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Pharmacokinetics of Iodoxamic Acid in Rhesus Monkey: Biliary Excretion, Plasma Protein Binding, and Enterohepatic Circulation

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Abstract □ The previously reported steady-state method allowed estimation of the capacity-limited pharmacokinetics of the cholangiographic agent, iodipamide. To circumvent the long time period required to establish each steady-state level, a dynamic method was applied to the study of the rate processes involved in the hepatic uptake and biliary excretion of a new cholangiographic agent, iodoxamic acid, in rhesus monkeys. The dynamic method has the advantage that the pharmacokinetic parameters involved in capacity-limited hepatic uptake or biliary excretion can be obtained from a single infusion experiment. The V_{max} was 1.03 ± 0.25 μmoles/kg/min (mean ± *SD*); K_m varied from animal to animal and ranged from 1.5 to 16.4 μ*M*. Protein binding was estimated using equilibrium dialysis. The Freundlich isotherm yielded a linear plot when the natural logarithm of unbound iodoxamic acid concentration in plasma was plotted against the natural logarithm of its blood concentration. The plasma protein binding data also could be fitted to the Langmuir isotherm, presuming two independent classes of binding.

Keyphrases □ Iodoxamic acid—biliary excretion, plasma protein binding, and enterohepatic circulation in rhesus monkeys □ Excretion, biliary—iodoxamic acid in rhesus monkeys □ Binding, plasma protein—iodoxamic acid in rhesus monkeys □ Enterohepatic circulation—iodoxamic acid in rhesus monkeys □ Pharmacokinetics—iodoxamic acid in rhesus monkeys □ Radiopaque media—iodoxamic acid, biliary excretion, plasma protein binding, and enterohepatic circulation in rhesus monkeys

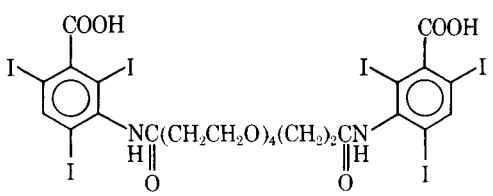
Cholecysto-cholangiographic agents bind significantly to plasma proteins (1–3), and this binding influences their biliary excretion. The role of serum albumin in the hepatic excretion of iodipamide was studied (4), and the binding of iodipamide to albumin retarded iodipamide transfer from plasma to the bile, probably because of competition between albumin and the anion binding protein of the liver.

Previous studies (5, 6) with a steady-state infusion method demonstrated that iodipamide is highly bound to plasma protein and has low blood clearance. Therefore, the unbound fraction of iodipamide is probably the major determinant in its renal and hepatic elimination (7). Although the steady-state method allows extensive analysis of the pharmacokinetics of iodipamide (5, 6), it has the disadvantages that: (*a*) a lengthy time is required to establish each steady-state level; (*b*) the time it takes to reach the steady state increases as one approaches saturation; and (*c*) a series of steady states is required in different experiments in the same animal, but the physiological status of an animal might change between experiments.

To circumvent some of these problems, a dynamic method was used to study the rate processes involved in the biliary excretion and hepatic uptake of a new cholecysto-cholangiographic agent, iodoxamic acid[1] (I), in rhesus monkeys. This method has the advantage that the pharmacokinetic parameters involved in the capacity-limited hepatic uptake or biliary excretion can be obtained from a single experiment.

EXPERIMENTAL

Studies were performed on three healthy rhesus monkeys (one male and two females), 4.5–9.1 kg, restrained in plastic chairs. They were



I

[1] Cholovue 40, 3% injection of the meglumine salt, E. R. Squibb and Sons, Inc., Princeton, N.J.

