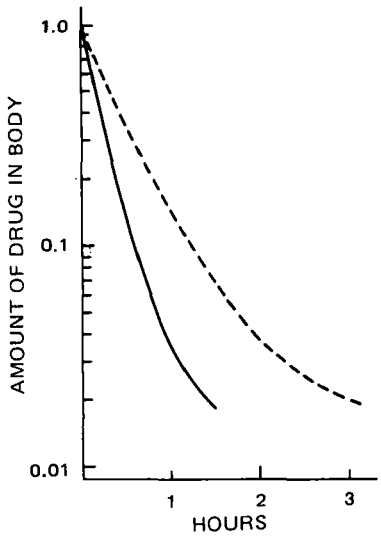

Figure 5—*Effect of reduced biliary excretion on the α-phase when* $k_{10}/k_{20} > 1$. *Key: —, control,* $k_{12} = 3.0$, $k_{21} = 0.1$, $k_{10} = 1.0$, *and* $k_{20} = 0.1\ hr^{-1}$; *and - - -, partial cholestasis,* $k_{12} = 1.0$, $k_{21} = 0.1$, $k_{10} = 1.0$, *and* $k_{20} = 0.1\ hr^{-1}$.

numerical analysis. The results of this analysis were quite clear over a wide range of rate constants. When $k_{10}/k_{20} > 1$, regardless of the value of k_{12}/k_{21}, a decrease in k_{12} produces an increase in β and a decrease in the half-life of the drug (Fig. 3). On the other hand, when $k_{10}/k_{20} < 1$, a decrease in k_{12} results in a decrease in β and an increased biological half-life (Fig. 4). Hence, the model in Scheme I suggests that cholestasis can either increase or decrease the biological half-life; the effect one observes depends on the relationship between the rate constants associated with nonbiliary elimination of the drug from the body and with "elimination" of the drug in the gut.

An interesting finding, made during the simulation and confirmed by numerical analysis of Eq. 2, suggests that an apparent decrease in drug elimination would be the most common observation in cholestasis. Regardless of the value of k_{10}/k_{20}, a decrease in k_{12} produces a decrease in α for any given set of rate constants. In other words, the half-life of the α-phase for a given drug will always be longer in cholestasis than under normal conditions. Limited computer studies suggest that this will also be the case when a multicompartment model is required to represent the body.

If, because of experimental design or assay limitations, the time course of drug concentrations in the plasma is followed for a period shorter than the time required to attain distribution equilibrium, *i.e.*, the time at which the β-phase becomes evident, one would conclude that the reduced biliary excretion increases the half-life of the drug, even if k_{10}/k_{20} were greater than 1 (Fig. 5). Although this conclusion is mathematically incorrect, it is realistic from a clinical and toxicological point of view. For example, it is highly likely that the LD_{50} of any drug subject to biliary excretion in the test species would be decreased in the bile duct-ligated animal compared to that observed in the control animal (8).

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 22, 1975, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214*

Accepted for publication November 21, 1975.

Supported in part by Grant GM-20852 from the National Institute of General Medical Sciences, National Institutes of Health.

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Biliary Secretion of Methotrexate in Rats and Its Inhibition by Probenecid

R. E. KATES ˣ **and T. N. TOZER**

Abstract □ The biliary secretion of methotrexate was investigated in rats under steady-state conditions. The transport system involved was saturable and displayed Michaelis–Menten-type kinetics. Values for the maximal rate of transport and a transport constant analogous to the Michaelis constant were 12 mg/hr and 32 mg/liter ($7 \times 10^{-5}\ M$), respectively. The inhibition of this transport mechanism by probenecid also was investigated, and the relationship between the plasma concentration of probenecid and the biliary clearance of methotrexate was elucidated. The value of K_i, the dissociation constant for the transport carrier–inhibitor complex, was 23 μg/ml ($8 \times 10^{-5}\ M$).

