BAGGOT, John Desmond, 1939-
A COMPARATIVE STUDY OF THE PHARMACOKINETICS AND Biotransformation of AMPHETAMINE.

The Ohio State University, Ph.D., 1971
Pharmacology

University Microfilms, A XEROX Company, Ann Arbor, Michigan
A COMPARATIVE STUDY OF THE PHARMACOKINETICS
AND BIOTRANSFORMATION OF AMPHETAMINE

DISSERTATION

Presented in Partial Fulfillment of the Requirement for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

John Desmond Baggot, B.Sc.,
M.V.B., M.V.M., M.R.C.V.S.

* * * * *

The Ohio State University
1971

Approved by
Lloyd E. Davis
Adviser
Department of Veterinary
Physiology and Pharmacology
ACKNOWLEDGMENTS

I am extremely grateful to Professor Lloyd E. Davis who was always prepared to discuss the progress of my work. Dr. Davis showed a very keen interest in the experimental observations and his constructive suggestions revealed his thorough understanding of comparative pharmacology and principles of physiology. Working with Dr. Davis was stimulating and the rapid progress of this research was largely due to his research experience and provision of experimental subjects and laboratory facilities. I must offer a special word of thanks to Drs. Philip W. Murdick, Richard S. Ray and James S. Noonan for their invaluable assistance with the analytical method for determination of amphetamine and the ready availability of the gas chromatography apparatus.

I wish to thank Dr. Thomas E. Powers for encouragement and general support during my memorable stay at The Ohio State University. His assistance to participate in the intensive course on the Uses of Radioisotopes in Medical Research at Oak Ridge Associated Universities is gratefully appreciated.

Mrs. Charlotte Mills and Miss Carol A. Neff deserve a great deal of praise for their technical assistance which was essential for successful progress. Mr. Walter Swanson was especially helpful with the collection of samples and the manipulation and restraint of animals.

Sincere thanks are due to Mrs. Karen Allen for the accuracy and high quality of her typing and for her complete
cooperation in drawing this work to a satisfactory conclusion. The unfailing assistance of Mrs. Lyn Brownlee in the Veterinary Medical Library was invaluable and gratefully appreciated.

Most sincere thanks are due to my wife Colette who supported my idea to pursue graduate studies in the United States. She provided encouragement and endured the trials and tribulations of this endeavor with unfailing understanding and assistance. My children, Siobhan and Jennifer, also made many sacrifices and I hope that I can repay them in the future.
VITA

October 11, 1939 . . . . Born - Abbyleix, Ireland
1962 . . . . . . . . . B. Sc., National University of Ireland, Dublin.
1966 . . . . . . . . M. V. B., National University of Ireland, Dublin.
1966 . . . . . . . . Member of the Royal College of Veterinary Surgeons, (London).
1966-1969 . . . . Lecturer in Pharmacology and Toxicology, College of Veterinary Medicine, Dublin.
1968 . . . . . . . . M. V. M., National University of Ireland, Dublin.
1969-1971 . . . . Teaching Associate, Department of Veterinary Physiology and Pharmacology, The Ohio State University, Columbus, Ohio.

PUBLICATIONS


FIELDS OF STUDY

Major Field: Pharmacology.

Comparative Pharmacology. Professor Lloyd E. Davis.

Pharmacokinetics. Professor Robert E. Notari.

Drug Absorption, Distribution, Metabolism and Excretion.
Professors Daniel Couri, Richard H. Reuning and Lloyd E. Davis.
Biometry. Professors Jean D. Hensel and Thomas E. Powers.

Radioisotopes in Medical Research. A.E.C., Oak Ridge
Associated Universities, Tenn.
# TABLE OF CONTENTS

ACKNOWLEDGMENTS ................................................. ii
VITA ........................................................................ iv
LIST OF TABLES ......................................................... viii
LIST OF FIGURES ......................................................... xi

Chapter

I. INTRODUCTION .................................................. 1

II. REVIEW OF THE LITERATURE ............................ 5

   The General Structure and Function of Cellular Membranes ........................................ 5
   The Movement of Molecules Across Cellular Membranes ........................................... 11
   The Passage of Drugs Across Various Body Membranes .......................................... 20
   Comparative Drug Metabolism .................................... 40
   The Elimination of Drugs ........................................ 49
   Amphetamine ....................................................... 61
       Physico-Chemical Properties .................................. 63
       Tissue Distribution of d-Amphetamine .................. 65
       Comparative Metabolism .................................... 66
   Factors Affecting the Rate of Disappearance of Amphetamine in Rats ...................... 71
   The Determination of Amphetamine .................................. 73
   The Philosophical Basis of Pharmacokinetics .............................................. 76