0022-3549/78/0700/0930$01.00/0

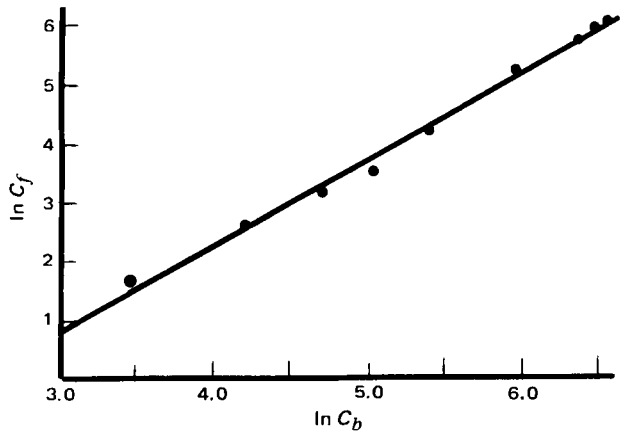


Figure 1—*Plasma protein binding of iodoxamic acid in Monkey A shown as the linear relationship between the natural logarithm of the unbound concentration (ln* C_f) versus *the natural logarithm of the blood concentration (ln* C_b) *of iodoxamic acid.*

prepared as previously described (8) with a T-tube in the common bile duct for bile collection and another T-tube in the duodenum for the recycling of bile salts. All three monkeys were cholecystectomized.

^{125}I-Labeled iodoxamic acid[2] was used in the plasma protein binding study. The ^{125}I-radiopurity was monitored by TLC and was more than 99% pure. Sodium taurocholate[3] was infused into the duodenum 1 hr prior to and throughout each infusion experiment at 5.56 μmoles/kg/min to replace the loss of bile salts from the collection of bile samples (9).

The iodoxamic acid concentrations in the blood, bile, and urine samples were determined by monitoring the iodine content using fluorescent excitation analysis (10), and the ^{125}I-radioactivity was measured using a scintillation spectrometer[4].

Plasma Protein Binding Studies—Appropriate amounts of ^{125}I-iodoxamic acid were added to blood samples obtained from each monkey to achieve the blood iodoxamic acid concentrations, C_b, of 42–912 μ*M*. After equilibration, the plasma was separated from blood cells by centrifugation. Each plasma sample was dialyzed against Krebs–Ringer buffer at 37° for 5 hr using an equilibrium dialysis technique[5]. The equilibrium ^{125}I-concentrations in the buffer and the plasma were measured to obtain the unbound and plasma concentrations of iodoxamic acid. The bound concentration of iodoxamic acid in the plasma was calculated by subtracting the unbound concentration from the plasma concentration of iodoxamic acid.

Lang and Lasser (1) showed that iodipamide binds to albumin but not to globulins in the plasma. Because iodoxamic acid and iodipamide have similar chemical properties, iodoxamic acid was assumed to bind only to albumin. Therefore, the moles of drug bound per mole of plasma protein, r, was calculated by dividing the bound concentration of iodoxamic acid by the albumin concentration in the plasma estimated by electrophoresis (11). A Scatchard plot was constructed by plotting r/C_f *versus* r, where C_f is the unbound concentration of iodoxamic acid in the plasma.

Steady-State Infusion Studies—After loading doses of 25.6 and 77.0 μmoles/kg, meglumine iodoxamate was infused into a monkey at the rates of 0.65 and 2.59 μmoles/kg/min for 120 and 160 min, respectively. In a separate experiment, meglumine iodoxamate was infused at the rates of 1.30 and 1.95 μmoles/kg/min for 160 min each into the same monkey. Blood and bile samples were collected every 20 min throughout the experiment.

Meglumine iodoxamate also was infused (after loading doses of 25.6, 124.9, and 85.0 μmoles/kg) into another monkey at the rates of 0.7, 4.17, and 6.53 μmoles/kg/min, respectively, for 2 hr each. In a separate experiment, meglumine iodoxamate was infused into the same monkey at the rate of 6.53 μmoles/kg/min for 140 min. Throughout each experiment, bile samples were collected at 20- or 30-min intervals. The total volume was recorded, and a 1.0-ml aliquot was used for determinations of biliary iodoxamic acid concentrations; the rest was infused back into the duodenum. In this monkey, no sodium taurocholate was infused during the infusion experiments.

