Biochemical Pharmacology, Vol. 21, pp. 1967–1976. Pergamon Press, 1972. Printed in Great Britain.

PHARMACOKINETIC STUDY OF AMPHETAMINE ELIMINATION IN DOGS AND SWINE

J. DESMOND BAGGOT and LLOYD E. DAVIS

Division of Comparative Pharmacology, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210, U.S.A.

(*Received* 26 *October* 1971; *accepted* 11 *February* 1972)

Abstract—The kinetics of amphetamine elimination were studied in healthy and nephrectomized mongrel dogs and healthy swine. A similar dose of *dl*-amphetamine sulfate (0·66 mg/kg, free base) was administered intravenously to each animal. The drug concentration in the biological fluids was determined by a sensitive and specific gas chromatographic method. Amphetamine was extracted from alkaline biologic fluid into cyclohexane. The trichloroacetamide derivative was prepared and detected by electron capture. The biological half-life of the drug (mean ± S. E. M.) was significantly shorter in swine (1·05 ± 0·05 hr) than in dogs (4·50 ± 0·24 hr) (Student's *t*-test, $P < 0·001$). This was believed to be due to the faster rate of biotransformation of the drug in swine. Diffusion equilibrium was attained rapidly in both species, and the apparent specific volumes of distribution were large. The disappearance of amphetamine from plasma and the appearance of unchanged drug in urine and bile were first-order processes. An appreciable amount of unchanged amphetamine was excreted in urine while the cumulative amount in bile was small. The renal clearance values in dogs (2·80–5·07 ml min^{-1} kg^{-1}) provide evidence that amphetamine probably undergoes glomerular filtration and tubular reabsorption. Extent of reabsorption was pH dependent. Anaesthesia with pentobarbital sodium (*ca.* 28·5 mg/kg) did not affect overall clearance (9·18 ml min^{-1} kg^{-1}) in intact dogs. The biological half-life of the drug (mean ± S. E. M.) was significantly longer in nephrectomized dogs (5·69 ± 0·20 hr) than in intact dogs (4·50 ± 0·24 hr) (Student's *t*-test, $P < 0·001$). Nephrectomy did not significantly change the extent of plasma protein binding or the apparent specific volume of distribution of the drug. The mean biliary clearance values (0·012 ml min^{-1} kg^{-1}) were similar in intact and nephrectomized dogs. The renal clearance value, the per cent of dose excreted unchanged in urine and the highly significant increase in half-life after nephrectomy provide evidence that in the dog renal excretion is important in the elimination of amphetamine. However, despite the importance of renal excretion, biotransformation appears to play the major role in the elimination of this drug.

AMPHETAMINE is eliminated from the body by excretion and biotransformation. A substantial portion of the dose was excreted in the urine of man unchanged,[1,2] and urinary pH was an important factor in determining the rate of excretion of this drug.[3–5] The metabolites of amphetamine isolated from urine, apart from the unchanged drug itself, were *p*-hydroxyamphetamine and benzoic acid and their conjugates.[6–10]

The objective of this work was to elucidate the contribution of renal excretion to elimination of amphetamine in the dog and the relative roles of renal and biliary excretion in dogs and swine.

MATERIALS AND METHODS

The experimental subjects were healthy mongrel dogs (10–25 kg), nephrectomized mongrel dogs (13–23 kg) and healthy swine (14–22 kg). A similar dose (0·66 mg/kg,

calculated as free base) of *dl*-amphetamine sulfate* was administered intravenously to each animal. Venous blood samples were collected at precisely timed intervals; dipotassium EDTA was the anticoagulant employed. Plasma was separated by centrifugation and the samples were stored at $-10°$ until assayed for amphetamine.

This study is most conveniently described as consisting of five experiments. The specific aim and procedure followed in each experiment will be described separately.

Experiment I. To determine the biological half-life of amphetamine and the apparent volume of distribution of the drug in normal dogs and swine. The experimental subjects were six healthy, adult, randomly selected mongrel dogs designated by the letters A–F, and six Yorkshire swine.

