

restricted in III as a result of the reduction of ring B to a five-membered ring. Thus, compounds III<sub>j</sub>, III<sub>l</sub>, and III<sub>n</sub> represent interesting rigid analogs of II. Furthermore, molecular model examination of III<sub>j</sub> and morphine shows the phenolic and tertiary amino groups, and the aromatic ring in both compounds to be almost stereosuperimposable. In addition, it was of interest to find a patent describing some 3-hydroxyspiro[indan-1,3'-pyrrolidine] derivatives as orally active analgesics (10). Profadol is thought to interact with the analgesic receptor in the half-chair conformation (11).

Although the spiro systems I and III represent structural analogs in which the profadol molecule is fixed in a conformation related to the morphine molecule, these compounds exhibit only weak or insignificant analgesic properties. Thus, other structural features appear to be important in profadol-receptor interaction. The inability of these spiro analogs to enhance or maintain the analgesic properties of profadol may be related to a decreased fit of these compounds at the receptor due to the rigid orientation of the B ring in these compounds, which represents a fixed, and perhaps undesirable, conformation of the 3-propyl group in II.

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# Quantitation of Hydroxyprogesterone Caproate, Medroxyprogesterone Acetate, and Progesterone by Reversed-Phase High-Pressure Liquid Chromatography

V. DAS GUPTA

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**Abstract** □ A high-pressure liquid chromatographic method for the quantitation of hydroxyprogesterone caproate, medroxyprogesterone acetate, and progesterone in pharmaceutical dosage forms was developed. The method gave accurate, precise, and reproducible results. The excipients present in the dosage forms did not interfere with the assay procedure except benzyl benzoate in progesterone injection. The percent relative standard deviations based on six injections were 1.6, 2.5, and 2.7% for hydroxyprogesterone caproate, medroxyprogesterone acetate, and progesterone, respectively. The stability of progesterone in ethanol-propylene glycol-water (10:50:40) was studied. The loss in potency of progesterone, even after 487 days of storage at 50°, was <10%.

**Keyphrases** □ Hydroxyprogesterone caproate—quantitation by reversed-phase high-pressure liquid chromatography □ Medroxyprogesterone acetate—quantitation by reversed-phase high-pressure liquid chromatography □ Progesterone—quantitation by reversed-phase high-pressure liquid chromatography □ High-pressure liquid chromatography—quantitation of hydroxyprogesterone caproate, medroxyprogesterone acetate, and progesterone

The pharmaceutical dosage forms of hydroxyprogesterone caproate (I), medroxyprogesterone acetate (II), and progesterone (III) are used extensively. The USP method (1) for the quantitative determination of I in dosage forms is based on a reaction with isoniazid. The color produced is measured spectrophotometrically.

For dosage forms of II, the USP method (2) requires normal phase high-pressure liquid chromatography (HPLC) with a porous silica column. The quantitation of III requires reversed-phase chromatography (3). The 1-m column for this procedure has to be packed with octade-

cylsilane chemically bonded to silica gel. The USP method (3) appears to be a modification of a procedure recommended previously (4).

This investigation attempted to develop a reversed-phase HPLC method (with prepacked column) suitable for the quantitation of I, II, and III in pharmaceutical dosage forms. The stability of III in some aqueous systems was also determined.

## EXPERIMENTAL

**Reagents and Chemicals**—All reagents and chemicals were ACS, USP, or NF grade and were used as received. 17-Hydroxyprogesterone<sup>1</sup>, hydroxyprogesterone caproate<sup>1</sup>, medroxyprogesterone acetate<sup>2</sup>, and progesterone<sup>3</sup> were used without further purification.

**Apparatus**—The chromatograph<sup>4</sup> was connected to a multiple-wavelength detector<sup>5</sup>, a recorder<sup>6</sup>, and an integrator<sup>7</sup>. All pH values were determined using a pH meter<sup>8</sup>.

**Column**—The column<sup>9</sup> (30 cm × 4-mm i.d.) was of a semipolar material consisting of a monomolecular layer of cyanopropylsilane permanently bonded by silicone-carbon bonds.

**Chromatographic Conditions**—The mobile phase contained 30%

<sup>1</sup> E. R. Squibb & Sons, Princeton, N.J.

<sup>2</sup> The Upjohn Co., Kalamazoo Mich.

<sup>3</sup> Aldrich Chemical Co., Milwaukee, Wis.

<sup>4</sup> Model ALC 202 equipped with a U6K universal injector, Waters Associates, Milford, Mass.