III. MATERIALS AND METHODS .................................. 79

   Experimental Procedure ......................................... 79
   Experimental Design and Animals Employed .............................................. 79
   Analytical Techniques ........................................... 83
   Determination of Amphetamine ................................... 83
   Reagents ............................................................. 84
   Analytical Procedure ............................................. 86
   Conditions for Gas-Liquid Chromatography .................................................. 88
   Determination of p-Hydroxyamphetamine .................................................. 90
   Estimation of p-Hydroxyamphetamine Conjugates ............................................. 91
   Plasma Protein Binding of $^3$H-d-Amphetamine ........................................... 92
   Sulphate .............................................................. 92
   Drug Concentrations Employed ........................................ 92
IV. RESULTS

Response of Electron Capture Detector to Amphetamine Derivative ........................................ 101
Plasma Amphetamine Concentration versus Time Profiles ........................................................ 107
Rate of Distribution of Amphetamine ......................................................... 114
Biological Half-life of Amphetamine in Various Species of Animals ...................... 117
Extent of Plasma Protein Binding .................................................................................... 120
Apparent Volume of Distribution and Pharmacokinetic Constants Describing Elimination of Amphetamine in Various Species of Animals .............................................. 120
Quantities of Amphetamine, p-Hydroxyamphetamine and Conjugates in Cumulative Urine of Different Species ............................................................................................................. 123
Extent of Plasma Protein Binding of Amphetamine in Dogs: in vivo and in vitro .... 138
Influence of Urinary pH upon Half-Life of Amphetamine in Dogs ................................................. 143
Cumulative Amounts of Amphetamine Excreted in Urine and Bile ......................... 143
Influence of Nephrectomy upon Half-Life of Amphetamine in Dogs ....................... 165
Clearance Values in Intact and Nephrectomized Dogs ................................................. 167
Apparent Volume of Distribution and Elimination of Amphetamine in Swine ................. 172
Renal and Biliary Excretion of Amphetamine in Swine ................................................. 175
Influence of Urinary pH upon Biological Half-Life of Amphetamine in Horses .......... 181
Diffusion of Amphetamine from Plasma into Rumen Liquor of Goats ................. 182
Statistical Analysis of Experimental Data ................................................................. 187

V. DISCUSSION ................................................................. 195

VI. SUMMARY AND CONCLUSIONS ............................................................ 220

BIBLIOGRAPHY ............................................................................... 225
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Partition Coefficients of d-Amphetamine.</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>Amphetamine Standard Solutions.</td>
<td>86</td>
</tr>
<tr>
<td>3</td>
<td>Conditions for Gas-Liquid Chromatography.</td>
<td>88</td>
</tr>
<tr>
<td>4</td>
<td>Biological Half-Life of Amphetamine in Various Species of Animals</td>
<td>118</td>
</tr>
<tr>
<td>5</td>
<td>Pharmacokinetic Constants Describing the Distribution and Elimination of Amphetamine in Various Species of Animals (1)</td>
<td>119</td>
</tr>
<tr>
<td>6</td>
<td>The Extent of Plasma Protein Binding of Amphetamine Under Conditions of Varying Drug Concentrations.</td>
<td>121</td>
</tr>
<tr>
<td>7</td>
<td>Total Plasma Protein Concentration and Extent of Protein Binding of Amphetamine in Various Species of Animals</td>
<td>122</td>
</tr>
<tr>
<td>8</td>
<td>Pharmacokinetic Constants Describing the Distribution and Elimination of Amphetamine in Various Species of Animals (2)</td>
<td>124</td>
</tr>
<tr>
<td>9</td>
<td>The Dose Administered and The Amounts of Amphetamine and Metabolites Recovered in 24-Hour Urine of Goats.</td>
<td>126</td>
</tr>
<tr>
<td>10</td>
<td>The Dose Administered and the Amounts of Amphetamine and Metabolites Recovered in 24-Hour Urine of Pigs.</td>
<td>127</td>
</tr>
<tr>
<td>11</td>
<td>The Dose Administered and the Amounts of Amphetamine and Metabolites Recovered in 24-Hour Urine of Rabbits.</td>
<td>128</td>
</tr>
<tr>
<td>12</td>
<td>Amounts of Amphetamine and Metabolites in Urine of Mares Following Administration of 180 mg Amphetamine Sulphate I, M.</td>
<td>129</td>
</tr>
<tr>
<td>13</td>
<td>The Dose Administered and the Amounts of Amphetamine and Metabolites Recovered in 24-Hour Urine of Ponies.</td>
<td>130</td>
</tr>
<tr>
<td>14</td>
<td>The Dose Administered and the Amounts of Amphetamine and Metabolites Recovered in 24-Hour Urine of Chickens.</td>
<td>131</td>
</tr>
<tr>
<td>15</td>
<td>The Dose Administered and the Amounts of Amphetamine and Metabolites Recovered in 24-Hour Urine of Dogs.</td>
<td>132</td>
</tr>
<tr>
<td>16</td>
<td>The Dose Administered and the Amounts of Amphetamine and Metabolites Recovered in 48-Hour Urine of Cats.</td>
<td>133</td>
</tr>
<tr>
<td>17</td>
<td>Percent Dose of Amphetamine and Some of its Metabolites in 24-Hour Urine of Different Species.</td>
<td>134</td>
</tr>
</tbody>
</table>
Table