Experiment II. To compare quantitatively the amphetamine excreted in urine and bile. A dose of pentobarbital sodium sufficient to produce medium surgical anaesthesia was administered intravenously. The dogs were labeled R, T, V, X and Z. The ureters and common bile duct were cannulated and the neck of the gall bladder was occluded by a ligature. After collecting blood, urine and bile samples which served as blank biological fluids for the experimental subject, the computed dose of amphetamine was administered intravenously. Urine and bile were collected continuously, the sample tubes were changed each half-hour during the experiment. The volumes of urine, bile and the urinary pH (pH Metre 26, Radiometer, Copenhagen) were measured, the tubes were then sealed. Blood samples were also collected at half-hour intervals.

A similar experiment was performed in three swine (PB, PC and PX).

Experiment III. To determine the rate of elimination of amphetamine in animals with no renal function. Five randomly selected mongrel dogs were bilaterally nephrectomized (NA–NE). Amphetamine was administered 42–48 hr after removal of the kidneys. Venous blood samples were collected at regular intervals.

Experiment IV. To determine the effect of nephrectomy upon biliary clearance of amphetamine. Under pentobarbital anaesthesia the common bile ducts were cannulated in three nephrectomized dogs (NF, NG and NL). Amphetamine was administered, bile was collected continuously and a blood sample was drawn each half-hour.

Experiment V. To determine quantitatively the proportion of the dose excreted as unchanged amphetamine in cumulative (24 hr) urine. The computed dose (1·32 mg/kg) was administered to six healthy dogs (11·5–23 kg); the animals were retained in metabolism cages for the duration of this experiment. The final volume and pH of the urine were measured.

The amphetamine concentration in the biological fluids was measured by a sensitive and specific gas chromatographic method.[11] Amphetamine was extracted from alkaline biologic fluid into cyclohexane. The trichloroacetamide derivative was prepared with trichloroacetyl chloride in the organic phase. This derivative was chromatographed on 3% OV-1 and detected by electron capture (Beckman Gas Chromatograph, model GC-5).

The concentration of *p*-hydroxyamphetamine in urine was determined by the colorimetric method of Axelrod.[6] The glucuronide and sulfate conjugates of *p*-hydroxyamphetamine were also assayed by the method of Axelrod[6] following incubation of urine with appropriate enzyme in a suitable buffer.

* Amfetasul, Pitman–Moore, Indianapolis, Ind.

Glucuronide conjugate. To 3 ml of 0·2 M acetate buffer (pH 4·6) was added 3 ml of urine and 50 mg β-glucuronidase (beef liver, salt free, 70,000–100,000 units per 10 g, (Nutritional Biochemicals Corp., Cleveland, Ohio). Sulfate derivative: to 3 ml of 0·2 M acetate buffer (pH 5·0) was added 3 ml of urine and 1 unit of sulfatase (Sulfatase, type III: from Limpets, Sigma Chemical Co., St. Louis, Mo). The mixtures were incubated for 1 hr at 37° in a Dubnoff metabolic shaking incubator. Following this period the hydroxyamphetamine concentration was determined in 2·5 ml of the incubated mixture.

The extent of plasma protein binding was determined *in vitro* by the equilibrium dialysis technique using ^{3}H-*d*-amphetamine (New England Nuclear Corp., Boston, Mass.) and Liquid scintillation counting (Packard TriCarb model 3380 liquid scintillation spectrometer). The specific activity of the amphetamine sulfate was 9·8 c/m-mole (radiochemical purity > 98 per cent). Extent of protein binding was determined at a drug concentration of 1×10^{-6} M.[12]

RESULTS AND DISCUSSION

The body may be described as a two-compartment open model, that is, a drug contained within the body behaves as though it were contained in two kinetically distinguishable compartments; the central compartment being the blood plasma and the other compartment the rest of the body tissues.

