standard curves in plasma and water, respectively, was 0.94 ± 0.03 with a coefficient of variation of 3%. Interassay variability of the slope and y-intercept of the five standard curves generated during the bioavailability study had coefficients of variation of 2.7 and 8%, respectively. A typical calibration regression line is $y = 0.229x + 0.0296$ and is linear over a 1–32-μg/ml concentration range. The good linearity between the peak area and acetaminophen concentration in plasma is indicated by the correlation coefficient of $r > 0.999$.

Accuracy—Table I shows the actual amounts with which 1-ml blank plasma samples were spiked and the amounts found when the plasma was analyzed according to the described method. The experiment was performed in duplicate on each of 5 days. The percent error for each unknown sample was calculated according to (17):

$$\text{percent error} = \frac{\text{amount added} - \text{amount found}}{\text{amount added}} \times 100 \quad \text{(Eq.1)}$$

The average percent error was 2.2%. In no case did it exceed 7.0%.

Reliability—A comparison was made of acetaminophen levels determined by the described method with levels determined simultaneously by a previously validated extraction procedure. Table II shows the acetaminophen levels found with both methods for 16 subjects who had been studied on a three-way crossover with samples taken at 10, 20, 40, and 60 min postdosing. The comparison gave a correlation coefficient of 0.993. In addition, the application of a paired Student's t test and the Wilcoxon-Mann-Whitney rank-sum test to the two sets of data indicated at the 95% confidence level that there was no significant difference between results obtained by the two methods.

Summary—The proposed method is simple and sensitive for the rapid determination of nonconjugated acetaminophen in plasma or blood at levels likely to be encountererd after a usual 650- or 1000-mg total dose. Plasma proteins are denatured by the addition of a saturated $Ba(OH)_2$ solution and then precipitated with 5% $ZnSO_4$ solution. The resulting clear supernatant is analyzed by HPLC. The reproducibility, sensitivity, and selectivity of the method make the use of an internal standard unnecessary.

Since the major biotransformation of acetaminophen in humans is direct conjugation with sulfate and glucuronic acid to form the sulfate and glucuronic metabolites, these polar metabolites do not interfere in the assay as they are eluted along with the solvent. The accuracy and reliability of this method have been proven by comparison of results obtained with an established extraction procedure in over 400 comparative determinations.

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Pharmacokinetics of Bretylium in Dogs and the Effect of Hemoperfusion on Elimination

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Abstract □ The pharmacokinetics of bretylium in dogs and the efficacy of hemoperfusion with a resin column in its removal from the body following intravenous administration of bretylium tosylate were investigated. Five mongrel dogs weighing 18–26 kg were given a bolus dose of 15 mg/kg. Serial blood samples were taken for 24 hr. Hemoperfusion, through a resin column, was then initiated and continued for 4 hr under pentobarbital anesthesia. During hemoperfusion, arterial and venous blood samples were collected several times; venous blood samples were then withdrawn for an additional 8 hr. Urine was collected from each dog in three portions for up to 48–54 hr. Pharmacokinetics of bretylium in dogs could be characterized by a two-compartment open model with a distribution half-life of 7 min and biological half-life of 15.9 ± 1.9 hr. Plasma levels declined rapidly from ~20 μg/ml at 6 min to <2 μg/ml within 1 hr. The ratio of intercompartmental rate constants, k_{12}/k_{21}, was 16.7, and the volume of the central compartment and apparent volume of distribution were 0.245 and 5.22 liter/kg, respectively, indicating a wide distribution of bretylium into the tissues. Plasma dialysis clearance averaged 29.7 ml/min, which is 30% of the total body clearance (98.8 ml/min). These data suggest that resin hemoperfusion may not be useful in the treatment of bretylium intoxication.

Keyphrases □ Pharmacokinetics—bretylium and the effect of hemoperfusion on elimination, distribution, dogs □ Bretylium—pharmacokinetics and the effect of hemoperfusion on elimination, distribution, dogs □ Distribution—pharmacokinetics of bretylium and the effect of hemoperfusion on elimination, dogs

Bretylium tosylate is a quaternary ammonium compound used in the treatment of ventricular tachycardia or ventricular fibrillation. It has been demonstrated that bretylium increases the action potential duration along the entire left ventricular conducting system (1).