18 Pharmacokinetic Constants Distribution and Elimination of Amphetamine in Intact and Nephrectomized Dogs.
19 Extent Plasma Protein Binding of Amphetamine in Dogs.
20 Influence of Urinary pH upon the Half-Life and Excretion of Amphetamine in Dogs.
21 Half-Hourly Excretion of Amphetamine in Urine of a Dog (R).
22 Half-Hourly Excretion of Amphetamine in Urine of a Dog (T).
23 Half-Hourly Excretion of Amphetamine in Urine of a Dog (V).
24 Half-Hourly Excretion of Amphetamine in Urine of a Dog (X).
26 The Cumulative Amounts of Amphetamine and p-Hydroxyamphetamine Excreted in Urine of Dog R.
27 The Cumulative Amounts of Amphetamine and p-Hydroxyamphetamine Excreted in Urine of Dog T.
28 The Cumulative Amounts of Amphetamine and p-Hydroxyamphetamine Excreted in Urine of Dog V.
29 The Cumulative Amounts of Amphetamine and p-Hydroxyamphetamine Excreted in Urine of Dog X.
30 The Cumulative Amounts of Amphetamine and p-Hydroxyamphetamine Excreted in Urine of Dog Z.
31 Half-Hourly Excretion of Amphetamine in Bile of Dog R.
32 Half-Hourly Excretion of Amphetamine in Bile of Dog T.
33 Half-Hourly Excretion of Amphetamine in Bile of Dog V.
34 Half-Hourly Excretion of Amphetamine in Bile of Dog X.
35 Half-Hourly Excretion of Amphetamine in Bile of Dog Z.
36 Half-Hourly Excretion of Amphetamine in Bile of Nephrectomized Dog NF.
37 Half-Hourly Excretion of Amphetamine in Bile of Nephrectomized Dog NG.
38 Half-Hourly Excretion of Amphetamine in Bile of Nephrectomized Dog NL.
39 The Apparent Specific Volumes of Distribution and the Rate of Elimination of Amphetamine in Intact and Nephrectomized Dogs.
40 Clearance Values of Amphetamine in Intact and Nephrectomized Dogs.
41 Pharmacokinetic Constants Describing the Elimination of Amphetamine in Intact and Nephrectomized Dogs.
<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>42 Pharmacokinetic Constants Describing the Distribution and Elimination of Amphetamine in Swine.</td>
<td>174</td>
</tr>
<tr>
<td>43 The Cumulative Amounts of Amphetamine Excreted in Urine and Bile of Swine.</td>
<td>176</td>
</tr>
<tr>
<td>44 Mean Cumulative Amount of Amphetamine in Biological Fluids of Swine.</td>
<td>177</td>
</tr>
<tr>
<td>45 Mean Cumulative Amounts of Amphetamine and Some Metabolites in Urine of Swine.</td>
<td>179</td>
</tr>
<tr>
<td>46 The pH Reaction of Horse Urine.</td>
<td>183</td>
</tr>
<tr>
<td>47 Pharmacokinetic Constants Describing the Distribution and Elimination of Amphetamine in Horses Producing Urine of Different pH Values.</td>
<td>184</td>
</tr>
<tr>
<td>48 The Concentrations of Amphetamine in Plasma and Rumen Liquor of Goats Following I. V. Injection of the Drug.</td>
<td>186</td>
</tr>
<tr>
<td>49 Student's &quot;t&quot; Test Comparing Mean Values of the Biological Half-Life of Amphetamine in the Various Species of Animals Taken Two at a Time.</td>
<td>188</td>
</tr>
<tr>
<td>50 Student's &quot;t&quot; Test Comparing Mean Values of Extent of Plasma Protein Binding of Amphetamine at a Drug Concentration of 10^-6 Molar in Various Species of Animals taken Two at a Time.</td>
<td>189</td>
</tr>
<tr>
<td>51 Student's &quot;t&quot; Test Comparing Mean Values of B^* for the Various Species of Animals taken Two at a Time.</td>
<td>190</td>
</tr>
<tr>
<td>52 ANOVA Table - Extent of Plasma Protein Binding of Amphetamine.</td>
<td>192</td>
</tr>
<tr>
