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THE PHARMACOKINETICS OF CHLORTETRACYCLINE IN TURKEYS

Iowa State University PH.D. 1984

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The pharmacokinetics of chlortetracycline in turkeys

by

Robert Alan Pollet

A Dissertation Submitted to the
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GENERAL INTRODUCTION

The antibiotic chlortetracycline (CTC) is administered frequently in veterinary medicine to treat infectious disease. Various investigators have found that divalent metal cations, present in animal feeds and drinking water, inhibit absorption. In addition, since CTC that enters the body is rapidly eliminated, achieving and maintaining therapeutic concentrations of drug in the blood/tissue can be difficult.

The primary objective of this work was to study the pharmacokinetics of orally administering CTC alone and with citric acid (a metal chelator) in healthy and diseased turkeys.

Explanation of Dissertation Format

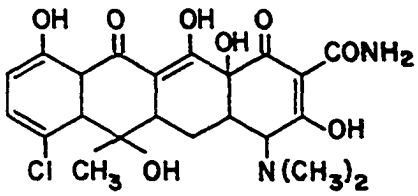
The work performed in this dissertation is presented in the form of two papers suitable for publication. The first paper, entitled "Oral Absorption of Chlortetracycline in Turkeys: Influence of Citric Acid and Pasteurella Multocida Infection", presents and discusses the experimental data collected. In the second paper, entitled "The Pharmacokinetics of Chlortetracycline Orally Administered to Turkeys: Influence of Citric Acid and Pasteurella Multocida Infection", a physiologically based pharmacokinetic model was used to study the absorption and elimination of CTC in the turkey. Reviews of general background information, of previous citric acid investigations and other pharmacokinetic studies with tetracyclines are found in this section and the introductions to the first and second papers, respectively. Following the papers, major conclusions are summarized and areas of future work are suggested.

Pharmacology

The antibiotic CTC is widely used in disease treatment, food preservation, feed supplementation, and crop control because of its broad antibacterial spectrum and relative nontoxicity (1).

Chemical properties

The chemical properties of CTC effects the processes of absorption, distribution, and metabolism. The chemical structure of CTC is shown in the following diagram. It is an amphoteric, yellow, crystalline



Chlortetracycline

substance, and is usually supplied in the hydrochloride form to provide greater solubility. Due to the acidic pH of aqueous CTC solutions, painful parenteral injections are usually avoided (2). The most common route of administration is oral.

Following oral administration of CTC, absorption of the antibiotic is highly dependent on the contents of the gastrointestinal tract. Albert and Rees (3) found that divalent and trivalent cations form CTC-metal complexes or chelates which have an inhibiting effect on absorption of CTC. Although the details are not discussed here, the chemical basis for the chelation of tetracyclines with divalent metal

ions are reviewed by Weinberg (4).

In addition, findings indicate that tetracyclines not only bind to metals, but are strongly bound to proteins (5). From 47-70% of the CTC *in vivo* is protein bound (6). Readers interested in the mechanism of CTC-protein binding will find details in an article by Popov *et al.* (7). Since bound tetracyclines form larger molecules which are less mobile, the processes of absorption, distribution, and elimination (glomerular filtration) are affected. In addition, most authorities agree that the bound drug is biologically inactive.

One of the primary routes of elimination of CTC is by chemical decomposition. Although the specific decomposition products depend largely on the pH of the solution, most degradation products of CTC have a low biological activity (0-43% of the original activity of CTC) (8).

Absorption

Since CTC is usually administered orally, the resulting blood levels of antibiotic depend largely on the absorption processes. Although the absorption processes have not been extensively studied in the fowl, it is believed that the absorption rate is faster in birds than in most mammals due to the fowl's greater metabolic rate, higher body temperature, and shorter circulation time (9).

In studies on dogs, tetracycline was found to be most rapidly absorbed from the duodenum and ileum (10). Peak blood levels were reached within one-half hour after administration into the duodenum or ileum. Little absorption occurred in the colon. Consequently, a high rate of absorption would be expected a short time after drug enters the

fowl's small intestine. The rate of absorption would decrease as the drug enters the large intestine.

According to the pH partition hypothesis, only the nonionized molecules are lipid-soluble and readily able to cross lipoidal membrane barriers. Although the tetracyclines are ionized throughout the physiological pH range, the highest level of zwitterion occurs at neutral pH values (11). The normal pH at various locations in the digestive tract of live chickens is shown in Table 1. By determining the

Table 1. pH of the digestive tract of chickens (12)

Location	pH
Mouth	6.75
Proventriculus	3.17
Gizzard	2.60
Small intestine	6.67
Large intestine	7.09

octanol/buffer partition coefficients (Table 2), Colaizzi and Klink (11) found that the maximum lipid solubility of CTC is between 3.9 and 6.6. Due to their neutral pH values, rapid absorption might be expected in the mouth, esophagus, small intestine, and large intestine. However, since 50% of ingested feed is excreted within 4-5 hours after consumption (9), unless absorption of drug is very fast, a large amount of orally administered drug will be excreted.

Table 2. Partition coefficients (octanol/aqueous buffer) of tetracycline (TC) and CTC hydrochloride (11)

	pH					
Analog	3.0	3.9	5.6	6.6	7.5	8.5
TC	.007	.044	.056	.052	.036	.010
CTC	.180	.270	.410	.320	.130	.071

Barber (13) indicates that the absorption of tetracyclines may be nonlinear. When increasing the oral dose of certain tetracyclines (oxytetracycline) in human subjects, the blood level obtained increases with the dose up to a dose of about 1.0 gram, but increasing the dose beyond this does not lead to significantly higher blood levels. Similarly, Mitscher (5), in reviewing the results of Finland and his colleagues, states that with repeated dosage, increasing the dose of tetracycline beyond .5 grams (administered every 6 hours) fails to give any higher blood levels. These results may be due to a saturation of the absorption route.

Distribution and elimination

Once in the blood stream, CTC enters the different tissues of the body at varying rates. Since a therapeutic level of drug is required at the site of infection, CTC must reach the infected tissue and/or fluids. When mice were given low dose intramuscular injections of CTC, the drug was found to concentrate primarily in the bone marrow, spleen, lymph nodes, liver, and kidney (6). At higher doses, CTC was found in

connective tissue, cartilage, and bone as well. Pleva and Schlee (14) studied residues of CTC in muscles and organs of chickens following the use of feed mixtures containing CTC. Although large CTC residues were found in the kidneys, a significantly lower concentration of CTC was found in the liver. Somewhat lower concentrations were found in the fowl's white and dark muscle.

A substantial amount of evidence indicates that a large proportion of CTC is excreted in the feces regardless of the route of administration. Eisner and Wulf (15) recovered 41% of the intravenous dose of CTC in the animal's (rats, dogs) feces. In addition, investigators (6) have found that the concentration of CTC in the bile is 8 to 16 times that observed in the serum. An enterohepatic circuit has been postulated whereby CTC is excreted into the intestine via the bile and then reabsorbed and recirculated. The proportion of the drug not reabsorbed is excreted via the intestines. Lanman et al. (16) discuss specific mechanisms of reabsorption. The result of interest is that the reabsorption process could perform an important role in the elimination of CTC.

The mechanism of excretion of tetracycline in the urine of chickens was studied by Pindell et al. (10). Using the circulation properties of the chicken (renal portal circulation), they concluded that tetracycline is passed into the urine by passive glomerular filtration. Large CTC complexes are not readily excreted into the urine. Thus, the rate of elimination by this route is dependent upon the amount of bound tetracycline. Considering these results, it was surprising to find that in uremic patients with renal failure the

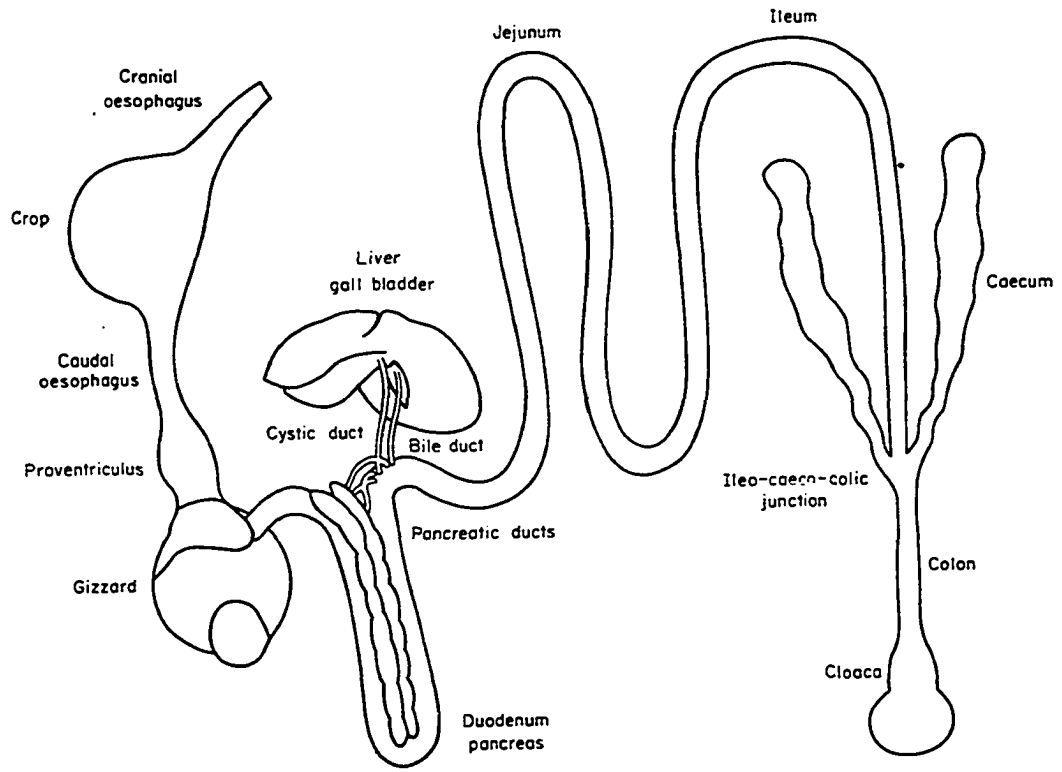
half-life of microbiologically active CTC ($t_{1/2}$ = 5.5 hrs) did not increase (17). Since fecal excretion of CTC is significant, there may have been an increased rate of biliary secretion.

Anatomy and Physiology of the Fowl

Although various experimental animals may be used in pharmacokinetic studies, turkeys were employed in this work because (1) absorption problems with CTC in fowl are reported, (2) many physiological processes in turkeys (e.g., enterohepatic circuit, glomerular filtration) resemble those in mammals, and (3) turkeys are inexpensive to purchase and maintain. Because the physiological processes largely effect absorption and disposition rates, the anatomy and physiology of the turkey is discussed. Most of this material was taken from avian physiology texts (18, 19).

Gastrointestinal tract

For materials poorly absorbed, the gastrointestinal flow rate largely effects the fraction absorbed. In fowl, the crop serves as a food reservoir or storage area. When the gizzard is empty, material bypasses the crop and enters the proventriculus directly. Material is rapidly transported from the crop, through the proventriculus, to the gizzard by peristaltic contractions (3-4 contractions/minute) originating up in the esophagus. Excitement retards crop contractions, while hunger stimulates contractions.



The general structure of the digestive tract of the fowl.

As material enters the gizzard, it is mixed with 6-21 ml of gastric juice. Although the gizzard grinds coarse material, finely divided foods pass through the gizzard to the upper portion (duodenum) of the small intestine in minutes. Consequently, an aqueous solution (containing drug) is quickly transported to the small intestine (primary site of absorption).

Materials pass through the intestines with peristalic and segmenting movements, thereby mixing the G.I. contents. In the turkey, 3-4 duodenal peristalic contractions occur per minute, with each contraction lasting 4.6-9.4 seconds. Duodenal flows of 40-60 ml/hr

have been measured in cockerels fed a mash diet. As material passes down the small intestine, net fluid secretion occurs in the duodenum, while absorption occurs in the jejunum and ileum. Regurgitation of the duodenal contents into the gizzard and crop have been reported, suggesting reverse peristalsis.

After material leaves the lower portion of the small intestine, it passes through the (relatively short) large intestine (where little absorption of CTC takes place), rectum, and cloaca. The urinary tract empties into the cloaca where urine is mixed with feces.

Liver and bile

The liver is bilobed with a hepatic duct leaving each lobe. The left duct leads directly to the duodenum, while the right duct has a branch going to the gall bladder for concentration and storage. Formation of bile by the liver is a continuous process and secretory rates of about 1 ml/kg/hr have been measured in fowl. The bile formation rate is important since CTC is concentrated in the liver and secreted in the bile.

Renal function

Although the fowl and mammalian kidneys differ anatomically, their basic function is similar. Renal elimination can occur by glomerular filtration and/or tubular secretion. Renal elimination of tetracycline in chickens occurs by glomerular filtration (10). Arterial blood is supplied to the glomeruli from the renal arteries and renal branches of the external iliac arteries. Reported glomerular clearance values

range from 1.5 to 3.0 ml/min kg.

Circulation

Drug is transported to the tissues through the circulatory system. The cardiac output in 14.5 kg male turkeys is 111 ml/min/kg with a heart rate of 149 beats/min. Blood flows to most of the organs or tissue regions are in parallel. An exception is the so-called "renal portal" system. The proventriculus, gizzard, and duodenum are drained by the gastroduodenal vein which empties into the hepatic portal vein (supplying the liver). The renal portal system collects blood from the hind part of the body (hind limbs, tail, hind gut) and delivers a portion of it to the kidney tubules. However, blood may be shunted from the kidney through the intestinal vessels to the liver or vice versa. Consequently, when a substance normally secreted by the tubules is injected into the leg vein, it is excreted first by tubules on the injected side before reaching the general circulation. This property is used to determine if a drug is excreted by tubular secretion.

Pharmacokinetic Models

Pharmacokinetic modeling may be defined as the mathematical description of the biochemical and physiological processes which determine tissue concentrations following drug therapy. The model can be used to provide mechanistic information, predict drug levels not experimentally determined (extrapolation), evaluate parameters difficult to measure (e.g., permeabilities), compare various drug formulations and preparations, schedule drug therapy (dosage size and frequency),

and/or define areas where existing experimental data are inadequate. Although a model describing physiological and biochemical events at the microscopic level seems natural, macroscopic descriptions can adequately describe tissue distributions and require considerably less information and detail.

Physiological modeling

In physiological modeling, the body is divided into subregions for which macroscopic mass balances are written. Each major subregion (compartment) contains tissues with similar pharmacological properties (e.g., rapidly equilibrated tissues) or defines an anatomical region (e.g., organ) of interest. The compartments are interconnected to best represent the natural physiology. Materials are carried into each compartment by blood flow, and distribution occurs between the intravascular, interstitial, and cellular space.

Most drugs are readily transported across the capillaries into the interstitial fluid (20). In some cases, there is no significant delay in complete equilibrium between blood plasma and the extravascular region. The time required for such lipid soluble drugs to reach equilibrium may only be limited by plasma flow (flow limited). That is, the tissue permeability (k_i) is much larger than the regional perfusion rate (Q_i). Consequently, plasma leaving the compartment is in equilibrium with the tissue and a thermodynamic distribution ratio exists (C_{plasma}/C_{tissue} = constant) (21). Flow limited compartments can be modeled as a single stirred tank.

However, in certain compartments transport limitations may exist across membrane (e.g., cellular) barriers (transport limited). In this case, the compartment must be subdivided (e.g., extracellular, intracellular). The intercompartmental flux can be defined by a saturable and/or passive process. As reliable transport parameters are difficult to obtain, flow limited conditions are too often assumed.

Classical modeling

The most commonly used pharmacokinetic model is the largely empirical "classical" model. Like the physiological model, this model assumes that various regions of the body can be represented by subregions or compartments. Within each compartment, property variations are ignored and perfect mixing is assumed. However, unlike the physiological model, the compartments are fewer and less well-defined.

The number of compartments used in the classical model is determined by fitting (curve fitting) a sum of exponentials to the experimental data. The number of compartments is equal to the number of exponentials required to fit the concentration-time curve. Intercompartmental transfer is assumed to take place by a linear law and the unsteady-state mass balances between compartments are of the form $\frac{dCi}{dt} = \Sigma\, k_{ij} C_i$. For constant k_{ij}, the solution is of the form

$$C_i = \Sigma\, A_{ij} e^{B_j t} \quad .$$

Classical pharmacokinetic models are simple and useful when comparing different drug formulations or preparations. However, unlike the physiologically based models, the parameters in the classical model

do not usually correspond to actual anatomic and physiological parameters. Consequently, the empirical parameters can be difficult to interpret, provide little mechanistic information, and cannot be modified to describe altered physiological function.

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SECTION I: ORAL ABSORPTION OF CHLORTETRACYCLINE IN TURKEYS: INFLUENCE OF CITRIC ACID AND *PASTEURELLA MULTOCIDA* INFECTION

INTRODUCTION

The antibiotic chlortetracycline (CTC) is frequently administered to turkeys and other fowl as a treatment for infectious diseases. There is, in general, a close correlation between the concentration of antibiotic in the animal's body fluids/tissues and the drug's clinical effectiveness. Since CTC is commonly administered in the bird's drinking water or feed, resulting blood levels of CTC are dependent upon the rates of absorption, distribution, metabolism, and excretion. Numerous authors report that divalent cations (e.g., calcium and magnesium) present in animal feeds and drinking water inhibit absorption (1-4). Consequently, there is considerable interest in developing methods to increase the amount of tetracyclines absorbed following oral administration.

Eisner et al. (5) found that the oral administration of certain organic acids (including citric acid) enhanced the absorption of CTC from the gastrointestinal tract of rats, guinea pigs, and dogs. It was postulated that the mechanism of action for these organic acids was that of binding to metallic ions, thereby preventing their interference with absorption of the tetracycline (6). Clary et al. (7), after having difficulty reaching therapeutic blood levels in turkeys, enhanced the absorption of CTC with citric acid by adding both in the drinking water. Similarly, Russell and Kruger (8) found that administering citric acid enhanced the absorption of oxytetracycline in chickens.

In a recent study, our laboratory determined the serum concentrations in chickens following the oral administration of CTC alone and with citric acid (9). The addition of citric acid produced

significantly higher serum levels than when CTC was administered alone.

The primary purposes of this study were to investigate the following in turkeys:

1. The dose versus plasma level relationship when CTC is administered orally.
2. The effect of citric acid on the absorption of CTC.
3. Whether infection with P. multocida influences the absorption of CTC.

MATERIALS AND METHODS

Animals

The animals used were small Beltsville white turkeys initially eight weeks old and weighing an average of 1.8 kilograms. In most of the experiments, the error caused by weight differences among the birds was removed by "blocking out" weight (10).

Drug Administration

The CTC hydrochloride used was 97.1% pure. All drug solutions were prepared in distilled, deionized water and administered orally by gavage directly into the crop. Food was withdrawn from the birds 17 hours prior to drug administration, but distilled, deionized water was supplied ad lib.

Sample Collection and Treatment of Samples

Immediately before drug administration, a 3.0 ml blood sample was drawn from each bird's jugular vein. This sample served as an analytic base line. After each drug administration, 3.0 ml blood samples were collected from each bird at various times over the 24-hour period following administration. The samples were placed in tubes containing 10.0 units of heparin (to prevent coagulation). However, during the first three weeks of the study, the birds were small; consequently, smaller 1.5 ml blood samples were collected and pooled from two birds of the same group and of similar weight.

The 3.0-ml blood samples were spun down in a clinical centrifuge at approximately 4000 rpm, and 1.5 ml of the resulting plasma was pipetted into a glass tube with a Teflon-lined cap. Tissue samples were washed of excess blood, placed in capped glass receptacles, and immediately put on ice. All biological samples were frozen at -15°C until the time of analysis to prevent CTC decomposition. Approximately 10 minutes elapsed between the time the birds were killed and the time their tissue samples were placed in the freezer. The CTC in the plasma and tissue samples was determined fluorimetrically by means of a Turner Model III filter fluorometer (11).

Treatment Descriptions

It is desirable to administer CTC at a dose high enough to produce therapeutic concentrations without saturating the fowl's absorption process. Consequently, the proper dosage regimen was determined by orally administering various doses (10, 15, or 20 mg/kg) of CTC to the turkeys.