In humans and rats, bretylium is primarily eliminated unchanged *via* the kidneys. No metabolites have been identified following administration of bretylium in humans (2–5). A half-life of 9.75 ± 4.19 (*SD*) hr in eight patients aged 48.4 ± 10.8 years has been reported (6). The longest

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Table I—Pharmacokinetic Parameters of Bretylium in Dogs

Parameter	Preperfusion Data			Pre- and Postperfusion Data		Mean	*SD*
	217	224	256	289	299		
α (hr^{-1})	7.61	5.11	5.30	6.93	6.76	6.34	1.09
$t_{1/2}$, α (min)	5.46	8.14	7.85	6.00	6.15	6.72	1.20
β (hr^{-1})	0.043	0.051	0.044	0.045	0.037	0.044	0.005
$t_{1/2}$, β (hr)	16.20	13.50	15.90	15.40	18.70	15.90	1.87
k_{12} (hr^{-1})	6.02	3.95	4.37	5.34	5.76	5.09	0.894
k_{21} (hr^{-1})	0.233	0.279	0.405	0.221	0.382	0.304	0.085
k_{10} (hr^{-1})	1.40	0.942	0.569	1.42	0.655	0.997	0.401
V_c (liter/kg)	0.189	0.273	0.294	0.204	0.265	0.245	0.046
$V_{d,\beta}$ (liter/kg)	6.18	5.01	3.84	6.40	4.68	5.22	1.07
TBC (liter/hr/kg)	0.265	0.258	0.167	0.289	0.174	0.231	0.056
Percent Dose in Urine							
Collection period, hours	54.0	48.0	48.0	56.0	54.0		
Recovery, %	102.2	72.6	73.6	72.0	74.1	78.9	13.1

half-lives were found in the oldest patients, which would be consistent with the reduction in the renal function known to occur with aging. More recently, an average half-life of 8.1 hr for normal subjects and 16.1 and 31.5 hr for two patients with creatinine clearance of 21.0 and 1.0 ml/min, respectively, has been reported (3, 4). As renal function declines, dosage alteration may be required to avoid toxicity. A half-life of 13.6 hr has been reported (5) for 10 normal volunteers given 5 mg/kg iv of bretylium.

The purpose of this investigation was to study the efficacy of hemoperfusion with a clinically used resin[1] column in the removal of bretylium from the body following intravenous administration of bretylium tosylate to dogs. This resin[1] is a macroreticular copolymer of styrene and divinyl benzene with a surface area of 750 m^2/g, a porosity of 51%, and an affinity for lipid-soluble materials. This column is used clinically to treat intoxication (7).

EXPERIMENTAL

Five mongrel dogs weighing 23.3 ± 3.1 (*SD*) kg were randomly selected. Each dog received a rapid intravenous bolus dose of 15 mg/kg bretylium tosylate[2]. Blood samples (3 ml each) were taken frequently during the first 2 hr for characterization of distribution phase and then every 2–4 hr for the next 14–18 hr and at 24 hr to characterize the elimination phase.

Twenty-four hours after administration of bretylium, the dogs were hemoperfused for 4 hr using clinically available columns[3] containing 312 ± 15 g (dry weight) of loosely packed resins[1]. Immediately prior to hemoperfusion, the animals were anesthetized with pentobarbital (30 mg/kg iv) and intubated with an endotracheal tube. A femoral artery and vein were cannulated, and 10,000 U of heparin was given. The extracorporeal circuit (~300 ml) was washed with 3 liters of normal saline containing 1000 U/liter of heparin and primed with the same solution. The primer was infused at the beginning of hemoperfusion. Blood was pumped through the circuit at a constant antigravity flow of ~112 ml/min as determined by timed collections of saline before and after each experiment.

During hemoperfusion samples of blood were withdrawn from the arterial (inlet) and venous (outlet) lines at 0.5, 1.0, 2.0, 3.0, and 4.0 hr following the start of hemoperfusion. At the conclusion of hemoperfusion, blood was returned to the dog by gravity. The artery and vein were then closed and blood sampled for an additional 8 hr. Immediately after the collection of blood samples, they were centrifuged and plasma was separated and frozen until assayed. The hematocrit of all blood samples taken during hemoperfusion was measured.

After the hemoperfusion, the column was eluted with methanol to remove all the adsorbed bretylium. The final volume was measured and an aliquot was stored for bretylium assay.

Quantitative urine samples were collected from each dog and divided into three time intervals: prehemoperfusion, during, and posthemoperfusion urine. Aliquots were frozen until assayed for bretylium.

Bretylium Assay—Plasma, urine, and methanol eluate of the hemoperfusion column were assayed for bretylium as described previously (8). The sensitivity of this procedure was not sufficient enough to determine the bretylium levels in the methanol eluate. Therefore, the same eluate was concentrated 10–50 times and assayed again.