Keyphrases □ Methotrexate—biliary secretion, effect of coadministration of probenecid, rats □ Probenecid—effect on biliary secretion of methotrexate, rats □ Biliary secretion—methotrexate, effect of coadministration of probenecid, rats □ Antineoplastic agents—methotrexate, biliary secretion, effect of probenecid, rats □ Uricosuric agents—probenecid, effect on biliary secretion of methotrexate, rats

The effectiveness of methotrexate as an anticancer drug was first reported 25 years ago. Since then, its mechanisms of action have been studied extensively to determine how it can be utilized effectively in treating neoplastic diseases. As with other anticancer drugs, methotrexate is a nonspecific agent which arrests cell

growth in normal and malignant tissues. For optimal effectiveness, the therapeutic effect (malignant cell kill) must be maximized while the effect on normal, nonmalignant tissues is minimized.

Numerous empirical dosage regimens have been suggested, but only a few have proven advantageous (1). Methotrexate has been used most effectively with an intermittent administration schedule, with each dose followed by leucovorin rescue (2). To design a better dosage scheme, the total body and individual tissue disposition kinetics of methotrexate have been studied (3–5). Attention has been focused both on the role of disposition phenomona in the delivery of drug to target tissues and on how drug disposition influences the incidence and severity of toxic side effects.

The pharmacokinetics of methotrexate were first investigated by Henderson *et al.* (6), and later an extensive pharmacokinetic model of the kinetics of distribution and elimination of methotrexate in several species was presented by Bischoff *et al.* (3). Both reports clearly demonstrated that biliary secretion plays an important role in the disposition kinetics of methotrexate in some species. Henderson *et al.* (6) reported that about 55% of an intravenous dose in rats was secreted into the bile. They found that biliary secretion played a smaller role in the mouse and monkey. In the monkey, about 15% of an intravenous dose was secreted into the bile. Although bile was not collected from mice, 20–40% of an intravenous dose was excreted in the feces. Whether methotrexate is substantially secreted into the bile of humans is currently unresolved. However, only about 5% of an intravenously administered dose is eliminated in the feces of humans.

The relationship between enterohepatic cycling of drugs and their toxicity was reviewed (7). For a drug like methotrexate, where the length of tissue exposure is a major factor determining the severity of toxicity, enterohepatic cycling can be important. With methotrexate, biliary secretion puts the drug in direct contact with the tissue that is a primary site of toxicity, the intestinal mucosa.

A main concern of the present study was the possible role of biliary cycling as a contributing factor to the toxicity of methotrexate. The kinetics of the biliary secretion of methotrexate and its inhibition by probenecid were investigated in rats.

THEORETICAL

The processes involved in the transport of drugs into the bile have been studied (8, 9). Although a passive filtration component may play a part in the transport of drugs into the bile, it is generally believed that active transport is the primary process (8).

For a drug to be transported into the bile, it must travel from the liver sinusoid through the space of Disse and then through the hepatic parenchymal cell before entering the bile canaliculus. The process by which a drug molecule travels from the sinusoid into the space of Disse is one of simple diffusion. Although the transport from the parenchymal cell into the bile canaliculus is generally believed to be an active transport process, the mechanisms involved in the uptake of drugs from the space of Disse by the parenchymal cell are not clearly understood. While some investigators reported that the uptake is due to physiological binding of drugs to cellular proteins (10), more recent studies suggested an active transport mechanism for drug uptake by the hepatic parenchymal cell (11). Although the nature of the uptake mechanism(s) is currently an unsettled issue, at least one active transport process is involved in the secretion of drugs into the bile.

Irrespective of location, the transport process that is rate limiting should be characterizable. For the purpose of simplifying this discussion, the rate-limiting step is assumed to be the transport of drug from the parenchymal cell into the bile canaliculus; however, no physiological identification of the actual site of transport is implied. One could, with equal justification, assume that the rate-limiting transport step occurs between the space of Disse and the hepatic parenchymal cell.

Since active transport processes are subject to saturation, the rate of transport is expected to be described by a relationship analogous to the equation of Michaelis and Menten:

$$T = \frac{T_{max}[D]}{K_t + [D]} \qquad \text{(Eq. 1)}$$

where T = rate of transport, T_{max} = maximal rate of transport, K_t = transport constant (concentration of drug resulting in a rate of transport equal to one-half of the maximal rate), and $[D]$ = concentration of drug at the site where transport occurs.