Two weeks later, after allowing the birds to physically recover, the fowl were orally dosed with varying amounts of citric acid (0, 75, 150, or 225 mg/kg) mixed with CTC (15 mg/kg). The drugs were dissolved in distilled, deionized water and the birds were allowed to drink only distilled, deionized water. As the birds were fasted 17 hours prior to drug administration, minerals in the gastrointestinal (GI) tract were minimized.

An experiment was conducted on 13-week-old birds to study the tissue distribution of CTC. The birds received an oral dosage of 15 mg/kg CTC and were killed by decapitation at one of 3 different times (2.5, 8.0, and 24 hours) after administration. Prior to decapitation, blood samples were collected for plasma. Tissue samples were collected from the red muscle, white muscle, liver, kidney and brain; these samples were subsequently frozen.

At 17 weeks of age, the birds were orally administered CTC (15 mg/kg) alone and mixed with citric acid (150 mg/kg). The drug (CTC) was dissolved in water containing 0.3 g/l Ca^{2+} (as $CaCl_2 \cdot 2H_2O$) and 0.1 g/l Mg^{2+} (as $MgSO_4$). The same levels of Ca^{2+} and Mg^{2+} were placed in the birds' drinking water. These concentrations of Ca^{2+} and Mg^{2+} are within the range of well water compositions reported in Iowa (12).

A common use for CTC is the treatment of fowl cholera. We were interested in ascertaining whether turkeys infected with P. multocida and subsequently dosed with a CTC (15 mg/kg) + citric acid (150 mg/kg) mixture had blood levels of CTC which differed from the same birds when they were similarly treated but healthy. Plasma levels of CTC were determined initially in 12 healthy turkeys as indicated above. One week later these same birds were infected by intramuscular injection with 2325 organisms of P. multocida in the right breast. Eighteen hours after the birds were infected, they were orally dosed with the CTC-citric acid mixture as described previously.

RESULTS

The plasma concentration curves representing turkeys orally dosed with 3 different amounts of CTC are presented in Figure 1. Plasma and tissue levels of CTC following the oral administration of 15 mg/kg CTC are presented in Table 1.

The plasma levels resulting from the oral administration of varying amounts of citric acid combined with 15 mg/kg CTC are presented in Figure 2. Citric acid does not appear to enhance the uptake of CTC when minerals in the GI tract are minimized. Figure 3 displays the plasma concentrations of CTC following the concomitant oral administration of CTC and citric acid when the CTC was dissolved in water that contained Ca^{2+} (0.3 g/l) and Mg^{2+} (0.1 g/l). The same levels of Ca^{2+} and Mg^{2+} were placed in the birds' drinking water. A statistical analysis (split plot (10)) indicates that, in the presence of minerals, the mean plasma level of the birds orally administered CTC + citric acid ($\bar{C}$ = 0.93) is significantly different (at the 5% level) than levels for the birds orally administered CTC alone ($\bar{C}$ = 0.35).

The data resulting from the oral administration of CTC combined with citric acid to turkeys that were initially healthy and later infected with P. multocida are presented in Figure 4. A statistical analysis (split plot (10)) indicates that the differences in the shape of the two plasma concentration curves is not significant at the 5% level, but the mean plasma concentration was significantly (at 5% level) higher when the birds were infected ($\bar{C}$ = 1.5) than when they were healthy ($\bar{C}$ = 0.98).

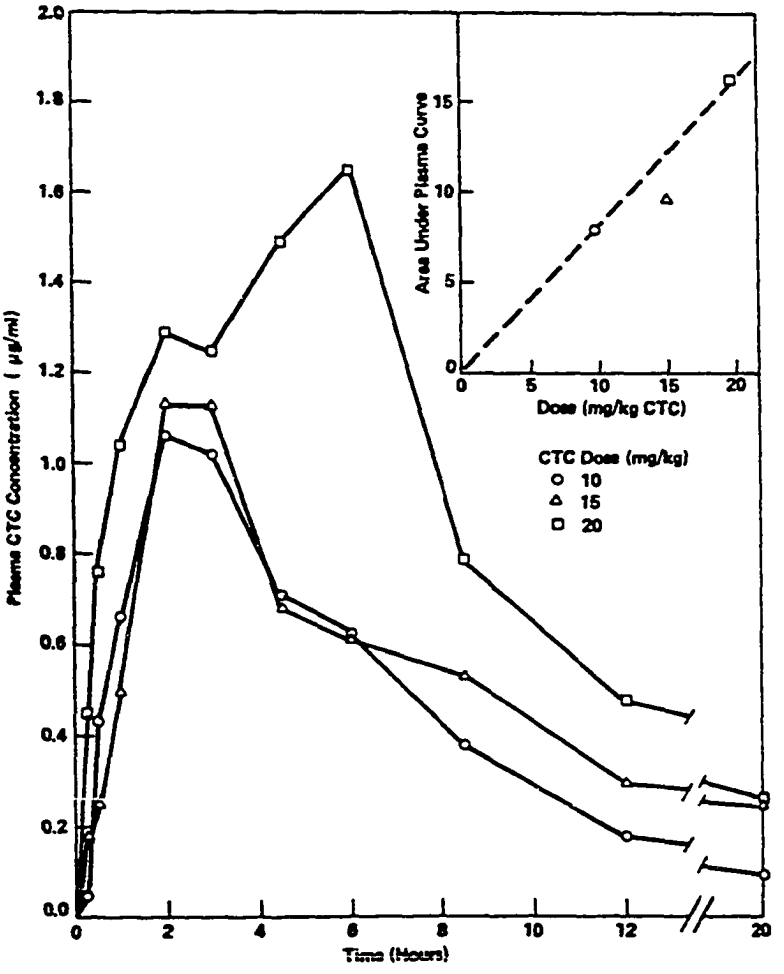

Figure 1. Average plasma concentrations of CTC in turkeys (8 weeks old, 1.8 kg) following the oral administration of different doses (10, 15, and 20 mg/kg) of CTC (n = 4 replications of each treatment)

Table 1. Concentration of CTC in tissue (μg/g) and plasma (μg/ml) samples following the oral administration of 15 mg/kg CTC to turkeys (n = 3). Results are expressed as the mean ± standard deviation of the mean. Birds were 13 weeks old and weighed an average of 3.3 kilograms

Time after administration (hr)	Region					
	Plasma	White muscle	Red muscle	Liver	Kidney	Brain
2.5	0.6 (±0.44)	0.09 (±0.06)	0.23 (±0.16)	3.05 (±2.01)	8.76 (±7.24)	0
8.0	0.44 (±0.25)	0.15 (±0.15)	0.28 (±0.17)	2.32 (±1.37)	10.84 (±8.19)	0.05 (±0.01)
24.0	0.11 (±0.05)	0.05 (±0.02)	0.05 (±0.04)	0.61 (±0.44)	2.57 (±0.35)	0.07 (±0.05)

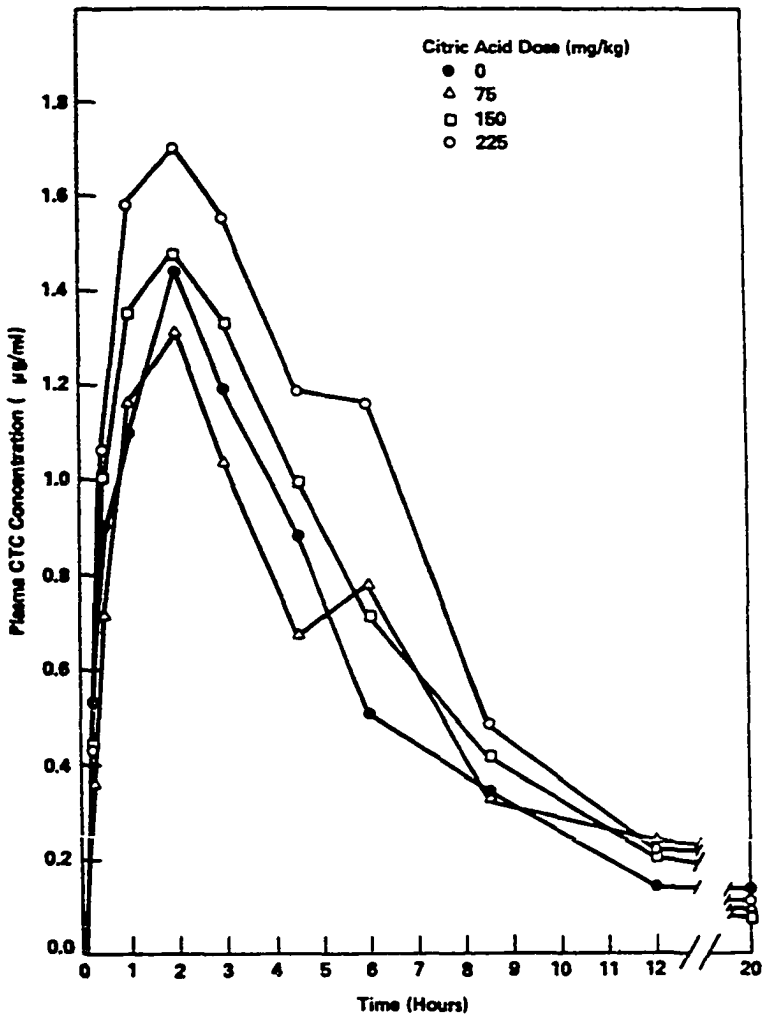

Figure 2. Average plasma concentrations of CTC in turkeys (10 weeks old, 2.3 kg) at various mean times following the oral administration of 15 mg/kg CTC combined with different amounts of citric acid (n = 4)

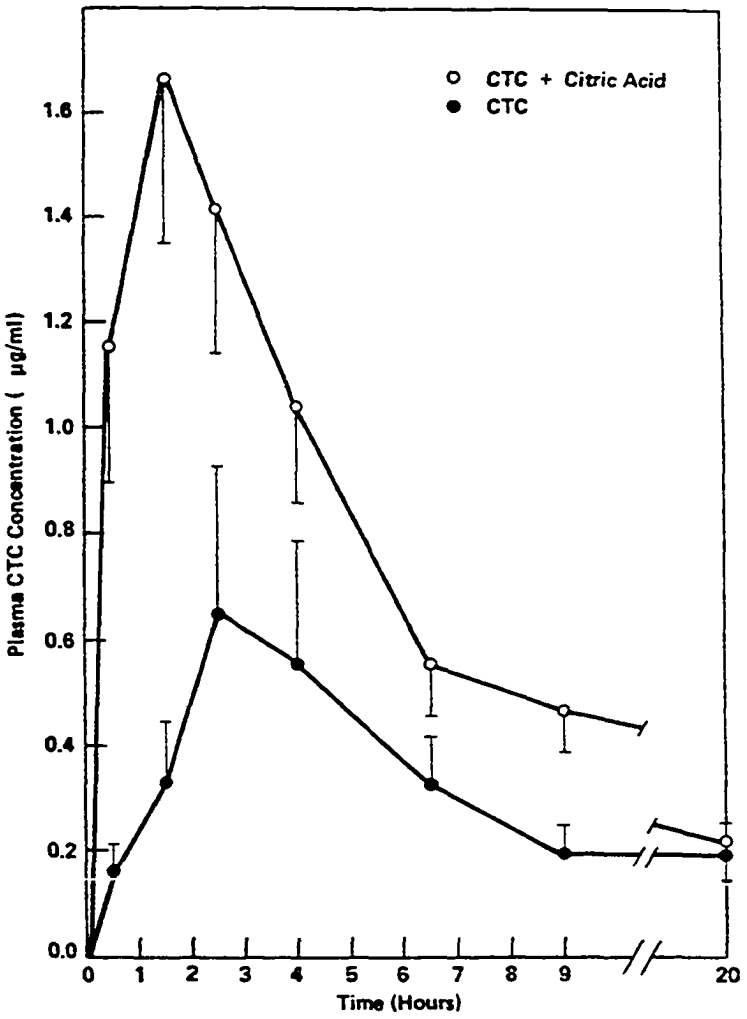

Figure 3. Average turkey (17 weeks old, 4.0 kg) plasma concentrations of CTC at various times following the oral administration of 15 mg/kg CTC alone (•) and with (o) 150 mg/kg citric acid. The CTC was dissolved in water containing calcium (0.3 g/liter) and magnesium (0.1 g/liter). Results are expressed as mean ± standard error of the mean ($\bar{y} \pm s/\sqrt{n}$), n = 12

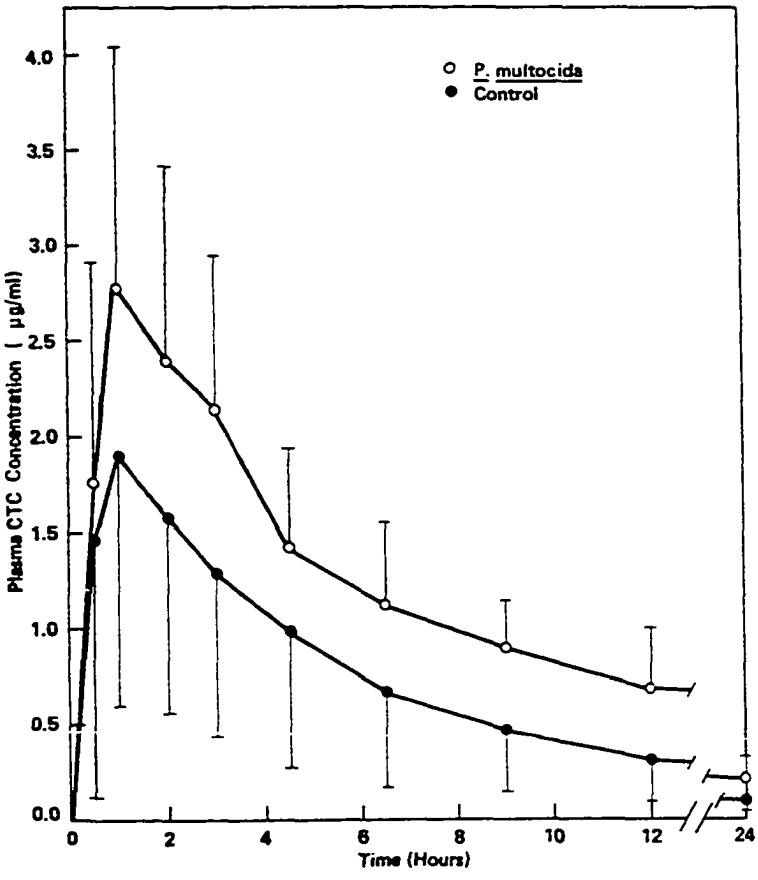

Figure 4. Plasma concentrations in the turkey (22 weeks old, 5.2 kg) following the oral administration of 15 mg/kg CTC combined with 150 mg/kg citric acid when the birds (n = 12) were healthy (●) and later infected (o) with P. multocida. Results expressed as mean ± standard deviation of the mean

DISCUSSION

When the oral dosage of CTC was varied, the results suggested that in the dosage regimens studied, plasma levels are nearly linearly related to dose (see Figure 1). Because a dose of 15 mg/kg CTC produces a peak level of CTC high enough to combat most microorganisms, this was the dose employed throughout the study.

It has been hypothesized that organic acids (such as citric acid) bind to divalent cations, thereby preventing their interference with the absorption of tetracyclines (6). This theory is supported by our data. When the amount of minerals present in the GI tract was kept at a minimum, the addition of citric acid to the dosing solution had little effect on the resulting CTC plasma levels. However, when Ca^{2+} and Mg^{2+} ions were present in the turkey's drinking water and in the CTC dosing solution, significantly higher drug levels were achieved with the addition of citric acid than when CTC was administered alone.

These results may be compared with earlier work when CTC (25 mg/kg) was administered to chickens, both alone and in conjunction with citric acid (125 mg/kg) (9). Although the CTC was dissolved in distilled, deionized water, the chickens were allowed to drink tap water ad lib. In this earlier study, significantly higher serum levels of CTC were achieved with the addition of citric acid. Apparently, the ions present in the tap water inhibited the absorption of CTC.

The tissue levels resulting from the oral administration of 15 mg/kg CTC (Table 1) provide valuable information regarding the distribution of CTC in the turkey. The low concentration of CTC in the

red and white muscle indicates that CTC does not readily enter muscle tissue. The high concentrations of CTC present in the kidney support earlier reports that the tetracyclines are secreted in the urine by glomerular filtration (13-16). The high levels of CTC present in the liver provide evidence that tetracyclines are concentrated in the liver and may be secreted in the bile (13, 14, 16). In addition, low levels of CTC in the brain are not surprising since most tetracyclines do not readily penetrate fatty tissue and the central nervous system (13).

When 12 birds were infected with P. multocida and dosed with CTC combined with citric acid, the plasma levels were significantly higher than when these same birds were healthy (Figure 4). Because fowl cholera can alter the normal physiology of the GI tract and liver (17), higher than normal plasma levels in the infected fowl may result from an alteration of the processes (gut motility, pH, biliary secretion, etc.) which control the absorption and elimination of CTC.

Of the twelve infected (2325 organisms/bird) birds orally dosed with CTC combined with citric acid, 5 birds died 3 days after administration of P. multocida and 2 birds died 5 days after administration. Five of the birds were alive and healthy on the sixth day. However, all 10 control turkeys, infected with 2200 organisms of the same P. multocida culture, died within 2 days. Consequently, it appears that, as reported, CTC lowers the mortality from infection with P. multocida (17).

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SECTION II: THE PHARMACOKINETICS OF CHLORTETRACYCLINE ORALLY ADMINISTERED TO TURKEYS: INFLUENCE OF CITRIC ACID AND *PASTEURELLA MULTOCIDA* INFECTION

INTRODUCTION

Chlortetracycline (CTC) is a broad spectrum bacteriostatic antibiotic frequently used in veterinary medicine to combat microbial growth. Since CTC is poorly absorbed (inhibited by minerals in the gastrointestinal tract) and rapidly eliminated, therapeutic blood and tissue levels can be difficult to achieve and maintain. In this work, a physiologically based pharmacokinetic model was developed and used to study the effects of both citric acid and fowl cholera on the absorption and elimination of CTC in the turkey.

The majority of previously reported pharmacokinetic studies involving tetracyclines have used the traditional two (1-5) or three (6) compartment models to describe serum or plasma profiles. In one earlier investigation in our laboratory, a two compartment model was used to describe the pharmacokinetics of orally administering CTC to chickens (7); the CTC was introduced both alone and with a metal chelator (citric acid). CTC increased the absorption rate; however, the hypothesized mechanism could not be verified with traditional modeling.

An alternate type of pharmacokinetic model is a physiologically based model. Physiological models have been used to accurately describe concentration profiles of numerous substances in various species (8). Of these previous studies, the work of Olanoff and Anderson (9) is of particular interest because of its focus on tetracycline delivery (in the rat). However, because the fowl differs anatomically, when compared to other species, there remains a need to describe the pharmacokinetics of CTC in the turkey.

EXPERIMENTAL

The purpose of the experimental work was to collect the data needed to 1) estimate pharmacokinetic model parameters in the healthy turkey, 2) test and evaluate the pharmacokinetic model, 3) determine the mechanism by which citric acid enhances absorption of CTC in the presence of minerals, and 4) evaluate the pharmacokinetics in the diseased turkey. Because many of the experimental details are reported elsewhere (10), only highlights and previously unreported experiments are described.

Animals

The animals used in this study were small Beltsville white turkeys initially eight weeks old and weighing an average of 1.8 kilograms.

Drug Administration

All drug solutions were prepared in distilled, deionized water. Food was withdrawn from the birds 17 hours prior to drug administration, but distilled, deionized water was supplied ad lib. CTC was orally dosed (by gavage) alone (15 mg CTC/kg) and in a mixture with citric acid (150 mg citrate/kg). CTC was given intravenously (IV) (1 mg CTC/kg) as a bolus injected into the right jugular vein.

Analytical Procedures

Phenolsulfonphthalein assay

The fecal samples, containing phenolsulfonphthalein, were mixed with distilled water (1:1) and centrifuged at 2500 rpm. Two milliliters of the supernatant were mixed with 2 ml of 1.0 N NaOH and the OD_{558} determined.

Radiochemical assay

Labeled polyethylene glycol (^{14}C - PEG, M.W. 4000) concentration in 0.2 ml gut samples was determined by liquid scintillation counting. Quenching was corrected by employing external standards.

CTC assay

Concentrations of CTC in plasma and tissue were determined fluorometrically (11).

Parameter Estimation

The parameters employed in the pharmacokinetic model are displayed in Tables 1 and 2.