Pharmacokinetic Analysis—The prehemoperfusion plasma concentration *versus* time profiles were fitted to a two-compartment open model using the NLIN procedure (9). Pharmacokinetic parameters were obtained for three of the five dogs (numbers 217, 224, and 256). For dogs 289 and 299, the best fit was obtained when the postperfusion plasma concentrations were used. Bretylium plasma dialysis clearance during hemoperfusion (Cl_d) was calculated as previously described (10):

$$Cl_d = Q_p(A - V)/A$$

where Q_p is plasma flow, and A and V are arterial and venous plasma concentrations of bretylium, respectively.

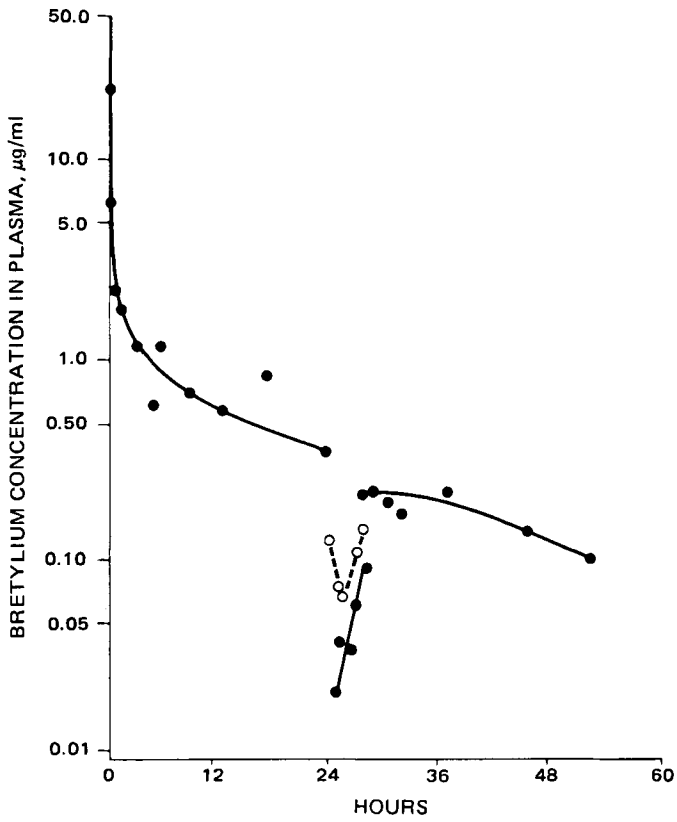

Figure 1—*Semilogarithmic plot of plasma concentrations of bretylium versus time following intravenous administration of bretylium tosylate, 15 mg/kg, in one dog (No. 217). Hemoperfusion was done 24 hr after administration of the drug. Key: (●) venous concentrations; (○) arterial concentrations; the solid line, through the first 24 hr, represents the least-squares computer fit.*

[1] Amberlite XAD-4, Extracorporeal Medical Specialties, Inc., King of Prussia, Pa.
[2] Bretylol, American Critical Care, McGaw Park, Ill.
[3] Extracorporeal Medical Specialties, Inc., King of Prussia, Pa.

Table II—Hemoperfusion Parameters in Dogs

Parameter	217	224	256	289	299	Mean	*SD*
Weight (kg)	24.9	26.1	18.1	24.0	23.6	23.3	3.1
Hematocrit	40.7	40.3	37.5	28.3	32.9	35.9	5.3
Blood flow (ml/min)	115.0	110.6	106.0	116.3	114.0	112.4	4.1
Plasma flow (ml/min)	68.2	66.0	66.3	83.4	76.5	72.1	7.63
Cl_d[a] (ml/min)	35.4	23.1	49.7	19.3	20.9	29.7	12.8
TBC[b] (ml/min)	110.2	112.4	87.4	115.8	68.4	98.8	20.4

[a] Plasma dialysis clearance during hemoperfusion. [b] Preperfusion total body clearance.

RESULTS AND DISCUSSION

The plasma concentration *versus* time plot, for a typical dog, is shown in Fig. 1. Immediately after the intravenous dose of 15 mg/kg, bretylium is distributed rapidly in the body. The plasma levels decreased from ~20 μg/ml at 6 min to <2 μg/ml within 1 hr. During the elimination phase, the levels declined more slowly with an average half-life of ~16 hr. In three dogs (217, 224, and 256), the preperfusion elimination phases were well defined with a half-life ranging from 13.5–16.2 hr. In dogs 289 and 299, the plasma concentrations could be best fitted to the model by using also the postperfusion data that were in line with the preperfusion levels.