The central compartment is open in the sense that elimination occurs from this compartment by excretion and/or biotransformation. Thus, the entire system is open since the drug passes reversibly from plasma to tissues. A drug administered intravenously will undergo simultaneous elimination and distribution processes. If the distribution of drug throughout the body is very rapid relative to the rate of elimination, then the disappearance of drug from plasma (i.e. the plasma-concentration time curve) may be approximated by a monoexponential expression: $Cp = Be^{-\beta t}$ where Cp is the concentration of drug in plasma, B is the zero time intercept of the data plotted on semilogarithmic paper and $-\beta$ the slope of the first-order plot. The term B is an estimate of the concentration of drug that would have been present initially in plasma if all the injected dose had been distributed in the volume of distribution ultimately attained. The apparent volume of distribution (Vd) may be defined as the volume of body fluids which holds the substance in solution at the same concentration as the plasma (dose/B). The apparent specific volume of distribution ($V'd$) is obtained by dividing the apparent volume of distribution by the weight of the animal, that is Vd/body wt. (l./kg). The distribution of amphetamine was rapid relative to the rate of elimination so the disappearance of the drug from plasma was considered monoexponential. The biological half-life ($T_{\frac{1}{2}}$) was calculated from the overall elimination rate constant (β). A clearance value may be defined as the volume of plasma cleared of drug by an elimination process during a given time interval. The clearance values were expressed on a unit weight basis (ml min^{-1} kg^{-1}). The apparent overall clearance value is an estimate of the sum of the metabolic and excretory clearances and was computed as follows: C' overall $= \beta \times V'd'$, where β is the overall elimination rate constant and $V'd'$ is the apparent specific volume of distribution corrected for extent of plasma protein binding. The latter correction is considered desirable as protein binding is reversible. Estimates of renal and biliary (i.e. excretory) clearance values

were obtained experimentally, whereas the apparent overall clearance value was computed. Constant intravenous infusion of the drug at a rate equal to the rate of elimination would lead to more precise renal clearance values. The concentration of drug in the blood would remain constant during infusion and therefore the value of P_t in the denominator of the equation:

$$C \text{ (ml/min)} = \text{(amount eliminated per unit time (mg/min))} \; t/P_t \text{ (mg/ml)}$$

could be calculated with greater accuracy. However, these experiments provide useful basic information and the groundwork for further investigation. It must be understood that the apparent overall clearance value was computed merely to indicate the relative magnitudes of metabolic and excretory clearances; the former may be obtained by difference.

Experiment I. The biological half-life (mean ± S. E. M. $n = 6$) under normal conditions of fluctuating urinary pH in this randomly selected group of healthy dogs was 4·56 ± 0·29 hr (Table 1). Extravascular distribution of the drug was complete in less than 30 min (Fig. 1), and the apparent specific volume of distribution was large (2·65 ± 0·06 l./kg). This indicates an ability of the tissues to concentrate and/or bind amphetamine. Axelrod[6] examined the distribution of *d*-amphetamine in representative tissues

TABLE 1. PHARMACOKINETIC CONSTANTS (MEAN ± S. E. M.) DESCRIBING DISTRIBUTION AND ELIMINATION OF AMPHETAMINE IN DOGS AFTER I.V. INJECTION OF *dl*-AMPHETAMINE SULFATE (0·66 mg/kg, FREE BASE)

Experiment	Dogs (no.)	B* (ng ml^{-1})	$T_{\frac{1}{2}}$† (hr)	β‡ (hr^{-1})	$V'd$§ (l. kg^{-1})
I	A–F (6)	275 ± 7·4	4·56 ± 0·29	0·152 ± 0·012	2·65 ± 0·06
II	R,T,V,X,Z (5)	248 ± 13·4	4·42 ± 0·44	0·157 ± 0·013	2·69 ± 0·15
III	NA–NE (5)	262 ± 4·47	5·81 ± 0·28	0·120 ± 0·005	2·53 ± 0·04
IV	NF,NG,NL (3)	251 ± 7·00	5·47 ± 0·30	0·127 ± 0·007	2·63 ± 0·07