Volume of gut lumen

The volume (V_{IL}) of the fowl's gastrointestinal lumen is highly dependent on the experimental conditions. The lumen volume was estimated with the following expression:

$$V_{IL} = M_T/\bar{C}_{IL} \qquad (1)$$

Table 1. Compartmental parameters used in the pharmacokinetic model

Parameter	Estimate
Bile to liver concentration ratio[a], B_R	20
Renal clearance[b,c], R_C	5.1 ml/min
Unbound fraction of CTC in plasma, bile, liver, carcass	0.59
Unbound fraction of CTC in intestinal lumen, F_{I5}	0.34-0.98
Decomposition rate constant[d], K_R	0.00015 1/min
Biliary flow[b,e], Q_B	0.05 ml/min
Fecal flow[b], Q_F	0.13 ml/min
Intestinal permeability[f,g], Region 1, k_{I2}	0.0047 ml/min
Intestinal permeability[f,g], Region 2, k_{I3}	0.0106 ml/min
Intestinal permeability[f,g], Region 3, k_{I4}	0.0059 ml/min
Intestinal permeability[f,g], Region 4, k_{I5}	0.0045 ml/min
Carcass permeability[f], k_C	5.5 ml/min
Liver transport parameter[f], K_L	4.7 ml/min

[a]Value estimated from Reference 12.

[b]Value scaled (13) to 3.3 kg turkey.

[c]Glomerular filtration rate for fowl (14).

[d]Calculated from decomposition data (15).

[e]Reference 12.

[f]Parameter providing best least squares fit to data.

[g]Intestinal permeabilities proportioned in accordance with Reference 16 (i.e. $k_{I2} = 0.44\ k_{I3} = 0.8\ k_{I4} = 1.04\ k_{I5}$).

Table 2. Physiological volumes and plasma flows used in the model[a]

Region	Volume (V_i)[b] or mass (M_i)	Plasma flow[c] (Q_i) ml/min
Plasma	116 ml	320
G.I. plasma	7.3 ml	36
G.I. lumen 1	7 ml	--
G.I. lumen 2-4	15.7 ml	--
Total lumen volume	54 ml	--
Liver plasma	24 ml	61
Liver tissue	22.8 g	--
Carcass[d] (rapid equil.)	483 ml	259
Carcass (slow equil.)	2358 g	--

[a]Values are scaled (13) for a 3.3 kg turkey.

[b]Plasma volumes were estimated from References 17 and 18.

[c]Plasma flows were estimated from References 19-22.

[d]Carcass volume corresponds to inulin space (23).

where M_T = initial dose of tracer (counts) and

$\bar{C}_{IL}$ = volume averaged tracer concentration in the G.I. lumen (counts/ml).

Since the thickness of the liquid layer on the inner surface of the gut was observed to be relatively constant throughout the gastrointestinal tract, a surface area ($\pi r^2 L$) averaged concentration was used to estimate $\bar{C}_{IL}$.

The experimental procedure involved orally dosing 6 fasted (16 hours) 3.8 kg turkeys with a predetermined amount of nonabsorbable (24) tracer (^{14}C - PEG). Each bird was killed one hour after drug administration and lumen samples were collected from the crop, duodenum, upper intestine, and lower intestine. The low concentrations of PEG found in the large intestine (Table 3) indicate that little tracer was lost in the feces.

Table 3. Concentrations of PEG in the gastrointestinal lumen of a turkey one hour after 7.4 ml of ^{14}C - PEG (63550 counts/ml) were placed in the bird's crop

Region	Concentration (counts/0.2 ml)
Crop	8145
Duodenum	3317
Upper small intestine	315
Lower small intestine and colon	20

Fecal flow rate

Although the rate at which material passes through the gut can be highly irregular (dependent on the fowl's emotional state and gut contents (25)), an average flow rate (Q_F) may be estimated by dividing the total volume of the gut lumen (V_{IL}) by the mean transit time (τ).

The transit time was estimated by orally dosing the birds with phenolsulfonphthalein (1 mg/kg of 0.5 mg/ml phenolsulfonphthalein) and

measuring the concentration of dye in the feces as a function of time. Phenolsulfonphthalein was used because it is poorly absorbed (26) and easy to assay colorimetrically. The resulting fecal concentration versus time curve is shown in Figure 1. The calculated (27) transit time, $\bar{t} = (\int_0^\infty tC_F dt)/(\int_0^\infty C_F dt)$, was 6.75 hours.

Binding

In the plasma, tetracyclines are reversibly bound to albumins, globulins, and lipoproteins (28). Reported values (29) of the fraction bound in plasma are from 47 to 70 percent; with the percent of bound tetracyclines found to be constant over a wide range of concentrations (.1 - 10 μg/ml) (30).

With our assay procedure, most bound CTC is not recovered. When plasma samples were spiked with CTC, 41% was not recovered, and hence, this value represents a lower limit on the fraction bound. As information is not available on CTC binding in turkey tissues, this conservative estimate (0.41) was used for the fraction bound in all tissues. Because the carcass compartment consists of several tissue types, this estimate represents the mass average of strong (e.g., bone (31)) and weak binding tissues.

CTC-Mineral-Citrate Binding

The acid-base and metal binding expressions for CTC and citric acid are presented in Figure 2. The mass balances describing a mixture of CTC, metal cations, and citric acid are given in Equations (2-5).

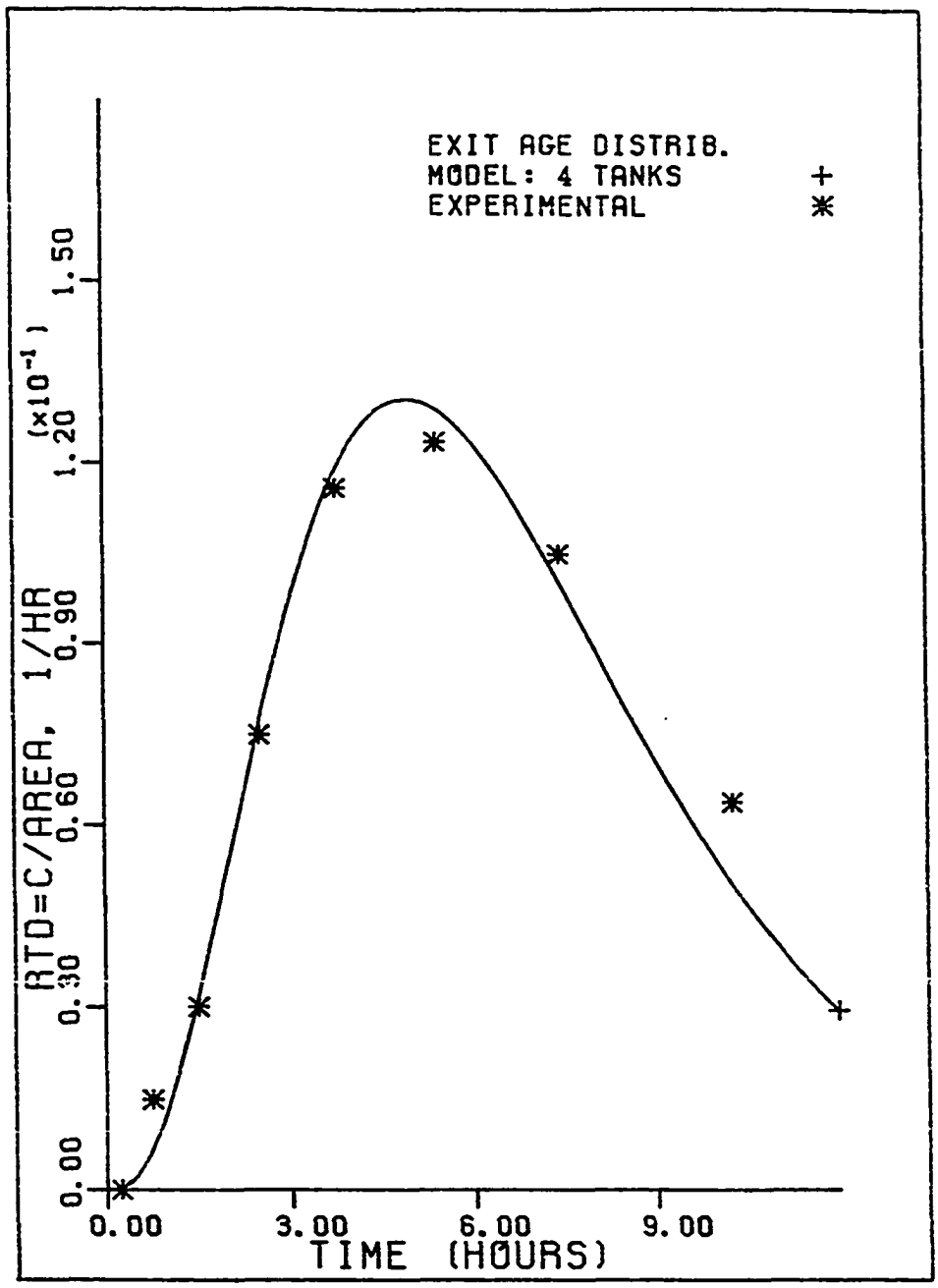

Figure 1. Distribution of phenolsulfonphthalein in turkey feces following oral administration

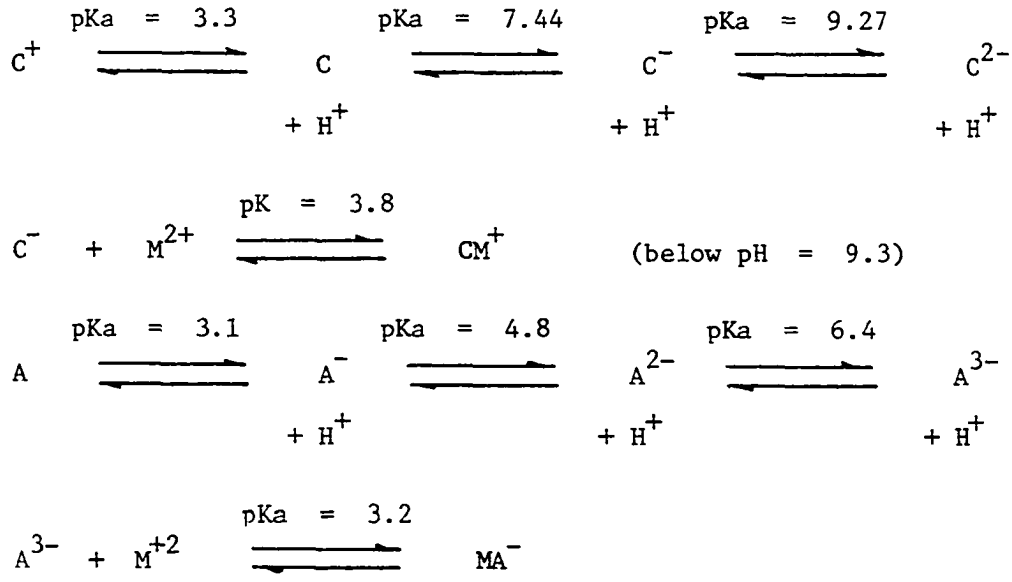

Figure 2. The ionization and chelation reactions reported in the literature. Ionization and stability constants are from References 32-35 (C = CTC; M = metal (Ca^{2+} or Mg^{2+}); A = citric acid)

CTC (C) Balance

$$[CTC]_{t=0} \doteq [C^+] + [C] + [C^-] + [CM^+] \quad (2)$$

Metal (M) Balance

$$[M^{2+}]_{t=0} = [M^{+2}] + [CM^+] + [MA^-] \quad (3)$$

Citric Acid (A) Balance

$$[A]_{t=0} = [A] + [A^-] + [A^{2-}] + [A^{3-}] + [MA^-] \quad (4)$$

H^+ Balance

$$[H^+] \doteq [H^+]_{t=0} + [C] + 2[C^-] + 2[CM^+] + [A^-]$$

$$+ 2[A^{2-}] + 3[A^{3-}] + 3[MA^-] \quad (5)$$

Assuming a constant pH, due to gastrointestinal buffering, Equations (2-5), combined with the appropriate ionization and stability expressions, leads to a relation describing the equilibrium concentrations of bound and free drug as a function of the initial concentrations of CTC, minerals, and citric acid. Solution of this relation gives Figure 3. For the experimental conditions, with and without citric acid, the free fractions of CTC are predicted to be 0.98 and 0.34, respectively.

Model Development

The following assumptions, described in previous or subsequent sections, were made in formulating the pharmacokinetic model:

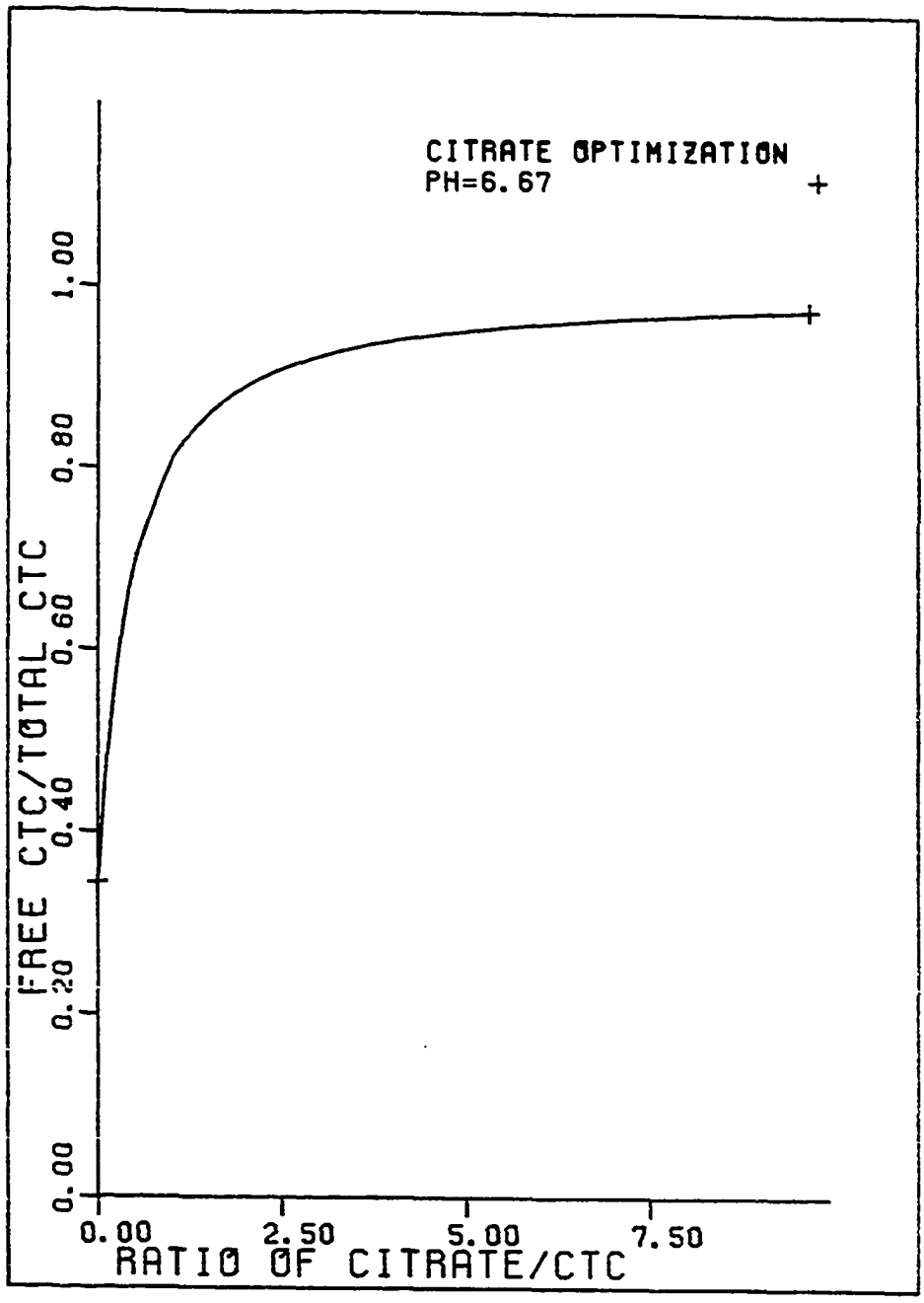

Figure 3. Theoretical relationship between the unbound fraction of CTC and the citrate/CTC ratio; pH = 6.67, 0.3 g/l Ca^{2+}, 0.1 g/l Mg^{2+}, and 7 g/l CTC

1) Tissues with similar properties may be lumped together.

2) Each lumped compartment may be represented by a well-mixed tank.

3) Transport and elimination occurs by first-order kinetics.

4) CTC readily crosses capillaries and equilibrates with easily diffusible interstitial fluids.

5) Only the free component of drug crosses membrane barriers or decomposes.

6) The free fraction of CTC in plasma/tissues remains constant.

The turkey is represented by 4 compartments (plasma, liver, gastrointestinal, and carcass) (see Figure 4). Each region is characterized by an average concentration varying in time. The compartmental macroscopic mass balances, describing the change in CTC levels within each compartment, are given in the Appendix A.

To determine if transport limitations exist, the liver and carcass compartments were each divided into rapidly (interstitial fluid and plasma) and slowly (intracellular) equilibrating fluid subregions. Mass transfer between carcass subregions was described as

$$\text{Net transfer} = k_c \, (F_P C_{C1} - F_{C2} C_{C2}) = A \, J_{12} \, .$$

Transfer of drug between the gastrointestinal lumen and plasma was described by a similar expression (see Appendix A).

Uptake of drug by the liver was modeled as a nonsaturable first-order process ($K_L F_P C_{L1}$). Because most materials are thought to enter the bile canaliculi after first entering the parenchymal cells (36, 37), transport into the bile was calculated as

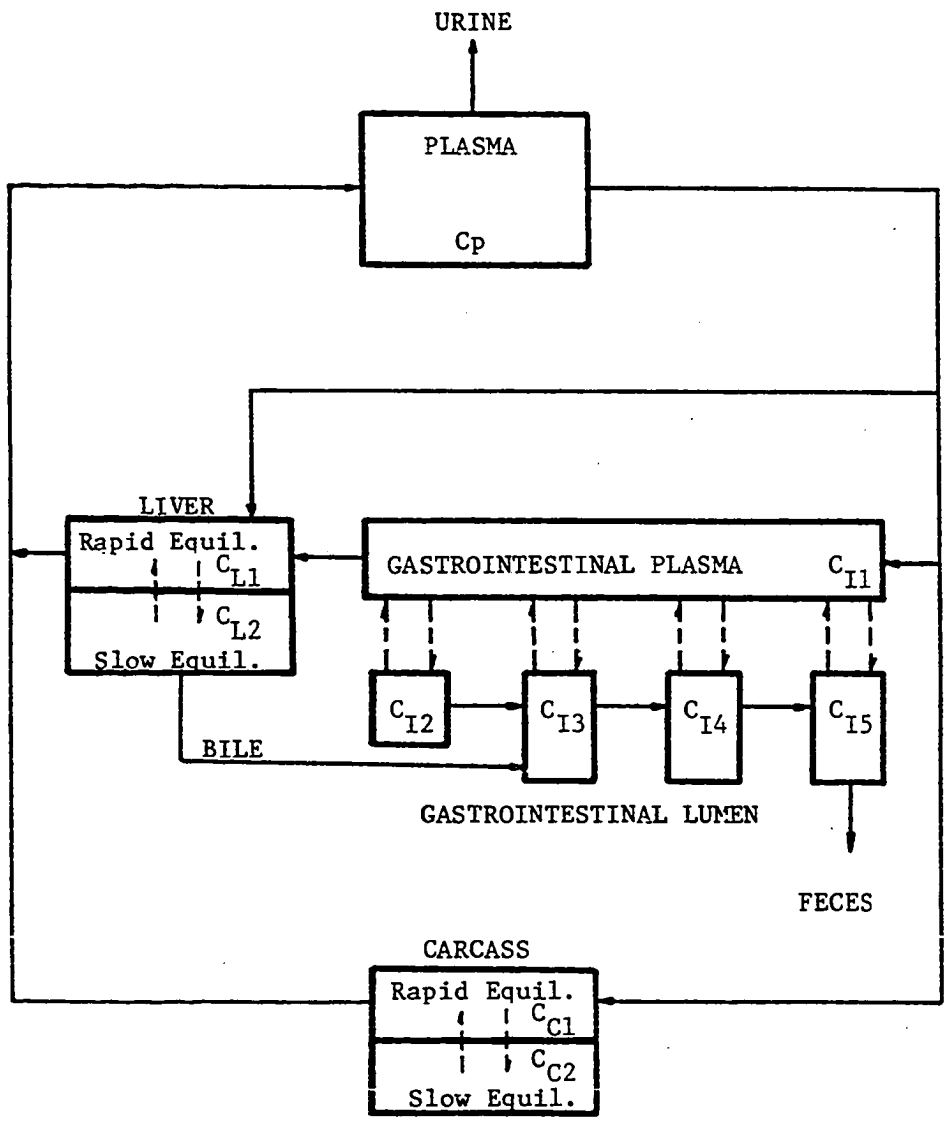

Figure 4. Diagram of the physiologically based pharmacokinetic model of CTC absorption and disposition in the turkey. Solid lines indicate flows between regions and dashed lines indicate regions where intercompartmental transfer is concentration dependent. Chemical decomposition occurs in all compartments

$$\text{Biliary transfer} \doteq Q_B B_R C_{L2} .$$

Elimination of CTC occurs by renal excretion, fecal excretion, and chemical decomposition. Chemical decomposition was described as a first-order rate process as observed in the dog (15). Since renal elimination of tetracycline in the fowl occurs by glomerular filtration (16), renal elimination was given by the glomerular clearance rate. For elimination via fecal flow, one must account for the mixing and dilution of the bolus dose as it moves down the gastrointestinal tract.

