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Effect of Crystal Forms on the Dissolution Behavior and Bioavailability of Tetracycline, Chlortetracycline, and Oxytetracycline Bases[1)]

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The two crystal forms of tetracycline (TC), chlortetracycline (CTC), and oxytetracycline (OTC) were isolated and characterized by X-ray diffraction, infrared spectroscopy, and thermal analysis. Results of elemental analysis and measurement of water contents indicated that all the crystal forms contained some water molecules.

The dissolution behavior of the two forms was determined in water, the simulated gastric fluid, and the simulated intestinal fluid. The crystals prepared from methanol were generally found to exhibit a higher solubility than the crystals from water.

In order to determine the effect of crystal forms on the bioavailability of the antibiotics, plasma levels in rabbits and blood levels in rats after intraduodenal administration were compared. The results indicated that crystalline states influence significantly the bioavailability of TC, but not CTC and OTC. The similar results were noted from the data of cumulative amounts of urinary excretion after oral administration of the two forms of TC to human subjects.

One of the most important factors influencing the bioavailability of drugs from solid dosage forms is the dissolution behavior which in turn is governed by solid states such as polymorphism,[3)] solvation,[4)] and crystallinity.[5)]

In the previous papers,[6,7)] the existence of two crystal forms of chlortetracycline hydrochloride (CTC-HCl) was confirmed by infrared (IR) spectroscopy and X-ray diffraction, and appreciable differences in the physico-chemical properties such as dissolution behavior and hygroscopicity were found between the two crystal forms. Furthermore, the absorption studies indicated that polymorphic states of CTC-HCl significantly influences its bioavailability.

In addition, the result of a similar investigation indicated that the amorphous form of tetracycline (TC) exhibits greater bioavailability than the crystalline form.[8)]

The present report is concerned with studies conducted to determine the differences in some of the physico-chemical properties of the two different crystal forms of TC, CTC, and oxytetracycline (OTC) bases. Furthermore, it is the purpose of this report to evaluate the effect of crystalline states on their bioavailability.

Experimental

Materials——TC and OTC bases were used, supplied by Taito Pfizer Co. CTC base was prepared by conversion of CTC-HCl obtained from Lederle of Japan to the free base.[9)]

1) A part of this work was presented at the 93rd Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1973.
2) Location: *Kita-12. Nishi-6, Kita-ku, Sapporo.*
3) A.J. Aguiar, J. Krc, Jr., A.W. Kinkel, and J.C. Samyn, *J. Pharm. Sci.*, **56**, 847 (1969).
4) J.W. Poole, G. Owen, J. Silverio, J.N. Freyhof, and S.B. Rosenman, *Current Therap. Res.*, **10**, 292 (1968).
5) J.D. Mullins and T.J. Macek, *J. Am. Pharm. Assoc. Sci. Ed.*, **47**, 657 (1958).
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Preparation of Two Crystal Forms——The Form I of OTC was prepared by recrystallization of the OTC from distilled water following the method described by Gans and Higuchi.[10] Both Form I's of TC and CTC were obtained by recrystallization from distilled water. All of the Form II's were prepared by recrystallization of the antibiotics from hot anhydrous methanol; the procedure was essentially the same as that previously used for the β form of CTC-HCl.[6]

Microscopic examination of the two forms of TC, CTC, and OTC showed that the crystal size was between 10 μ and 40 μ.

Measurement of IR Spectra and of X-Ray Diffraction Patterns——IR spectra were obtained with a JASCO IR-S infrared spectrophotometer. X-ray powder diffraction patterns were obtained with a Rigaku Denki D-9C X-ray Diffractometer.

Thermogravimetric (TG) and Differential Scanning Calorimetry (DSC)——Simultaneous measurements of DSC and TG were carried out with a Rigaku Denki CN-8085 Thermobalance and Differential Scanning Calorimeter. Samples weighing 7—10 mg were used in an ordinary aluminum pan and a scan speed of 10°/min was employed.

Procedure for Dissolution Studies——Dissolution behavior of crystalline powder was determined as previously reported[6] at 37° in distilled water, the simulated gastric fluid at pH 1.2, and the simulated intestinal fluid at pH 7.5 described in J.P. VIII. The concentrations of antibiotics in solution were measured by ultraviolet (UV) absorption method following appropriate dilution with 0.1 N sulfuric acid. The wavelengths chosen for spectral analyses were : TC, 270 nm; CTC, 265 nm; OTC, 268 nm. All absorbance measurements were made with a Hitachi Type-139 spectrophotometer.