* Theoretical drug concentration in plasma at time zero, assuming instantaneous distribution.
† Biological half-life.
‡ Overall elimination rate constant.
§ Apparent specific volume of distribution.

of a dog which was given 10 mg/kg of the drug intraperitoneally. The animal was sacrificed 1 hr after the drug was administered. The drug was found to be distributed in most organ tissues but only negligible amounts were present in fat and bile. Similar tissue distributions of this drug were reported in the mouse,[13] cat[14] and rat.[15] Most of the drug was shunted toward tissues of high blood perfusion, that is, kidneys, lungs, brain and liver.

Experiment II. The dose of amphetamine administered, the urinary pH, the biological half-life of the drug, and the cumulative amounts of unchanged amphetamine excreted in urine and bile for the duration of the experiment in individual dogs are tabulated (Table 2). No attempt was made to control urinary pH, the observations are within the range of normal values for the canine species. Despite individual

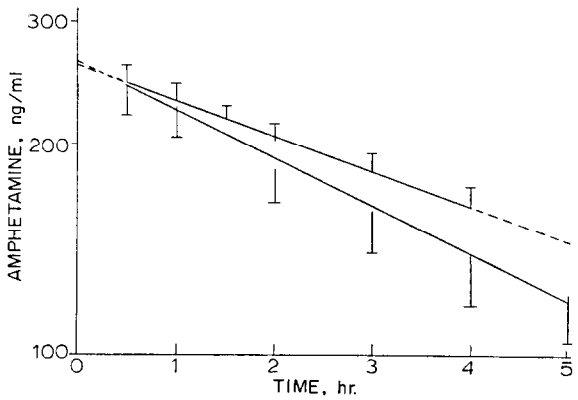

FIG. 1. Disappearance of amphetamine from the blood plasma of intact (lower line) and nephrectomized (upper line) dogs following the intravenous injection of amphetamine sulfate (0·66 mg/kg, free base). Each point represents the mean plasma amphetamine concentration and each vertical bar is 1 S. D. The mean values of the pharmacokinetic parameters were calculated: intact dogs (n = 11), B = 263 ng ml^{-1}, $T_{\frac{1}{2}}$ = 4·50 hr, $V'd$ = 2·67 l. kg^{-1}, C' overall = 9·18 ml min^{-1} kg^{-1}; nephrectomized dogs (n = 8), B = 258 ng ml^{-1}, $T_{\frac{1}{2}}$ = 5·69 hr, $V'd$ = 2·57 l. kg^{-1}, C' overall = 6·88 ml min^{-1} kg^{-1}.

TABLE 2. DOSE OF AMPHETAMINE INJECTED I.V., THE BIOLOGICAL HALF-LIFE OF THE DRUG AND THE CUMULATIVE AMOUNTS OF AMPHETAMINE EXCRETED IN URINE AND BILE OF DOGS

Subject (dog)	Total dose (mg)	Urine pH mean value (range)	$T_{\frac{1}{2}}$ (hr)	Cumulative amount of amphetamine (4 hr)	
				Urine (mg)	Bile (μg)
R	13	7·50 (7·38–7·76)	6·13	1·25	3·79
T	10	5·96 (5·92–6·00)	3·67	2·10	5·00*
V	10	6·55 (6·48–6·61)	4·25	1·77	5·50
X	10	6·51 (6·48–6·54)	4·18	1·80	6·87
Z	13	6·28 (6·20–6·38)	3·85	3·25	6·50

* Three hr.

variation in biological half-life there appears to be a direct relationship between urinary pH and the half-life of this drug, the more acidic the urine the shorter the biological half-life. The urinary pH determines the proportion of drug in the renal tubular fluid which exists in the nonionized form and thus the amount of this lipid-soluble fraction which is available for reabsorption by passive nonionic diffusion. The lipid-solubility of the neutral form of amphetamine is reflected in the so-called true partition coefficient (heptane–water, 1·88, chloroform–water, 146).[16]