Four tanks in series (Figure 4) were required to describe the exit age distribution (Figure 1) determined from nonabsorbable tracer studies. Tank 1 represents the volume (and concentration) of tracer administered to the birds at time = 0. Transport of drug occurs between each tank and the gastrointestinal plasma. Since the highest rate of absorption of tetracycline occurs in the upper portion of the small intestine (16), higher permeabilities were used in the first two tanks.

An alternative model considered was a single tank with variable volume. As solute is transported throughout the gut, it distributes into a larger volume ($V_{IL}(t)$) with a larger surface area for absorption. Fecal elimination (Q_F) does not begin until the drug reaches the end of the large intestine (at $t = t_E$). As an approximation to the distribution process, $V_{IL}(t)$ and $k_I(t)$ were described by linear functions. For $t < t_E$,

$$V_{IL}(t) = V_{IL}^{o} + (t/t_E)(V(t_E) - V_{IL}^{o})$$

$$k_I(t) = k_I \, V_{IL}(t)/V_{IL}(t_E)$$

where V_{IL}^{o} = volume of dose. Finally, for $t \geq t_E$:

$$V_{IL}(t) = V_{IL}(t_E)$$

$$Q_F(t) = Q_F$$

$$k_I(t) = k_I \, .$$

Solution

When the 'tanks-in-series' model is used to describe flow through the gut, the entire physiological model is described by a system of first-order differential equations of the form:

$$C' = AC \qquad C_{t=0} = B$$

where C' = vector of concentration derivatives,

A = constant coefficient matrix,

C = vector of compartmental concentrations, and

B = vector of concentrations at time = 0.

The analytical and numerical solutions, available elsewhere (38), were solved on a digital computer, employing various routines in the IMSL library (39). Permeabilities were estimated with an (IMSL) iterative least squares routine.

RESULTS AND DISCUSSION

Oral Administration

Figures 5a, 5b, and 5c display the fitted (tanks in series model) and observed concentration profiles following the oral dosage of 15 mg/kg of CTC to healthy turkeys. The permeabilities used in the model were shown in Table 1.

The low permeabilities relative to the regional perfusion rates ($k_i/Q_i << 1$) and existence of a nonlinear relationship between experimental tissue and plasma concentrations (Table 1 of Reference 10) suggest transport limitations from lumen to plasma and between certain tissues. Low permeabilities would be consistent with the observation that CTC occurs predominantly in an ionized (membrane insoluble) state (40, 41).

Simulations which model the gut lumen as one variable volume stirred tank are shown in Figures 6a, 6b, and 6c. Permeability values (ml/min) for the gut, liver, and carcass are 0.034, 4.7, and 5.5, respectively. The fit is inferior to that of Figures 5a, 5b, and 5c, but could be improved were the higher permeability gut region incorporated.

A simulated comparison of all routes of drug transport and elimination was made by integrating the appropriate transport term over time (Table 4). As the literature suggests (15), biliary secretion accounts for a significant portion of CTC elimination. Chemical decomposition accounts for less than 5% of elimination. However, the

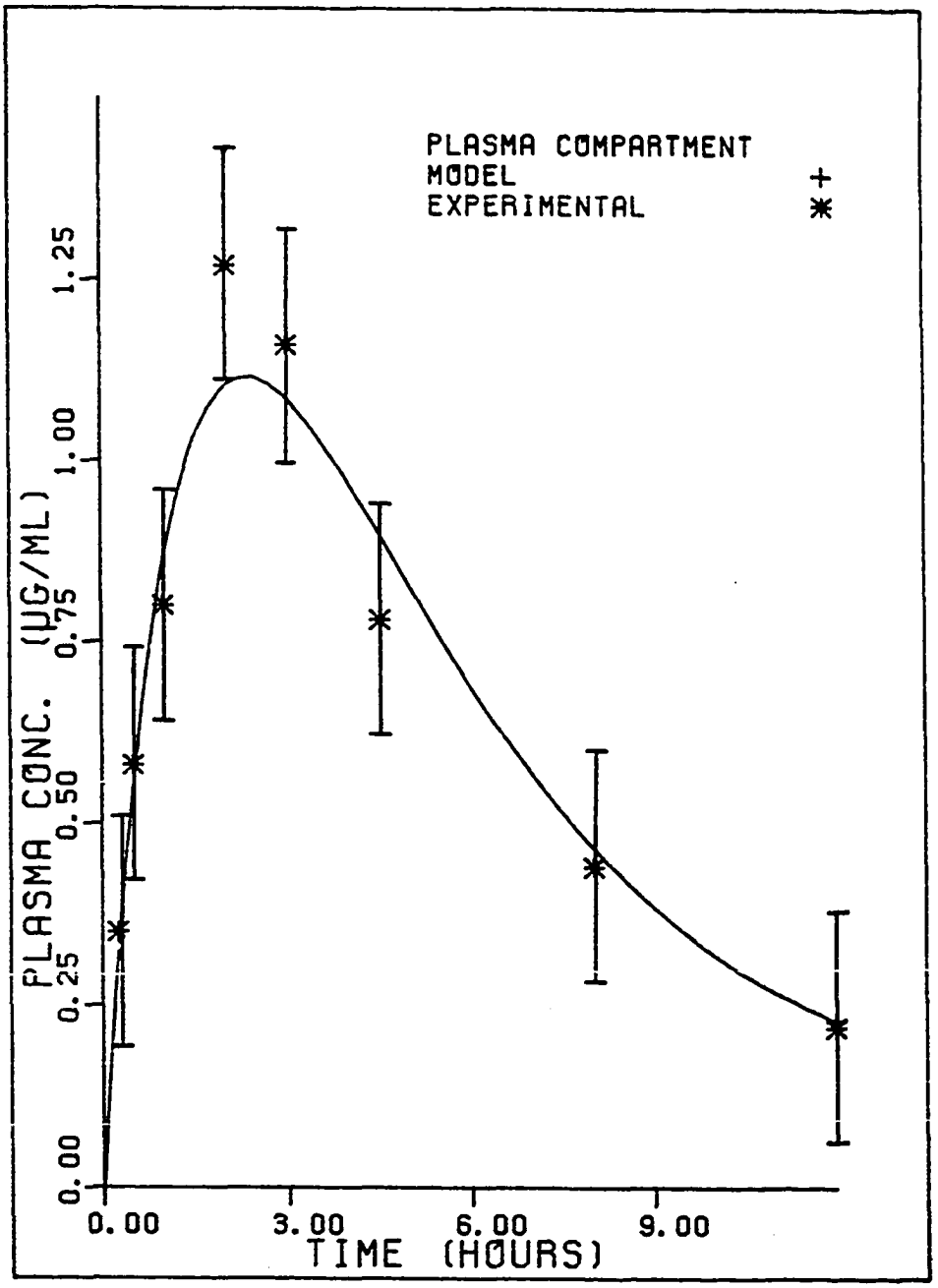

Figure 5a. Concentration profiles of unbound CTC in the plasma following oral administration (15 mg/kg). Experimental and simulated (lumen modeled as tanks in series) concentrations at 24 hours are 0.10 and 0.08, respectively. Error bars represent the standard error of the mean (SEM) = $\sqrt{S^2/n} = 0.16$

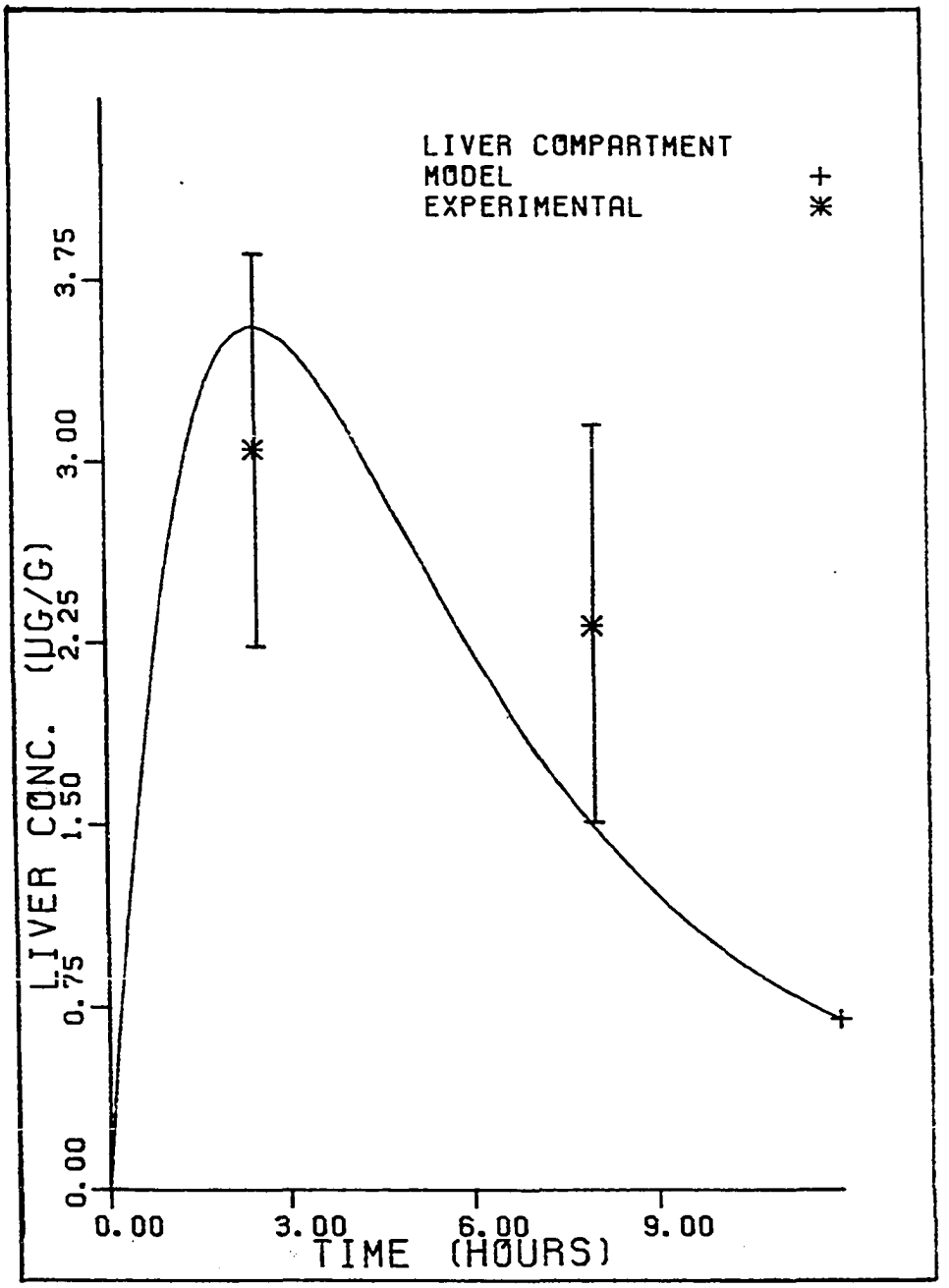

Figure 5b. Concentration profiles of total CTC in the liver following oral administration (15 mg/kg). Experimental and simulated (lumen modeled as tanks in series) concentrations at 24 hours are 0.61 and 0.23, respectively. SEM = 0.82

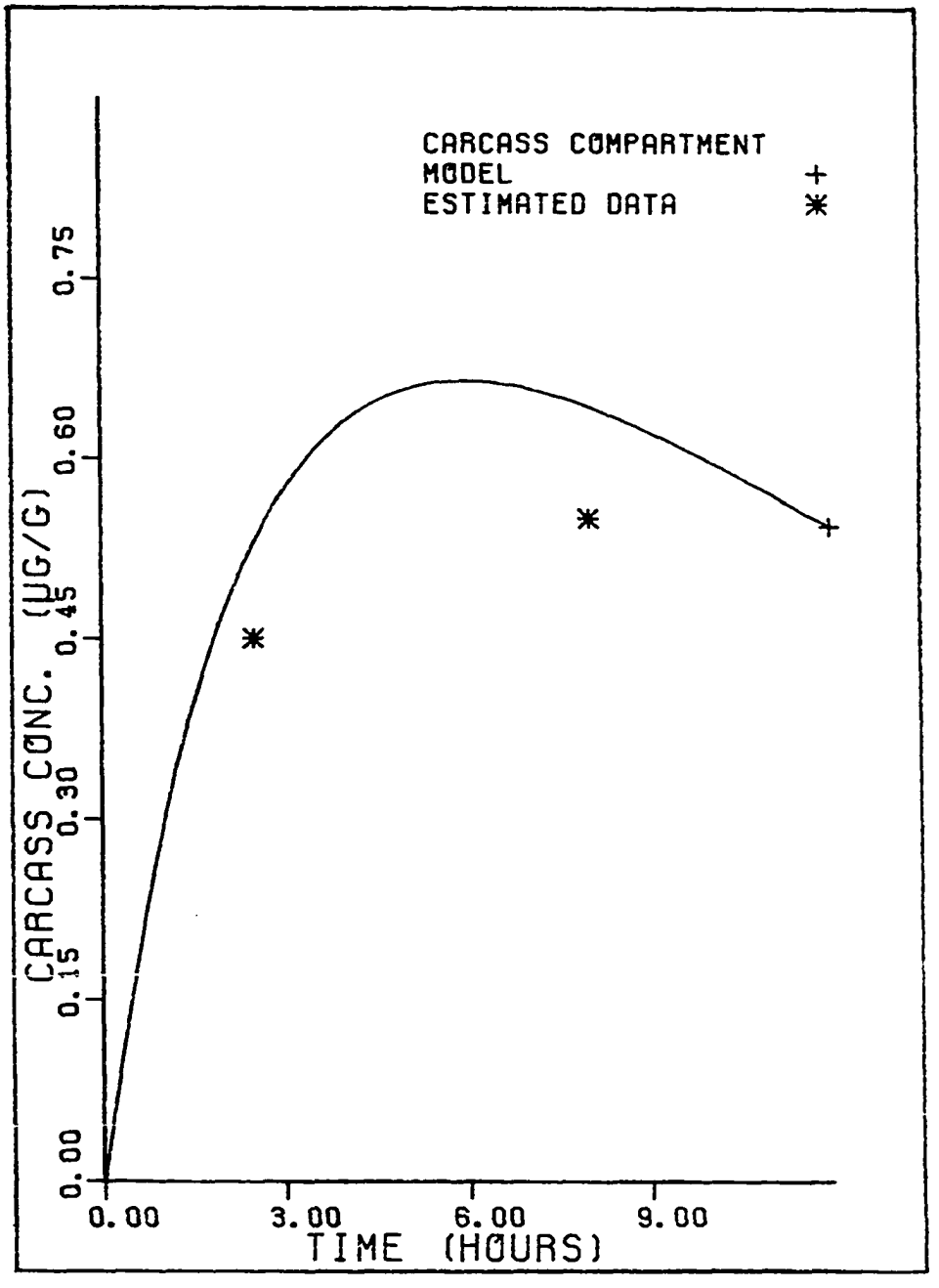

Figure 5c. Concentration profiles of total CTC in the carcass tissue following oral administration (15 mg/kg). Estimated and simulated (lumen modeled as tanks in series) concentrations at 24 hours are 0.29 and 0.28, respectively.

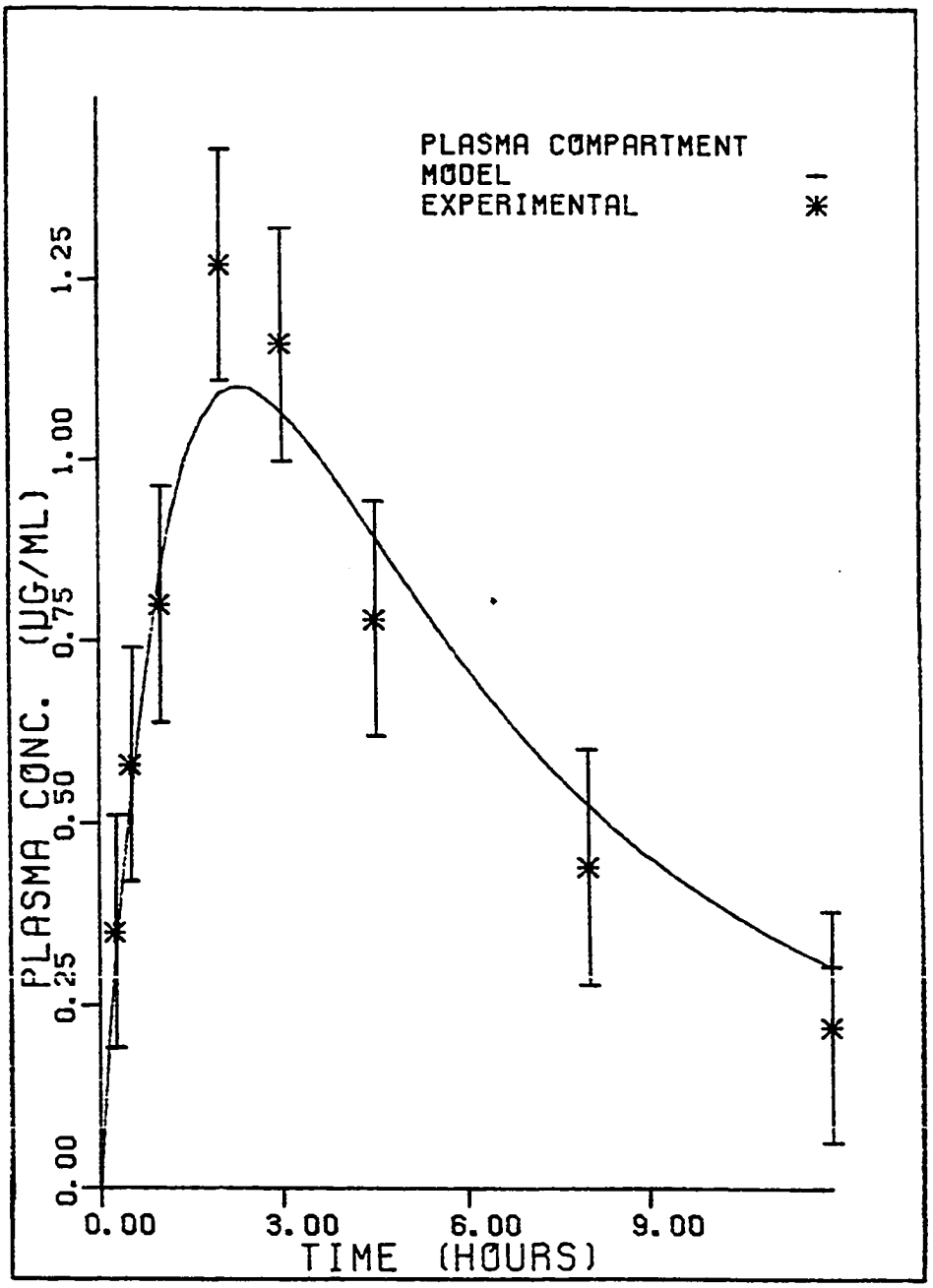

Figure 6a. Concentration profiles of unbound CTC in the plasma following oral administration (15 mg/kg). Experimental and simulated (lumen modeled as variable volume) concentrations at 24 hours are 0.10 and 0.10, respectively. SEM = 0.16