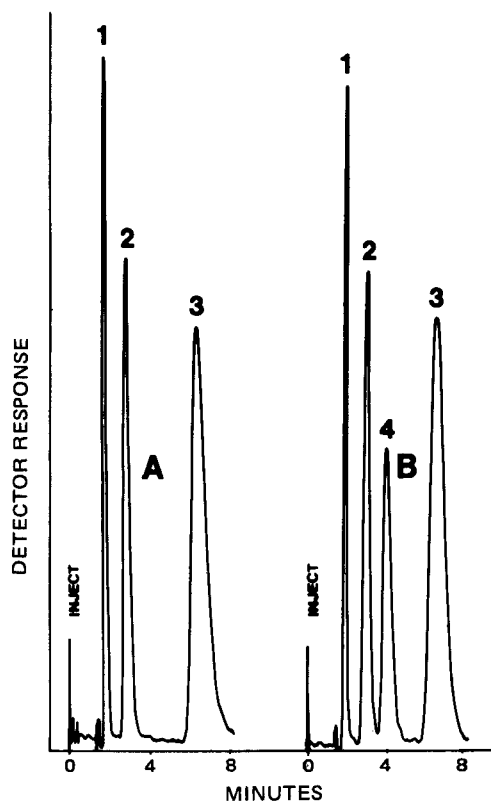
<sup>5</sup> Spectroflow monitor SF770, Schoeffel Instruments Corp., Westwood, N.J.

<sup>6</sup> Omniscrite 5213-12, Houston Instruments, Austin, Tex.

<sup>7</sup> Autolab minigrator, Spectra-Physics, Santa Clara, Calif.

<sup>8</sup> Model 4500 digital pH meter, Beckman Instruments, Irvine, Calif.

<sup>9</sup> μBondapak/CN, Waters Associates, Milford, Mass.



**Figure 1**—Sample chromatograms. Peaks 1–3 are from the solvent, internal standard (17-hydroxyprogesterone) and I, respectively. Peak 4 in chromatogram B is from benzyl benzoate. Key: A, standard solution; and B, injection of I. The chromatographic conditions are given in the text.

by volume (40% by volume for I) of methanol in 0.02 M aqueous solution of monobasic potassium phosphate. The temperature was ambient and the flow rate was 2.0 ml/min. The detector was set at 0.04 (254 nm) and the chart speed was 30.5 cm/hr.

**Solution Preparation**—The stock solutions of I, II, and III were prepared by dissolving 100.0 mg of the compound in enough ethanol to make 100.0 ml. The standard solutions were prepared as needed by diluting the stock solutions either with ethanol or dilute ethanol (50% by volume in water). Before final dilution, an appropriate quantity of the internal standard (17-hydroxyprogesterone for I and hydrocortisone for II and III) was added. The final concentration of the internal standard was always 20 µg/ml. The stock solutions of internal standards were also prepared by dissolving 100.0 mg of the substance in enough ethanol to make 100.0 ml.

**Preparation of Progesterone Solutions for Stability Studies**—A 10.0-ml quantity of the III solution (10.0 mg/ml in ethanol) was mixed with 50.0 ml of propylene glycol and the mixture was brought to volume (100.0 ml) with either water or a 0.125 M phosphate buffer of an appropriate pH value.

After the initial assays, the solutions were divided into two parts and stored in amber bottles<sup>10</sup> (60-ml capacity) at 25 and 50°.

**Assay Solutions**—One milliliter of the commercially available hydroxyprogesterone caproate injection (250 mg/ml) was diluted to 100.0 ml with ethanol. In addition to I, the injection also contained 46% of benzyl benzoate and castor oil *q.s.*; a 1.2 ml of this solution was mixed with 1.0 ml of the internal standard (a 1.0 mg/ml solution of 17-hydroxyprogesterone in ethanol) and the mixture was brought to volume (50.0 ml) with ethanol.

