Table I—Activities of Glycosaminoglycan Sulfates after Incubation in Human Digestive Juices

Substance	Time of incubation, min	Residual activity[a] Lipasemic	Anticoagulant
	In Human Gastric Juice, pH 1.4		
Heparin	0	100	100
	5	97	—
	15	95	97
	60	93	100
	180	93	98
Standardized extracts of glycosaminoglycans	0	100	100
	5	—	—
	15	97	107
	60	98	110
	180	105	109
	In Human Duodenal Juice, pH 8.5		
Heparin	0	100	100
	5	—	—
	15	95	97
	60	—	—
	180	98	92
Standardized extracts of glycosaminoglycans	0	100	100
	5	87	102
	15	92	94
	60	98	100
	180	95	96

[a] The values are expressed as percent of initial activity.

fluence of dilution, stability tests were carried out in which the concentrations of heparin and the standardized mixture of glycosaminoglycan sulfates were 19 and 23.8 USP/ml, respectively. Anticoagulant and lipoprotein–lipase-inducing activities were measured in relation to the time of incubation by means of *in vitro* and *in vivo* quantitative methods. Lipoprotein–lipase activity was measured by a turbidimetric method[3].

Anticoagulant action *in vivo* was evaluated by thrombin time with an aliquot of the same plasma used for the determination of lipoprotein–lipase releasing action *in vivo* by the ediol method. All coagulation times were determined automatically.

The biological activity pattern of heparin and other glycosaminoglycan sulfates remained unchanged during the experimental time period, although some variability in biological assessment occurred (Table I).

The confirmed biological activity shows that the nature and conformation of even the more labile chemical groups of glycosaminoglycan sulfates remain unaltered, despite being incubated in chemically and enzymatically aggressive environments such as digestive juices.

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[3] This method is based on the capability of rat plasma, treated with glycosaminoglycan sulfates previously (10 min before), to clear an artificial fatty ediol–water suspension. The clearing effect is proportional to the administered amount of glycosaminoglycan sulfates (10).

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Adsorption of Methotrexate onto Glassware and Syringes

Keyphrases □ Methotrexate—adsorption onto glassware and syringes □ Adsorption—methotrexate onto glassware and syringes

To the Editor:

Methotrexate, a potent antifolate, is a widely used antineoplastic agent (1). With the advent of high-dose therapy and citrovorum-factor rescue, monitoring of plasma concentrations of methotrexate is recommended (1–4). However, the potential interference of active metabolites, such as 7-hydroxymethotrexate and 4-amino-4-deoxy-N^{10}-methylpteroic acid, renders many assay methods nonspecific (5). A need for re-evaluation of past pharmacokinetic studies has been advocated (6). Hence, development of specific and sensitive assay methods is required. In our recent development of a high-performance liquid chromatographic (HPLC) assay for methotrexate and its metabolites in biological fluids (7), erratic results were often found when series dilutions of methotrexate in water-miscible organic solvents such as methanol were made. These organic solvents were used to enhance the aqueous solubility of methotrexate (8).

Additional studies were carried out to explore the potential interaction between methotrexate and glassware or syringes. This communication reports the results of our preliminary adsorption studies, and discusses its implication in quantitative analysis.

Stock solutions (0.1 mg/ml) of methotrexate were prepared in distilled water, methanol, or 80% ethanol (with 20% water, v/v) using 100-ml volumetric flasks[1]. Series dilutions were made to concentrations of 50, 10, and 1 μg/ml. After mixing and equilibration overnight, the methotrexate concentration was determined directly by the HPLC method described earlier (7). A cation-exchange

[1] Kimax, Fisher Co., Chicago, Ill.

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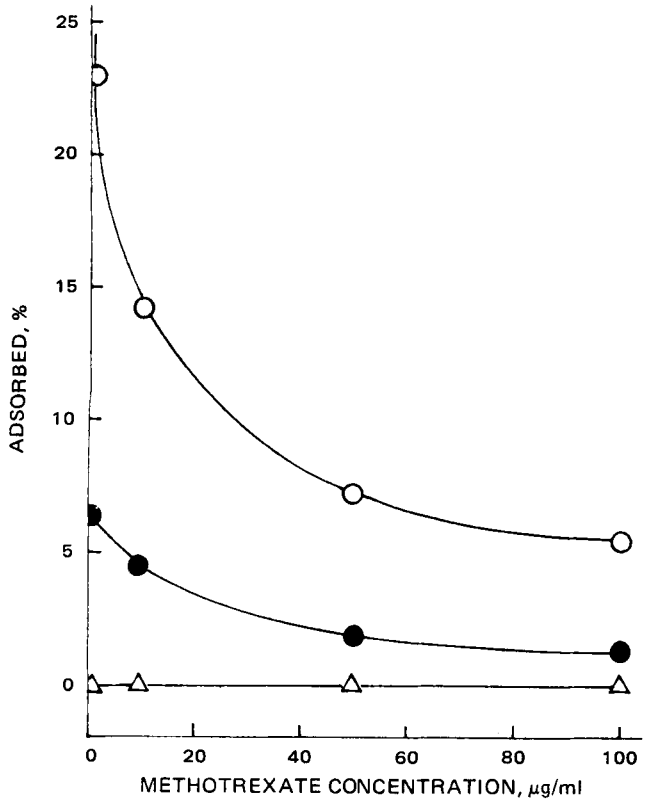

