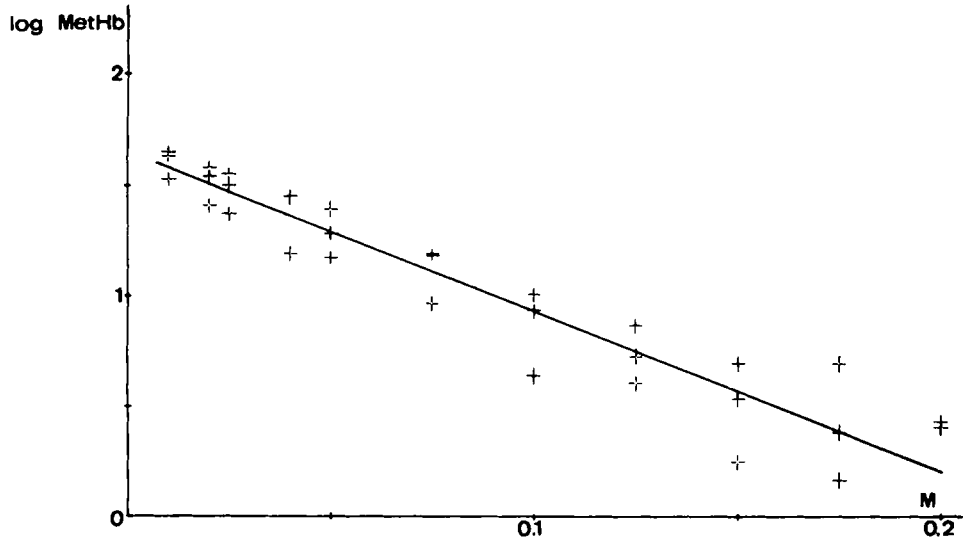

Figure 4—*Linear regression between the log of methemoglobin (%) and molar concentration (0.01 to 0.20 M) of* DL-*panthenol.*

such protectors "modify" the structure of globin as has been described for some proteins and glycerol by Gekko and Timasheff (14).

The changes in the proportion of methemoglobin as a function of concentration of the protective agent always fitted a hyperbola that could be linearized by a logarithmic transformation, giving a straight line with a negative slope. The three correlation lines thus obtained had slopes of the same order of magnitude, which indicates that these three model compounds are fairly similar in effectiveness. However, they are much less effective, during both freeze-drying and storage, than the amino acid salts that we have studied previously. This difference in behavior, which undoubtedly derives from quite specific physicochemical properties of the protective agents, remains unexplained.

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Disposition Kinetics of Ethambutol in Nephrectomized Dogs

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Abstract □ The effect of nephrectomy on the disposition of ethambutol was investigated in seven adult mongrel dogs: five were nephrectomized and two served as the control. Each dog was intravenously administerd 500 mg ethambutol, followed by blood sample collection for 12 h. Total urine was collected over 24 h from the normal control dogs. Ethambutol contents in plasma and urine were assayed by a GC method. The nephrectomized group and the control group exhibited differences in the following pharmacokinetic parameters: half-life, 5.0 *versus* 4.1 h (significant at $p < 0.1$); total body clearance, 8.4 *versus* 13.2 mL/min/kg (significant at $p < 0.1$); and volume of distribution, 2.7 *versus* 3.8 L/kg (significant at $p < 0.1$). Comparison of pharamacokinetic parameters among rabbits, dogs, and humans revealed distinct interspecies differences with regard to total body clearance, renal clearance, volume of distribution, and fractional renal excretion. One comparable parameter shared by all species is the β-phase half-life.

Keyphrases □ Ethambutol—disposition, pharmacokinetics in the dog, effect of nephrectomy, interspecies comparison □ Pharmacokinetics—ethambutol disposition in the dog, effect of nephrectomy, interspecies comparison □ Distribution—ethambutol in the dog, effect of nephrectomy, pharmacokinetics, interspecies comparison

Ethambutol (I), an antitubercular agent, is used alone or in combination with other drugs for the treatment of tuberculosis. The absorption and excretion of I has been studied in rats and mice (1), dogs (2, 3), and human subjects (4–6). In mice receiving single oral doses of I, peak serum concentrations were reached within 1 h of administration, and the compound

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Table I—Mean Plasma Ethambutol Concentrations in Five Nephrectomized Dogs and Two Normal Control Dogs [a]