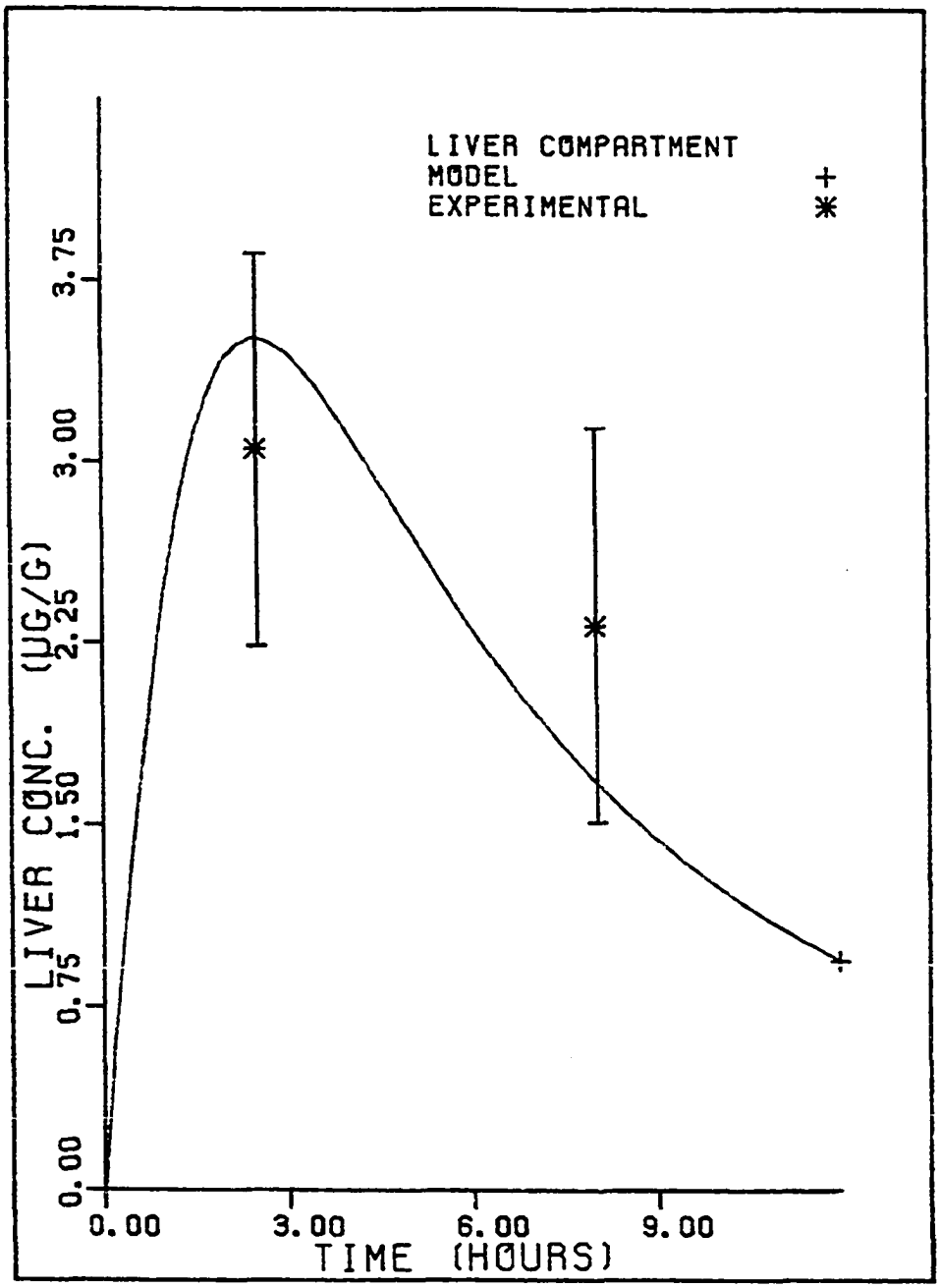

Figure 6b. Concentration profiles of total CTC in the liver following oral administration (15 mg/kg). Experimental and simulated (lumen modeled as variable volume) concentrations at 24 hours are 0.61 and 0.30, respectively. SEM = 0.82

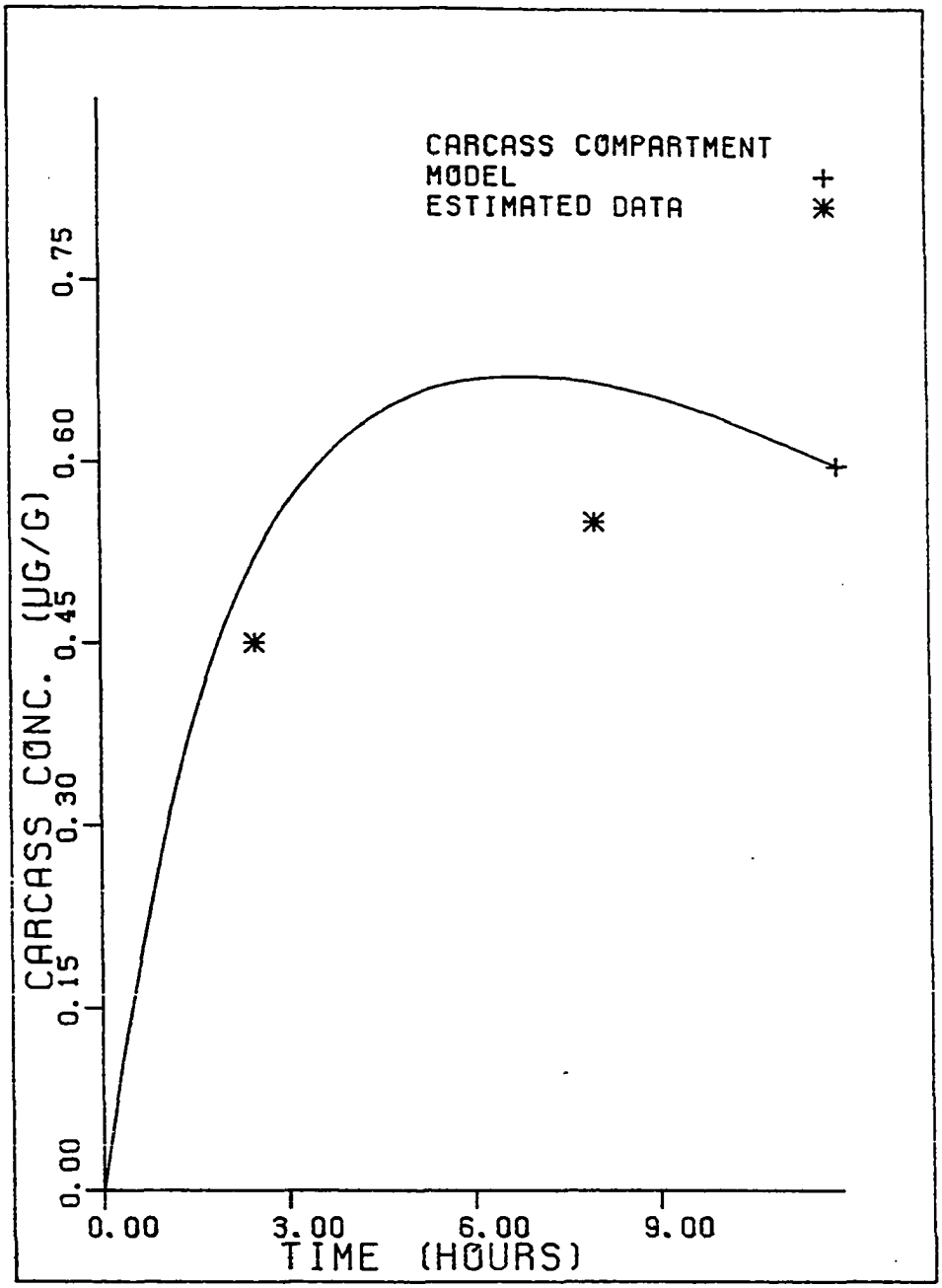

Figure 6c. Concentration profiles of total CTC in the carcass tissue following oral administration (15 mg/kg). Estimated and simulated (lumen modeled as variable volume) concentrations at 24 hours are 0.29 and 0.33, respectively

Table 4. Percent of CTC absorbed and eliminated in the presence of minerals (0.1 g/l Mg^{2+}, 0.3 g/l Ca^{2+}), as based on model calculations

Treatment	Percent of oral dose absorbed	Percent of absorbed drug eliminated by:		
		Reaction	Renal	Biliary
CTC alone	6	4	50	46
CTC + citric acid	16	4	49	47

reactive rate constant used in the model describes the decomposition of CTC in dog urine; constants may differ in other tissues.

Intravenous Administration

Simulation of the IV data is displayed in Figure 7. The good agreement of the basic model with the first data point (t = 2 min) indicates that the model adequately describes the early, rapid distribution process. However, at later times, the experimental plasma data show a more rapid decline of drug than the model predicts.

Although an alternate route of administration can provide a rigorous test for a model, it is not uncommon for the simulation to inadequately describe the data. Toutain and Raymaud (3) found that the elimination half-life ($t_{\frac{1}{2}(\beta)}$) following the IV dosing of OTC to cattle was shorter than $t_{\frac{1}{2}(\beta)}$ for intramuscular (IM) administration. They hypothesized that only a small fraction of the IM dose is immediately available for transport, suggesting that a model with rapid and slow release compartments is required.

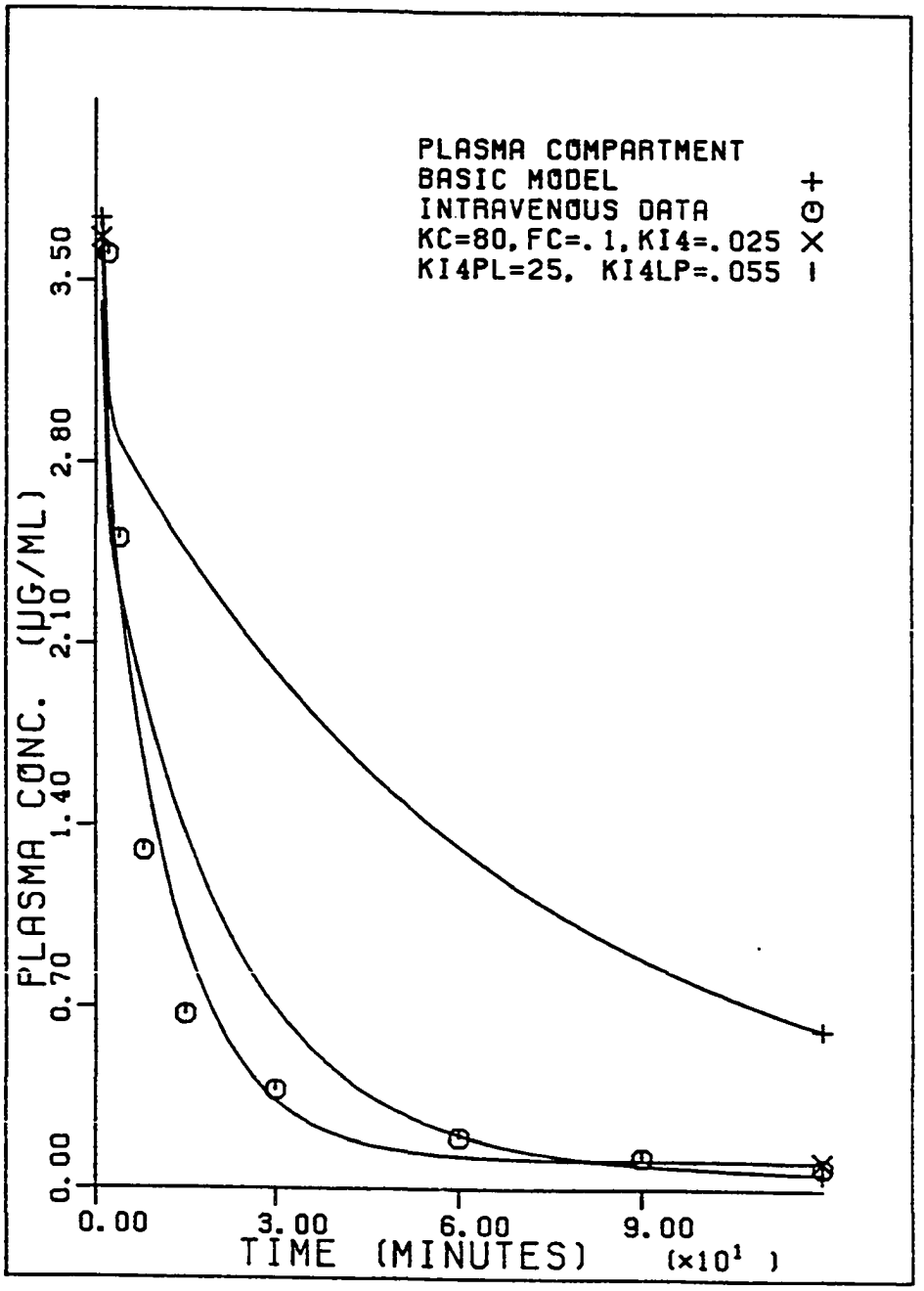

Figure 7. Experimental and simulated concentration profiles of unbound CTC in the plasma following intravenous administration (1 mg/kg)

Possible reasons this model does not adequately describe both the IV and oral data are the following:

1) Rapid IV administration of high bolus doses may induce conditions (e.g., nonlinear binding) that invalidate certain model assumptions.
2) A rapid distribution phase with strong binding may exist in regions (e.g., bone) not sampled.
3) The gastrointestinal transport parameter may be higher when drug is transported from plasma to lumen than from lumen to plasma.

Items 2 and 3 above were tested by adjusting parameters of the existing model.

When a highly bound (F_c = .1) rapid distribution (k_c = 80 ml/min) phase is incorporated into the model, the result is as shown in Figures 7, 8a, and 8b. Although the intravenous plasma fit (Figure 7) is improved, the oral simulation (Figure 8a) peaks early with high levels at longer times. However, this model assumed linear, concentration dependent binding. If CTC located in the rapid distribution region was tightly bound, then the material would be released more slowly into the plasma. However, the predicted carcass concentrations (Figure 8b) with this hypothesis are unrealistically high.

Although certain studies (42, 43) indicate that intestinal secretion may account for a significant portion of tetracycline elimination, other investigators (15) indicate that little CTC is transported from plasma to lumen. When the coefficients describing

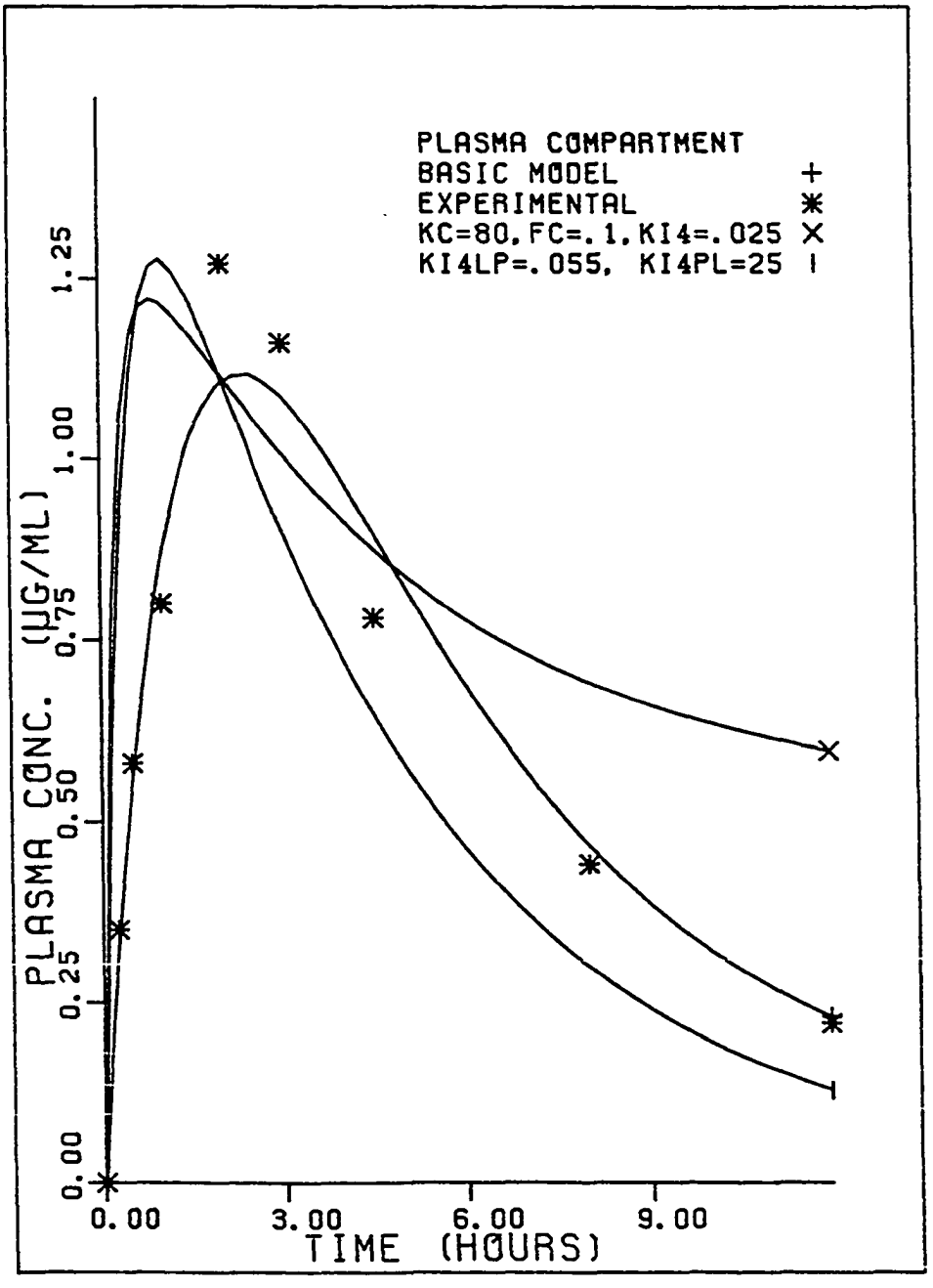

Figure 8a. Concentration profiles of unbound CTC in the plasma following oral administration (15 mg/kg). Simulations use intravenous parameters

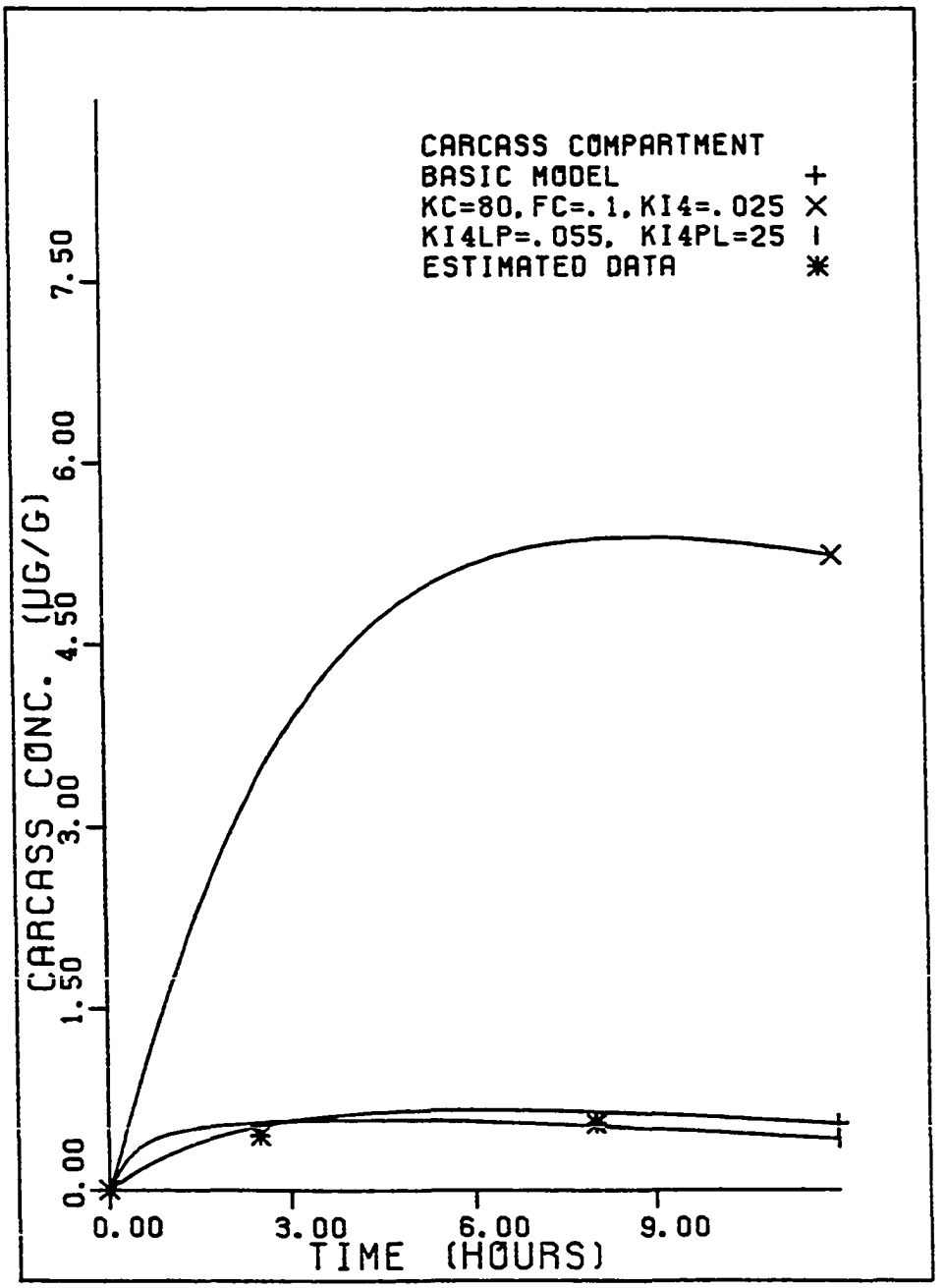

Figure 8b. Concentration profiles of total CTC in the carcass tissue following oral administration (15 mg/kg). Simulations use intravenous parameters

transport from plasma to lumen (i.e. $K_{I2-5}(PL)$ = 20.0, 45.0, 25.0 and 19.3, respectively) are assumed higher than from lumen to plasma (i.e. $K_{I2-5}(LP)$ = 0.044, 0.099, 0.055 and 0.042, respectively), the IV fit is improved (Figure 7), but description of the oral data (Figures 8a and 8b) is inadequate.