Table I—Freundlich Equation[a] Parameters for Iodoxamic Acid in Monkeys

Monkey	m	A	r^2
A	1.479 (2.9)[b]	−3.671 (6.3)	0.993
B	1.402 (1.6)	−1.370 (2.8)	0.999
C	1.473 (3.0)	−4.366 (5.7)	0.996

[a] $C_f = AC_b^m$. [b] Coefficient of variation in percent.

Dynamic Infusion Studies—In all three monkeys, meglumine iodoxamate was infused at rates varying from 1.20 to 2.60 μmoles/kg/min for 120 min (except in one experiment in one monkey where infusion was for 95 min). To estimate the lag time between the appearance of the drug in the bile canaliculi and at the sampling site, the bile samples were collected every 2 min during the first 30 min. Thereafter, bile samples were collected at 5–15-min intervals until 3–4 hr after the infusion.

Enterohepatic Circulation Studies—A 150-μmole/kg bolus dose of iodoxamic acid was administered into the duodenum of each of two monkeys, and the bile samples were collected every 2 hr for 6 hr.

Pooled bile containing iodoxamic acid collected from infusion experiments was administered as a 75-μmole/kg bolus dose into the duodenum of another monkey. Bile samples were collected every 80–~90 min over 715 min. Sodium taurocholate was infused into the duodenum as previously noted.

Biotransformation Studies—The blood, bile, and urine samples were analyzed for iodoxamic acid and possible metabolites by the following methods.

1. Due to the high iodoxamic acid concentration in the bile and urine samples, these samples were spotted directly on a TLC plate[6] containing a fluorescent pigment[7] and were compared with standard iodoxamic acid and blank bile and urine. The TLC plate was developed with 2-propanol–28% ammonium hydroxide (4:1 v/v) and air dried, and then the spots were visualized under short wavelength UV light to locate iodoxamic acid and possible metabolites by the use of the UV absorption characteristics of the aromatic ring.
2. Blood samples were acidified with 0.1 *N* HCl and extracted with ether. The ether extract was evaporated to dryness with purified nitrogen at room temperature. The residue was redissolved in 20 μl of methanol and spotted on a TLC plate as described for Method 1.

RESULTS

Plasma Protein Binding Studies—Iodoxamic acid was highly bound to plasma protein. As the blood iodoxamic acid concentration increased from 42 to 912 μ*M*, the fraction unbound varied from 6.1 to 41.2%. By using the Freundlich isotherm approach (12), a linear relationship was found when ln C_f was plotted against ln C_b. The data for Monkey A are shown in Fig. 1, and the composite for all monkeys is given in Table I.

When the data were plotted according to the Scatchard equation, a concave descending curve was obtained (Fig. 2), indicating the existence of more than one class of binding sites for iodoxamic acid. By presuming two classes of binding sites that behave independently, r was fitted to C_f according to the Langmuir isotherm equation using the FITFUN program of the PROPHET computer system[8]:

$$r = \frac{n_1 k_1 C_f}{1 + k_1 C_f} + \frac{n_2 k_2 C_f}{1 + k_2 C_f} \qquad \text{(Eq. 1)}$$

where n_1 and n_2 are the number of binding sites for first and second class binding, respectively, and k_1 and k_2 are the association constants of each class of binding. The results are shown in Fig. 3 for the relationship between r and C_f. The computer-estimated binding parameters of the three monkeys are given in Table II. After these parameters were obtained, a theoretical curve was drawn for the relationship of r/C_f *versus* r in the Scatchard plot (Fig. 2), which was resolved into two linear components representing each class of binding.