There is an inverse relationship between the renal clearance values observed and the biological half-lives obtained (Table 3). While the renal clearance values of amphetamine were all within the normal glomerular filtration rate range in dogs, there appeared

TABLE 3. CLEARANCE VALUES OF AMPHETAMINE IN INTACT AND NEPHRECTOMIZED DOGS*

Subject (dog)	$T_{\frac{1}{2}}$ (hr)	C' renal (ml/min/kg)	C' biliary (ml/min/kg)
R	6·13	2·80	0·006
T	3·67	4·27	0·012
V	4·25	4·38	0·013
X	4·18	4·03	0·017
Z	3·85	5·07	0·011
NF	6·03		0·012
NG	5·37		0·013
NL	5·02		0·010

*Renal clearance (ml min^{-1}) =

$$\frac{\text{amphet. conc. in urine } (\mu g\ ml^{-1}) \times \text{urine vol. (ml } min^{-1})}{\text{amphet. conc. in plasma } (\mu g\ ml^{-1})}$$

to be a relationship between urinary pH and amphetamine clearance. The clearance values obtained (2·80–5·07 ml min^{-1} kg^{-1}) suggest that amphetamine probably undergoes glomerular filtration and tubular reabsorption. The extent of reabsorption appeared to increase with increasing urinary pH. The clearances in adult dogs of inulin, creatinine and *p*-aminohippurate (PAH) were 3·77 (1·74–5·86),[17] 4·29 (2·15–8·32)[18] and 13·5 (8·05–22·4)[18] ml min^{-1} kg^{-1} respectively. Anesthesia with pentobarbital sodium in dogs had no influence on the glomerular filtration rate,[19–21] while the effective renal plasma flow (i.e. PAH clearance) was unchanged[20] or slightly decreased.[19] In contrast, barbiturates or other anesthetics when used in the human caused a considerable reduction both in glomerular filtration and renal blood flow.[22–24] In man, amphetamine was cleared from blood more rapidly than could be accounted for by glomerular filtration under acid conditions, but when urinary pH fluctuated, clearance of the drug could be accounted for by this route.[25] The cumulative amounts of amphetamine in urine and bile varied among dogs; however, the

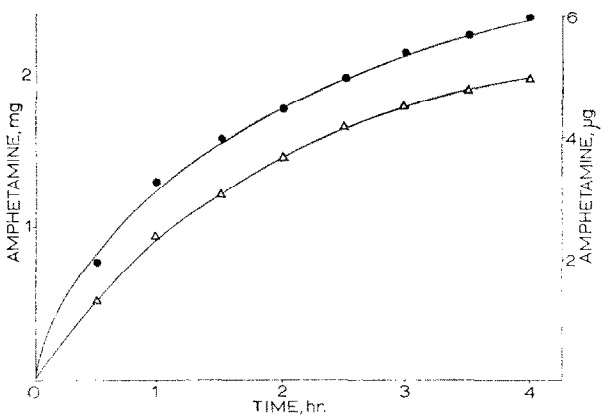

FIG. 2. The cumulative appearance of amphetamine in urine △——△ (mg) and bile ●——● (μg) of dogs following the intravenous injection of amphetamine sulfate (0·66 mg/kg, free base). Each point represents the mean amount of amphetamine in the biological fluid of five dogs.

amount of unchanged drug in urine was approximately 500 times the amount present in bile in the same experimental subject (Table 2). The appearances of amphetamine in urine and bile are shown (Fig. 2). The biliary clearance values (Table 3) indicate that this route of elimination, or perhaps distribution, plays a minor role in the overall pharmacokinetics of the drug. The cumulative amount of drug recovered in bile (Table 2) supports this hypothesis. The mean apparent overall clearance value in intact dogs was 9·18 ml min^{-1} kg^{-1}. Thus, the metabolic clearance in intact dogs varied from 4·10 to 6·37 ml min^{-1} kg^{-1}. Anesthesia with pentobarbital sodium (*ca.* 28·5 mg/kg, i.v.) did not affect overall clearance in intact dogs.