Figure 1—*Effect of concentration and diluent on methotrexate adsorption onto a volumetric flask. Key: ○, methanol; ●, 80% ethanol; and △, water.*

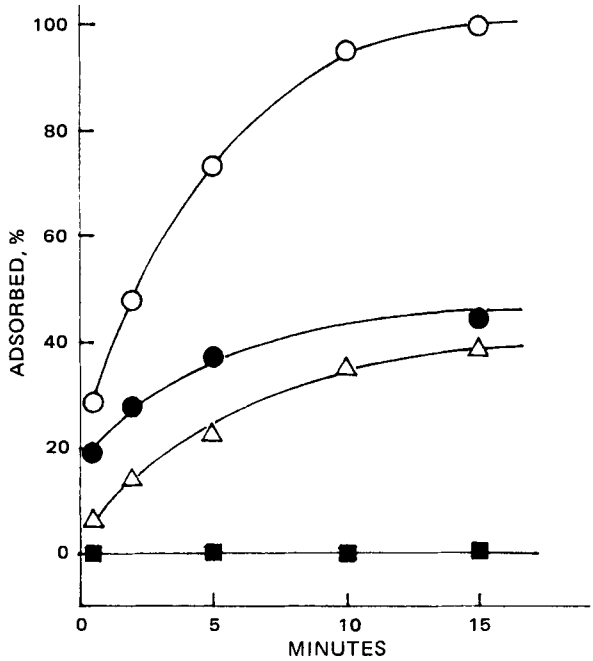

Figure 2—*Effect of exposure time on methotrexate adsorption by the syringe. Key: ○, methanol (0.1 μg/ml); ●, methanol (10 μg/ml); △, 80% ethanol (10 μg/ml); and ■, water (0.1 μg/ml).*

column[2] was used. The mobile phase was prepared by mixing 10 parts of acetonitrile with 90 parts of 0.02 *M* monobasic ammonium phosphate solution acidified with phosphoric acid (0.2%) to pH 2.7. The flow rate was set at 2 ml/min; the retention time for methotrexate was 7 min. *p*-Aminobenzoic acid was used as an internal standard to ensure the proper series dilution and HPLC injection; it was dissolved together with methotrexate in the preparation of stock solutions. No adsorption problem with *p*-aminobenzoic acid was encountered. Experiments were run at least in duplicate.

Figure 1 shows that the extent of adsorption was solvent and concentration dependent. The aqueous solution had no adsorption problem. However, methotrexate showed significant adsorption onto the flask in methanol or 80% ethanol. For example, the adsorption of methotrexate at 1 μg/ml was ~23% in methanol and 7% in 80% ethanol. As shown in Fig. 1, the percentage of methotrexate lost to the volumetric flask increased as the concentration decreased. It is possible that the extent of adsorption may be much greater when the concentration is <1 μg/ml (9).

Further evidence for methotrexate adsorption onto the glassware was supported by the following study. After adsorption equilibration overnight, various drug solutions were withdrawn into syringes[3]. The methotrexate concentrations in the syringes were then measured as a function of time. As shown in Fig. 2, the adsorption of methotrexate increased with the length of contact time in the 15-min period of the study. Again, methotrexate showed a higher degree of adsorption in methanol as compared with the 80% ethanol solution. The extent of adsorption also increased as methotrexate concentration decreased. It appears that the phenomenon of drug adsorption onto syringes used for the HPLC analysis has never been reported in the literature. Adsorption of butaperazine onto quartz cells was reported earlier (10).

A previous study (9) showed that the pH of the solution may influence the magnitude of adsorption. Our preliminary results showed that the adsorption of methotrexate in alcoholic solutions was reduced at lower (pH 2–4) or higher (pH 8–9) pH values.

It should be pointed out that the syringes used in this study were used not only in the preparation of drug standard solutions but also for sample delivery in HPLC assays. If methotrexate is prepared in solvents other than water, serious errors in the determination of the drug concentrations may result. Our preliminary findings also showed that 4-amino-4-deoxy-N^{10}-methylpteroic acid, a methotrexate metabolite (8), had a similar adsorption problem. The results of the present study clearly indicate that the potential adsorption of methotrexate and its metabolites onto glassware and other contact surfaces should be considered as a source of variability in quantitative analysis.