Steady-State Infusion Studies—The results from steady-state infusion studies are shown in Table III. The data from two independent experiments in each monkey are listed. The biliary excretion rate appears

[2] ^{125}I-meglumine iodoxamate, E. R. Squibb and Sons.
[3] ICN Pharmaceuticals Inc., Cleveland Ohio.
[4] Auto-γ-scintillation spectrometer, model 1185, Searle Analytic Inc., Des Plaines, Ill.
[5] Dianorm, a multiple-equilibrium dialysis system, Innovativ-Medizin AG, Ch-Esslingen, Switzerland.
[6] Silica gel F-254.
[7] EM reagent, West Germany.
[8] The PROPHET system is a specialized computer resource developed by the Chemical/Biological Information Handling Program of the National Institutes of Health. A detailed description of the system features appears in *Proc. Natl. Comput. Conf. Exposition*, **43**, 457 (1974).

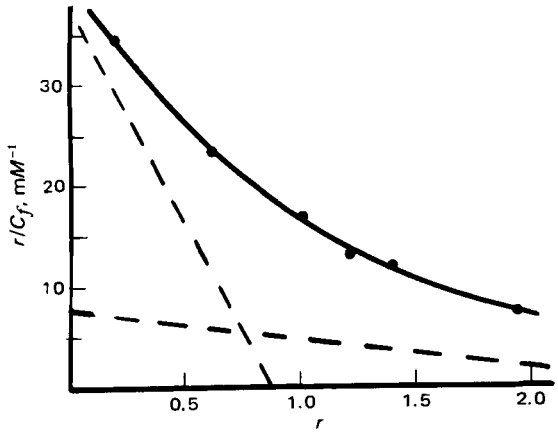


Figure 2—*Scatchard plot of the binding of iodoxamic acid to plasma albumin in Monkey B. Key: ●, data points; —, computer-fitted line presuming two independent binding classes; and – –, computer-resolved linear components representing each class of binding.*

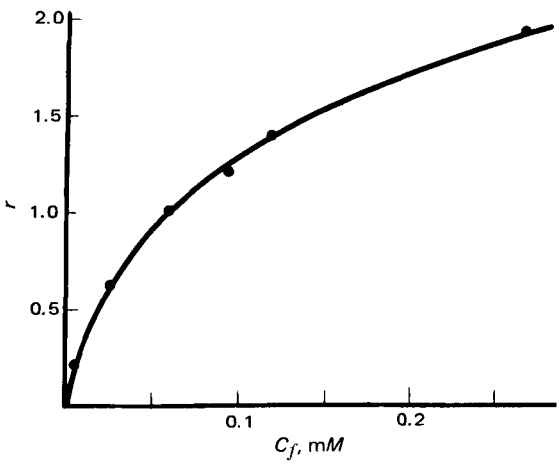


Figure 3—*Relationship between* r *and* C_f *in Monkey C. Key: ●, data points; and —, computer-fitted line presuming two classes of binding according to Eq. 1.*

to increase approximately in proportion to the infusion rates when the low value is compared to the next value listed. However, when intermediate and high infusion rates were utilized, the biliary excretion rates in Monkey C were 1.20, 1.60, and 1.40 μmoles/kg/min; in Monkey D, they were 1.72, 1.12, and 1.44 μmoles/kg/min. Although the data showed variation in biliary excretion, they support the contention that the biliary excretion process or a rate-limiting step prior to excretion appears to be saturable.

Dynamic Infusion Studies—A lag time existed for the bile to flow from the canaliculi to the sampling site, and it was estimated by plotting the cumulative amount of iodoxamic acid excreted in the bile in the first 30 min against time (Fig. 4). The lag time was estimated by extrapolating the initial linear portion to the abscissa; it varied from 5 to ~7 min.

The biliary excretion rate data were plotted against the midpoint of the collection time after correcting for the lag time (Fig. 5).

The blood iodoxamic acid concentration was plotted against time on the same graphs (Fig. 5). Even though two different doses (2.2 and 2.6 μmoles/kg/min) were infused, the maximum biliary excretion rate did not increase proportionately but reached essentially the same plateau value. This result is an indication of the saturation of the biliary excretory mechanism or, alternatively, a hepatic uptake mechanism.