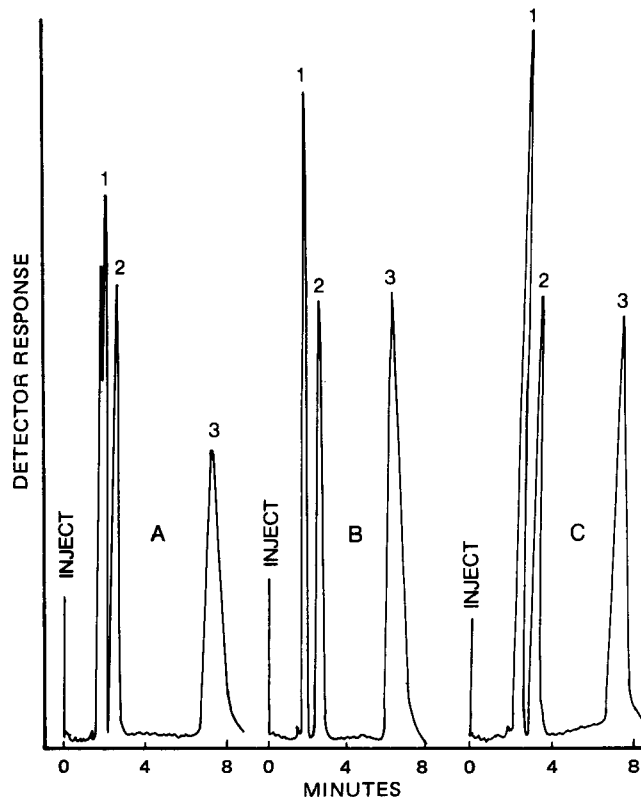
**Medroxyprogesterone Acetate Suspension (400 mg/ml)**—A 2.0-ml quantity of the well-shaken suspension was diluted to 1000.0 ml (to 250 ml when the concentration of II in suspension was 100 mg/ml) with ethanol. Then 2.5 ml of the clear mixture was further diluted to 50.0 ml (before diluting, 1.0 ml of the internal standard was added) with ethanol. In addition to II, the suspension contained (per milliliter) polyethylene glycol 4000, 20.3 mg; sodium sulfate anhydrous, 11 mg; and myristyl-γ-picolinium chloride, 1.7 mg.

**Table I**—Assay Results of Dosage Forms

Type of Dosage Form	Active Ingredient	Percent of the Label Claim Found <sup>a</sup> (Proposed HPLC Method)	USP XX–NF XV Method
Injection	I	99.2	100.1
Injection (different lot)	I	100.8	100.7
Synthetic mixture similar to above injection	I	100.2	100.1
Suspension	II	98.6	— <sup>b</sup>
Suspension (different lot)	II	99.2	— <sup>b</sup>
Tablets	II	99.1	— <sup>b</sup>
Tablets (different lot)	II	100.7	— <sup>b</sup>
Injection	III	132.8 <sup>c</sup>	— <sup>b</sup>
Suspension	III	95.6	— <sup>b</sup>
Suspension (different lot)	III	96.3	— <sup>b</sup>
Synthetic mixture similar to above suspension	III	99.7	— <sup>b</sup>

<sup>a</sup> Average of three assays. Reproducibility on different days was within ±1.5%.  
<sup>b</sup> Not assayed using USP–NF method which is also based on HPLC. <sup>c</sup> Results are high because of interference from benzyl benzoate with the assay procedure.

**Medroxyprogesterone Acetate Tablets (10 mg/Tablet)**—Ten tablets were ground to a fine powder and stirred with ~50 ml of ethanol. The mixture was transferred to a 100-ml volumetric flask and more ethanol rinses were added to bring it to volume. The mixture was filtered<sup>11</sup>, 20 ml of the filtrate was rejected and then 2.0 ml of the filtrate was mixed with 1.0 of the internal standard (hydrocortisone solution in



**Figure 2**—Sample chromatograms. Peaks 1 and 2 are from the solvent and the internal standard (hydrocortisone), respectively. Peak 3 in chromatogram A is from II and in chromatograms B and C from III. Key: A, suspension of II; B, standard solution of III in ethanol; and C, suspension of III. The chromatographic conditions are given in the text.

<sup>10</sup> Made of Sera glass, Brockway Glass Co., Brockway, Pa.

<sup>11</sup> Whatman No. 1 filter paper.

**Table II—Assay Results<sup>a</sup> of Aqueous Solutions of Progesterone Compounded for Stability Studies**

Solution <sup>b</sup>	pH (±0.1) <sup>c</sup>	Assay Results after (days) Based on 100% on Day 0				
		48	120	240	360	487
1 (Unbuffered)	5.7	99.3	100.1	99.7	98.9	99.4
2 (Buffered)	5.0	99.9	99.5	98.9	97.4	92.5
3 (Buffered)	5.6	100.2	100.1	100.1	99.8	94.9
4 (Buffered)	6.6	99.9	101.0	100.1	99.1	95.2
5 (Buffered)	7.5	100.8	100.8	99.7	98.1	95.2

<sup>a</sup> For samples stored at 50°. The results of samples stored at 25° did not indicate any decomposition, even in 487 days. <sup>b</sup> All other solutions were diluted with 0.125 M KH<sub>2</sub>PO<sub>4</sub> solution of an appropriate pH value except solution 1, which was diluted with water. <sup>c</sup> pH values of the solutions.

ethanol, 1 mg/ml), and the mixture was brought to volume (50.0 ml) with ethanol. The list of the excipients was not disclosed on the label.