These simulations indicate that the present model is not capable of describing both oral and IV administration. Apparently, certain model assumptions (e.g., linear binding, well-mixed) become invalid with rapid IV administration. However, the model does provide a good description of the oral data.

Effect of Citric Acid

Figure 3, representing the unbound fraction of CTC (in the gut lumen) with increasing citric acid, indicates that a considerably higher unbound fraction of CTC is available with citric acid (F_I = 0.98) than without (F_I = 0.34). When these unbound fractions (F_I) are substituted into the model (without altering the other model parameters), the simulation (Figure 9) predicts the plasma profiles well. Thus, these simulations support our previous hypothesis that citric acid increases the rate of absorption by providing a larger fraction of unbound CTC for absorption.

The addition of citric acid increased the fraction of drug absorbed from 6 to 16 percent (Table 4). Because disposition parameters were not altered when simulating the citrate data, these parameters do not appear to be significantly affected by citric acid.

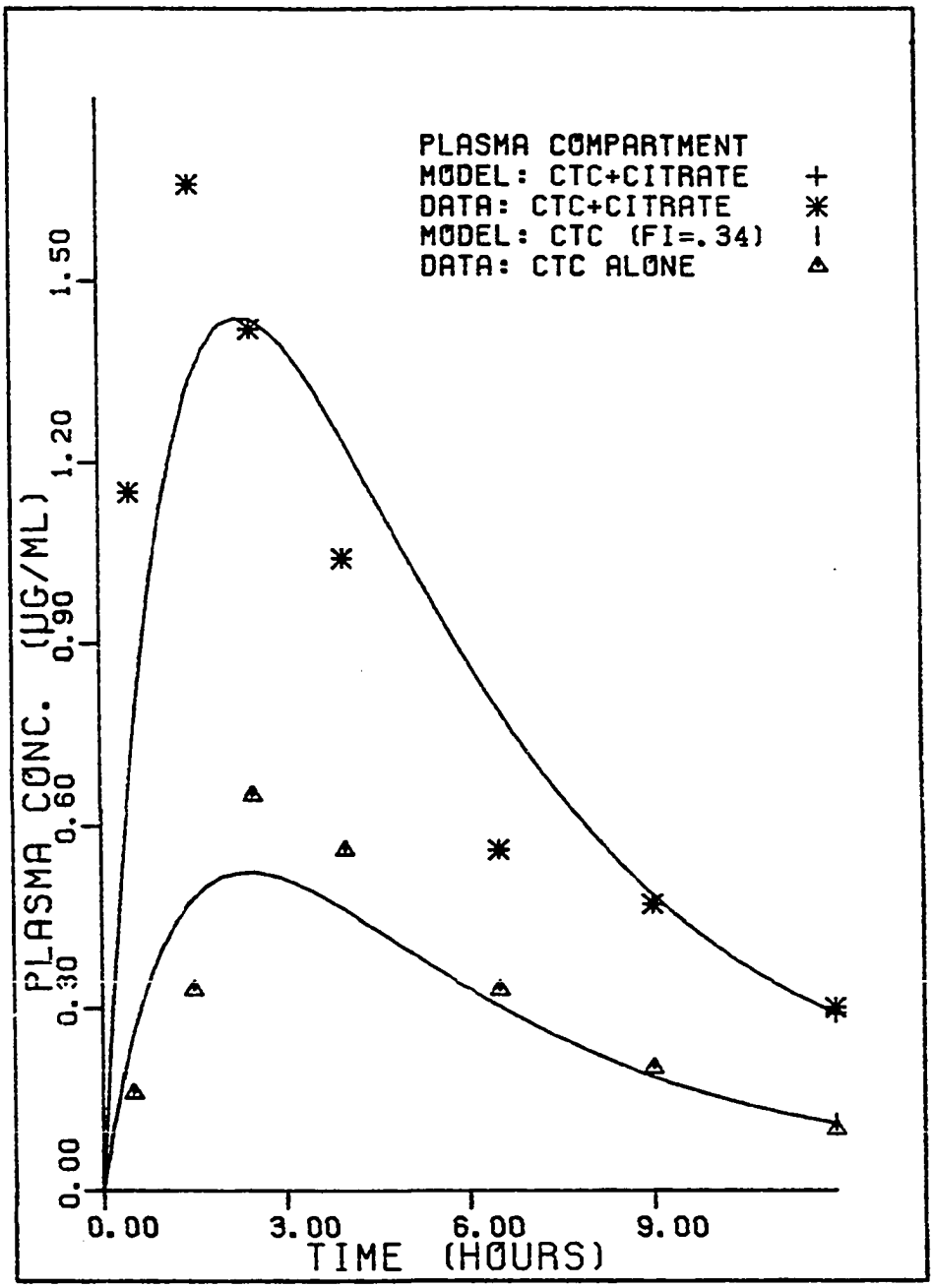

Figure 9. Experimental and simulated concentration profiles of unbound CTC in the plasma following the oral administration of CTC alone (15 mg/kg) and with citric acid (150 mg/kg). SEM = 0.35

Effect of Fowl Cholera

Pharmacokinetics frequently change in the diseased state due to alterations in plasma perfusion, hepatic function, drug binding, drug transport, and/or renal function (44). Consequently, the pharmacokinetics (of CTC) in infected (P. multocida) turkeys were compared with those in healthy birds.

The birds used in the diseased study were older and larger than those used in the other studies; consequently, older control (healthy) birds were used. Following the oral administration of CTC, the plasma profiles in older (5.2 kg), healthy turkeys peaked earlier than those in younger (3.3 kg) birds. The investigation of this phenomenon with the pharmacokinetic model indicates that the original (simulation A, Table 5) gastrointestinal permeabilities appear to be modified, with the upper portion of the gut contributing more to absorption (simulation B, Table 5). The altered gut permeabilities appear to represent an age-induced change in the physical characteristics of the gut (e.g., Δ pH, more surface area, etc.).

When plasma profiles of CTC in diseased birds were compared to profiles in healthy birds, concentrations of CTC were consistently higher in the diseased birds (Figure 10). Although fowl cholera can produce a wide range of physiological changes (45), only those changes that could increase plasma levels were simulated (Table 5, Figure 10). When gastrointestinal permeabilities are increased (simulation C, Table 5), the early time data is described well; however, simulated concentrations are low at later times. On the other hand, lowering the hepatic

Table 5. Model parameters in healthy and infected (P. multocida) turkeys (see text for simulation descriptions)

Simulation	Age (weeks)	Condition	Parameters (ml/min)						
			k_{I2}	k_{I3}	k_{I4}	k_{I5}	k_c	K_L	Q_F
A	13	healthy	0.0047	0.0106	0.0059	0.0045	5.5	4.7	0.13
B	22	healthy	0.0164	0.0080	0.0074	0.0057	9.5[a]	7.0[a]	0.20[a]
C	22	diseased	0.0249	0.0121	0.0113	0.0087	9.5[a]	7.0[a]	0.20[a]
D	22	diseased	0.0164	0.0080	0.0074	0.0057	9.5[a]	0.0	0.20[a]
E	22	diseased	0.0200	0.0097	0.0090	0.0069	9.5[a]	1.7	0.20[a]

[a]Parameters scaled (13) to describe 5.2 kg turkeys.

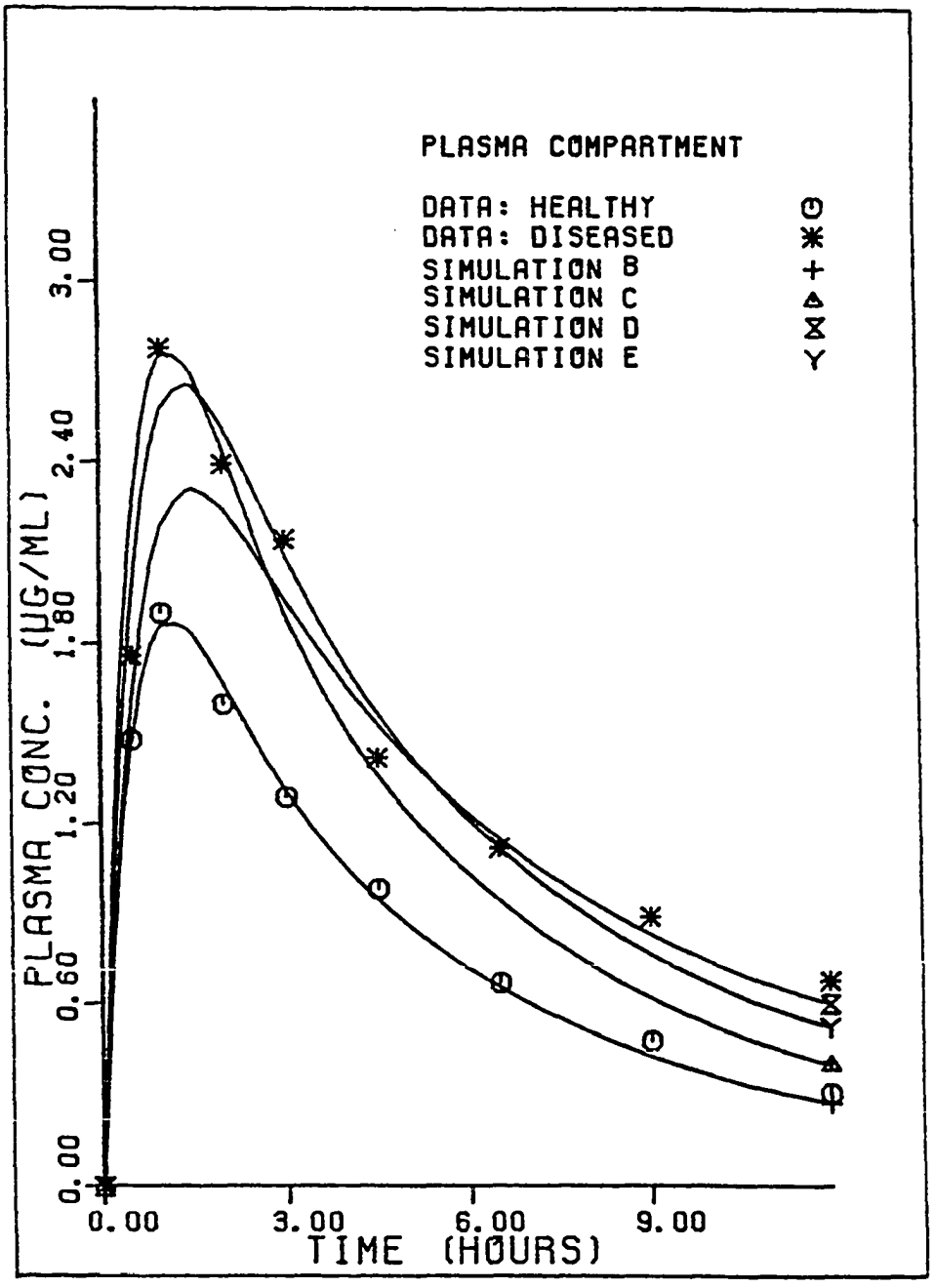

Figure 10. Experimental and simulated concentration profiles of unbound CTC in the plasma following the oral administration of CTC (15 mg/kg) + citric acid (150 mg/kg) to healthy and diseased turkeys. SEM = 0.29

clearance (simulation D, Table 5) produces low concentrations at early times, but higher concentrations at later times. When both the gastrointestinal permeabilities and hepatic clearance were optimized (simulation E, least squares best fit), the plasma data were adequately described.

Because lesions of the liver are a clinical manifestation of fowl cholera, a decrease in hepatic clearance appears likely. A possible explanation for disease increasing gastrointestinal permeabilities is that the presence of pathogenic bacteria in the gastrointestinal tract may alter the pH of the lumen, thereby changing the fraction of unionized (absorbable) CTC. An alternate explanation is that certain chemicals (i.e. histamine, endotoxins), released by damaged tissues and bacteria, increase capillary permeabilities. If these substances also increased the permeabilities of surrounding, uninfected, tissues, then the overall gastrointestinal permeability could increase.

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APPENDIX A: MODEL EQUATIONS

Plasma:

$$V_P \frac{d\,C_P}{dt} = Q_L C_{L1} + Q_C C_{C1} - (Q_P + F_P R_C + V_P K_R F_P)\, C_P$$

Gastrointestinal plasma, region 1:

$$V_{I1} \frac{d\,C_{I1}}{dt} = Q_I C_P + k_{I2}(F_{I2} C_{I2} - F_P C_{I1}) + k_{I3}\,(F_{I3} C_{I3} - F_P C_{I1})$$

$$+ k_{I4}\,(F_{I4} C_{I4} - F_P C_{I1}) + k_{I5}\,(F_{I5} C_{I5} - F_P C_{I1})$$

$$- (Q_I + V_{I1} K_R F_P)\, C_{I1}$$

Gastrointestinal lumen, region 2:

$$V_{I2} \frac{d\,C_{I2}}{dt} = k_{I2}\,(F_P C_{I1} - F_{I2} C_{I2}) - (Q_F + V_{I2} K_R F_{I2})\, C_{I2}$$

Gastrointestinal lumen, region 3:

$$V_{I3} \frac{d\,C_{I3}}{dt} = Q_B B_R C_{L2} + k_{I3}\,(F_P C_{I1} - F_{I3} C_{I3}) + Q_F C_{I2}$$

$$- (Q_F + V_{I3} K_R F_{I3})\, C_{I3}$$

Gastrointestinal lumen, region 4:

$$V_{I4} \frac{d\,C_{I4}}{dt} = k_{I4}\,(F_P C_{I1} - F_{I4} C_{I4}) + Q_F C_{I3} - (Q_F + V_{I4} K_R F_{I4})\, C_{I4}$$

Gastrointestinal lumen, region 5:

$$V_{I5} \frac{d\, C_{I5}}{dt} = k_{I5} (F_P C_{I1} - F_{I5} C_{I5}) + Q_F C_{I4} - (Q_F + V_{I5} K_R F_{I5})\, C_{I5}$$

Liver plasma:

$$V_{L1} \frac{d\, C_{L1}}{dt} = Q_I C_{I1} + (Q_L - Q_I)\, C_P - (K_L F_P + Q_L + V_{L1} K_R F_P)\, C_{L1}$$

Liver tissue:

$$M_{L2} \frac{d\, C_{L2}}{dt} = K_L F_P C_{L1} - (Q_B B_R + M_{L2} K_R F_{L2})\, C_{L2}$$

Carcass (rapidly equilibrating):

$$V_{C1} \frac{d\, C_{C1}}{dt} = Q_C (C_P - C_{C1}) + k_C (F_{C2} d_C C_{C2} - F_P C_{C1}) - V_{C1} K_R F_P C_{C1}$$

Carcass (slowly equilibrating):

$$M_{C2} \frac{d\, C_{C2}}{dt} = k_C (F_P C_{C1} - F_{C2} d_C C_{C2}) - M_{C2} K_R F_{C2} C_{C2}$$

APPENDIX B: NOMENCLATURE

General

A	Surface area perpendicular to transport, cm^2
B_R	Concentration ratio of bile to liver (C_B/C_{L2})
C	Total tissue concentration, µg/ml or µg/g
d_C	Density of carcass tissue, g/ml
F	Fraction of CTC unbound
J	Mass flux, µg/min cm^2
k	Permeability, ml/min
K	First order transport coefficient, ml/min
K_R	Decomposition rate constant, 1/min
M	Mass of compartment, g
Q	Flow rate, ml/min
Q_C, Q_I, Q_L, Q_P	Flow rates of plasma in carcass, intestine, liver, and plasma, respectively, ml/min
Q_B	Flow rate of bile, ml/min
Q_F	Flow rate of feces, ml/min
R_C	Renal clearance rate, ml/min
t	Time, min
V	Volume of compartment, ml

Subscripts

C	Carcass
C1	Rapidly equilibrating carcass tissue and plasma
C2	Slowly equilibrating carcass tissue
I	Gastrointestinal

I1	Gastrointestinal equilibrium plasma, region 1
I2	Gastrointestinal lumen, region 2
I3	Gastrointestinal lumen, region 3
I4	Gastrointestinal lumen, region 4
I5	Gastrointestinal lumen, region 5
IL	Total gastrointestinal lumen
L	Liver
L1	Rapidly equilibrating liver tissue and plasma
L2	Slowly equilibrating liver tissue
P	Arterial and venous plasma

RECOMMENDATIONS

The physiologically based pharmacokinetic model adequately described concentration profiles following oral administration of CTC (+ citrate); however, the IV data could not be described by the same model. To adequately represent the fowl's natural physiology, the existing model may require certain refinements (e.g., additional compartments, nonlinear binding, and/or nonlinear transport). In this section, additional experiments are suggested that will provide the information necessary to ameliorate the present model so that it more closely represents physiological reality.

Tissue sampling: In this study, selected tissues were sampled at 3 different times after drug administration. Although the tissue groups sampled comprise the majority of the bird's total weight, tissues not sampled (e.g., bone) could function as reversible sinks. Future experiments should include a study of CTC partitioning in all turkey tissues. The assumption that compartments are well-mixed could be tested with intratissue sampling. The sampling frequency is important because nonlinearities cannot be discovered or described without sufficient tissue sampling.

Binding: Although this study assumes linear tissue binding, there are circumstances when nonlinear binding may exist. For example, high concentrations (e.g., IV dosing) of CTC could cause binding sites to become saturated. Since information describing binding of tetracyclines in turkey tissues is unavailable, binding experiments are needed. In particular, the concentration of CTC at which saturation occurs should be

determined.

Elimination routes: Model estimates of the fraction of CTC eliminated by various routes compare favorably with experimental data reported for dogs and rats. However, because birds differ anatomically from dogs and rats, similar experiments need to be conducted in turkeys. In addition, since CTC chemically decomposes, reaction rates must be estimated in tissues and wastes (urine and feces). These experiments would provide information on drug elimination and data to test for conservation of mass.

Gastrointestinal processes: In the present model, the gastrointestinal lumen was represented by a series of well-mixed tanks. Lumen flow between tanks was assumed constant. However, results from previous investigations indicate that the flow rate of gut contents decreases from the proximal to distal portions of the gastrointestinal tract. Such studies need to be carried out in the fowl; they require data on tracer (nonabsorbable) distribution as a function of time and position. Another parameter that varies with position along the gastrointestinal tract is the permeability. Experiments should be conducted to determine gut permeabilities in different segments of the turkey's gastrointestinal tract.