The biliary excretion rate data in the postinfusion period were fitted to the Michaelis–Menten equation against the corresponding unbound concentration of iodoxamic acid at each time using the HYPERBOLIC program of the PROPHET computer system[8] (Fig. 6):

$$RB = \frac{V_m C_f}{K_m + C_f} \qquad \text{(Eq. 2)}$$

where RB is the biliary excretion rate at a given time and V_m and K_m are the maximal biliary excretion rate and the Michaelis–Menten constant, respectively. The parameter estimates are given in Table IV.

Enterohepatic Circulation Studies—The percentages of iodoxamic acid recovered in the bile over 6 hr after an intraduodenal bolus dose of 150 μmoles/kg of meglumine iodoxamate solution were 0.05 and 0.20% in two monkeys. In another monkey, 11.9 hr after a bolus dose of bile containing 75 μmoles/kg, the percent recovery was 1.1%.

Biotransformation Studies—With TLC, iodoxamic acid was found to exist in the blood as the unchanged species. No metabolites were found in the bile. In the urine, two additional spots in very small quantity were found on the thin-layer chromatogram in addition to unchanged iodoxamic acid. These two metabolites had R_f values of 0.48 and 0.23, whereas iodoxamic acid had R_f 0.35. Due to the minute quantity obtained, no attempts were made to identify these metabolites.

DISCUSSION

The goal of the present plasma protein binding studies was to develop a method of estimating the C_f values at various blood or plasma concentrations of iodoxamic acid. The Freundlich empirical relationship that relates the bound and unbound drug concentrations fit the data when log C_f was plotted *versus* log C_b, where C_b is the total blood iodoxamic acid concentration. Normally, one would plot the plasma concentration of the bound drug. However, since the assay results in the measurement of C_b, it is convenient to use this representation so that a direct conversion from C_b to C_f can be made. A virtually identical plot was obtained when log C_f was plotted *versus* log C_p, where C_p is the total plasma iodoxamic acid concentration.

Application of the Freundlich isotherm to the analysis of plasma protein binding data (Table I) shows a relatively small coefficient of variation of the parameter estimates. When analyzed using the Langmuir isotherm, a reasonably good fit was obtained presuming two classes of binding sites and the drug bound solely to plasma albumin (Table II). Two separate studies of iodoxamic acid binding were used with Monkey A, which might have contributed to the observed large coefficients of variation. The data from Monkey B are plotted in Fig. 3 according to the Langmuir equation (Eq. 1) and in Fig. 2 according to the Scatchard relationship. The dashed lines represent the two classes of binding sites.

Rosati *et al.* (13) studied the plasma protein binding of three radiopaques and showed that iodoxamic acid binds slightly less than iodipamide to dog plasma protein. In human serum albumin, the association constant of iodoxamic acid was one-tenth that of iodipamide. The data (13) indicate that iodoxamic acid binds to at least two classes of binding sites in plasma albumin. However, no calculations were reported.

Although the whole blood clearance of drugs varies with dose in capacity-limited processes, the whole blood clearance of iodoxamic acid in the monkeys was calculated by dividing the dose by the area under blood concentrations with time curves in the infusion experiments. The blood clearance of iodoxamic acid was estimated to vary from 1.9 to 14.9 ml/kg/min. These values were much lower than the average hepatic blood flow: 44–50 ml/kg/min (14). Since iodoxamic acid has a high degree of plasma protein binding and low blood clearance, the unbound concentration of iodoxamic acid probably is the major determinant in its hepatic elimination. Therefore, the unbound concentration rather than the blood or plasma concentration of iodoxamic acid was used in the computer fitting of biliary excretion rate data (Eq. 2).

This situation is analogous to comparing the urinary excretion rate to the unbound plasma drug concentration. Even if the blood drug concentration–time profile indicated multiple compartments, the relationship in Eq. 2 nevertheless is applicable and is independent of the distribution into peripheral tissues other than the liver and kidneys.