**Progesterone Aqueous Suspension (50 mg/ml)**—The suspension was shaken well, and 2.0 ml was transferred to a 100-ml volumetric flask and brought to volume with ethanol. The mixture was filtered<sup>11</sup> if necessary, and 2.0 ml of the clear solution was mixed with 1.0 ml of the internal standard (1 mg/ml solution of hydrocortisone in ethanol) and brought to volume (50.0 ml) with ethanol. In addition to III, the suspension contained (per milliliter) sodium carboxymethylcellulose, 2 mg; methylcellulose, 0.3 mg; dioctyl sodium sulfosuccinate, 0.15 mg; and thimerosal, 0.08 mg.

**Progesterone Injection (50 mg/ml)**—A 2.0-ml quantity of the injection was extracted with ethanol (85% by volume in water) according to the procedure recommended by King *et al.* (4), and the extracts were brought to volume (100.0 ml) with 85% ethanol. A 2.0-ml quantity of the clear solution was further diluted to 50.0 ml with dilute ethanol (50% by volume in water). Before diluting, 1.0 ml of the internal standard was added as already described. In addition to III, the injection contained 20% benzyl benzoate, 0.5% phenol, and peanut oil *q.s.*

**Progesterone Solutions for Stability Studies**—A 2.0-ml quantity of the solution was mixed with 1.0 ml of the internal standard and the mixture was brought to volume (50.0 ml) with dilute ethanol.

**Assay Procedure**—A 20.0-μl aliquot of the assay solution was injected into the chromatograph using the described conditions. For comparison, an identical volume of the appropriate standard solution was injected after the assay solution eluted. The standard solutions contained identical concentrations of the drug (based on label claim of the assay) and the internal standard and were diluted using the identical solvent (ethanol or dilute ethanol).

Preliminary studies were conducted to determine the interferences from the excipients with the assay procedure. No interference was found except from benzyl benzoate present in progesterone injection.

**Calculations**—Since preliminary investigations indicated that peak heights (also the peak areas) were directly related to the concentrations (ranges tested were 0.4–1.6 μg for I and 0.4–1.2 μg for II and III), the results were calculated using

$$\text{percent of label claim} = \frac{(R_{ph})_a}{(R_{ph})_s} \times 100 \quad (\text{Eq. 1})$$

where  $(R_{ph})_a$  is the ratio of the peak heights of the drug in the assay solution and the internal standard and  $(R_{ph})_s$  for the standard solution of identical concentrations. The results are presented in Tables I and II and Figs. 1–3.

**Other Experiments**—A 2.0-ml quantity of the stock solution of III was mixed with either 2.0 ml of ~0.1 N NaOH or 0.1 N HCl solution and allowed to stand for 24 hr. The internal standard was then added, and the mixture was brought to volume (50.0 ml) with dilute ethanol and assayed.

In another experiment, 2.0 ml of the stock solution of III was mixed with 1.0 ml of ~1 N NaOH solution and 20 ml of dilute ethanol. The mixture was boiled for ~5 min on a hot plate and cooled to room temperature. The internal standard was then added, and the mixture was brought to volume and assayed. The result is presented in Fig. 3C.

## RESULTS AND DISCUSSION

**Hydroxyprogesterone Caproate**—The assay results (Table I) indicate that the proposed HPLC method can be adopted for the quantitative determination of I in injectables. The castor oil and benzyl benzoate present in the injection did not interfere with the assay procedure. Benzyl benzoate gave a separate peak between the internal standard and the drug (peak 2 in Fig. 1B).

No preliminary procedure to extract I from the injectable solution was required. The percent relative standard deviation based on six injections was 1.6. The method was reproducible.

**Medroxyprogesterone Acetate**—The assay results (Table I) indicate that HPLC is a reliable method for the quantitation of II in suspension and tablets. A very simple procedure was required to extract II from the tablets, while the suspension did not require preliminary extraction. None of the excipients present in suspension (polyethylene glycol 4000, sodium sulfate, and myristyl-γ-picolinium chloride) interfered with the assay procedure (Fig. 2A). The chromatogram obtained from tablets was similar to Fig. 2A.