The disappearance of amphetamine from blood plasma of swine was exponential (Fig. 3). The biological half-life of the drug (mean ± S. E. M.) was significantly

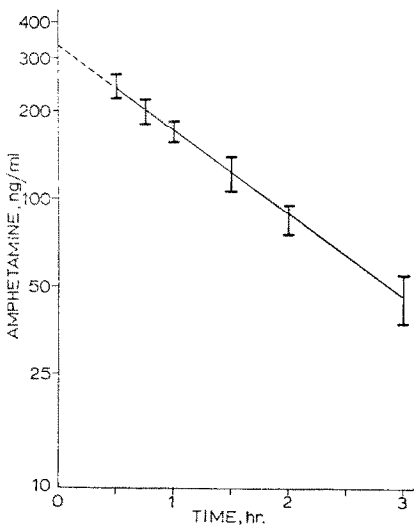

FIG. 3. Disappearance of amphetamine from the blood plasma of swine following the intravenous injection of amphetamine sulfate (0·66 mg/kg, free base). Each point represents the mean ($n = 9$) plasma amphetamine concentration and each vertical bar is ± 1 S. D. The mean values of the pharmacokinetic parameters were calculated: $B = 330$ ng ml^{-1}, $T_{\frac{1}{2}} = 1{\cdot}05$ hr, $V'd = 1{\cdot}93$ l. kg^{-1}, C' overall = 40·6 ml min^{-1} kg^{-1}.

shorter in swine (1·05 ± 0·05 hr) than in dogs (4·50 ± 0·24 hr) (Student's *t*-test, $P < 0{\cdot}001$). The apparent overall clearance of the drug in swine was 40·6 ml min^{-1} kg^{-1}. The clearances of inulin, endogenous creatinine, urea and PAH in swine were 21 (18–25), 22 (15–34), 12 (9–18) and 64 (52–84) ml/min/10 kg respectively.[26] Sedation with pentobarbitone had no effect on the clearances of these substances in swine.[27] Assuming similar renal clearance values in the animals used in these experiments, it is apparent from the overall clearance that metabolic clearance was dominant. The dose of amphetamine administered, the urinary pH, the biological half-life and the cumulative amounts of amphetamine in urine and bile of individual swine are tabulated (Table 4). The mean cumulative amounts of unchanged amphetamine excreted in urine and bile were 8·18 and 0·12 per cent of the dose respectively. The appearance of the drug in urine and bile followed first-order kinetics (Fig. 4).

Experiments III and IV. The plasma amphetamine concentration vs. time profiles in intact and nephrectomized dogs are shown (Fig. 1). The half-life (mean ± S. E. M.)

TABLE 4. DOSE OF AMPHETAMINE INJECTED I.V., THE BIOLOGICAL HALF-LIFE OF THE DRUG AND THE CUMULATIVE AMOUNTS OF AMPHETAMINE EXCRETED IN URINE AND BILE OF SWINE

Subject (pig)	Total dose (mg)	Urine pH mean (range)	$T_{\frac{1}{2}}$ (hr)	Cumulative amount of amphetamine 3 hr)	
				Urine (mg)	Bile (μg)
PB	13·2	5·87 (5·71–6·05)	0·93	1·08	14·4
PC	16·2	5·82 (5·61–5·76)	1·14	1·36	20·3
PX	11·8	6·07 (5·88–6·34)	0·92	0·93	14·9