Procedure for Absorption Studies——Measurements of plasma levels in rabbits (male albino rabbits weighing 2.4 to 2.8 kg) after intraduodenal administration and cumulative amounts excreted in human subjects after oral administration of the two crystal forms were carried out following the method described previously.[6]

The method used in studying blood levels after intraduodenal administration to rats followed closely that of rabbits. Male Wistar rats weighing 230 to 280 g were anesthetized with urethane (1.3 g/kg) injected intraperitoneally. The upper part of the small intestine was exposed and 30 mg (TC, CTC) or 60 mg (OTC) of the sample dispersed in 3 ml of 0.9% NaCl was injected by means of a syringe near an opening of biliary duct in the duodenum. Blood collections were made at various times during the experiment through polyethylene catheter inserted to the carotid artery. The specimen were analysed fluorometrically following Kohn's method.[11]

Result and Discussion

Characterization of Two Crystal Forms

Two crystal forms of the tetracycline antibiotics were characterized by the following procedures; IR spectroscopy, X-ray diffraction, elemental analysis, measurements of water contents, and thermal analysis.

The X-ray powder diffraction patterns are shown in Fig. 1. In the cases of TC and OTC, the diffraction patterns of Form I were distinctly different from those of Form II and there may be appreciable differences in the crystal structure between the two forms.

The IR spectra, in the 3000—3600 cm^{-1} and 1500—1700 cm^{-1} regions, are shown in Fig. 2. From these spectra, in which Forms I and II of TC and OTC exhibited the features different from each other, these distinguishing features may be utilized for the identification of a crystal form. On the other hand, no difference in the spectra was observed between two forms of CTC.

Results of elemental analysis and measurement of water contents by the Karl Fisher method are shown in Table I. These data indicate that all the crystal forms contain some water molecules. Repeated elemental analysis and moisture determinations by Karl Fisher method or thermal analysis on various samples of each crystal form revealed the hygroscopic nature. Form I's of TC and OTC were analysed as trihydrate and dihydrate, respectively and these results are in agreement with the hydrate described in "The Merck Index."[12]

10) E.H. Gans and T. Higuchi, *J. Am. Pharm. Assoc. Sci. Ed.*, **46**, 458 (1957).
11) K.W. Kohn, *Anal. Chem.*, **23**, 862 (1961).
12) "The Merck Index," 8th ed., P.G. Stecher, Ed., Merck & Co., Inc., Rahway, N.J., 1968, p. 776, 1024.

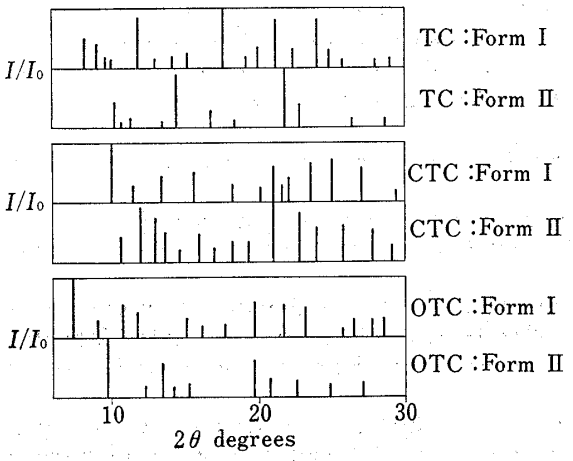

Fig. 1. X-Ray Diffraction Patterns of Forms I and II of Tetracycline, Chlortetracycline, and Oxytetracycline

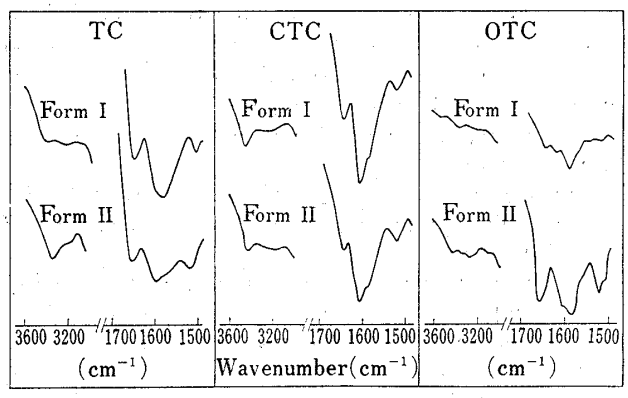

Fig. 2. Infrared Absorption Spectra of Forms I and II of Tetracycline, Chlortetracycline, and Oxytetracycline in Nujol