This model assumes the formation of a complex, CD, between a transport carrier, C, and the drug, D. This complex is assumed to form in the hepatic parenchymal cell or in the cell membrane, and this complex crosses the membrane to deposit the drug into the bile canaliculus. The carrier then returns to the interior of the cell, where it complexes with more drug (Scheme I).

hepatic parenchymal cell | bile canaliculus

$$D + C \rightleftarrows CD \rightarrow D$$

Scheme I

The rate-limiting step is neither the association nor the dissociation of the complex but the translational event between them (12).

To characterize this transport mechanism adequately, it is necessary to determine both the value of the maximal rate of transport and the value of the transport constant. These values can be determined by measuring the rate of methotrexate transport into the bile as a function of the drug concentration at the transport site. Since this concentration cannot be determined directly, the blood methotrexate concentration is assumed to be representative of, or proportional to, the concentration at the transport site.

Furthermore, the rate at which methotrexate appears in the bile duct may not be equal to the transport rate into the bile if there is a significant lag between the time methotrexate is secreted into the bile and the time it can be detected in the bile duct. The studies reported here were carried out under conditions of steady state, thereby obviating the effect of a lag time. The methotrexate concentrations at the transport site and in the blood also were assumed to be at steady state. Since it is not possible to determine whether the concentration at the actual transport site is different than the blood concentration, the K_t for this system is defined relative to the concentration of drug in the blood.

To determine the values of the transport maximum, T_{max}, and the transport constant, K_t, the data obtained by measuring the rate of biliary secretion of methotrexate as a function of its concentration in the blood can be treated in three ways:

$$\frac{1}{T} = \frac{K_t}{T_{max}[D]} + \frac{1}{T_{max}} \qquad \text{(Eq. 2)}$$

$$T = \frac{-K_t T}{[D]} + T_{max} \qquad \text{(Eq. 3)}$$

$$\frac{[D]}{T} = \frac{K_t}{T_{max}} + \frac{[D]}{T_{max}} \qquad \text{(Eq. 4)}$$

Equations 2–4 are linearized rearrangements of the Michaelis–Menten equation.

Dowd and Riggs (13) analyzed these three linear transformations of the Michaelis–Menten equation and reported that they were not equivalent when used to estimate values of K_t and T_{max}. In Eqs. 2 and 4, the reciprocal of T tends to give unwarranted emphasis to the smallest values of T, and these values are most likely to have the greatest percent error in their measurement. According to Dowd and Riggs (13), the plot using Eq. 2 is by far the least reliable method. Furthermore, the plot using Eq. 4 is superior to the plot using Eq. 3 when the error in T is small; the reverse is true when the error in T is large. The data obtained in this study were analyzed by each of these treatments to facilitate comparison of the values.

Inhibition of active transport is generally classified as being com-

petitive or noncompetitive. In competitive inhibition, the inhibitor forms a reversible complex with the transport carrier and essentially competes with the substrate, in this case methotrexate, for the transport carrier. The actual rate at which a drug or substrate is transported depends on the relative concentrations of both the substrate and the inhibitor. In noncompetitive inhibition, there is no relationship between the degree of inhibition and the substrate concentration. Inhibition depends only on the inhibitor concentration (14).

The two relationships that describe systems subject to competitive and noncompetitive inhibition are:

$$T = \frac{T_{max}[D]}{K_t(1 + [I]/K_i) + [D]} \quad \text{(Eq. 5)}$$

and:

$$T = \frac{T_{max}[D]}{(K_t + [D])(1 + [I]/K_i)} \quad \text{(Eq. 6)}$$

respectively, where $[I]$ = inhibitor concentration, K_i = dissociation constant of the enzyme–inhibitor complex, and the other symbols are as previously described.

In competitive inhibition (Eq. 5), the maximal transport rate is unaffected by the inhibitor, but the apparent transport constant is increased by a factor of $(1 + [I]/K_i)$. The anticipated effect of a noncompetitive inhibitor (Eq. 6) is to decrease the maximal transport rate by a factor equal to the reciprocal of $(1 + [I]/K_i)$.