		Plasma Concentrations (μg/mL) at Time (min)														
		5	30	60	90	120	150	180	210	240	300	360	420	480	600	720
Nephrectomized dogs (n = 5)	Mean	33.18	13.93	10.43	9.14	7.91	6.35	5.91	5.30	4.27	3.75	3.26	2.26	1.88	1.55	1.21
	SD	6.26	5.79	4.44	3.62	2.95	2.75	3.43	2.33	2.64	2.51	2.49	1.34	1.18	0.92	0.81
Normal dogs (n = 2)	Mean	--	18.84	12.41	3.35	2.73	1.57	1.45	1.01	1.07	0.91	0.79	0.76	0.63	0.42	0.33
	SD	—	13.67	13.85	2.23	1.40	0.57	0.21	0.28	0.54	0.18	0.17	0.25	0.36	0.24	0.18

[a] Ethambutol was administered to each dog at a dose of 500 mg iv.

was rapidly excreted (1). The medium lethal dose of I varied considerably for the different routes of drug administration to mice (1), suggesting a first-pass metabolism of the drug when administered nonparenterally. The first-pass effect was demonstrated for I using a rabbit model in which a low bioavailability of 28% was observed[1], in comparison with the nearly 80% oral bioavailability in humans (5).

After a single oral 100-mg/kg dose of I to dogs, absorption was rapid and the drug was cleared from the blood with a half-life of 1.5 h (2). Within 8 h of an intravenous injection of [^{14}C]I, 57% of the radioactivity appeared in the urine as the unchanged drug, determined after separation of metabolites by counter-current distribution (3). Arnaud *et al.* (7) reported a substantial urinary excretion of the unchanged I in two dogs, up to 50% of the dose within 2 h of a rapid intravenous injection. Limited studies of I in dogs make it difficult to relate the pharmacokinetic similarities between dogs and humans, especially with respect to renal excretion. This study was intended to investigate the effect of nephrectomy on the disposition of I in dogs and to compare the pharmacokinetics of I in rabbits, dogs, and humans.

$$HOCH_2CH(R)NH(CH_2)_2NHCH(R)CH_2OH$$

I R = C_2H_5
II R = CH_3

EXPERIMENTAL

Materials—Ethambutol[2] and the methyl analogue of ethambutol (II)[2] were supplied as the dihydrochloride salts. Trifluoroacetic anhydride[3] and pyridine[3] were used as derivatizing agents. Chromatographic-grade chloroform[4] and benzene[4] were used as supplied.

Animal Experiments—Seven adult mongrel dogs weighing 13.6–24.5 kg were used in the study. Five dogs were bilaterally nephrectomized, while two normal dogs served as the control. Dogs were surgically prepared after being anesthetized with 50 mg/kg of pentobarbital. The jugular and anticubital veins were cannulated for blood drawing and drug infusion, respectively. Normal dogs were allowed sufficient time to recover from anesthesia before drug administration. In the nephrectomized group, the drug study was not initiated until 2 d after the nephrectomization. During the waiting period, dogs were allowed limited access to food and water. Sham operations were not performed on the normal control dogs; otherwise, both groups of dogs were similarly treated prior to and during the experiment. Each dog was intravenously administered 500 mg of I *via* the anticubital vein of the forelimb over a 3-min period. Blood samples were collected at various times for 12 h. In the control dog study, total urine was collected by natural voiding over a 24-h period and aliquots were pooled. Plasma and urine samples were frozen at −20°C until analysis.

Analytical Procedure—Plasma and urine samples, to which a specific quantity of II (the internal standard) was added, were extracted with chloroform, derivatized with trifluoroacetic anhydride, and subjected to a GC analysis (8). A gas chromatograph[5] equipped with a ^{63}Ni electron-capture detector, a 61-cm × 32-mm i.d. glass column, and a 5% liquid[6] on solid support[6] stationary phase was used. The carrier gas was nitrogen at 20 mL/min; temperatures for the oven, injector, and detector were 155°C, 210°C, and 240°C, respectively.

Pharmacokinetic Analysis—Plasma data were fitted to a two-compartment open body model using the NONLIN program (9). The β-phase half-life ($t_{1/2,\beta}$) and the total body clearance (CL_t) were determined as:

$$t_{1/2,\beta} = \ln 2/\beta \quad \text{(Eq. 1)}$$

$$CL_t = D_0/AUC_\infty \quad \text{(Eq. 2)}$$

where β is the slope of the log-linear phase of the plasma decay curve, D_0 is the dose, and AUC_∞ is the area under the plasma concentration (C_p) *versus* time curve, calculated by the trapezoidal rule in conjunction with an area extrapolation method. The steady-state volume of distribution (Vd_{ss}) was determined using the model-independent expression of Benet and Galeazzi (10):

$$Vd_{ss} = D_0 \cdot (AUMC_\infty)/(AUC_\infty)^2 \quad \text{(Eq. 3)}$$

where AUMC is the area under the first moment of the plasma curve.

