base (6.09±0.78 μg/ml) was twice that after the hydrochloride (2.47±0.69 μg/ml, $p<0.01$). The results of this comparison suggest that in rats CTC base is better absorbed than the hydrochloride.

The mean plasma levels of DMCT and MOTC at 2 hr are shown in Fig. 2. The plasma levels of DMCT base and the hydrochloride were essentially identical in this absorption study.

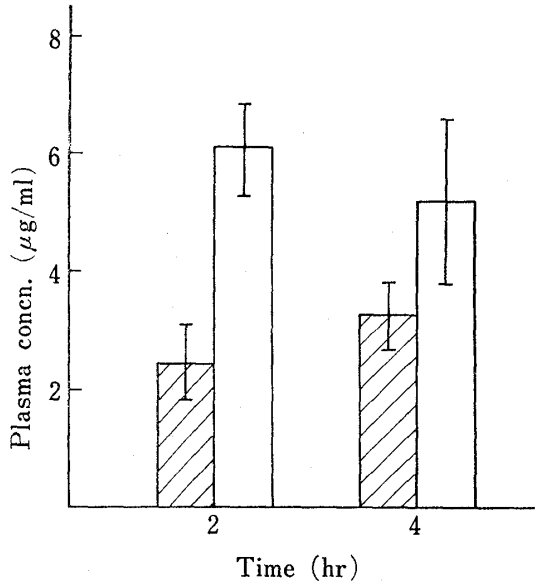


Fig. 1. Mean Plasma Levels after Oral Administration of the Free Base and the Hydrochloride of Chlortetracycline to Rats

□ : free base
▨ : hydrochloride salt
Values are given as the mean ± S.E. of five experiments. All data are expressed in terms of the free base equivalent.

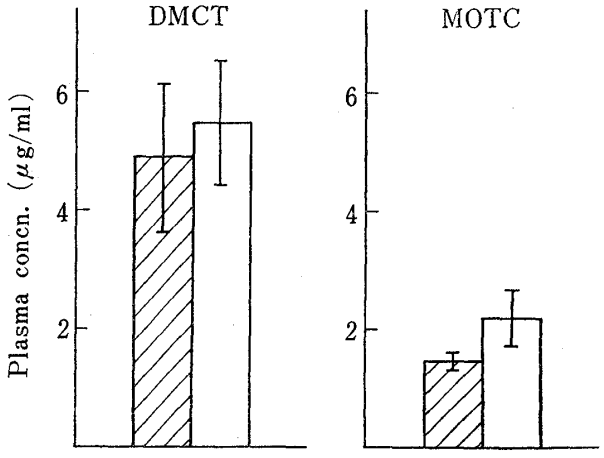


Fig. 2. Mean Plasma Levels at 2 Hour after Oral Administration of the Free Base and the Hydrochloride of Demethylchlortetracycline and Methacycline to Rats

□ : free base
▨ : hydrochloride salt
Values are given as the mean ± S.E. of four experiments. All data are expressed in terms of the free base equivalent.

6) K.W. Kohn, *Anal. Chem.*, 23, 862 (1961).

On the basis of dissolution studies,[3] distinct difference in the plasma concentrations of MOTC base and the hydrochloride after oral administration would be expected. However, the results obtained in this study indicate that the plasma level after the administration of the base was slightly higher than that after the administration of the hydrochloride (2.20±0.46 μg/ml and 1.48±0.12 μg/ml, respectively, for the base and the hydrochloride). No significant difference in plasma concentration was demonstrated between the two species.

Since distinct differences in the plasma levels after oral administration of CTC base and the hydrochloride were found in rats, the cumulative excretion of CTC in urine after the oral administration of the two species was investigated in humans. Similar investigation was carried out with MOTC, whose base exhibited much greater solubility than the hydrochloride in the hydrochloric acid solutions.[3]

The cumulative excretion of CTC after oral administration of the base and the hydrochloride is shown in Table I. The average cumulative excretion of CTC is shown in Fig. 3, in which the cumulative amounts of CTC are plotted as the function of time. These three experiments showed that greater amounts of CTC base were absorbed from the gastrointestinal tract than the hydrochloride for all three subjects. These results are in accord with those reported by Welch, *et al.*[4] in their study on the serum concentrations following oral administra-