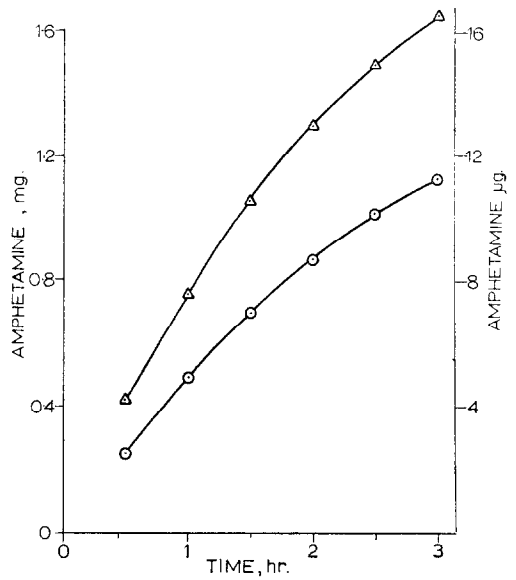

FIG. 4. The cumulative appearance of amphetamine in urine ○——○ (mg) and bile △——△ (μg) of swine following the intravenous injection of amphetamine sulfate (0·66 mg/kg, free base). Each point represents the mean amount of amphetamine in the biological fluid of three animals.

was significantly longer in nephrectomized dogs (5·69 $\pm$ 0·20 hr) than in intact dogs (4·50 $\pm$ 0·24 hr) (Student's *t*-test, $P < 0{\cdot}001$). The pharmacokinetic parameters describing distribution and elimination of amphetamine in intact and nephrectomized dogs are tabulated (Table 1). Nephrectomy did not significantly alter the apparent specific volume of distribution of the drug. The mean biliary clearance values were similar (0·012 ml min^{-1} kg^{-1}) in intact and nephrectomized animals (Table 3). The low biliary clearance supports the observation that interruption of the potential enterohepatic cycle by cannulation of the common bile duct had no significant effect upon the biological half-life of the drug. The apparent overall clearance in nephrectomized dogs (6·88 ml min^{-1} kg^{-1}) reflects mainly metabolic clearance.

The extent of protein binding was independent of amphetamine concentration within the range of plasma levels observed *in vivo* (25–400 ng ml^{-1}).[12] The per cent of

TABLE 5. AMOUNT OF AMPHETAMINE AND METABOLITES IN 24-hr URINE OF DOGS

Dog	Dose (mg/kg)	Urine pH	Amphetamine (mg)	*p*-Hydroxyamph. + conjugates		
				p-OH (mg)	Glucuronide (mg)	Sulfate (mg)
A	15	7·75	4·49	1·04	0·46	0·61
B	20	7·60	5·13	2·85	1·20	0·88
C	15	7·67	4·74	2·96	0·72	0·49
D	15	7·48	4·26	0·70	0·21	0·33
E	25	6·65	7·44	2·79	0·93	0·62
F	30	7·56	10·32	3·20	1·00	0·70
Mean	20	7·45	6·07	2·26	0·76	0·61
S. E. M.			1·2	2·2	0·6	0·4
% of dose			30	11	4	3

amphetamine protein bound (mean ± S. E. M.) was 39·6% ± 3·0 in swine plasma. The extent of protein binding (mean ± S. E. M.) in intact (23·1% ± 1·7) and nephrectomized uremic (24·3% ± 1·5) dogs was not significantly different.[12]

Experiment V. The quantities of unchanged amphetamine, *p*-hydroxyamphetamine and its conjugates were measured in 24-hr urine samples. This time period represents five half-lives of amphetamine in the dog. The amount of each fraction recovered is tabulated (Table 5). Thirty per cent of the dose administered was excreted unchanged in urine; this finding is an agreement with that of Axelrod.[6] The relatively low per cent of dose (48 per cent) recovered may be explained by the fact that the dog was shown to have two major pathways for biotransformation of amphetamine, namely *p*-hydroxylation and deamination.[6,8–10]

In conclusion, the renal clearance values, the per cent of dose excreted unchanged in urine and the highly significant increase in biological half-life following nephrectomy provide evidence that in the dog renal excretion is important in the elimination of amphetamine. However, metabolic clearance, represented by the apparent overall clearance minus the renal clearance in intact dogs and the apparent overall clearance in nephrectomized dogs, appears to play the major role in the elimination of this drug.