Probenecid was chosen as a potential inhibitor of the biliary secretion of methotrexate. Probenecid inhibits the active transport of organic acids at several sites in the body. To measure the inhibition of an active transport system, it is usually necessary to determine the transport rate of the substrate at several different concentrations while maintaining the inhibitor concentration at a constant level.

The elimination rate of probenecid, however, is subject to large intersubject variability. This variability makes it impractical to produce the same steady-state plasma probenecid concentration in different experimental animals. This problem was overcome by using methotrexate concentrations that were well below the value of K_t. If the methotrexate concentration, $[D]$, is much less than K_t, then the drug concentration term can be dropped from the denominator of both Eqs. 5 and 6, giving:

$$T = \frac{T_{max}[D]}{K_t(1 + [I]/K_i)} \quad \text{(Eq. 7)}$$

Dividing by $[D]$ and taking the reciprocal of both sides of Eq. 7 give:

$$[D]/T = K_t/T_{max} + [K_t/(T_{max}K_i)][I] \quad \text{(Eq. 8)}$$

The reciprocal of clearance is $[D]/T$. A plot of the reciprocal of the biliary clearance of methotrexate *versus* probenecid concentration theoretically should give a straight line with an intercept on the reciprocal clearance axis of K_t/T_{max} and a slope of $K_t/(T_{max}K_i)$. From this plot, the value of K_i for probenecid as an inhibitor of the biliary transport of methotrexate can be determined.

EXPERIMENTAL

Materials—Tritiated methotrexate[1] was diluted with the nonradioactive drug[2] and purified by the method of Oliverio (15). Probenecid was obtained as a powder[3]. A methotrexate solution for injection was prepared by dissolving a weighed amount of the purified drug in a few drops of 0.1 *M*, pH 8.2 ammonium bicarbonate buffer, and diluting to the desired volume with normal saline. A probenecid solution for injection was prepared similarly. A weighed amount of powder was dissolved in a few drops of 1.0 *N* NaOH, and the pH was then adjusted to 7.4 with a pH 7.0 phosphate buffer. This solution was diluted to the desired volume with normal saline.

All studies were conducted on male Sprague-Dawley rats, 400–500 g.

Analytical Procedures—Liquid scintillation counting techniques were used for the determination of the methotrexate concentration in blood and bile samples. Samples were collected in sacks prepared from sections of dialysis tubing. Nine centimeter lengths of 0.63-cm diameter cellulose dialysis tubing were tied at one end and used for the collection of blood samples; 10-cm lengths of 1.88-cm tubing were used for bile collection. The casings used for blood collection were weighed before and after being filled with approximately 0.5 ml of blood. The exact volume of the blood was determined by dividing the net weight of the blood sample by 1.05, the average specific gravity of rat blood (16).

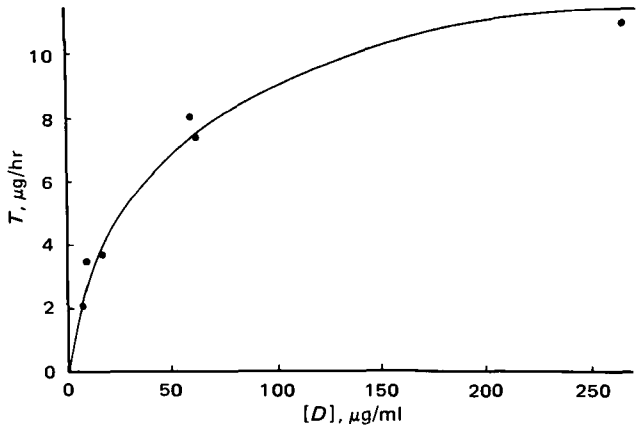

Figure 1—*Plot of the biliary secretion rate,* T, *against the blood concentration of methotrexate,* [D].

The samples were dried prior to being combusted on a sample oxidizer[4] in preparation for scintillation counting. A commercially prepared scintillation cocktail[5] was used.

All samples were counted on a liquid scintillation spectrometer[6]. Quenching was corrected for by means of an automatic internal standard. Since the total radiolabel in blood and bile samples was measured, a few selected samples were chromatographed by a previously reported procedure (17) to determine if a significant fraction of the radiolabel was associated with nonmethotrexate species.

The concentration of probenecid in plasma samples were determined by the spectrophotometric method of Dayton *et al.* (18).