Table I. Cumulative Urinary Excretion Data after Oral Administration to Human Subjects[a] of 300 mg Chlortetracycline

| Hours | Cumulative amount, mg[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Subject A | | Subject B | | Subject C | | Mean values | |
| | HCl | Base | HCl | Base | HCl | Base | HCl | Base |
| 2 | 0 | 5.08 | 5.25 | 3.98 | 1.92 | 4.98 | 2.39 | 4.68 |
| 4 | 8.57 | 24.49 | 17.49 | 19.39 | 16.56 | 27.76 | 14.21 | 23.88 |
| 6 | 17.94 | 44.29 | 27.96 | 36.87 | 26.10 | 43.57 | 24.00 | 41.35 |
| 8 | 24.57 | 59.32 | 35.61 | 47.46 | 32.42 | 55.64 | 30.81 | 54.14 |
| 10 | 29.53 | 70.20 | 41.86 | 55.18 | 34.51 | 63.32 | 35.30 | 62.90 |

*a*) subject A: male, 28 yr., 65 kg; subject B: female, 22 yr., 49 kg; subject C: male, 27 yr., 54 kg.
*b*) All data are expressed in terms of the free base equivalent.

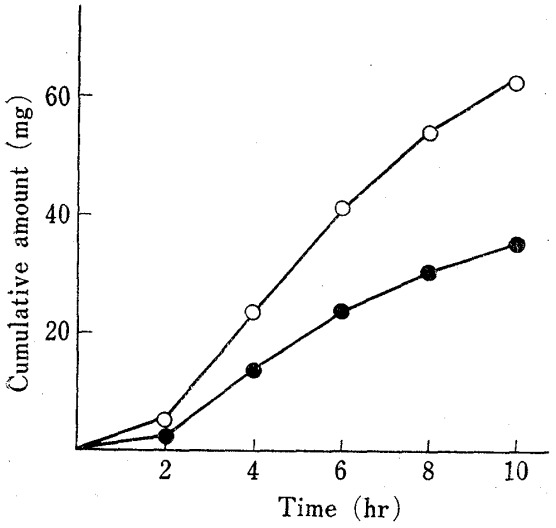


Fig. 3. Cumulative Excretion after Oral Administration of the Free Base (○) and the Hydrochloride (●) of Chlortetracycline to Human Subjects

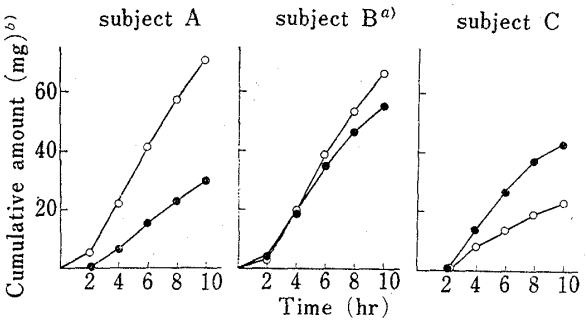


Fig. 4. Cumulative Excretion after Oral Administration of the Free Base (○) and the Hydrochloride (●) of Methacycline[c] to Human Subjects[d]

*a*) Administered in capsule form, while administered in powder form in other subjects.
*b*) All data are expressed in terms of the free base equivalent.
*c*) The doses were 400 mg of subject A, and 300 mg of subjects B and C.
*d*) Subjects A and B are the same subjects as in Table I. Subject C: male, 25 yr., 66 kg.

tion of capsules of CTC base and the hydrochloride. In this study, the urinary recovery in 0 to 10 hr averaged 22.1% of the administered dose in the case of the base, but only 12.6% for the hydrochloride.

Urinary excretion data for MOTC after oral administration of the base and the hydrochloride are shown in Fig. 4. In subjects A and B, MOTC base gave greater cumulative amounts than the hydrochloride, thus indicating the superior absorption of the base in these two subjects. On the other hand, subject C showed reduced excretion when the base was administered; amounts excreted from the base and the hydrochloride are reversed.

## Discussion

The administration of CTC base gave higher plasma concentrations in rats than those after the administration of the hydrochloride. Similar results were noted in urinary excretion after oral administration to human subjects. These results indicate that CTC base is more efficiently absorbed from the gastrointestinal tract of rats and human subjects than the hydrochloride. The differences between the bioavailability of the base and the hydrochloride are due to the differences in solubility characteristics at the gastric pH.[3]

As the absorption of tetracycline antibiotics takes place chiefly from the duodenum, it is important that it should reach the intestine in a dissolved or readily soluble form. It appears that the solubility of CTC in the stomach is of decisive importance for the absorption since the solubility is markedly reduced in the pH range of 4—5.[3,7] The results of our studies would indicate that after oral administration, more of the base is available in a dissolved form for absorption in the intestine than the hydrochloride since the hydrochloride is poorly soluble in the acidic solutions. The readily soluble compound of CTC base in the gastric pH leads to the best conditions of absorption, and the CTC levels in the body fluids obtained after the administration of the base are higher than those of the hydrochloride.