Rosati *et al.* (13) reported that iodoxamic acid displayed a maximum biliary transport (V_m) value twice that of iodipamide in anesthetized dogs

Table II—Langmuir Equation[a] Parameters for Iodoxamic Acid in Monkeys

Monkey	n_1	k_1, M^{-1}	n_2	k_2, M^{-1}	r^2
A	0.56 (64)[b]	4×10^4 (118)	29.9 (1253)	7.7×10^1 (1351)	0.9850
B	0.46 (34)	5.3×10^4 (31)	4.2 (7)	3.2×10^3 (24)	0.9999
C	0.87 (68)	3.7×10^4 (67)	2.7 (17)	29×10^3 (100)	0.9995

[a] See Eq. 1. [b] Coefficient of variation in percent.

Table III—Relationships among Infusion Rate, R^0, Steady-State Blood Concentration, C_b, Bile Flow, Bile Concentration, and Biliary Excretion Rate, RB, of Iodoxamic Acid

Study	R^0, μmoles/kg/min	C_b[a], μM	Bile Flow[b], ml/min	Bile Concentration[b], mM	RB[b], μmoles/kg/min
Monkey C					
Study 1	0.65	37	0.19	15.1	0.65
	2.60	677[c]	0.27	23.4	1.40
Study 2	1.30	64	0.28	19.3	1.20
	1.95	286	0.32	22.6	1.60
Monkey D[d]					
Study 1	0.71	—[e]	0.14	10.8	0.34
	4.17	—[e]	0.41	18.9	1.72
	6.53	—[e]	0.25	20.1	1.12
Study 2	6.53	—[e]	0.33	19.6	1.44

[a] Mean of at least three determinations. [b] Mean of at least four determinations. [c] The blood concentration in this instance was still increasing at about 10%/hr; however, the biliary excretion rate did not reflect the increase and appeared to be at a steady state. [d] Monkey D died after these two studies. [e] No blood samples were obtained due to dysfunction of the arterial catheter.

(0.91 *versus* 0.48 μmole/kg/min). Evil and Benness (15) also reported that the biliary output of iodoxamic acid (0.74 μmole/kg/min) was more than 50% higher than the iodipamide output (0.46 μmole/kg/min). However, Berk *et al.* (16), using a steady-state infusion method, showed that the biliary transport maximum of 1.60 μmoles/kg/min for iodoxamic acid was only 25% greater than that for iodipamide (1.27 μmoles/kg/min).

In the Rosati *et al.* (13) study, anesthetized dogs were used with no replacement of bile salts, which probably explains why the maximal excretion rates for both iodoxamic acid and iodipamide were lower than those of the Berk *et al.* (16) studies in which unanesthetized dogs with bile salts replacement were used. The Evil and Benness (15) studies also used unanesthetized dogs with bile salts replacement. However, it was not certain whether saturation of biliary excretion was ever reached. In all cases, however, it seems that iodoxamic acid had a higher biliary excretion rate than iodipamide at the same dose levels.

Similar to the previous experiments with dogs (13, 15, 16), the present steady-state infusion experiments in monkeys also demonstrated that the biliary excretion of iodoxamic acid is saturable (Table III). In Monkey C, the infusion of 1.95 μmoles/kg/min resulted in the biliary excretion rate of 1.60 μmoles/kg/min, whereas the infusion of 2.59 μmoles/kg/min resulted in the biliary excretion rate of 1.40 μmoles/kg/min. The fact that slightly lower biliary excretion was obtained following higher infusion rates suggests that biliary excretion was saturated. The reason that the biliary excretion rate following the higher dose was slightly lower than that following the lower dose is unclear. It could be due to day-to-day variations of the animal or the toxicity following the higher dose. Sperber and Sperber (17) showed that the biliary excretion of iodipamide was depressed following excessively high doses in rats.