The fraction of renal excretion (f_e) in normal dogs was calculated as

$$f_e = (A_{e,24} + CL_r \int_t^\infty C_p dt)/D_0 \quad \text{(Eq. 4)}$$

where $A_{e,24}$ is the amount of I excreted in the 24-h urine sample and CL_r is the renal clearance determined by A_e/AUC using parameter values of up to 12 h. The integration term actually represents the extrapolated urinary recovery of the drug from the last plasma sampling point to infinite time. In this study, the last plasma datum at 24 h was extrapolated since the assay sensitivity could not allow accurate detection of plasma concentration <0.25 μg/mL.

Difference in mean pharmacokinetic parameters of the control and nephrectomized dogs was tested for significance by nonpaired Student's t statistics.

RESULTS AND DISCUSSION

Table I records the mean plasma data obtained for the five nephrectomized and two normal dogs. The normal group appeared to maintain higher plasma levels of I during the first hour of intravenous drug injection, whereas the nephrectomized group retained substantially greater plasma concentrations after 1.5 h. At the end of the blood sampling period of 12 h, the mean plasma concentrations observed were 1.21 μg/mL for the nephrectomized group and 0.33 μg/mL for the control group. Figure 1 depicts the time profiles for mean plasma levels in the normal and nephrectomized dogs. A distinct distribution phase was noted in the normal control dogs, and the distribution equilibrium was not reached until 2.5 h after the drug injection. In the nephrectomized

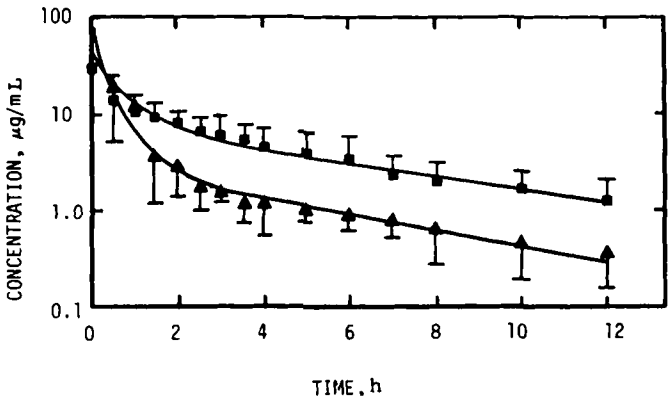

Figure 1—*Mean plasma concentration* versus *time plots for nephrectomized* (■) *and normal dogs* (▲) *over a 12-h period following single 500-mg iv doses of ethambutol.*

[1] C. S. Lee *et al.*, unpublished results.
[2] Lederle Laboratories, Pearl River, N.Y.
[3] Pierce Chemical, Rockford, Ill.
[4] MCB, Norwood, Ohio.
[5] Hewlett-Packard, Model 5730 A.
[6] OV-101 on Gas Chrom Q 100/120 mesh; Applied Science, State College, Pa.

Table II—Half-life, Total Body Clearance, and Volume of Distribution of Ethambutol in Five Nephrectomized and Two Normal Dogs

Dog	Weight, kg	$t_{1/2,\beta}$, h	CL_t, mL/min/kg	Vd_{ss}, L/kg
Nephrectomized				
1	17.0	4.29	11.7	3.2
2	20.0	5.18	12.4	3.6
3	13.6	4.73	6.2	2.3
4	16.3	4.79	5.0	1.8
5	20.2	5.93	6.7	2.4
Mean	17.4	4.98	8.4	2.7
SD	2.8	0.62	3.4	0.7
Normal				
6	24.5	3.52	16.5	4.4
7	19.8	4.72	10.0	3.2
Mean	22.2	4.12	13.2	3.8
SD	3.3	0.85	4.6	0.9

group, the distribution phase was apparent, but less extensive. The rapid and extensive distribution phenomena of I have also been noted in rabbits[1] and in human subjects (5).

Since a distinct distribution phase was noted after drug injection, plasma concentrations of I were subjected to computer analysis using a two-compartment open body model. Table II lists the pharmacokinetic parameters, and Table III compares the mean parameter values. Half-lives in the nephrectomized group varied from 4.29 to 5.93 h and those of the normal dogs were 3.52 and 4.72 h. The difference in the mean half-life of the two groups represented a 20.9% prolongation of half-life, presumably caused by nephrectomization, and was statistically significant ($p < 0.1$). Mean total body clearances of the nephrectomized (8.4 ± 3.4 mL/min/kg) and the normal dogs (13.2 ± 4.6 mL/min/kg) showed a significant difference of 36.33% ($p < 0.1$). In the limited number of dogs studied, nephrectomy induced a general trend for the increased half-life and reduced total body clearance of I, reflecting the renal excretory feature of the drug. The effect of nephrectomy on the elimination of I has not been reported in dogs, perhaps because such an effect is generally regarded as obvious. In humans, chronic renal failure caused a reduction of total body clearance from 500 mL/min to <10 mL/min (4). A prolongation of the half-life of I to ~10 h was also reported in renal failure (11).