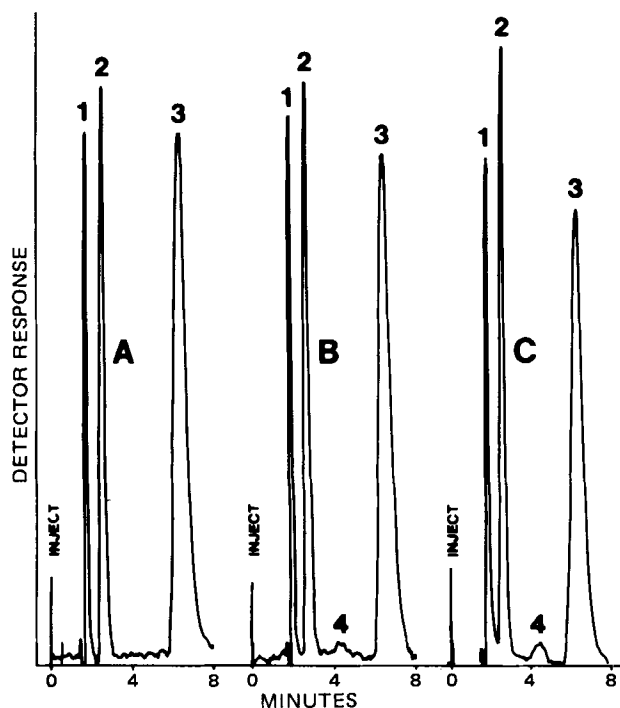
The percent relative standard deviation based on six injections was 2.5% and the assay method was reproducible.

**Progesterone**—The assay results of suspensions (Table I) indicate that the HPLC method can be used for the quantitation of III in suspension. However, in the case of injection, the benzyl benzoate interfered. It had the same retention time as of III; therefore, the assay results were high (Table I).

The excipients present in the suspension (sodium carboxymethylcellulose, methylcellulose, dioctyl sodium sulfosuccinate, and thimerosal) did not interfere with assay procedure (Fig. 2C). The percent relative standard deviation based on six injections was 2.7% and the method was reproducible.

The stability results (Table II) indicate that progesterone is a very stable compound in the vehicle (ethanol, 10%; propylene glycol, 50%; and water, 40%) studied. Even in 487 days of storage at 50°, the decomposition in all the solutions was <10%. This is equal to at least 5 years of storage at room temperature. The samples which were stored at room temperature did not decompose at all, even in 487 days.

In other experiments, the progesterone did not decompose in 24 hr on treating with 0.1 N HCl and with 0.1 N NaOH, the loss in potency was ~4%. On boiling with 1 N NaOH for 5 min, the sample lost slightly more than 16% of the potency. An additional peak between the peaks of the



**Figure 3**—Sample chromatograms. Peaks 1–3 are from the solvent, hydrocortisone (internal standard), and III, respectively. Peak 4 in chromatograms B and C is from the decomposition product of III. Key: A, standard solution of III in 50% ethanol; B, solution 3 (Table II) after 487 days of storage at 50°; and C, solution of III after boiling with 1 N NaOH. The chromatographic conditions are given in the text.

internal standard (hydrocortisone) and III was obtained (Fig. 3C) in the chromatogram.

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## A Time-Lag Model for Pharmacokinetics of Drugs Subject to Enterohepatic Circulation

JEAN-LOUIS STEIMER<sup>\*\*</sup>, YVES PLUSQUELLEC<sup>‡</sup>, ANNE GUILLAUME<sup>\*</sup>, and JEAN-FRANÇOIS BOISVIEUX<sup>\*</sup>

Received August 1, 1980, from the <sup>\*</sup>INSERM U 194, Département de Biomathématiques, 91, Bd de l'Hôpital, F-75634 Paris Cedex 13, France and the <sup>‡</sup>Laboratoire d'Algèbre, Département de Mathématiques, Université Paul-Sabatier, F-31000 Toulouse, France. Accepted for publication June 29, 1981.