Pharmacokinetic parameters, derived constants, and the means, are listed in Table I. The parameters were in good agreement in all dogs. As seen from Table I, bretylium is widely distributed in the body, with a volume of distribution of 5.22 liter/kg (±1.07) and a very small volume of central compartment of 0.245 ± 0.046 liter/kg. The average distribution half-life was 6.72 min. The intercompartmental rate constants, k_{12} and k_{21}, were 5.09 and 0.304 hr^{-1}, respectively. The extremely large volume of distribution and the very high ratio of k_{12}/k_{21} (16.7) signify strong affinity of bretylium to the tissues in the peripheral compartment. Earlier studies in dogs and rats (11, 12, and unpublished data) have shown an extremely high affinity of bretylium to the myocardium, with heart to plasma concentration ratio >20. The heart was found to be a part of the peripheral compartment in rats (12). The elimination rate constant, k_{10}, averaged 1.0 hr^{-1}, with a total body clearance (TBC) of 0.231 liter/hr/kg or 3.85 ml/min/kg.

The percentage of dose recovered in urine collected during the study period is also shown in Table I. An average of 79% of the dose was recovered unchanged in urine 48–54 hr following intravenous administration, and only an insignificant amount was cleared during hemoperfusion through the column. Available data on glomerular filtration rate in dogs (13) show that it ranges from 3.33–4.75 ml/min/kg. Total body clearance of bretylium in the dogs was 3.85 ml/min/kg and the renal clearance was ~3.0 ml/min/kg. These values indicate that bretylium might not be eliminated by an active mechanism in dogs. In humans and rats, bretylium is excreted extensively by active tubular secretion (4, 12).

A hemoperfusion 24 hr after the bretylium tosylate dose caused an immediate decrease (twofold–tenfold) in the arterial as well as venous plasma levels within 1 hr; this was followed by a gradual increase and reached a normal expected level by the completion of hemoperfusion in all animals (Fig. 1). Pertinent hemoperfusion parameters for each dog are given in Table II. Plasma dialysis clearance (Cl_d) averaged 29.7 ± 12.8 ml/min or 30% of the endogenous (preperfusion) TBC. The actual amount of bretylium removed from each dog *via* hemoperfusion and, consequently, recovery clearance (14) could not be calculated.

The rapid fall in the plasma concentration (central compartment), due to perfusion clearance, causes a shift in the equilibrium between the two compartments resulting in redistribution of bretylium from the peripheral compartment to the central compartment. When the rate of this redistribution exceeds the clearance from the central compartment, ~1 hr following the initiation of hemoperfusion, the plasma concentration rises (Fig. 1).

The efforts to determine the concentration of bretylium in the methanol eluate of the perfusion column did not succeed because the concentration was below the detection limit of 10 ng/ml. Concentrating the eluate, 10- to 50-fold, did not indicate a significant removal of bretylium by the column. Because of low bretylium concentrations and high background, the gas chromatogram did not provide any meaningful data. Even assuming a 10-ng/ml concentration of the eluate, the total amount removed would not be >10 μg total. Theoretically, the rate of removal of drug from plasma depends on the concentration in plasma. Because of the wide distribution in the tissues, *i.e.*, very large k_{12}/k_{21}, the plasma concentrations are very low and the rate of removal is very slow. These data are quite similar to those after digoxin in dogs and confirm that the rate-limiting step may be the clearance from tissues and not from plasma (15).

After the hemoperfusion was stopped, the plasma levels increased further till a new equilibrium was reached. Thereafter, levels declined exponentially as shown in Fig. 1. The plasma levels in the postperfusion equilibrium phase were very close to the levels that could be predicted by extrapolation of the curve fitted to the preperfusion data. This fact indicates that (resin) hemoperfusion may not be capable of substantially increasing the total body clearance of bretylium in subjects with normal renal function. Similar increase in the plasma levels after hemoperfusion has also been demonstrated for digoxin (15) and has been attributed to reequilibration of digoxin between two compartments.

In patients with severely impaired renal function, in whom the plasma concentrations are relatively high and the total body clearance of bretylium is markedly reduced, it is not clear whether resin hemoperfusions will be of any benefit in removing the drug. However, marked differences may exist in the ability of different commercially available hemoperfusion devices to remove a specific drug (16). Therefore, the results of this study should not be taken to indicate that hemoperfusion with other devices will produce identical results.

This study describes the pharmacokinetics of bretylium in dogs and demonstrates that hemoperfusion with a resin column does not remove bretylium equilibrated in the body. These data suggest that resin hemoperfusion may not be useful in the treatment of human bretylium intoxication.

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