Diseased state: Although the effects fowl cholera has on pharmacokinetics can be hypothesized, the theories cannot be verified without further experimentation on infected birds. Tissue concentrations in diseased birds need to be determined so that permeabilities can be estimated. Whenever possible, other model parameters should be

determined by model independent methods. For example, the fecal flow, renal clearance, and fraction bound can be determined by tracer experiments, urine collection, and equilibrium dialysis, respectively.

CONCLUSIONS

The principal conclusions were the following:

1) The plasma response, measured as the area under the concentration-time curves, indicated that the amount of CTC absorbed was nearly linearly related to the dose (10, 15, 20 mg CTC/kg orally).

2) The concentration of CTC was considerably higher in the liver and kidney than in the muscle and brain at all sample times (2.5, 8.0, 24.0 hrs).

3) The time-course of CTC in the plasma and tissues following the oral administration of CTC to turkeys was adequately described by a physiologically based pharmacokinetic model.

4) The low values of permeabilities (k_i), relative to the regional plasma flows (Q_i), indicate that tissue permeability limits CTC distribution.

5) Based on model simulations, the fractions of dose eliminated by renal excretion, biliary secretion, and chemical decomposition are 50%, 46%, and 4%, respectively.

6) The addition of citric acid to an oral dosage mixture (7 g/l CTC, 70 g/l citrate, 0.3 g/l Ca^{2+}, 0.1 g/l Mg^{2+}) produces significantly higher plasma levels than when citrate is omitted. The higher plasma concentrations were accounted for by a model of competitive binding of CTC and citrate to metal cations. Citric acid appears to bind to metal cations, thereby preventing their interference with the absorption of CTC.

7) Based on model simulations, the addition of citric acid to a CTC (7 g/l) mixture containing Ca^{2+} (0.3 g/l) and Mg^{2+} (0.1 g/l) increases the unbound fraction of CTC in the lumen from 0.34 to 0.98, and the fraction of dose absorbed from 0.06 to 0.16.

8) Following oral administration of CTC, birds infected with P. multocida had significantly higher plasma levels than healthy birds.

9) Although the effects that fowl cholera has on the pharmacokinetics were not fully characterized, the model indicates that intestinal permeability is higher in infected birds and, in addition, renal and/or hepatic clearances are reduced.

10) Concentration profiles in the plasma following intravenous administration of CTC declined more rapidly than those predicted by the model.