Animal Preparation—To facilitate frequent sampling of blood and bile, the bile duct and the vena cava of each rat were cannulated. A cannula also was inserted into the aorta *via* the left carotid artery, and both methotrexate and probenecid were infused through this site. The carotid and vena cava cannulas were exteriorized through a small subcutaneous incision on the dorsal side of the head, between the ears. This procedure prevented the rat from pulling at or chewing on the cannulas and also permitted the study to be conducted without disturbing the rat each time a blood sample was drawn.

The rats were anesthesized with pentobarbital in preparation for surgery but were allowed to recover following surgery before a study was begun. During the study period, the rats were kept in restraining cages and allowed water *ad libitum*.

Procedures—Two studies were conducted. In one, the rats were infused with methotrexate alone; in the other, the rats were infused with both methotrexate and probenecid. The drugs were infused into the aorta by a constant infusion pump[7] connected to the carotid cannula. When both methotrexate and probenecid were infused, the carotid cannula was connected to two pumps by a four-way stopcock.

At the beginning of each study, loading doses were administered and constant infusions were then maintained for 6–8 hr. Each rat was infused at a different rate to produce a range of blood methotrexate concentrations. Bile was collected over 1-hr intervals, and a blood sample was obtained at the midpoint of each collection period. At 1.5, 4, and 7 hr after initiation of the infusion, plasma samples were obtained from rats receiving probenecid for the purpose of determining the probenecid concentration.

RESULTS AND DISCUSSION

The blood methotrexate concentration in the rats approached a steady state during the infusion period. The values of the blood con-

[1] Amersham/Searle.
[2] Nutritional Biochemicals Corp.
[3] Merck Sharp and Dohme Research Laboratories.
[4] Packard model 305.
[5] Insta Gel, Packard Corp.
[6] Packard Tri-Carb model 3775.
[7] Harvard model 975.

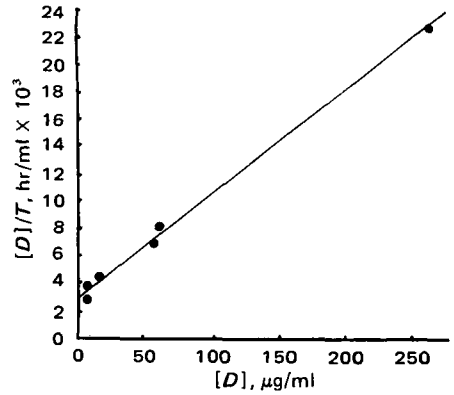

Figure 2—*Plot of the reciprocal of biliary clearance,* ([D]/T), *against the blood concentration of methotrexate,* [D].

centration and biliary excretion rate of methotrexate during the final 3 hr of each study were sufficiently constant to be averaged. In these studies, the total radioactivity in the blood was used to calculate the methotrexate concentration.

In a previous report (17), a fraction of the radioactivity in the blood was associated with nonmethotrexate species following a bolus injection of methotrexate to rats. The fraction of total label representing this nonmethotrexate component increased with time. In this study, however, the fraction of nonmethotrexate radiolabel in both blood and bile was undetectable.

There are two possible explanations for a significant nonmethotrexate fraction of the radiolabel in rat blood following a bolus injection but not when methotrexate is administered by constant infusion. Following a bolus injection, methotrexate is rapidly eliminated from the blood; the nonmethotrexate component, being very slowly removed, becomes the increasingly dominant species present. The nonmethotrexate component after a single bolus gains significance with time due to its retention in the blood. Under conditions in which a constant blood level is maintained, the concentration of the nonmethotrexate component is insignificant in relation to the concentration of the unchanged drug.

Another possible explanation arises from the fact that methotrexate has been shown to be metabolized by the bacteria of the lower intestine (19, 20). The nonmethotrexate species present in the blood could be an absorbed bacterial metabolite. In the current studies, however, the bile ducts of the rats were cannulated, preventing access of the drug to the intestinal lumen for bacterial metabolism. Whatever the actual reason, in these studies total radiolabel measurements adequately reflected levels of unchanged methotrexate.