Present dynamic infusion studies also demonstrated the saturation of biliary excretion of iodoxamic acid (Table IV and Fig. 5). The computer-estimated maximal biliary excretion rate was 0.90 μmole/kg/min (Fig. 6) for Monkey A, a value very close to the actual maximal biliary excretion rate of 1.0 μmole/kg/min (Fig. 5) obtained experimentally. The Michaelis–Menten parameters remained relatively constant following different doses of each animal, *i.e.*, independent of dose (Table IV). The V_m value of 0.66 μmole/kg/min for Monkey A following an excessively high dose (2.60 μmoles/kg/min) was somehow lower than the value of 0.90 μmole/kg/min following an intermediate dose (2.20 μmoles/kg/min) in the same animal. Again, this result could be due to the toxicity following the high dose. The maximum doses in other experiments were below 2.0 μmoles/kg/min. The dynamic method has the important advantage that the pharmacokinetic parameters involved in capacity-limited hepatic uptake or biliary excretion can be obtained from a single experiment.

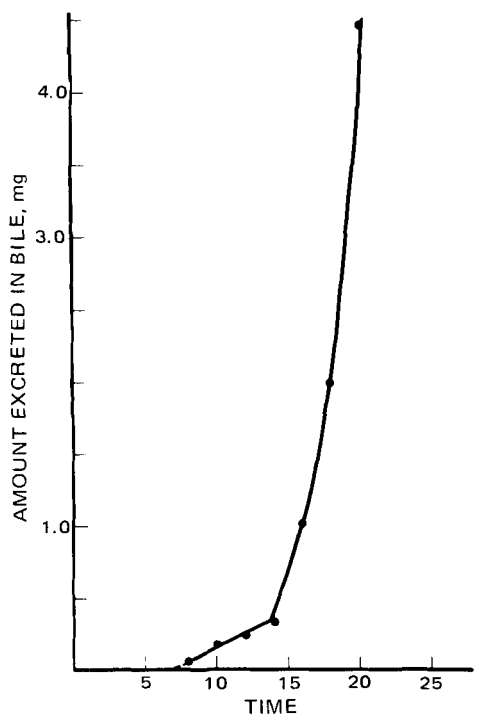


Figure 4—*Cumulative amount of iodoxamic acid excreted in the bile during the initial 20 min in one infusion study in Monkey A.*

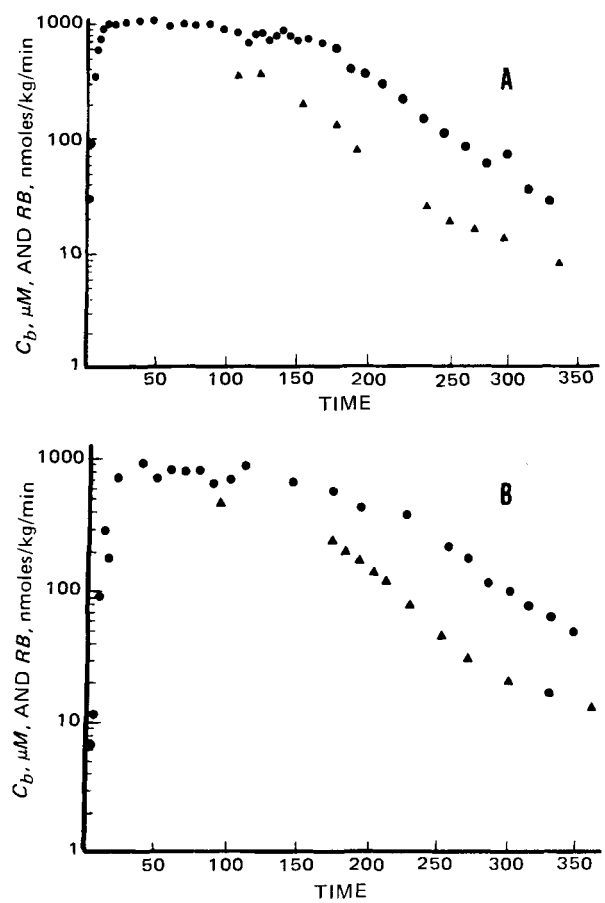


Figure 5—*Semilog plot of the blood concentration (▲) and biliary excretion rate (●) of iodoxamic acid with time following the infusion of 2.20 (A) and 2.60 (B) μmoles/kg/min into Monkey A.*