Acknowledgement—The authors gratefully acknowledge the surgical assistance of Mr. Richard B. Ford, senior student in Veterinary Medicine. This investigation was supported in part by Grants from the National Institutes of Health, Bethesda, Md., U.S.A. (GM 17244, FR 05463 and 1-FO3-MH 51940-01-DA).

REFERENCES

1. A. H. BECKETT and M. ROWLAND, *J. Pharm. Pharmac.* **17,** 628 (1965).
2. M. ROWLAND, *J. pharm. Sci.* **58,** 508 (1969).
3. A. M. ASATOOR, B. R. GALMAN, J. R. JOHNSON and M. D. MILNE, *Br. J. Pharmac. Chemother.* **24,** 293 (1965).
4. A. H. BECKETT, M. ROWLAND and P. TURNER, *Lancet* **1,** 303 (1965).
5. M. ROWLAND and A. H. BECKETT, *Arzneimittel-Forsch.* **16,** 1369 (1966).
6. J. AXELROD, *J. Pharmac. exp. Ther.* **110,** 315 (1954).
7. J. J. ALLEVA, *J. med. Chem.* **6,** 621 (1963).
8. L. G. DRING, R. L. SMITH and R. T. WILLIAMS, *J. Pharm. Pharmac.* **18,** 402 (1966).
9. T. ELLISON, L. GUTZAIT and E. J. VANLOON, *J. Pharmac. exp. Ther.* **152,** 383 (1966).
10. L. G. DRING, R. L. SMITH and R. T. WILLIAMS, *Biochem. J.* **116,** 425 (1970).

11. J. S. NOONAN, P. W. MURDICK and R. S. RAY, *J. Pharmac. exp. Ther.* **168,** 205 (1969).
12. J. D. BAGGOT, L. E. DAVIS and C. A. NEFF, *Biochem. Pharmac.* **21,** 1813 (1972)
13. R. W. FULLER and C. W. HINES, *Biochem. Pharmac.* **16,** 11 (1967).
14. M. SIEGEL, T. ELLISON, A. G. SILVERMAN and R. OKUN, *Proc. west. Pharmac. Soc.* **11,** 90 (1968).
15. J. BRZEZINSKI, *Acta Pol. pharm.* **25,** 91 (1968).
16. T. B. VREE, A. T. MUSKENS and J. M. VAN ROSSUM, *J. Pharm. Pharmac.* **21,** 774 (1969).
17. A. ASHEIM, F. PERSSON and S. PERSSON, *Acta physiol. scand.* **51,** 150 (1961).
18. C. R. HOUCK, *Am. J. Physiol.* **153,** 169 (1948).
19. K. F. GLAUSER and E. E. SELKURT, *Am. J. Physiol.* **168,** 469 (1952).
20. A. C. CORCORAN and I. H. PAGE, *Am. J. Physiol.* **140,** 234 (1943).
21. J. P. GILMORE and L. L. MICHAELIS, *Am. J. Physiol.* **216,** 1367 (1969).
22. D. V. HABIF, E. M. PAPPER, H. F. FITZPATRICK, P. LOWRANCE, C. MCC. SMYTHE and S. E. BRADLEY, *Surgery, St. Louis* **30,** 241 (1951).
23. S. PAPPER and E. M. PAPPER, *Clin. Pharmac. Ther.* **5,** 205 (1964).
24. S. DEUTSCH, E. C. PIERCE, JR. and L. D. VANDAM, *Anesthesiology* **28,** 547 (1967).
25. A. H. BECKETT, J. A. SALMON and M. MITCHARD, *J. Pharm. Pharmac.* **21,** 251 (1969).
26. N. GYRD-HANSEN, *Acta vet. scand.* **9,** 183 (1968).
27. N. GYRD-HANSEN, *Acta vet. scand.* **11,** 443 (1970).