A plot of the biliary secretion rate, T, of methotrexate as a function of blood concentration, $[D]$, is shown in Fig. 1. The biliary secretion rate approached saturation when blood levels were increased to over 250 μg/ml. The data obtained were treated by each of the three linear transformations of the Michaelis–Menten equation; each point represented the average of three sampling times from each individual rat study. The data were fit to each of the three equations using a nonweighted least-squares fitting program.

The plot resulting from Eq. 4 is shown in Fig. 2. A summary of the values obtained for the transport constant and the maximal transport rate is given in Table I, along with the correlation coefficient for each plot. The averages of the values obtained for K_i and T_{max} from the two "more reliable" plots (Eqs. 3 and 4) are 32 mg/liter or 7×10^{-5} M and 12 mg/hr, respectively. These values are surprisingly close to those obtained from the reportedly "less reliable" plot of Eq. 2.

The value of the biliary clearance for blood methotrexate concentrations, which are well below the value of the transport constant, is obtained by dividing T_{max} by K_t. When using the average values for T_{max} and K_t, respectively, a value for biliary clearance of 6.33 ml/min is obtained. A value of 3 ml/min was reported previously (3), but the average size of the rats used in that study was 200 g. In this study, the average weight was 420 g. If these values are normalized for body weight, they are in good agreement: 15 ml/min/kg from Ref. 3 and 15.1 ml/min/kg from this study.

Table I—Values of T_{max} and K_t for the Biliary Secretion of Methotrexate

Parameter	Equation 2, $1/T$ *versus* $1/[D]$	Equation 3, $[D]/T$ *versus* $[D]$	Equation 4, T *versus* $T/[D]$
K_t, mg/ml	0.0325	0.0376	0.0254
T_{max}, mg/hr	12.09	12.97	10.96
Correlation coefficient	0.940	0.997	0.858

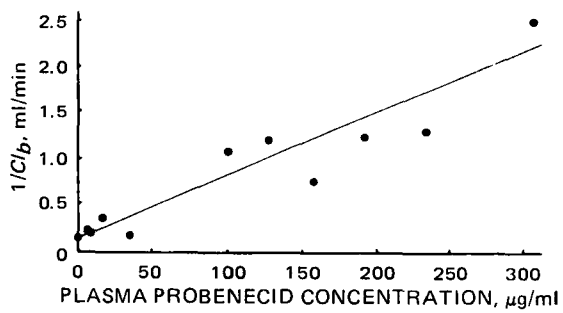

Figure 3—*Plot of the reciprocal of biliary clearance of methotrexate* Cl_b, *as a function of the plasma probenecid concentration.*

The efficiency of removal of methotrexate from the blood by the liver can be evaluated by relating the biliary clearance of methotrexate to the liver blood flow. In the adult rat, the blood flow to the liver is reported to be 79 ml/min/100 g of tissue, and the weight of the liver is reported to represent 3.35% of the total body weight (21). From these parameters, a value of 26.5 ml/min/kg can be calculated for the blood flow to the liver. The value of the biliary clearance of methotrexate divided by the liver blood flow gives an extraction ratio of 0.53. Therefore, the efficiency of removal of methotrexate from the blood by the liver is 53%. At high blood methotrexate concentrations, where saturation of the biliary secretion mechanism is approached, the extraction efficiency decreases.

The effect of probenecid on the biliary secretion of methotrexate was significant. Figure 3 is a plot of the reciprocal of the biliary clearance of methotrexate as a function of the plasma concentration of probenecid. With the exception of the point on the reciprocal clearance axis, each point represents the average of steady-state data obtained from individual rats. The value for the reciprocal of clearance when the probenecid concentration was zero is the average of four different studies. This value was weighted (times four) for purposes of fitting the data with a least-squares program.

As discussed previously, to utilize this simplified treatment of the data, the blood concentration of methotrexate in all rats was maintained at a level (<1 μg/ml) substantially lower than the value of K_t (30 μg/ml). The value of K_i obtained from this plot was 22.7 μg/ml or 8.1×10^{-5} M. The data were fit to Eq. 8 using a least-squares fitting program. The correlation coefficient was 0.95.

