TABLE III. Tissue Distribution of Tritium after Injection of Labeled PES to Rats.*

Pooled tissue	Tissue radioactivity (% of dose) 24 hr	14 days	48 hr after 4 injections
Urine	52.8	0	40.0
Plasma	0	0	0
Liver	19.6	20.0	40.6
Kidney	8.3	2.0	3.7
Skeletal muscle†	7.0	17.8	10.4
Fat (perirenal & genital)‡	4.1	4.4	3.4
Intestine (small & large)	3.8	3.7	3.8
Spleen	.3	.3	.4
Heart	.2	.2	.2
Lungs	.2	.5	.7
Total retained by tissues	43.5	48.9	63.2

* Tritiated PES given I.V. (60 mg/kg) to 6 rats; 3 sacrificed after 24 hr; 3 sacrificed after 14 days. Four successive daily I.V. (60 mg/kg) injections of labeled PES given 4 additional rats; sacrificed 48 hr after last inj.

† Taken from foreleg; total skeletal muscle calculated as 50% of total rat wt (wet).

‡ Calculated as representative of total fat considered 12.6% of total rat wt (wet)(8).

ethylene sulfonate (PES) nor heparin affected the rate of oxidation of intravenously administered C^{14}-1-palmitic acid in rats whereas both agents increased the oxidation of C^{14}-1-tripalmitin. These findings are interpreted as indicating that PES and heparin increase the rate of oxidation of triglyceride fatty acids indirectly by increasing the rate of hydrolysis to fatty acids, which in turn are rapidly oxidized. When tritiated PES was given to dogs and rats, only a fraction of the dose was found in the urine. In rats about 50% was excreted in the urine and the remainder could be accounted for in various tissues; liver and skeletal muscle contained the most.

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Comparative Serum Binding, Distribution and Excretion of Tetracycline and a New Analogue, Methacycline. (27501)

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Of the many tetracycline analogues, only 4, chlortetracycline, oxytetracycline, tetracycline (TC), and demethylchlortetracycline (DMCT), are currently available for clinical use. These analogues share virtually the same antimicrobial spectrum, differing only in relative activity and in properties such as stability, binding to serum proteins, relative distribution in body and renal excretion(1,2). Recently, a new analogue, 6-methylene oxytetracycline, generic name methacycline (MC), has been reported to have antimicrobial and pharmacologic properties similar to DMCT(3,4).

In a previous report dealing with various penicillin analogues(5), it was pointed out that oral absorption studies provide only limited information concerning the effect of serum protein binding on blood levels. Some workers have attributed higher blood concen-

trations achieved after oral doses of similar compounds to the superior gastrointestinal absorption of one of them, and have tended to ignore differences in distribution and excretion which may, at times, be more critical than absorption in determining blood levels.

This report presents comparative data on serum concentration and urinary recovery of MC and TC following oral and intravenous administration. Evidence is presented to indicate that the somewhat higher blood levels of MC over TC observed after oral doses are due to factors governing distribution and excretion rather than to more efficient absorption of MC.

Materials and methods. Single oral and intravenous doses of 500 mg of tetracycline (TC) and methacycline (MC)* were given in rotation to 4 healthy young men while in the fasting state. Intravenous preparations were diluted in 100 ml of 5% glucose in water, and injected over a period of 15 minutes. Doses were separated by at least one week. Venous blood samples were obtained at 0, 1, 2, 4, 6, 8, 10, and 12 hours after each dose. Serums were separated as soon as possible, and stored at −20°C until time of assay. All urine passed at timed intervals during 72 hours following each dose was collected in sterile vessels, and immediately refrigerated; aliquots from each collection period were kept frozen at −20°C until time of assay. Specimens of serum and urine were assayed by the cup plate method employing *Sarcina lutea* as the test organism, and levels were expressed in μg/ml based on comparison with standard drug. These tests were performed under code, employing standards of *both* drugs, in the laboratory of the Charles Pfizer Co. Codes were not broken until all results were reported.