Table IV—Michaelis-Menten Parameters [a] of Biliary Excretion of Iodoxamic Acid in Monkeys

Parameter	Monkey A	Monkey B	Monkey C
V_m, μmoles/kg/min	0.90 (15) [b]	1.01 (7)	1.28 (20)
	0.66 (6.8)	1.00 (25.5)	1.30 (21.6)
K_m, μM	16.4 (55)	3.5 (30)	1.5 (64)
	11.1 (23.4)	3.10 (54.4)	1.79 (43.9)

[a] Parameter values from two independent studies following different doses in each monkey are listed together. [b] Coefficient of variation in percent.

The overall average maximal biliary excretion rate (V_m) obtained (1.03 μmoles/kg/min) for iodoxamic acid in rhesus monkeys was only slightly (8.4%) higher than the 0.95 μmole/kg/min reported by Wittenberg *et al.* (18) for iodipamide in the same animal species. However, in the cited report (18), there was one study in which an exceptionally high biliary excretion rate was observed (almost three times higher than the other five studies). If this particular study is excluded from the results, the maximal biliary excretion rate becomes 0.64 μmole/kg/min, which is about 61% lower than that of iodoxamic acid in the present studies. In both cases, more iodoxamic acid was excreted in rhesus monkeys than was obtained with iodipamide.

The percentages of iodoxamic acid excreted in the bile following introduction of a meglumine iodoxamate solution into the duodenum indicates that the enterohepatic circulation of iodoxamic acid is insignificant. When the dose of 75 μmoles/kg was given to Monkey C (as the bile containing iodoxamic acid), the percentage of recovery over 11.9 hr was 1.1%. This result is not surprising because of the large molecular weight and polar characteristics of the molecule. Whitney and Cambell (19) reported that the interruption of enterohepatic circulation has no effect on iodipamide excretion in the bile of the rhesus monkey.

TLC revealed that iodoxamic acid existed in the blood and bile as the unchanged species. In the urine, two metabolites were found, one with higher and one with lower polarity. Qualitatively, this finding is consistent with the report of Mutzel *et al.* (20) concerning humans. However, the two metabolites constituted about half of the iodinated compounds in the Mutzel *et al.* (20) studies whereas only very small amounts of these metabolites were found in the urine. Therefore, the metabolism of iodoxamic acid in monkeys is probably closer to that in humans than to that in dogs.

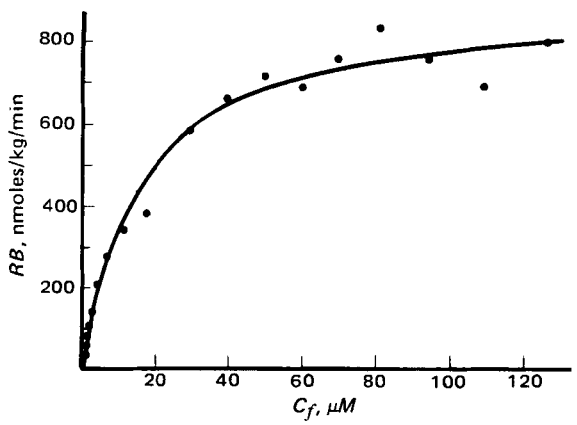


Figure 6—*Relationship between the biliary excretion rate and the unbound concentration of iodoxamic acid in the plasma during the postinfusion period following an infusion of 2.20 μmoles/kg/min into Monkey A. Key: ●, data points; and —, computer-fitted line according to Eq. 2.*

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