with time, as is seen with quinidine (1), it is important to realize the difference in the time to approach equilibrium between adding drug to plasma and buffer.

For the following discussion, the associating and dissociation rates are assumed to be fast in comparison to the diffusion across the dialysis membrane. Furthermore, it is also assumed that there is the same volume V on both sides of the dialysis membrane, and no net flux of water takes place. In addition, the rate of transfer across the membrane is assumed to be covered by a clearance term Cl_T, which is the same in both directions, and the unbound concentration on each side. The binding is assumed to be linear and no net loss of drug occurs during dialysis.

The rate of change in the buffer side can be expressed by:

$$\frac{dA_B}{dt} = -Cl_T C_B + Cl_T \alpha C_p \qquad \text{(Eq. 1)}$$

where A_B is the amount on the buffer side, α is the unbound fraction in plasma, C_B the buffer concentration, and C_p the plasma concentration of drug. The rate of change in the plasma side is expressed by:

$$\frac{dA_p}{dt} = -Cl_T \alpha C_p + Cl_T C_B \qquad \text{(Eq. 2)}$$

where A_p is the amount on the plasma side.

When the drug initially is placed on the plasma side, the concentration on the buffer side is then described by:

$$C_B = \frac{C_0 \alpha}{1+\alpha}\left[1 - e^{-(k_T\alpha + k_T)t}\right] \qquad \text{(Eq. 3)}$$

where t is time, C_0 initial concentration, and k_T the ratio Cl_T/V.

When the drug initially is placed on the buffer side, the buffer side concentration is described by:

$$C_B = \frac{C_0}{1+\alpha}\left[\alpha + e^{-(k_T\alpha + k_T)t}\right] \qquad \text{(Eq. 4)}$$

In both cases the concentration approaches the equilibrium concentration $(C_0\,\alpha)/(1+\alpha)$ when $t \to \infty$. One can determine from Eqs. 3 and 4 the time that is needed to reach a value that is only a fraction, δ, away from the equilibrium concentration; *i.e.*, the time required to reach a buffer concentration of $[(C_0\,\alpha)/(1+\alpha)]\,(1-\delta)$ if the drug is placed initially on the plasma side and $[(C_0\,\alpha)/1+\alpha]\,(1+\delta)$ if it is placed initially on the buffer side. The ratio of the times to reach these concentration is then:

$$R = \frac{t_B}{t_p} = \frac{\ln\left\{\left[\left(\frac{C_0\alpha}{1+\alpha}\right)(1+\delta)\Big/\frac{C_0}{1+\alpha}\right] - \alpha\right\}}{-(k_T\,\alpha + k_T)} \qquad \text{(Eq. 5)}$$

$$\frac{\ln\left\{\left[-\frac{C_0\,\alpha}{1+\alpha}(1-\delta)\Big/\frac{C_0\,\alpha}{1+\alpha}\right] + 1\right\}}{-(k_T\,\alpha + k_T)} \qquad \text{(Eq. 5)}$$

where t_p and t_B are the times to reach equilibrium when the drug is initially added to the plasma and buffer side, respectively. Cancelling the common terms gives:

$$R = \frac{t_B}{t_p} = \frac{\ln\delta + \ln\alpha}{\ln\delta} = 1 + \frac{\ln\alpha}{\ln\delta} \qquad \text{(Eq. 6)}$$

Equation 6 indicates that the closer to the true equilibrium value one wants to be, the closer the ratio is to unity. The stronger the binding and the larger the deviation from the true equilibrium concentration, the greater the advantage of spiking the plasma side.

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Stability of Heparin and Other Fractions of Glycosaminoglycan Sulfates in Human Digestive Juices

Keyphrases □ Heparin—stability in human digestive juices □ Glycosaminoglycan sulfates—stability in human digestive juices □ Absorption, intestinal—stability of heparin and glycosaminoglycan sulfates

To the Editor:

The oral administration of heparin has been a subject of controversy (1) because of its uncertain absorption in the GI tract and its stability in digestive juices. Its N-sulfate groups are easily hydrolyzed in an acidic medium, with consequent impairment of biological activity. Some authors (2) also affirmed that intestinal flora degrades and metabolizes heparin.

However, according to recent studies (3, 4), some fractions of glycosaminoglycan sulfates, strictly correlated to heparin, exerted antithrombotic and hypolipidemic activities after oral administration. Furthermore, it was proved that intestinal absorption took place when some fractions of glycosaminoglycan sulfates, labeled with fluorescein (5), were administered intraduodenally (6).

Special excipients to suppress ionization of the functional groups of glycosaminoglycan sulfates and, thus, to promote their GI absorption (7) were studied. It was found that, if biopolymer is administered in micellar suspension with monoolein and bile salts, the intestinal absorption of heparin greatly increases with increasing mucosa permeability (8). For example, the rectal administration to rats of heparin and other glycosaminoglycan sulfates in a 2-mg/kg dose in an oily emulsion prolonged the time of coagulation; only 1 mg/kg, administered by the same route, activated the lipoprotein lipase-inducing system (9).

To evaluate if and to what extent human digestive juices can degrade glycosaminoglycan sulfates, we investigated the stability and biological activity of heparin and a standardized mixture[1] of fractions of glycosaminoglycan sulfates, containing a heparin-like substance with a low molecular weight and condroitin sulfate B, in cat and human gastric and duodenal juices.

Glycosaminoglycan sulfates and heparin were incubated at concentrations of 190 and 238 USP/ml, respectively, for up to 3 hr in digestive juices[2] at 37°. To evaluate the in-

[1] Sulodexide.
[2] Gastric juice of the cat was obtained by a Heidenhain gastric pouch. Human gastric juice was obtained from a probe of five volunteers who had previously been given an injection of 3 U of cholecystokinin kg/hr iv.

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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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