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Pharmacokinetic and Biopharmaceutic Parameters During Enterohepatic Circulation of Drugs

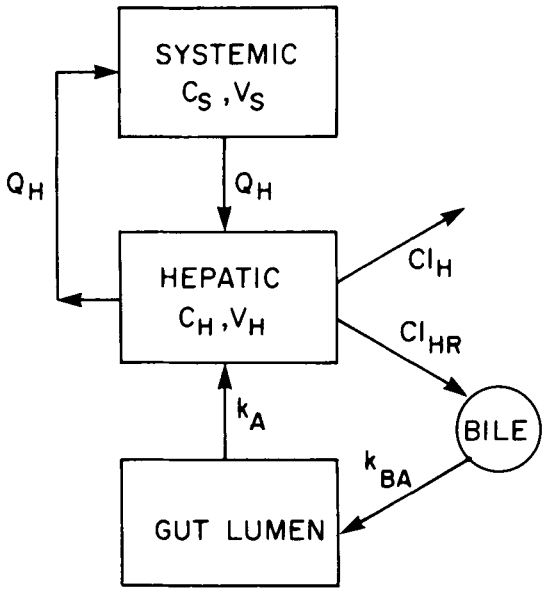
Keyphrases □ Enterohepatic circulation—model, evaluation of pharmacokinetic parameters □ Pharmacokinetics—evaluation of parameters, enterohepatic circulation model

To the Editor:

As research in the area of enterohepatic circulation continues in this laboratory, several aspects of its impact on the interpretation of pharmacokinetic and biopharmaceutic studies have become apparent. Simulation techniques have been used to investigate the effect of enterohepatic circulation on various pharmacokinetic and biopharmaceutic parameters under various conditions.

The simple first-pass perfusion model in Scheme I was used for the simulations. In the model, V_H and V_S are the effective hepatic and systemic volumes[1], Q_H is the blood flow to the liver, and Cl_H and Cl_{HR} are the irreversible and recirculating hepatic organ clearances, respectively. The biliary excretion component was assumed to be continuous in the present model to avoid the complexities associated with gallbladder storage and subsequent excretion into the duodenum. The more complex model was addressed previously (1) and will be investigated further, but the concepts presented here are generally applicable to both situations. For the purpose of the oral absorption simulations, the absorption rate constant, k_A, was set at 0.693 hr^{-1}. Simulations were performed under conditions of intact enterohepatic circulation (k_{BA} = 1.0) and under conditions of bile duct cannulation, *i.e.*, interrupted enterohepatic circulation (k_{BA} = 0.0).

Blood concentration–time data following intravenous and oral doses were simulated using several sets of parameter values. Representative examples from the total number of simulations are presented in Tables I–III. The



Scheme I—Pharmacokinetic first-pass perfusion model used to describe the systemic blood concentrations of drugs that undergo enterohepatic recycling. Key: Q_H, hepatic blood flow; Cl_H and Cl_{HR}, irreversible and recirculating hepatic organ clearances, respectively; and k_A and k_{BA}, the absorption and bile transport processes, respectively.

effect of enterohepatic circulation on blood clearance (Cl_B), oral clearance (Cl_O), elimination half-life ($t_{1/2\beta}$), and the volume of distribution ($V_{d_{area}}$) are presented in Table I. The influence of enterohepatic circulation on the time (T_{max}) of the maximum observed blood concentration (C_{max}) and the elimination half-life following oral dosing are presented in Table II. Table III contains the blood clearance (Cl_B) and oral clearance (Cl_O) parameters along with the absolute bioavailability under intact (F_I) as well as bile duct-cannulated (F_C) conditions. In addition, the ratio of the area under the curves following intravenous doses (AUC_{IV}) under intact and bile-duct-cannulated conditions (R_{IV}) as well as the ratio of the area under the curves following oral doses (AUC_O) under intact and bile duct-cannulated conditions (R_O) are presented in Table III.

The results of these simulations indicate that enterohepatic circulation increases the apparent volume distribution and prolongs the half-life of elimination when compared to identical blood clearances without it. The more extensive the recycling, the more prolonged the half-life and greater the volume increase (Table I). The data presented in Table II indicate that the pharmacokinetic complexities associated with enterohepatic circulation are affected by the effective hepatic and systemic volumes of distribution. The time (T_{max}) of the maximum observed blood concentrations (C_{max}) following oral doses are a function of both the interrelationship of systemic to hepatic effective volumes of distribution as well as the extent of enterohepatic circulation (Table II).

For example, comparison of cases G–I with cases J–L in Table II indicates that when the effective systemic volume of distribution is smaller than the effective hepatic volume of distribution, more extensive recycling results in longer elimination half-lives and lower C_{max} values, but results

[1] Effective volume is the product of the physiological volume of the organ or pooled tissues times its retention factor.

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