Qualitatively, it can be seen from Fig. 3 that the biliary secretion of methotrexate is sensitive to inhibition by probenecid. A plasma probenecid concentration of 50 μg/ml reduced the biliary clearance of methotrexate to about 4.4 ml/min/kg. This rate represents a significant reduction in the efficiency of the biliary extraction of methotrexate from the blood. At this low plasma probenecid concentration, the extraction efficiency was 17% or a reduction of 70%.

The value of the Y-intercept in Fig. 3 is equal to the reciprocal of biliary clearance in the absence of an inhibitor and should be equal to K_t/T_{max}. When using the values of 12 mg/hr and 32 mg/liter for T_{max} and K_t, respectively, a value for K_t/T_{max} of 0.158 min/ml was calculated. The actual intercept was 0.14 min/ml, in close agreement with the calculated value.

CONCLUSIONS

The biliary secretion of methotrexate in the rat is mediated by an active transport process which can be characterized by Michaelis–Menten kinetics, and this process is inhibited by the coadministration of probenecid. Whether accumulation of methotrexate in the intestinal lumen due to biliary secretion is a contributing factor to GI toxicity of methotrexate and whether the inhibition of this secretory process can be utilized to design a safer therapeutic dosage regimen remain to be determined.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 12, 1975, from the *School of Pharmacy, University of California Medical Center, San Francisco, CA 94143*

Accepted for publication November 21, 1975.

Supported in part by Public Health Service Training Grant GM-00728-13.

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GLC Determination of Nylidrin in Human Urine Samples

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Abstract □ A method for the detection of nanogram quantities of nylidrin in human urine is described. The method involves β-glucuronidase hydrolysis, extraction with chloroform, derivatization by silylation, and GLC determination. The suitability of the method was tested by analysis of urine samples of subjects after oral ingestion of nylidrin hydrochloride.

Keyphrases □ Nylidrin—GLC analysis, urine □ GLC—analysis, nylidrin in urine □ Vasodilators, peripheral—nylidrin, GLC analysis in urine

Nylidrin hydrochloride[1], 1-(*p*-hydroxyphenyl)-2-(1′-methyl-3′-phenylpropylamino)-1-propanol hydrochloride, is a sympathomimetic agent that acts predominantly by β-adrenergic receptor stimulation (1–3). It is a vasodilator used in the treatment of some peripheral vascular disorders (4, 5). The recommended human oral dose of nylidrin hydrochloride is 3–12 mg three or four times a day.

Two colorimetric analytical methods for measurement of nylidrin have been reported (6, 7), but they do not provide specificity or sufficient sensitivity for the determination of nylidrin in biological fluids.

Walle and Ehrsson (8, 9) developed a GC procedure for various alcoholic and amino compounds, using heptafluorobutyric anhydride and trimethylamine to prepare suitable derivatives for electron-capture detection. However, application of their method in this laboratory for the derivatization of nylidrin was only partially successful, since two peaks were observed in the chromatogram. Mass spectral analysis revealed that the major peak was the triheptafluorobutyryl derivative and the minor peak was the diheptafluorobutyryl derivative of nylidrin. Whether the diheptafluorobutyryl derivative was due to incomplete reaction of nylidrin with heptafluorobutyric anhydride as a result of steric hindrance at the secondary amino group of nylidrin or due to reverse hydrolysis of the triheptafluorobutyryl derivative is not clear at this time. An alternative approach was taken to provide a suitable method of determining urinary nylidrin in human subjects.

Preliminary studies performed on dogs in this laboratory showed that nylidrin is excreted in the urine as the free base and its glucuronide. The present method was designed to measure the total amount of nylidrin in urine after the urine was subjected to β-glucuronidase hydrolysis.

EXPERIMENTAL

Reagents—An aqueous standard stock solution was prepared by dissolving 5.00 mg of nylidrin hydrochloride[2] (equivalent to 89%

[1] Nylidrin hydrochloride is marketed by the USV Pharmaceutical Corp. under the trade name of Arlidin as 6- and 12-mg tablets. Subjects in this study received the 6-mg tablet orally.

[2] Nylidrin hydrochloride and nylidrin base were prepared in the Organic Chemistry Laboratories, USV Pharmaceutical Corp., Tuckahoe, NY 10707