The pH of the gastric fluid is said to range usually from 1 to 3.[8] In addition, NaCl exists in body fluid in a high concentration, which is also unfavorable for dissolution of CTC hydrochloride due to the common ion suppression of the solubility product equilibrium.[3] In such a way, perhaps, these factors create conditions less favorable for the dissolution of the hydrochloride in gastric fluid; as a consequence, the bioavailability would be influenced. Indeed, the solubility of base at 37° (7.38 mg/ml) was shown to be slightly higher than that of the hydrochloride (6.25 mg/ml) in pH 2.1 dilute simulated gastric fluid composed from HCl and NaCl: whereas, in pH 2.1 hydrochloric acid solution, CTC hydrochloride showed higher solubility than the base as described previously[3] (9.42 and 3.43 mg/ml, respectively, for the hydrochloride and the base).

In order to eliminate as many variables as possible, however, the subjects were requested to fast for at least 12 hr before the administration of the samples. Likewise, they were permitted-only 20 ml of water at the time of administration. Consequently, the artificial conditions under which these observation were made will render it hazardous to draw conclusions concerning similar values which might be anticipated under clinical conditions.

In the experiments comparing the cumulative amounts of MOTC base and the hydrochloride after oral administration to human subjects, in two of the 3 subjects, MOTC base produced greater cumulative amounts than the hydrochloride: these 2 subjects were also the 2 who showed greater excretion of CTC when CTC base was administered. However, one subject (subject C) exhibited lower excretion when the base was administered. This may be due to the differences in gastric acidity. In order to evaluate the possible influence of gastric acidity on absorption, the gastric juice of each subject should be analyzed for free

7) W.H. Barr, J. Adir, and L. Garrettson, *Clin. Pharmacol. Ther.*, **12**, 779 (1971).
8) T.H. Wilson, "Intestinal Absorption," W.B. Saunders Company, Philadelphia, 1962, p. 250.

hydrochloric acid and pH value in the fasting state before administration of the base and before administration of the hydrochloride.

Furthermore, it should be stressed that the limited data presented in this report on DMCT cannot exclude the possibility that DMCT base can exert similar effect on the absorption to greater cumulative amounts of CTC base after oral administration to human subjects. More extensive studies are desirable with regard to absorption in this case.

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# Effect of Fenitrothion on Hepatic Microsomal Components of Drug Metabolizing System in Mice[1]

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To examine the mechanisms of the inhibition of drug metabolizing enzyme activity by organophosphate insecticides, microsomal components of drug metabolizing system were determined after treatment with fenitrothion. Mice given intraperitoneal dose of fenitrothion exhibited the rapid decrease in cytochrome P-450 content and slight inhibition of NADPH-cytochrome c reductase activity. Administration of fenitrothion to phenobarbital-treated mice decreased in N-demethylation of aminopyrine as well as cytochrome P-450 content. The actual decrease in cytochrome P-450 content caused by fenitrothion in phenobarbital-treated mice was greater than non-treated mice.

The results presented here strongly suggest that the inhibition of drug metabolizing enzyme by fenitrothion was due to the decrease in cytochrome P-450.

In a preceding paper,[3] we reported that some organophosphate insecticides inhibit hepatic drug metabolizing enzymes both *in vivo* and *in vitro*, although the mechanism whereby organophosphates inhibit drug metabolizing enzymes has not been clarified except that the inhibition originated from microsomes other than soluble components. It must be noted, however, that the *in vivo* inhibition by these thiophosphate insecticides occurs very rapidly, that is, within a few hours after the administration of thiophosphates.

This paper deals with the *in vivo* effect of fenitrothion, O,O-dimethyl O-3-methyl-4-nitrophenyl phosphorothioate, on the components of microsomal drug metabolizing system of mouse liver.

## Result and Discussion

Effect of fenitrothion on mouse liver microsomal cytochrome P-450 content and NADPH-cytochrome c reductase activity is shown in Table I. It is obvious that microsomes from fenitrothion-treated mice showed the lowered cytochrome P-450 content in comparison with that of control mice.

1) This work was presented at the Sixth Symposium on Drug Metabolism and Action, Tokyo, November 1974.
2) Location: *Aobayama, Sendai.*
3) M. Uchiyama, T. Yoshida, K. Homma and T. Hongo, *Biochem. Pharmacol.*, **24**, 1221 (1975).