**Abstract** □ A two-compartment model with time lag is proposed to describe the pharmacokinetics of drugs subject to enterohepatic circulation. The basic model, including two compartments for body and GI tract, respectively, with elimination occurring from both compartments, was previously proposed. The assumption that the reabsorption of a drug molecule is delayed after its biliary excretion is expressed by the addition of a time lag in the transfer from the first to the second compartment. Computer simulation of the model for intravenous bolus injection and oral intake of the drug was performed through first-order numerical integration. Several qualitative results concerning changes in pharmacokinetics due to modifications in biliary excretion, in reabsorption, or in elimination are identical with predictions using the basic model. However, several qualitative and quantitative results were significantly different. The pharmacokinetics, though remaining linear, are no longer biexponential. Initial decay after intravenous injection was not affected by modifications in reabsorption or elimination from intestine. Predictions based on the time-delay model agree with existing experimental evidence concerning pharmacokinetics of substances undergoing enterohepatic cycling. Delayed recirculation may lead to rebounds in plasma level profiles as well as after intravenous and oral administration. The half-life of the drug is significantly prolonged even when the kinetic processes involved in recirculation remain unchanged.

**Keyphrases** □ Pharmacokinetics—time-lag model, enterohepatic drug circulation □ Models, pharmacokinetic—enterohepatic drug circulation, time-lag model □ Enterohepatic circulation—drugs, pharmacokinetics, time-lag model

When a substance is taken up by the liver and excreted into the bile, it may be either eliminated through the GI tract or reabsorbed and carried to the liver *via* the portal blood stream. This second process is known as enterohepatic cycling. This phenomenon affects both endogenous and exogenous substances.

#### BACKGROUND

A two-compartment model was previously developed (1) representing the body and the GI tract. Qualitative modifications in pharmacokinetic time-profiles and parameters for drugs subject to enterohepatic cycling due to changes in biliary excretion and reabsorption rates are well depicted by this model.

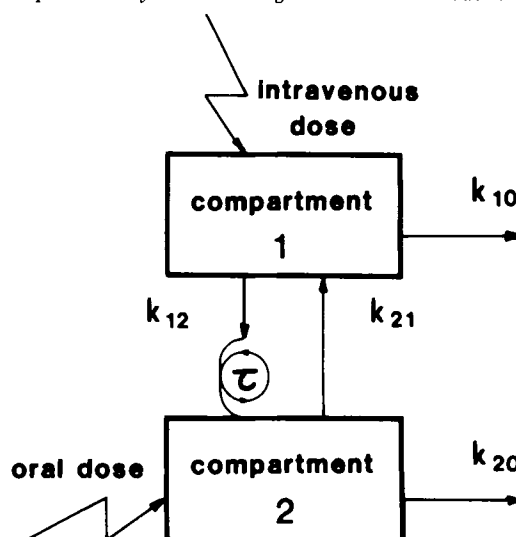
Predictions of the two-compartment model agree with previous experimental results (1, 2). However, some discrepancies, such as the evidence of a secondary peak in the time course of serum concentrations, remain. Such a secondary peak was reported (3) appearing after intravenous, oral, and intraportal administration of morphine in rats. An eight-compartment model for morphine pharmacokinetics was proposed. The GI tract compartment of Harrison and Gibaldi (1) is split into a catenary system of three compartments forming a loop connected with

the central compartment. Experimental evidence for a secondary peak in plasma radioactivity time profiles, as well as subsequent very slow elimination kinetics exist for labeled vitamin D<sub>3</sub> in humans (4) and rats (5). The main active metabolite of vitamin D<sub>3</sub> undergoes enterohepatic cycling (6).

A modification of the two-compartment enterohepatic recirculation model is proposed to take into account these previous experimental findings. The basic assumption is that a time-delay exists between the excretion of a given molecule of the substance into bile and its reabsorption from the intestine. This time lag may be due to delayed biotransformation in the liver, to the storage of the substance in the gall-bladder (*e.g.*, in humans), or simply to its transport in bile, at a limited flow-rate, from the site of excretion to the site of reabsorption. The corresponding time-delay model proposed here exhibits qualitative agreement with the two basic observations on pharmacokinetics of drugs subject to enterohepatic circulation: first, the occurrence of "rebounds" in serum level profiles after intravenous and oral administration of drug; and second, the slow terminal kinetics of the drug when recirculation occurs, even when the processes of deconjugation and reabsorption are not rate-limiting.

#### THEORETICAL

**Mathematical Formulation**—The model presented in Scheme I is a two-compartment system differing from the basic model of Harrison



Scheme I—Time-lag pharmacokinetic model for a drug subject to enterohepatic circulation. Compartment 1 represents the body including the liver. Compartment 2 represents the GI tract. Existence of a time delay is assumed after biliary excretion, before reabsorption can occur.