Serum protein binding determinations were performed by suspending cellophane bags† (holding 5 ml of Krebs-Ringer phosphate buffer, pH 7.4) in individual tubes containing 5 ml of serum obtained 2 hours following intravenous injection of TC or MC. Bags containing buffer were also placed in tubes with 5 ml of buffer containing 25 μg/ml of each drug in order to demonstrate free permeability of the membrane to the drugs. Dialysis was conducted for 48 hours at 8°C in a slowly rotating drum. Serum and buffer were removed at completion of incubation, stored at −20°C and assayed as above.

Analysis of protein binding, half-life, relative distribution volume, and tests of significance were conducted as previously reported (1). Calculations of renal clearance were performed by the standard formula, employing data from urine collected during 3 consecutive 2-hour periods—beginning 2 hours after completion of intravenous injection—and serum concentrations midway between these periods derived from a semilogarithmic plot of the individual serum decay curves.

Results. Serum binding and kinetic studies. MC was found to be bound by serum about 2 times more than TC in each of the 4 subjects given both drugs; mean values were 78.5% and 35.6% respectively, Table I. Almost identical values were found in bag and surrounding buffer when drug in buffer was allowed to dialyze in the absence of serum, demonstrating free permeability of the membrane to both MC and TC. Calculated predialysis serum concentrations are similar but not identical to those shown for 2-hour blood levels in Fig. 2 since samples were assayed at different times and were treated differently.

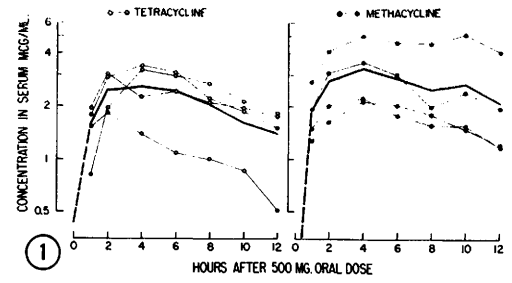
The concentration of TC and MC achieved in serum following single oral doses of 500 mg in the same 4 subjects is shown in Fig. 1. Considerable variation was encountered with both drugs, but in general, higher and more

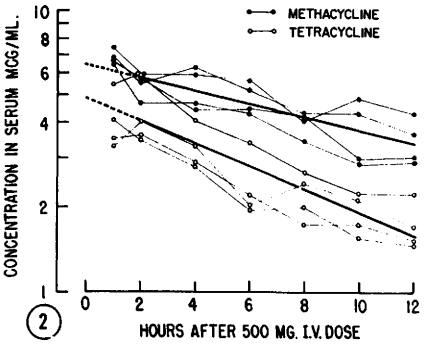
TABLE I. Human Serum Protein Binding of Tetracycline and Methacycline.

Analogue	Subject	Post-dialysis Serum μg/ml	Post-dialysis Buffer μg/ml	% bound
Tetracycline	A	2.55	1.60	37.3
	B	2.50	1.50	40.0
	C	3.05	2.05	32.8
	D	2.80	1.90	32.1
	Mean			35.6
Methacycline	A	3.60	.78	78.3
	B	3.60	1.00	72.2
	C	5.20	1.30	75.0
	D	7.50	.87	88.4
	Mean			78.5

* Kindly supplied with assay potency by Dr. Robert Riggio, Charles Pfizer Co. Capsules contained 260 mg of TC and 253 mg of MC as the hydrochloride.

† Obtained from Visking Co., Chicago, Ill.





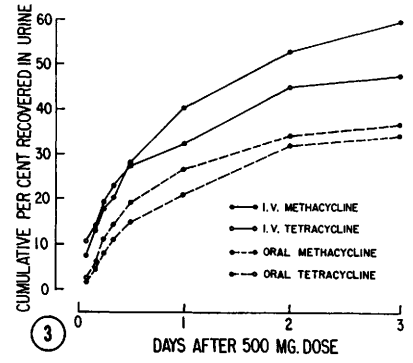


FIG. 1. Individual and mean concentrations of tetracycline and methacycline attained in serum of 4 healthy young men following a 500 mg oral dose of each.