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APPENDIX: COMPUTER PROGRAMS

```
C--------------    PROGRAM #1     ----------------------
C
C
C   ORAL MODEL: G.I. TRACT MODELED BY 4 TANKS IN SERIES
C
C            WRITTEN BY: BOB POLLET
C
C         PROGRAM IS WRITTEN IN WATFIV
C
C          REFERENCE:  ELEMENTARY DIFFERENTIAL EQUATIONS & BOUNDRY
C                      VALUE PROBLEMS, 1977
C                      W.E. BOYCE   R.C. DIPRIMA          PP304-321
C
C
C
C   EXPLICIT DECLARATION OF ALL VARIABLES
       INTEGER FLAG(12),I,IA,IDGT,IER,IJOB,IZ,J
       INTEGER LUNFLO,M,N,NUM,P
       DOUBLE PRECISION A(11,11),B(11,1),BR,CL,CLT(10)
       DOUBLE PRECISION CMT(10),COSI(11),CP(10),DM,E(11),EE(11)
       DOUBLE PRECISION F,F2,F3,F4,F5,F6,FB,FI,FL,FM,IW(11),K(11)
       DOUBLE PRECISION KI,KI2,KI3,KI4,KI5,KI6,KL,KM,KR,ML2,MM2
       DOUBLE PRECISION PAR(11),POWER(11),PR(11),QB,QF,QI,QL,QM,QP
       DOUBLE PRECISION RW(22),RZ(242),SINE(11),SSQ,SSQL,SSQM,SSQP,T
       DOUBLE PRECISION TEST(11),VI1,VI2,VI3,VI4,VI5,VI6
       DOUBLE PRECISION V(11,11),VL1,VM1,VP
       DOUBLE PRECISION WK(143),WKAREA(154),WT(11),X(11)
       REAL*8 DREAL,DIMAG
       COMPLEX*16 W(11),Z(11,11),ZN
       EQUIVALENCE (W(1),RW(1)),(Z(1,1),RZ(1))
       NUM=11
C   PREVENTS EXPONENTIAL UNDERFLOW
       LUNFLO=100
       CALL TRAPS(0,0,LUNFLO,0,0)
C
C   VALUES OF PARAMETERS
C
C    DENSITY OF CARCASS COMPARTMENT
       DM=1.05D0
C    FRACTION OF CTC UNBOUND IN PLASMA
       F=.59D0
C    UNBOUND FRACTION IN BILE
       FB=.59D0
C    UNBOUND FRACTION IN LIVER TISSUE
       FL=.59D0
C    UNBOUND FRACTION IN CARCASS TISSUE
       FM=.59D0
C    UNBOUND FRACTION IN INTESTINAL LUMEN
       FI=.75D0
C    COEFFICIENT DESCRIBING TRANSPORT INTO LIVER TISSUE,ML/MIN
```

```
      KL=4.7D0
C     BILIARY FLOW RATE, ML/MIN
      QB=.05D0
C     RATIO OF BILE CONC. TO LIVER CONC., CB/CL2
      BR=20.D0
C     RENAL CLEARANCE, ML/MIN
      CL=5.1D0
C     FECAL FLOW RATE, ML/MIN
      QF=.133D0
C     DECOMPOSITION RATE CONSTANT, 1/MIN
      KR=.00015D0
C     INTESTINAL PERMEABILITY IN MIDDLE PORTION OF SMALL INTESTINE
C     ML/MIN
      KI=.00587D0
C     RATIO OF INTES. PERMEABILITY IN LUMEN 1 TO LUMEN3
C     KI2/KI4
      F2=.8D0
C     KI3/KI4
      F3=1.8D0
C     KI4/KI4
      F4=1.D0
C     KI5/KI4
      F5=.77D0
C     PROGRAM SET UP WITH AN EXTRA LUMEN TANK; SINCE ONLY 4 TANKS
C      REQUIRED TO DESCRIBE EXIT AGE DISTRIBUTION IN FECES, LAST
C     TANK NOT USED---- THEREFORE F6=KI6=0
      F6=0.D0
C     DETERMINE PERMEABILITIES IN EACH LUMEN REGION (RATIOS OF EACH
C     OTHER) , ML/MIN
      KI2=F2*KI
      KI3=F3*KI
      KI4=F4*KI
      KI5=F5*KI
      KI6=0.D0
C     CARCASS PERMEABILITY, ML/MIN
      KM=5.5D0
C     MASS OF SLOW EQUIL. LIVER TISSUE, G
      ML2=22.8D0
C     MASS OF SLOW EQUIL. CARCASS TISSUE, G
      MM2=2358.D0
C     INTESTINAL PLASMA FLOW RATE, ML/MIN
      QI=36.2D0
C     LIVER PLASMA FLOW RATE, ML/MIN
      QL=60.7D0
C     CARCASS PLASMA FLOW RATE, ML/MIN
      QM=258.8D0
C     TOTAL PLASMA FLOW RATE,ML/MIN
      QP=319.5D0
C     VOLUME OF PLASMA IN INTESTINE, ML
      VI1=7.3D0
```

```
C     VOLUME OF LUMEN REGIONS
        VI2=7.D0
        VI3=15.67D0
        VI4=15.67D0
        VI5=15.67D0
        VI6=15.67D0
C     VOLUME OF RAPIDLY EQUIL. LIVER TISSUE+PLASMA, ML
        VL1=24.D0
C     VOLUME OF RAPIDLY EQUIL. CARCASS TISSUE+PLASMA, ML
        VM1=482.6D0
C     VOLUME OF PLASMA IN ARTERIES AND VEINS
        VP=115.6D0
C
C
C    THE SYSTEM, C'=AC   C0=B , IS TO BE SOLVED
C
C       SINCE MANY OF THE CHARACTORS IN MATRIX A EQUAL ZERO,
C       INITIALLY SET A=0
        DO 20 I=1,11
        DO 10 J=1,11
        A(I,J)=0.D0
 10     CONTINUE
 20     CONTINUE
C
C      MASS BALANCES WERE PERFORMED ON FREE COMPONENT OF
C      CP,CI1-CI6,CL1,CM1;    TOTAL COMPONENT OF CL2,CM2
C
C
C           VALUES IN MATRIX A DESCRIBING:
C
C      TRANSPORT OF CTC IN PLASMA FLOW FROM LIVER TO PLASMA COMP.
        A(1,4)=QL/VP
C      XPORT TO PLASMA FROM CARCASS
        A(1,6)=QM/VP
C      XPORT OUT OF PLASMA COMP.
        A(1,1)=-(QP+(CL*F)+(VP*KR*F))/VP
C      XPORT TO INTESTINAL PLASMA FROM ARTERIES
        A(2,1)=QI/VI1
C      XPORT INTO INTES. PLASMA FROM LUMEN SEGMENT 1
        A(2,3)=KI2*F/VI1
C      XPORT & ELIM. FROM INTESTINAL PLASMA
        A(2,2)=-((F*(KI2+KI3+KI4+KI5+KI6))+QI+(F*VI1*KR))/VI1
C      XPORT INTO INTES. PLASMA FROM LUMEN SEGMENTS 2-4
        A(2,8)=KI3*F/VI1
        A(2,9)=KI4*F/VI1
        A(2,10)=KI5*F/VI1
        A(2,11)=KI6*F/VI1
C      XPORT INTO UPPER SEGMENT OF G.I. LUMEN FROM INTES. PLASMA
        A(3,2)=KI2*FI/VI2
C      XPORT & ELIM. FROM UPPER INTES. LUMEN
```

```
      A(3,3)=-(((QF+(VI2*KR*FI))/VI2)+(KI2*FI/VI2))
C     XPORT BY PLASMA FLOW INTO RAPIDLY EQUIL. (RE) LIVER TISSUE
      A(4,2)=QI/VL1
      A(4,1)=(QL-QI)/VL1
C     XPORT & ELIM. FROM RE LIVER TISSUE
      A(4,4)=-((F*KL)+QL+(VL1*KR*F))/VL1
C     XPORT TO SLOWLY EQUIL. (SE) LIVER TISSUE FROM RE LIVER TISSUE
      A(5,4)=KL/ML2
C     XPORT & ELIM. FROM SE LIVER TISSUE
      A(5,5)=-((KR*FL)+((QB*BR)/ML2))
C     XPORT INTO RE CARCASS TISSUE BY PLASMA FLOW
      A(6,1)=QM/VM1
C     XPORT INTO RE CARCASS TISSUE FROM SE CARCASS TISSUE
      A(6,7)=(KM*F*FM*DM)/VM1
C     XPORT & ELIM. FROM RE CARCASS TISSUE
      A(6,6)=-(QM+(F*KM)+(F*VM1*KR))/VM1
C     XPORT INTO SE CARCASS FROM RE CARCASS
      A(7,6)=KM/MM2
C     XPORT & ELIM. FROM SE CARCASS TISSUE
      A(7,7)=-(((FM*KM*DM)/(MM2))+((MM2*KR*FM)/MM2))
C     XPORT INTO INTES. LUMEN SEGMENT 2 FROM LUMEN PLASMA
      A(8,2)=KI3*FI/VI3
C     XPORT INTO LUMEN SEGMENT 2 FROM SEGMENT 1 BY FECAL FLOW
      A(8,3)=QF/VI3
C     BILIARY XPORT (FLOW) INTO LUMEN 2
      A(8,5)=(QB*BR*FI)/VI3
C     XPORT & ELIM. FROM INTES. LUMEN SEG. 2
      A(8,8)=-((QF/VI3)+(KR*FI)+(KI3*FI/VI3))
C     XPORT INTO LUMEN 3 FROM PLASMA
      A(9,2)=KI4*FI/VI4
C     XPORT INTO LUMEN 3 BY FECAL FLOW
      A(9,8)=QF/VI4
C     XPORT & ELIM. FROM INTES. LUMEN 3
      A(9,9)=-((QF/VI4)+(KR*FI)+(KI4*FI/VI4))
C     XPORT & ELIM. INTO LUMEN 4 FROM PLASMA
      A(10,2)=KI5*FI/VI5
C     XPORT INTO LUMEN 4 BY FECAL FLOW
      A(10,9)=QF/VI5
C     XPORT. & ELIM. FROM INTES. LUMEN SEGMENT 4
      A(10,10)=-((QF/VI5)+(KR*FI)+(KI5*FI/VI5))
C     THE LAST 3 TERMS OF MATRIX 'A' DESCRIBE A 5TH INTES.
C     LUMEN SEGMENT; SINCE ONLY 4 LUMEN SEGMENTS ARE USED TO
C     DESCRIBE FECAL XPORT IN THE TURKEY GUT THESE TERMS ARE NOT
C     IMPORTANT IN THE MODEL (I.E. KI6=0)
      A(11,2)=KI6*FI/VI6
      A(11,10)=QF/VI6
      A(11,11)=-((QF/VI6)+(KR*FI)+(KI6*FI/VI6))
C
C
C     THE FIRST STEP IN SOLVING THE SYSTEM OF LINEAR FIRST
```

```
C     ORDER HOMOGENEOUS ORDINARY DIFF. EQUATIONS WITH
C     CONSTANT COEFFICIENTS IS TO SOLVE MATRIX 'A' FOR IT'S
C     EIGENVALUES & EIGENVECTORS,  (A-RI)V=0  R=EIGENVALUES
C     V=EIGENVECTORS.  WITH NO REPEATED OR COMPLEX ROOTS THE SOLN.
C     IS OF THE FORM: X=SUM K*V*EXP(R*T),  K=CONSTANT
C
C      IMSL ROUTINE EIGRF IS USED TO DETERMINE THE EIGENVALUES OF
C      MATRIX 'A'
C
C
C      ORDER OF MATRIX 'A'
       N=NUM
C      ROW DIMENSION OF MATRIX 'A'
       IA=NUM
C      INPUT OPTION PARAMETER: CALC. EIGENVALUES,EIGENVECTORS
       IJOB=2
C      ROW DIMENSION OF OUTPUT MATRIX
       IZ=NUM
       CALL EIGRF(A,N,IA,IJOB,RW,RZ,IZ,WK,IER)
C      THE EIGENVECTORS ARE STORED IN  MATRIX 'V'
       DO 40 J=1,NUM
       DO 30 I=1,NUM
       V(I,J)=Z(I,J)
 30    CONTINUE
 40    CONTINUE
C
C
C     WARNING:  THIS PROGRAM IS NOT DESIGNED TO HANDLE REPEATED ROOTS
C     UNLESS ALL THE EIGENVECTORS ARE LINEARLY (SUM CV=0) INDEPENDENT
C
C
C     IF MATRIX 'A' (OF C'=AC) IS REAL, ANY COMPLEX EIGENVALUES
C     MUST OCCUR IN CONJUGATE PAIRS, R1=F+GI   R2=F-GI
C     IN ADDITION, THE CORRESPONDING EIGENVECTORS ARE COMPLEX
C     CONJUGATES,  V1=L+MI    V2=L-MI
C     THEREFORE, THE SOLUTIONS ARE COMPLEX CONJUGATES OF EACH OTHER.
C     THE FORM OF THE PORTION OF A SOLUTION CORRESPONDING TO A PAIR
C     OF COMPLEX CONJUGATES IS=K1*(EXP(F*T))*(L*COS(G*T)-M*SIN(G*T))
C                             +K2*(EXP(F*T))*(L*SIN(G*T)+M*COS(G*T))
C     SINCE COPLEX EIGENVALUES LEAD TO A SOLUTION CONTAINING SINE
C     AND COSINE TERMS, FLAGS ARE USED TO CHECK FOR COMPLEX ROOTS
C       FLAG=0 :REAL ROOT;  FLAG=1 :COMPLEX ROOT;
C       FLAG=2 :COMPLEX CONJUGATE ROOT
C
        FLAG(1)=0
        DO 60 I=1,NUM
        P=I+1
        IF(FLAG(I).NE.2)THEN
C          EXTRACT IMAGINARY PORTION OF EIGENVALUE USING LIBRARY
C          FUNCTION 'DIMAG'
```

```
        IW(I)=DIMAG(W(I))
        TEST(I)=DABS(IW(I))
          IF(TEST(I).GT.0.0000001D0)THEN
           FLAG(I)=1
           DO 50 J=1,NUM
           V(J,I)=DREAL(Z(J,I))
           V(J,P)=DIMAG(Z(J,I))
           FLAG(P)=2
50         CONTINUE
          ELSE
           FLAG(P)=0
          END IF
        ELSE
           FLAG(P)=0
        END IF
60      CONTINUE
C
C
C     AT TIME=0 THE EXPONENTIAL TERMS IN THE SOLUTION ARE ZERO;
C     THEREFORE THE SOLUTION AT T=0 IS OF THE FORM: VK=B
C     V=MATRIX OF EIGENVECTORS, B=VECTOR OF INITIAL CONDITIONS
C     THE CONSTANTS(K) MUST BE SOLVED FOR BY SOLVING THE LINEAR
C     SYSTEM VK=B ------IMSL ROUTINE LEQT2F WAS EMPLOYED
C
C      M=# OF RIGHT HAND SIDES OF VK=B
        M=1
C      INPUT OPTION: PERFORMS ACCURACY TEST
        IDGT=3
C      SET BOUNDRY CONDITIONS: CONCENTRATIONS AT TIME = 0 IN ALL
C      REGIONS EXCEPT INTES. LUMEN 1 EQUAL 0
        DO 70 I=1,NUM
70      B(I,1)=0.D0
        B(3,1)=(49500.D0*FI)/VI2
        CALL LEQT2F(V,M,N,IA,B,IDGT,WKAREA,IER)
C
C
C
C       ON OUTPUT B=CONSTANTS
C       REDEFINE CONSTANTS (DESCRIBED ABOVE AS K)
C
        DO 75 I=1,NUM
        K(I)=B(I,1)
75           CONTINUE
C
C
C
C    CALCULATE CONCENTRATIONS AT VARIOUS TIMES
C
C      DEFINE TIMES (T) AT WHICH SOLUTIONS ARE DESIRED--------
C      STEPPING OFF TIME
```

```
      DO 95 M=1,10
        IF(M.EQ.1)THEN
          T=0.D0
        ELSE
          IF(M.EQ.2)THEN
            T=15.D0
          ELSE
            IF(M.EQ.3)THEN
              T=30.D0
            ELSE
              IF(M.EQ.4)THEN
                T=60.D0
              ELSE
                IF(M.EQ.5)THEN
                  T=2.5D0*60.D0
                ELSE
                  IF(M.EQ.6)THEN
                    T=3.D0*60.D0
                  ELSE
                    IF(M.EQ.7)THEN
                      T=4.5D0*60.D0
                    ELSE
                      IF(M.EQ.8)THEN
                        T=8.D0*60.D0
                      ELSE
                        IF(M.EQ.9)THEN
                          T=12.D0*60.D0
                        ELSE
                          T=24.D0*60.D0
                        END IF
                      END IF
                    END IF
                  END IF
                END IF
              END IF
            END IF
          END IF
        END IF
C
C
C
        DO 80 I=1,NUM
C     FOR IMAGINARY ROOTS, SEPARATE ROOTS INTO REAL AND IMAGINARY
C     PARTS USING LIBRARY FUNCTIONS 'DIMAG'(PERFORMED EARLIER=IW(I))
C     AND 'DREAL' RESPECTIVELY.  BOTH REAL & IMAG. PORTIONS ARE THEN
C     MULTIPLIED BY TIME (T).  SINE, COSINE, & EXPONENTIAL FUNCTIONS
C     OF TIME ARE ALSO SOLVED SINCE NEEDED IN TOTAL SOLUTION
C     DESCRIBED EARLIER.
C
        IF (FLAG(I).EQ.1)THEN
```

```
          PR(I)=DREAL(W(I))
          POWER(I)=PR(I)*T
          PAR(I)=IW(I)*T
C     PREVENT EXPONENTIAL UNDERFLOW
        IF(POWER(I).LT.-150.D0)THEN
          POWER(I)=-150.D0
        ELSE
          CONTINUE
        END IF
          EE(I)=DEXP(POWER(I))
          SINE(I)=DSIN(PAR(I))
          COSI(I)=DCOS(PAR(I))
        ELSE
        IF(FLAG(I).EQ.0)THEN
          WT(I)=W(I)*T
C     PREVENT EXPONENTIAL UNDERFLOW
        IF(WT(I).LT.-150.D0)THEN
          WT(I)=-150.D0
        ELSE
          CONTINUE
        END IF
          E(I)=DEXP(WT(I))
        ELSE
          CONTINUE
        END IF
        END IF
 80     CONTINUE
C
C
C    EXPRESSIONS TO CALCULATE CONCENTRATIONS (X(I)) AT VARIOUS TIMES;
C     PARTIAL SOLUTIONS ARE SUMMED UP TO GET SOLUTION(CONCENTRATIONS)
C     AS A FUNCTION OF TIME (AT TIMES REQUESTED).
C
        DO 90 I=1,NUM
        X(I)=0.D0
        DO 85 J=1,NUM
        P=J+1
        IF (FLAG(J).EQ.0)THEN
          X(I)=K(J)*Z(I,J)*E(J)+X(I)
        ELSE
          IF(FLAG(J).EQ.1)THEN
            X(I)=X(I)+(K(J)*EE(J)*(V(I,J)*COSI(J)-V(I,P)*SINE(J)))+
     *           (K(P)*EE(J)*(V(I,J)*SINE(J)+V(I,P)*COSI(J)))
          ELSE
            CONTINUE
          END IF
        END IF
 85     CONTINUE
 90     CONTINUE
C
```

```
C     COMPARTMENTS: CONC PLASMA=X(1), CONC INTESTINAL PLASMA=X(2),
C         CONC. INTES. LUMEN1=X(3), CONC. RAPID EQUIL. LIVER=X(4),
C         CONC. SLOW EQUIL. LIVER=X(5), CONC. RAPID EQUIL. CARCASS=
C         X(6), CONC. SLOW EQUIL. CARCASS=X(7), CONC. INTES. LUMEN
C         SEGMENT 2=X(8), CONC. INTES. LUMEN SEG.3=X(9)
C         CONC. INTES. LUMEN SEG. 4=X(10)
C
C     SINCE EXPERIMENTAL TISSUE DATA CONTAINS PLASMA, MODEL TISSUES
C     (LIVER, CARCASS) MUST BE THE VOLUME AVERAGE OF PLASMA & TISSUE.
C
       CP(M)=X(1)
       CLT(M)=((ML2*X(5))+(28.8D0*X(4)/F))/53.D0
       CMT(M)=((MM2*X(7))+(VM1*X(6)/F))/(MM2+(VM1*DM))
C
C
       PRINT, 'AT TIME=',T
       PRINT,'CP=',X(1),'CIP=',X(2),'CIC=',X(3),'CLP=',X(4)
     * ,'CLT=',CLT(M),X(5),'CMP=',X(6),'CMT=',CMT(M),X(7)
       PRINT,'CI3=',X(8),'CI4=',X(9),'CI5=',X(10),'CI6=',X(11)
 95    CONTINUE
C
C
C    CALCULATE SUM OF SQUARES
C
C    PLASMA SSQ=SSQP; LIVER SSQ=SSQL;  CARCASS SSQ=SSQM
C
       SSQP=(.35D0-CP(2))**2+(.58D0-CP(3))**2+(.8D0-CP(4))**2
     * +(1.1D0-CP(5))**2+(1.16D0-CP(6))**2+(.74D0-CP(7))**2+
     * (.44D0-CP(8))**2+(.23D0-CP(9))**2+(.11D0-CP(10))**2
       SSQL=(3.05D0-CLT(5))**2+(2.32D0-CLT(8))**2+(.61D0-CLT(10))**2
       SSQM=(.23D0-CMT(5))**2+(.28D0-CMT(8))**2+(.05D0-CMT(10))**2
       SSQ=SSQP+SSQL+SSQM
       PRINT,'SSQP=',SSQP,'SSQL=',SSQL,'SSQM=',SSQM,'SSQ=',SSQ
       STOP
       END
$ENTRY
```

```
C-------------------   PROGRAM #2   ------------------------------
C
C
C   NUMERICAL SOLUTION TO THE ORAL MODEL: G.I. TRACT MODEL AS ONE
C                  VARIABLE VOLUME STIRRED TANK
C
C                    WRITTEN BY: BOB POLLET
C                 PROGRAM IS WRITTEN IN WATFIV
C
C
C
C   EXPLICIT DECLARATION OF ALL VARIABLES
      INTEGER I,IER,INDEX,IWK(7),M,METH,MITER,N
      DOUBLE PRECISION A(7,7),BR,C(7),CL,CL2,CM2,DM
      DOUBLE PRECISION F,FB,FI,FL,FM,H,KI,KL,KM,KR
      DOUBLE PRECISION ML2,MM2,QB,QF,QI,QL,QM,QP,T,TAU,TOL
      DOUBLE PRECISION VI1,VI2,VL1,VM1,VP,WK(126),X,XEND,Y(7)
      EXTERNAL FCN,FCNJ
      COMMON/VAR/A,F,FI,KI,KR,ML2,MM2,QF,QI,VI1,VI2,VL1,VM1,VP
C
C
C   VALUES OF PARAMETERS
C
C    DENSITY OF CARCASS COMPARTMENT
       DM=1.05D0
C    FRACTION OF CTC UNBOUND IN PLASMA
       F=.59D0
C    UNBOUND FRACTION IN BILE
       FB=.59D0
C    UNBOUND FRACTION IN LIVER TISSUE
       FL=.59D0
C    UNBOUND FRACTION IN CARCASS TISSUE
       FM=.59D0
C    UNBOUND FRACTION IN INTESTINAL LUMEN
       FI=.75D0
C    COEFFICIENT DESCRIBING TRANSPORT INTO LIVER TISSUE,ML/MIN
       KL=4.7D0
C    BILIARY FLOW RATE, ML/MIN
       QB=.05D0
C    RATIO OF BILE CONC. TO LIVER CONC., CB/CL2
       BR=20.D0
C    RENAL CLEARANCE, ML/MIN
       CL=5.1D0
C    FECAL FLOW RATE, ML/MIN
C    MATERIAL IS NOT ELIMINATED IN FECES UNTIL LEAVING LARGE INTEST.
C    (T.GT.TAU);CONSEQUENTLY, AT T=0 QF=0
       QF=0.D0
C    DECOMPOSITION RATE CONSTANT, 1/MIN
       KR=.00015D0
C    INTESTINAL PERMEABILITY IN GASTROINTESTINAL TRACT AT T=0
```

```
C     INITALLY DRUG COVERS ONLY A PORTION OF G.I. TRACT SURFACE AREA
C     ML/MIN
        KI=.034D0*7.D0/54.D0
C     CARCASS PERMEABILITY, ML/MIN
        KM=5.5D0
C     MASS OF SLOW EQUIL. LIVER TISSUE
        ML2=22.8D0
C     MASS OF SLOW EQUIL. CARCASS TISSUE, G
        MM2=2358.D0
C     INTESTINAL PLASMA FLOW RATE, ML/MIN
        QI=36.2D0
C     LIVER PLASMA FLOW RATE, ML/MIN
        QL=60.7D0
C     CARCASS PLASMA FLOW RATE, ML/MIN
        QM=258.8D0
C     TOTAL PLASMA FLOW RATE,ML/MIN
        QP=319.5D0
C     VOLUME OF PLASMA IN INTESTINE, ML
        VI1=7.3D0
C     INITIAL VOLUME OF LUMEN REGION
        VI2=7.D0
C     VOLUME OF RAPIDLY EQUIL. LIVER TISSUE+PLASMA, ML
        VL1=24.D0
C     VOLUME OF RAPIDLY EQUIL. CARCASS TISSUE+PLASMA, ML
        VM1=482.6D0
C     VOLUME OF PLASMA IN ARTERIES AND VEINS
        VP=115.6D0
C
C
C    THE SYSTEM, M'=AM   M0=B , IS TO BE SOLVED
C
C       SINCE MANY OF THE CHARACTORS IN MATRIX A EQUAL ZERO,
C       INITIALLY SET A=0
        DO 20 I=1,7
        DO 10 J=1,7
        A(I,J)=0.D0
  10    CONTINUE
  20    CONTINUE
C
C      MASS BALANCES WERE PERFORMED ON TOTAL CTC (M) IN EACH
C      COMPARTMENT
C
C      SYSTEM:  M'=AM  M=VECTOR DESCRIBING MASS OF DRUG IN EACH COMP.
C
C
C           VALUES IN MATRIX A DESCRIBING:
C
C      TRANSPORT OF CTC IN PLASMA FLOW FROM LIVER TO PLASMA COMP.
        A(1,4)=QL/VL1
C      XPORT TO PLASMA FROM CARCASS
```

```
      A(1,6)=QM/VM1
C     XPORT & ELIM. FROM PLASMA COMPART.
      A(1,1)=(QP+(CL*F)+(VP*KR*F))/VP
C     XPORT TO INTESTIAL PLASMA FROM PLASMA COMPARTMENT
      A(2,1)=QI/VP
C     XPORT INTO INTES. PLASMA FROM LUMEN
      A(2,3)=(KI*FI)/VI2
C     XPORT & ELIM. FROM INTESTINAL PLASMA
      A(2,2)=((F*KI)+QI+(F*VI1*KR))/VI1
C     XPORT BY BILIARY FLOW FROM LIVER TO INTES. LUMEN
      A(3,5)=(QB*BR)/ML2
C     XPORT INTO G.I. LUMEN FROM INTES. PLASMA
      A(3,2)=(KI*F)/VI1
C     XPORT & ELIM. FROM G.I. LUMEN
      A(3,3)=((KI*FI)+QF+(VI2*KR*FI))/VI2
C     XPORT BY PLASMA FLOW INTO RAPIDLY EQUIL.(RE) LIVER TISSUE
      A(4,2)=QI/VI1
      A(4,1)=(QL-QI)/VP
C     XPORT & ELIM. FROM RE LIVER TISSUE
      A(4,4)=((F*KL)+QL+(VL1*KR*F))/VL1
C     XPORT TO SLOWLY EQUIL. (SE) LIVER TISSUE FROM RE LIVER TISSUE
      A(5,4)=KL*F/VL1
C     XPORT & ELIM. FROM SE LIVER TISSUE
      A(5,5)=((KR*FL*ML2)+(QB*BR))/ML2
C     XPORT INTO RE CARCASS TISSUE BY PLASMA FLOW
      A(6,1)=QM/VP
C     XPORT INTO RE CARCASS TISSUE FROM SE CARCASS TISSUE
      A(6,7)=(KM*FM*DM)/MM2
C     XPORT & ELIM. FROM RE CARCASS TISSUE
      A(6,6)=(QM+(F*KM)+(F*VM1*KR))/VM1
C     XPORT INTO SE CARCASS FROM RE CARCASS
      A(7,6)=(KM*F)/VM1
C     XPORT & ELIM FROM SE CARCASS TISSUE
      A(7,7)=((FM*KM*DM)+(MM2*KR*FM))/MM2
C
C
C     IMSL ROUTINE 'DGEAR' USED FOR NUMERICAL INTEGRATION
C
C
C   SPECIFICATION PARAMETERS FOR IMSL ROUTINE 'DGEAR'
C
C     INPUT ITERATION INDICATOR ( MITER=2, CHORD METHOD USED WITH
C       THE JACOBIAN CALCULATED INTERNALLY BY FINITE DIFFERENCES)
      MITER=2
C     INPUT AND OUTPUT PARAMETER INDICATING THE TYPE OF CALL TO
C       SUBROUTINE (INDEX=1, IMPLIES FIRST CALL FOR THIS PROBLEM)
      INDEX=1
C     INPUT BASIC METHOD INDICATOR (METHOD=2, IMPLIES THE STIFF
C       METHODS OF GEAR, OR THE BACKWARD DIFFERENTIATION FORMULAE
C       ARE TO BE USED)
```

```
       METH=2
C      NUMBER OF FIRST ORDER DIFFERENTIAL EQUATIONS
       N=7
C      INDEPENDENT VARIABLE (TIME)
C        ON INPUT, X SUPPLIES THE INITIAL VALUE OF TIME
C        ON OUTPUT, X REPLACED WITH CURRENT VALUE OF OF INDEP.
C          VARIABLE AT WHICH INTEGRATION HAS BEEN COMPLETED.
       X=0.D0
C      INPUT RELATIVE ERROR BOUND
       TOL=.0001D0
C      STEP SIZE:  ON INPUT, INITIAL STEP SIZE IN X
C                  ON OUTPUT, STEP SIZE USED LAST
       H=.00001D0
C
C
C    DEPENDENT VARIABLES (MASS OF DRUG IN EACH COMPARTMENT)
C      ON INPUT Y(I) SUPPLIES INITIAL VALUES
C      ON OUTPUT Y(I) REPLACED WITH COMPUTED VALUE AT XEND
C
C    INITIALLY  (T=0) MASS OF CTC IN ALL COMPARTMENTS EXCEPT
C      GUT LUMEN (Y(3)) EQUALS ZERO.
C
       Y(1)=0.D0
       Y(2)=0.D0
       Y(3)=49500.D0
       Y(4)=0.D0
       Y(5)=0.D0
       Y(6)=0.D0
       Y(7)=0.D0
C
C
C    TIMES (T) AT WHICH SOLUTIONS ARE DESIRED.....
C      STEPPING OFF TIME
C
       DO 30 M=1,11
           IF(M.EQ.1)THEN
             T=15.D0
           ELSE
             IF(M.EQ.2)THEN
               T=30.D0
             ELSE
               IF(M.EQ.3)THEN
                 T=60.D0
               ELSE
               IF(M.EQ.4)THEN
                T=90.D0
               ELSE
                IF(M.EQ.5)THEN
                 T=120.D0
                ELSE
```

```
                IF(M.EQ.6)THEN
                  T=2.5D0*60.D0
                ELSE
                  IF(M.EQ.7)THEN
                    T=3.D0*60.D0
                  ELSE
                    IF(M.EQ.8)THEN
                      T=4.5D0*60.D0
                    ELSE
                      IF(M.EQ.9)THEN
                        T=8.D0*60.D0
                      ELSE
                        IF(M.EQ.10)THEN
                          T=12.D0*60.D0
                        ELSE
                          T=24.D0*60.D0
                        END IF
                      END IF
                    END IF
                  END IF
                END IF
              END IF
              END IF
            END IF
C
C   XEND=VALUE OF X (TIME) AT WHICH SOLUTION IS DESIRED
      XEND=T
C
C   IMSL NUMERICAL DIFFERENTIAL EQUATION SOLVER 'DGEAR' EMPLOYED
C
      CALL DGEAR(N,FCN,FCNJ,X,H,Y,XEND,TOL,METH,MITER,INDEX,IWK,WK,
     * IER)
C
C
C   CONCENTRATIONS (MASS/VOLUME) OF UNBOUND OR TOTAL CTC CALCUL.
C     FOR EACH COMPARTMENT
C
      C(1)=Y(1)*F/VP
      C(2)=Y(2)*F/VI1
      C(3)=Y(3)*FI/VI2
      C(4)=Y(4)*F/VL1
      C(5)=Y(5)/ML2
      C(6)=Y(6)*F/VM1
      C(7)=Y(7)/MM2
C   SINCE EXPERIMENTAL DATA CONTAINS PLASMA, MODEL TISSUES
C     (LIVER,CARCASS) MUST BE THE VOLUME AVERAGE OF PLASMA & TISSUE.
C
      CL2=((ML2*C(5))+(28.8D0*C(4)/F))/53.D0
```

```
      CM2=((MM2*C(7))+(VM1*C(6)/F))/(MM2+(VM1*1.02D0))
C
C
C   PRINT CONCENTRATIONS AS A FUNCTION OF TIME.....
      PRINT,'AT TIME=',X
      PRINT,'CP=',C(1),'CI1=',C(2),'CI2=',C(3),'CL1=',C(4),'CL2=',
     * C(5),'CM1=',C(6),'CM2=',C(7),'MI2=',Y(3)
      PRINT,'IER=',IER,'CL2 EXPER=',CL2,'CM2 EXPER=',CM2
 30   CONTINUE
      STOP
      END
C
C
C ---------------------------------------------------------------
C
C   SUBROUTINE FOR EVALUATING FUNCTIONS (Y'(I)=FUNCT(T), I=1,7)
C
C
      SUBROUTINE FCN(N,X,Y,YPRIME)
      INTEGER N
      DOUBLE PRECISION A(7,7),BR,CL,DL2,F,FB,FI,FL,FM
      DOUBLE PRECISION KI,KL,KM,KR,ML2,MM2,QB,QF,QI,QL,QM,QP
      DOUBLE PRECISION TAU,VI1,VI2,VL1,VM1,VP,X,Y(N),YPRIME(N)
      COMMON/VAR/A,F,FI,KI,KR,ML2,MM2,QF,QI,VI1,VI2,VL1,VM1,VP
C   VALUES OF PARAMETERS
C
C    TIME (MIN) REQUIRED FOR CTC ADMINISTERED IN CROP TO REACH
C      THE END OF THE GASTROINTESTINAL TRACT (LEADING EDGE)
      TAU=60.D0
C
C   AS CTC DISTRIBUTES THROUGHOUT THE G.I. TRACT LUMEN, DRUG
C     DISTRIBUTES IN TO A LARGER LUMEN VOLUME (VI2), WITH A
C     LARGER SURFACE AREA FOR TRANSFER (SINCE KI IS PROPORTIONAL
C     TO THE SURFACE AREA; INCREASING AREA INCREASES KI)
C     FECAL FLOW BEGINS WHEN MATERIAL REACHES END OF INTES. TRACT.
C   INCREASE IN KI AND VI2 DESCRIBED BY LINEAR FUNCTIONS OF TIME.
C
      KI=.034D0
      IF(X.LE.TAU)THEN
        VI2=7.D0+((47.D0*X)/TAU)
        KI=(KI*VI2)/54.D0
        QF=0.D0
      ELSE
        QF=.182D0
        VI2=54.D0
      END IF
C
C   TRANSPORT OR ELIMINATION PARAMETERS WHICH VARY WITH TIME
C   -----DESCRIBED EARLIER
      A(2,3)=(KI*FI)/VI2
```

```
      A(2,2)=((F*KI)+QI+(F*VI1*KR))/VI1
      A(3,2)=(KI*F)/VI1
      A(3,3)=((KI*FI)+QF+(VI2*KR*FI))/VI2
C
C   CHANGE (DERIVATIVE) IN MASS OF CTC IN EACH COMPARTMENT
C     WITH TIME.
C
C       PLASMA
      YPRIME(1)=A(1,4)*Y(4)+A(1,6)*Y(6)-A(1,1)*Y(1)
C       GASTROINTESTINAL PLASMA
      YPRIME(2)=A(2,1)*Y(1)+A(2,3)*Y(3)-A(2,2)*Y(2)
C       G.I. LUMEN
      YPRIME(3)=A(3,5)*Y(5)+A(3,2)*Y(2)-A(3,3)*Y(3)
C       RAPIDLY EQUILIBRATING LIVER TISSUE
      YPRIME(4)=A(4,2)*Y(2)+A(4,1)*Y(1)-A(4,4)*Y(4)
C       SLOWLY EQUIL. LIVER TISSUE
      YPRIME(5)=A(5,4)*Y(4)-A(5,5)*Y(5)
C       RAPIDLY EQUIL. CARCASS TISSUE
      YPRIME(6)=A(6,1)*Y(1)+A(6,7)*Y(7)-A(6,6)*Y(6)
C       SLOWLY EQUIL. CARCASS TISSUE
      YPRIME(7)=A(7,6)*Y(6)-A(7,7)*Y(7)
      RETURN
      END
C
C
C  ---------------------------------------------------------------
C
C   DUMMY SUBROUTINE TO  COMPUTE JACOBIAN MATRIX OF PARTIAL DERIV.
C
C
      SUBROUTINE FCNJ(N,X,Y,PD)
      INTEGER N
      DOUBLE PRECISION A(7,7),F,FI,KI,KR,ML2,MM2,PD(N,N),QI,QF
      DOUBLE PRECISION VI1,VI2,VL1,VM1,VP,Y(N),X
      COMMON/VAR/A,F,FI,KI,KR,ML2,MM2,QF,QI,VI1,VI2,VL1,VM1,VP
      RETURN
      END
```