TABLE I. Analyses of the Composition of Forms I and II of Tetracycline, Chlortetracycline, and Oxytetracycline[a)]

Tetracyclines	Form	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N	H_2O (%) Calcd.	Found[b)]
TC	Form I	$C_{22}H_{24}O_8N_2(3H_2O)$	53.01	6.08	5.62	53.22	5.81	5.54	10.8	9.8
	Form II	$C_{22}H_{24}O_8N_2(2H_2O)$	55.00	5.89	5.84	55.10	5.61	5.73	7.5	4.1
CTC	Form I	$C_{22}H_{23}O_8N_2Cl(5/2H_2O)$	50.38	5.34	5.34	50.24	4.93	5.54	8.6	8.9
	Form II	$C_{22}H_{23}O_8N_2Cl(3/2H_2O)$	52.17	5.14	5.53	51.89	5.03	5.43	5.3	5.4
OTC	Form I	$C_{22}H_{24}O_9N_2(2H_2O)$	53.22	5.68	5.64	53.14	5.63	5.48	7.3	6.7
	Form II	$C_{22}H_{24}O_9N_2(1/2H_2O)$	56.24	5.33	5.97	56.07	5.23	5.66	1.9	2.6

a) Samples for elemental analyses and measurement of water contents were not always the same.
b) Karl Fisher method

The thermal behavior was determined by simultaneous measurements of DSC and TG. The solid and the dotted curves in Fig. 3 are DSC and TG curves, respectively. In the DSC curves of TC, both Forms I and II show endothermic peak over the temperature range 100—150° suggesting the presence of water and subsequent exotherm corresponding to decomposition. In the TG curves, both forms start to lose weight at about 80° and the amount of the weight loss were approximately equal to the water contents in Table I within the limits of experimental error. A similar thermal behavior was found with the two forms of CTC and OTC, a broad endothermic peak in the DSC curves and the gradual weight loss in the TG curves over the entire heating range corresponding to dehydration. Both forms of CTC display an additional endothermic peak at about 170° in the DSC curves suggesting the sintering and/or water volatilization.[13)] This peak is also found to a small extent in the DSC curves of the two forms of TC and OTC.

Although the nature of water contained in the crystal is not clear, these results suggest that the water, especially in the two forms of CTC and OTC, can be considered as adsorbed water or free water, but not water of crystallization.

The chemical identity of all the crystal forms was examined by paper chromatography.[14)] A descending chromatography on Toyo Roshi No. 51A paper impregnated with edetic acid

13) D.L. Simmons, R.J. Ranz, P. Picotte, and S. Szabolcs, *Can. J. Pharm. Sci.*, **5**, 49 (1970).
14) R.G. Kelly and D.A. Buyske, *Antibiotics and Chemotherapy*, **10**, 604 (1960).

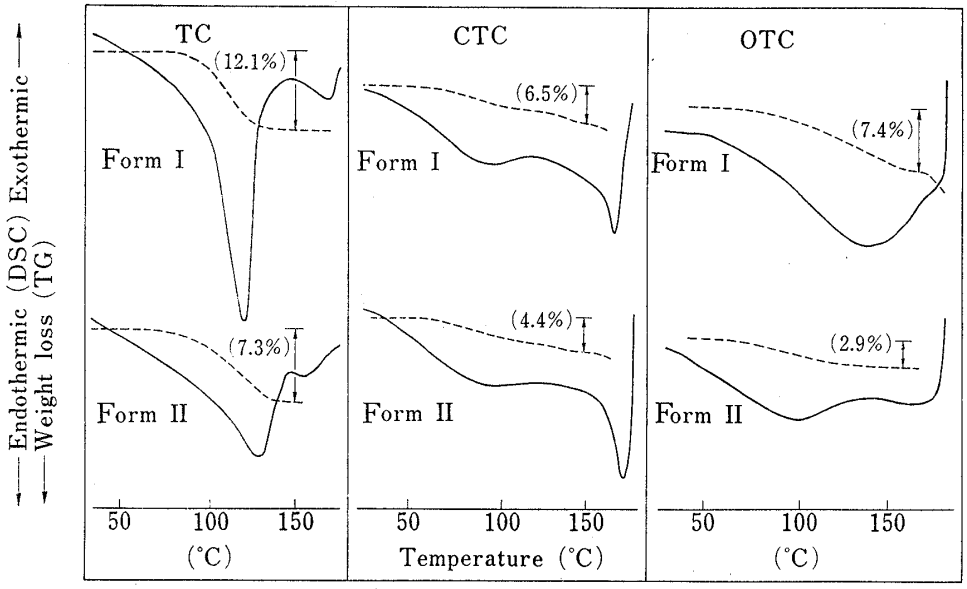

Fig. 3. DSC-TG Curves of Forms I and II of Tetracycline, Chlortetracycline, and Oxytetracycline