FIG. 2. Individual and mean concentrations of tetracycline and methacycline attained in serum of 4 healthy young men following 500 mg of each injected intravenously.

FIG. 3. Cumulative 72-hour urinary recovery of tetracycline and methacycline in 4 healthy young men given 500 mg of each by oral and intravenous routes.

prolonged levels were achieved with MC. Note, however, (Fig. 2) that serum concentrations were also higher and better sustained in these subjects following the same dose given intravenously. A distinct separation of the decay curves, with the exception of only 2 points, was obtained with the 2 analogues.

The half-life in serum of TC was only 57% of MC, 8.2 and 14.3 hours respectively (Table II). Calculation of the relative volume distribution for the 2 drugs revealed that MC was diluted in a relative volume 27% smaller than TC, 79.4 and 108.7 liters respectively.

Slightly greater urinary recovery up to 72 hours was achieved following intravenously administered MC (59.8%); TC (47.8%), Fig. 3, Table II. Urinary recovery was about the same for both drugs following oral doses. The ratio of oral/intravenous urinary recoveries shown in Table II was higher with TC than MC, 77.2% and 58.0%, respectively, suggesting that oral adsorption was more complete with TC than MC.

Renal clearance of TC was 38% greater than MC, 50.2 ml/min versus 31.0 ml/min, respectively; the differences noted were significant at the $<.01$ level.

Untoward reactions to drugs. Seven healthy young men were initially selected to participate in this study; this report, however, deals only with the results obtained in 4 who were able to complete it. One subject failed to complete the study because of vomiting which occurred shortly after oral ingestion of 500 mg of MC. Four other subjects complained of burning epigastric pain after taking the same drug, but did not vomit; only one such reaction was noted with oral TC.

A 27-year-old man, who had previously tolerated well single oral doses of TC and MC and an intravenous injection of TC, developed severe bilateral flank pain within 15 minutes after intravenous injection of MC. The pain persisted for 48 hours. There were no chills, fever, leukocytosis or other complaints. Examination of the urine within a few hours after onset of pain revealed marked proteinuria, and 30-40 white blood cells in the sediment. Urine culture was sterile. Blood urea was elevated to 87 mg% and creatinine was 4.5 mg% on the day after onset of reaction; these gradually fell to normal levels during the next week. Pyurea and proteinuria cleared within a week, but microscopic hematuria persisted for 2 months. Examination of the urine prior to receipt of drug revealed no proteinuria, nor was there proteinuria or similar reaction to this drug in 4 other subjects who received the same lot at the same time.

TABLE II. Distribution and Excretion of Tetracycline and Methacycline in 4 Normal Men Following Single Oral or Intravenous 500 mg Doses.

	Tetracycline	Methacycline
Half-life following IV injections, (hr)	8.2	14.3
RVD* (liters)	108.7	79.4
RVD/kg (liters/kg)	1.33	.97
Urinary Recovery, Oral (% of dose)	36.9	34.7
" " IV (% of dose)	47.8	59.8
" " Oral/IV (%)	77.2	58.0
Renal Clearance† (ml/min/1.73m²)	50.2 ± 14.4	31.0 ± 11.0
Serum Protein Binding (%)	35.6	78.5

* Relative volume distribution.
† Differences noted are significant at <.01 level.

Four months before participating in the study, this subject had suffered an acute episode of right sided, uncomplicated, pyelonephritis, which had responded well to sulfonamide therapy. There was no proteinuria or urea elevation during that episode, and intravenous pyelograms were entirely normal. In view of this reaction, further injections of MC were discontinued.

Discussion. The data indicate that MC is more slowly cleared by the kidneys than is TC. This property is probably due to more marked serum binding of MC, and accounts for the more sustained blood levels noted after oral and intravenous administration of the new analogue. In all likelihood, serum binding also accounts for the smaller relative body volume in which MC is distributed and for the higher blood levels achieved. MC closely resembles DMCT in these properties(1). The serum binding of TC noted here was somewhat higher than previously observed when the drug was added to human plasma *in vitro*(1). In the current studies, serum obtained from subjects who had been given each drug was employed in the dialysis experiments. Kirby *et al.*(4) and Remington and Finland‡ have found the extent or serum binding of MC and DMCT to be very similar and quite close in value to that noted for MC in the present study. The ratios of oral/IV urinary recovery of TC and MC suggest that gastrointestinal absorption of TC is superior even though TC blood levels are lower.