Nephrectomized dogs were found to have a smaller volume of distribution (mean, 2.7 L/kg) than normal dogs (mean, 3.8 L/kg); the difference was statistically significant ($p < 0.1$). Nephrectomy would presumably cause a decreased volume of distribution due, in part, to the organ removal and the ligation of the vascular system. Chronic renal failure has been reported to alter the apparent volume of distribution for a number of drugs, notably digoxin (12) and phenytoin (13). It should be noted, however, that the nephrectomized dogs used in this study could only be deemed as approaching the chronic uremic state, since the drug investigation was initiated 2 d after the surgical preparation. Had the study been carried out at the steady state of chronic uremia, the change of pharmacokinetic parameters would have been more pronounced. Nevertheless, one can generally conclude that nephrectomy increased the half-life, decreased the total body clearance, and decreased the volume of distribution of I in dogs.

In a recent study of the effect of aluminum hydroxide on the absorption of I in rabbits[1], a substantially lower bioavailability of 28% was observed in comparison with the 80% bioavailability in humans, suggesting a first-pass metabolism of I in rabbits. With respect to renal excretion, rabbits excreted <5% of the intravenous dose as the unchanged compound in 24 h; the equivalent values were 30% for normal dogs and 70% in healthy human volunteers (Table IV). The observations of renal excretion of I in rabbits and dogs are contradictory to the general belief that I is predominantly excreted unchanged in all animal species. Arnaud *et al.* (7) reported a substantial urinary excretion of up to 50% for I; however, the urine assay was performed by a relatively

Table III—Comparison of Mean Pharmacokinetic Parameter Values for Half-Life, Total Body Clearance, and Volume of Distribution Between the Nephrectomized and Normal Dogs

Dog	$t_{1/2,\beta}$, h	CL_t, mL/min/kg	Vd_{ss}, L/kg
Nephrectomized	4.98	8.4	2.7
Normal	4.12	13.2	3.8
Percent change[a]	20.9	36.3	28.9
t value	1.54	1.51	1.81

[a] All differences are significant at $p < 0.1$.

Table IV—Comparison of Half-life, Total Body Clearance, Renal Clearance, Volume of Distribution, and Fractional Renal Excretion among Rabbits, Dogs, and Humans

Parameter	Rabbit[a]	Dog[b]	Human
$t_{1/2,\beta}$ (h)	3.87	4.12	4.21[c]
CL_t (mL/min/kg)	43.7	13.2	8.6[c]
CL_r (mL/min/kg)[d]	2.2	4.0	6.0
Vd_{ss} (L/kg)	8.5	3.8	3.9[c]
f_e (%)	5	30	70[c,e]

[a] C. S. Lee *et al.*, unpublished results. [b] Present study with normal dogs. [c] From Ref. 5. [d] Calculated as f_e-CL_t based on the f_e values listed. [e] From Ref. 3.

nonspecific and less sensitive colorimetric method (14). With regard to volume of distribution, rabbits exhibited the highest value (13.6 L/kg), followed by dogs (4.6 L/kg), and humans had the smallest distribution volume (2.3 L/kg) of the three species. In all cases, the volume of distribution exceeded total body weight, suggesting a localized distribution of I in the dogs. Pujet and Pujet (15) have noted a preferential distribution of I in the deep layers of the lung. In addition, I has been demonstrated, both *in vivo* and *in vitro*, to have a preference for partition into red blood cells (3, 16).

Total body clearance followed the same rank order as that of distribution volume: 43.7 mL/min/kg in rabbits, 13.2 mL/min/kg in dogs, and 8.6 mL/min/kg in humans, respectively. Since all clearance values listed in Table IV were calculated independent of a pharmacokinetic model ($CL = D_0/AUC$), compartmentalization would not have contributed to the difference in clearances observed among the three species. Assuming a renal excretion of 5% in rabbits, 30% in dogs, and 70% in humans, the respective renal clearances can be calculated as $CL_r = f_e \cdot CL_t$ and values of 2.2, 4.0, and 6.0 mL/min/kg would be obtained. In all cases, the renal clearance would exceed the glomerular filtration rate normalized to the body weight, indicating a net renal tubule secretion of the drug in all species. The active secretory mechanism of I has been demonstrated in humans, whose renal clearance was ~400 mL/min (4, 16). Since half-life is a function of both clearance and volume of distribution ($t_{1/2} = 0.693V/CL$), the three species actually shared similar half-lives—3.87 h in rabbits, 4.12 h in dogs, and 4.21 h in humans. However, there were distinct differences in other pharmacokinetic parameters, including total body clearance, renal clearance, volume of distribution, and fractional renal excretion.

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