——: DSC curves, ------: TG curves
range: DSC 1 mV, TG 10 mV; atmosphere: air
The number in parentheses indicates the amount of the weight loss.

was accomplished with *n*-butanol: acetic acid: water (4: 1: 1) and *n*-butanol: ammonium hydroxide: water (4: 1: 5) as solvents. The two forms of TC, CTC, and OTC exhibited each *Rf* value identical with that of reference sample; this suggests that both forms show the same chemical nature in solution. Consequently, it can be concluded that the existence of the two crystal forms of TC, CTC, and OTC is attributed to the difference in the crystalline states.

Dissolution Behavior of Two Crystal Forms

The dissolution curves of the two forms of TC, CTC, and OTC from crystalline powder in water at 37° are shown in Fig. 4, where the concentrations of drugs in solution are plotted as a function of time under constant agitation. Each curve is drawn through points obtained during more than two experimental runs.

The distinct difference in dissolution behavior was observed between the two forms of TC. Form II is more soluble than Form I and yields a concentration supersaturated with respects to Form I, indicating that dissolution behavior may be influenced by the crystalline structure. On the other hand, only a small variation was found in the dissolution behavior of the two forms of CTC. In the case of OTC, the concentration of Form II in an initial stage of dissolution is quite high. However, a decrease in the amounts of the drug dissolved was observed within a relative short period. The limiting value of this decrease was found to be the solubility of Form I. The transformation of Form II to I was confirmed by IR spectrum of the crystal isolated from the medium after equilibrium.

The dissolution behavior of the two forms in the simulated gastric fluid at pH 1.2 was also determined since the behavior in acidic solutions is more relevant to the bioavailability after oral administration. The dissolution curves obtained in the acidic solution are shown in Fig. 5. A similar difference was found to that in water for TC, indicating that the apparent solubility of Form II is about 1.3 times of Form I (27.7 and 35.0 mg/ml respectively for Forms I and II). The dissolution curves of the two forms of OTC were virtually identical in the acidic solution. This may be considered to be due to the rapid transformation to a stable form.

During the dissolution studies of CTC, a decrease in the concentration of both forms in solution was observed due to the conversion from the base to the hydrochloride salt. The

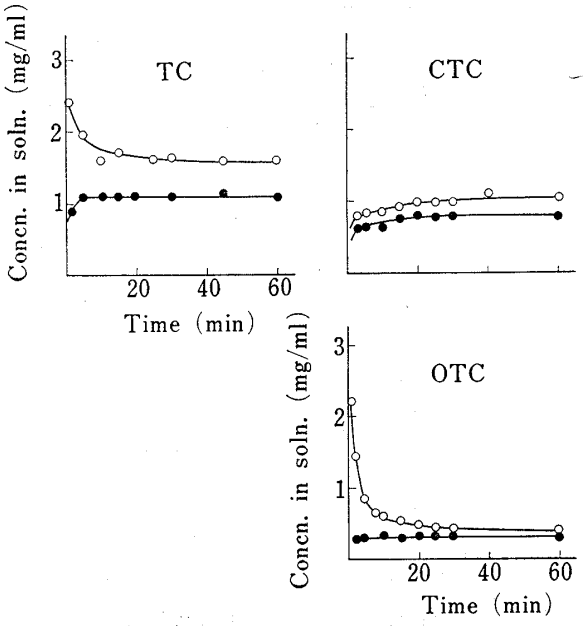

Fig. 4. Dissolution Curves of Forms I (●) and II (○) of Tetracycline, Chlortetracycline, and Oxytetracycline in Water at 37°