The gastrointestinal side effects noted with MC when given in 500 mg doses are of interest, since oral DMCT has also been reported to be less well tolerated than TC when given in large doses by this route(6-9). The severe renal reaction to intravenously administered MC in one subject who had previously had pyelonephritis remains unexplained. The lot of MC used had been found to be pyrogen-free prior to use, and was well tolerated by 4 other subjects. It is possible that this reaction was due to rapid lysis of residual coliform organisms in the kidney giving rise to a bacterial endotoxin-like response reported to occur in pyelonephritis(10). This appears unlikely since the same subject had no reaction when given oral TC and MC and intravenous TC during the 3 preceding weeks.

Final judgment regarding the place of MC in the therapeutic armamentarium must await further reports of relative *in vitro* activity and clinical efficacy. Interpretation of oral absorption studies, however, must take into account the pharmacologic properties of this drug reported here, particularly serum binding.

Summary. The absorption, distribution, excretion, and serum binding of tetracycline and methacycline, a new analogue, have been compared. The higher and more sustained levels of the latter drug noted after oral and intravenous administration in man appear to be due to delayed renal excretion and more limited distribution rather than to enhanced gastrointestinal absorption. These differences are thought to be a reflection of greater serum binding of methacycline.

‡ Personal communication.

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The Site of Vitamin B_{12}-Intrinsic Factor Complex "Releasing Factor" Activity in the Rat Small Intestine. (27502)

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It has recently been reported(1) that an aqueous extract prepared from rat whole small intestine will break the bond between much of the radioactive vit. B_{12} bound to rat stomach extract rendering the vitamin dialyzable. However, less of the radioactivity bound to human gastric juice, and none of that bound to a hog intrinsic factor concentrate was rendered dialyzable during incubation at pH 7 and 37° C. The effect was minimal below pH 4.0. Subsequently, it was determined (2) that small intestinal extract prepared from human ileum mucosa would release vit. B_{12} preferentially from human intrinsic factor at pH 7.4. It was speculated that this apparently species-related "releasing factor" may play a role in the physiologic mechanism for absorption of vit. B_{12}, by releasing vit B_{12} from intrinsic factor at or within the intestinal mucosal wall.

A seemingly less species-related intestinal extract "releasing factor" has also been found by others(3) in homogenates obtained from rat small intestine. This factor was equally active in splitting the bond between vit. B_{12} and intrinsic factor preparations from human, rat or hog sources. It is possible that the apparent lack of specificity of this particular rat small intestine homogenate was due to its concentration being so high that species specificity might have been obscured. Still other workers(4) have demonstrated that extracts from human small intestine, kidney, liver, thyroid, heart muscle, brain, spleen and lung were all capable of splitting off vit. B_{12} previously bound to human gastric juice, serum or plasma. The identity or lack thereof of these various releasing factors remains to be determined.

The present study was undertaken to define the site of releasing factor activity in the rat small intestine by means of extracts prepared by our previous method(1).

Materials and Methods. Adult Sprague-Dawley rats were sacrificed. The pylorus was transected and the small intestine washed through with 0.9% NaCl. It was then stripped free from its mesenteric attachment, cut into 10 pieces of approximately equal length, each of which was put into a separate beaker containing 10 ml of 0.9% NaCl. The contents of each beaker were minced, homogenized for 50 seconds in a Waring blendor and centrifuged for 10 minutes at 3,000 r.p.m. The supernatant fluid was then aspirated and frozen at −17° until used as a source of "releasing factor."

Rat intrinsic factor concentrate (RIFC) was prepared as previously described(1). Two ml of RIFC resulted from each stomach. The

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