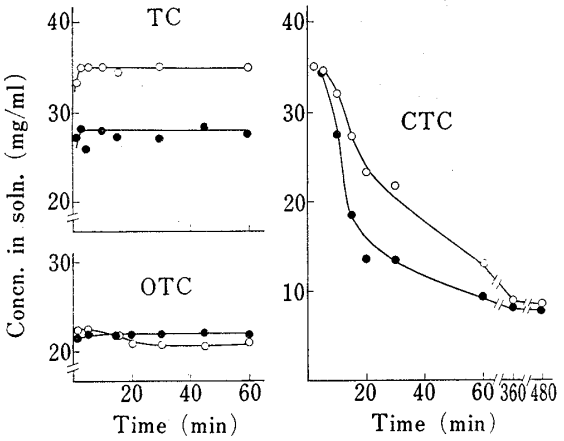

Fig. 5. Dissolution Curves of Forms I (●) and II (○) of Tetracycline, Chlortetracycline, and Oxytetracycline in pH 1.2 Simulated Gastric Fluid at 37°

confirmation of the conversion of both forms of CTC to the hydrochloride was made by IR spectra and thermal analysis of the coverted product. These results suggest that the oral administration of the free base of CTC may exhibit greater bioavailability than the hydrochloride since CTC hydrochloride is poorly soluble in acidic solutions.[15]

In addition, the dissolution curves in the simulated intestinal fluid at pH 7.5 are illustrated in Fig. 6. Similar dissolution curves were obtained to those in water.

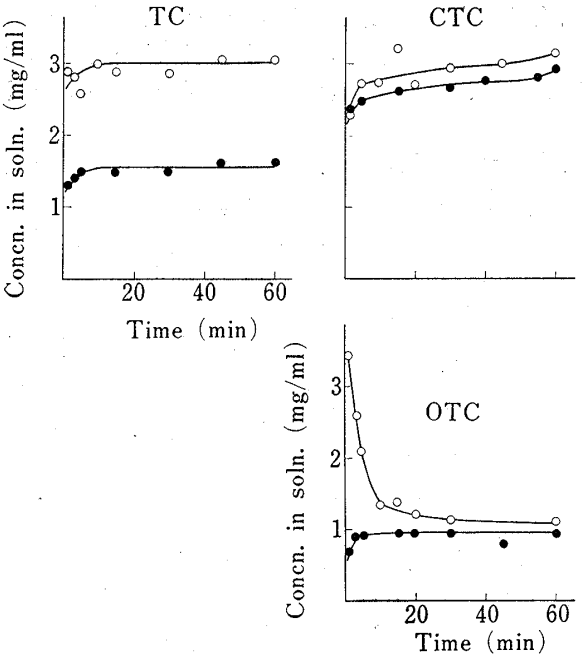

Fig. 6. Dissolution Curves of Forms I (●) and II (○) of Tetracycline, Chlortetracycline in pH 7.5 Simulated Intestinal Fluid at 37°

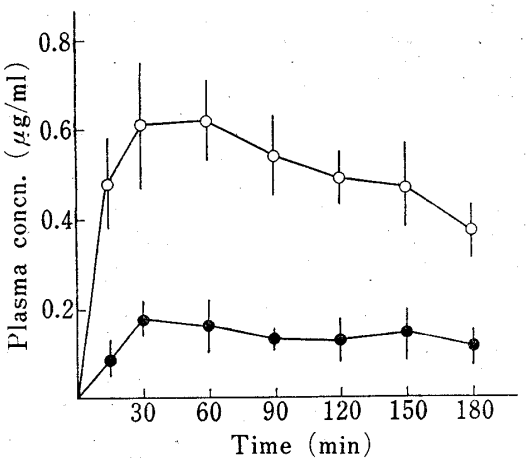

Fig. 7. Plasma Levels after Intraduodenal Administration of Forms I (●) and II (○) of Tetracycline to Rabbits

dose: 200 mg
Points are given as the mean ± S.E. of four experiments.

15) S. Miyazaki, M. Nakano, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), accepted.

Effect of Crystal Forms on Bioavailability

A study was instituted to determine the effect of the observed solubility difference on the bioavailability of tetracycline antibiotics from solid dosage form. It is known that the drug absorption is affected by foods and gastric emptying.[16] In order to exclude these factors, the absorption after intraduodenal administration was studied first in rabbits and rats.

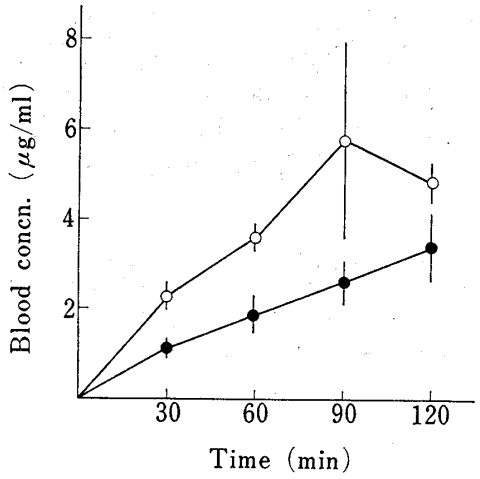

Fig. 8. Blood Levels after Intraduodenal Administration of Forms I (●) and II (○) of Tetracycline to Rats

dose: 30 mg
Points are given as the mean±S.E. of four experiments except three experiments of 120 min of Form I and 90 min of Form II.

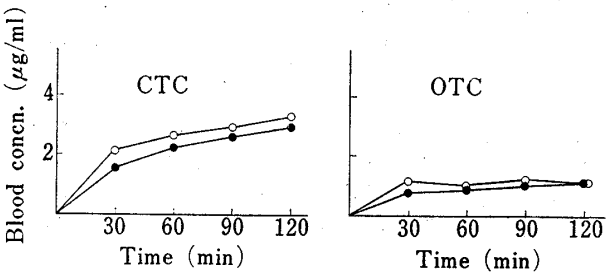

Fig. 9. Blood Levels after Intraduodenal Administration of Forms I (●) and II (○) of Chlortetracycline and Oxytetracycline to Rats

dose: 30 mg (CTC), 60 mg (OTC)
Points are given as the mean of two experiments.

Figure 7 shows the mean plasma levels of the two forms of TC after intraduodenal administration to rabbits. The administration of Form II gave higher plasma levels than those after the administration of Form I during actual absorption studies and the differences in the bioavailability are thought to be due to the differences in solubility of the two forms in aqueous solutions. The similar results were noted in blood levels after intraduodenal administration to rats, as shown in Fig. 8. These results indicate that Form II of TC is more efficiently absorbed from the gastrointestinal tract of rabbits and rats than Form I.

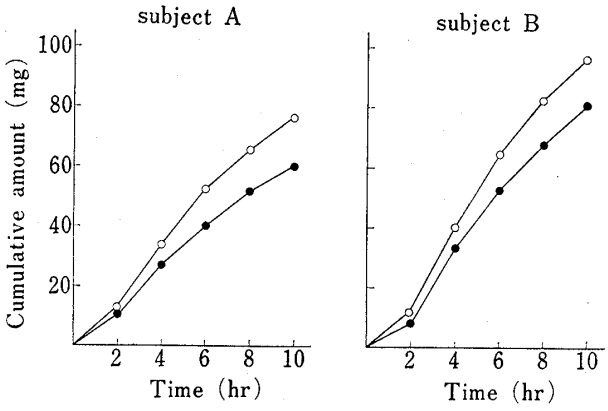

Fig. 10. Cumulative Excretion Curves after Oral Administration of Forms I (●) and II (○) of Tetracycline to Human Subjects

The 250 mg of sample was given in oblate with 100 ml of water.

The blood levels after intraduodenal administration of the two forms of CTC and OTC to rats are shown in Fig. 9. Only a minor difference was observed in blood levels between Forms I and II of CTC; this may reflect the small difference in the *in vitro* dissolution behavior in aqueous solutions. On the other hand, on the basis of dissolution studies, some differences in the blood levels of Forms I and II of OTC after intraduodenal administration would be expected. However, the results obtained in this study indicate that there is no difference

16) R.R. Levine, *Digestive Diseases*, **15**, 171 (1970).

in the blood levels after the administration of the two forms. This may be explained by the rapid transformation from Form II to I in gastrointestinal tract, but further work is required to clarify this point.

Since distinct differences in the plasma or blood levels after intraduodenal administration of two forms of TC to the animals were found, the cumulative excretion of TC in urine after the oral administration of the two forms to human subjects were also investigated. The results of this study are shown in Fig. 10, in which the cumulative amounts of TC are plotted as the function of time. These two experimental data also indicate that Form II gave greater cumulative amounts than Form I indicating greater bioavailability of Form II than Form I.

In conclusion, these results suggest that the solubility difference between the two forms, as was demonstrated in the case of TC, affects significantly the bioavailability of TC. On the other hand, the small difference, as was seen with CTC and OTC, do not appear to affect the bioavailability of the drugs.

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