<td>53 ANOVA Table - Y-axis Intercept Values Corrected For Extent of Plasma Protein Binding (B^*)</td>
<td>193</td>
</tr>
<tr>
<td>54 ANOVA Table - Biological Half-Life of Amphetamine</td>
<td>194</td>
</tr>
<tr>
<td>55 The Overall Elimination Rate Constant of Amphetamine in Various Species of Animals.</td>
<td>198</td>
</tr>
<tr>
<td>56 The Biological Half-Life of Amphetamine in Various Species of Animals.</td>
<td>200</td>
</tr>
<tr>
<td>57 Classification of Animal Species on the Basis of Magnitude of k_excretion.</td>
<td>202</td>
</tr>
<tr>
<td>58 Protein Binding of Amphetamine in Dogs: in vitro versus in vivo.</td>
<td>210</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Routes of Drug Metabolism.</td>
<td>43</td>
</tr>
<tr>
<td>2</td>
<td>Response of Electron Capture Detector to Standard Solutions of Amphetamine.</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>Linear Response of Electron Capture Detector to Standard Solutions of Amphetamine.</td>
<td>102</td>
</tr>
<tr>
<td>4</td>
<td>Consistent Response of Electron Capture Detector to a Number of Similar TCAA Injections.</td>
<td>103</td>
</tr>
<tr>
<td>5</td>
<td>Response of Electron Capture Detector to TCAA Prepared from Plasma Extracts.</td>
<td>104</td>
</tr>
<tr>
<td>6</td>
<td>Response of Electron Capture Detector to TCAA Prepared from Plasma Extracts.</td>
<td>105</td>
</tr>
<tr>
<td>7</td>
<td>Plasma Amphetamine Concentration versus Time Profile in Goats.</td>
<td>107</td>
</tr>
<tr>
<td>8</td>
<td>Plasma Amphetamine Concentration versus Time Profile in Swine.</td>
<td>108</td>
</tr>
<tr>
<td>9</td>
<td>Plasma Amphetamine Concentration versus Time Profile in Ponies.</td>
<td>109</td>
</tr>
<tr>
<td>10</td>
<td>Plasma Amphetamine Concentration versus Time Profile in Dogs.</td>
<td>110</td>
</tr>
<tr>
<td>11</td>
<td>Plasma Amphetamine Concentration versus Time Profile in Cats.</td>
<td>111</td>
</tr>
<tr>
<td>12</td>
<td>Plasma Amphetamine Concentration versus Time Profile in Rabbits.</td>
<td>112</td>
</tr>
<tr>
<td>13</td>
<td>Plasma Amphetamine Concentration versus Time Profile in Chickens.</td>
<td>113</td>
</tr>
<tr>
<td>14</td>
<td>Disappearance of Amphetamine from the Blood Plasma of the Feline, Canine, Avian, Lapine, Equine (Pony), Porcine and Caprine Species following the I, V, Injection of Amphetamine Sulphate (0.66 mg/kg, calculated as free base).</td>
<td>115</td>
</tr>
<tr>
<td>15</td>
<td>The Body: A Two Compartment Open Model</td>
<td>116</td>
</tr>
<tr>
<td>16</td>
<td>The Percent of Dose of Amphetamine, p-Hydroxy-amphetamine and Conjugates Recovered in Cumulative Urine of Different Species.</td>
<td>135</td>
</tr>
<tr>
<td>17</td>
<td>The Total Recovery of Amphetamine, p-Hydroxy-amphetamine and Conjugates in Cumulative Urine of Different Species.</td>
<td>136</td>
</tr>
<tr>
<td>18</td>
<td>Plasma Amphetamine Concentration versus Time Profiles in Intact and Nephrectomized Dogs.</td>
<td>139</td>
</tr>
<tr>
<td>19</td>
<td>Response of Electron Capture Detector to TCAA Prepared from Extracts of Plasma, Cerebrospinal and Ocular Fluids.</td>
<td>141</td>
</tr>
<tr>
<td>20</td>
<td>Response of Electron Capture Detector to TCAA Prepared from Extracts of Dog Urine.</td>
<td>145</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>21</td>
<td>Mean Cumulative Appearances of Amphetamine in Urine and Bile, and p-Hydroxyamphetamine in Urine of Dogs.</td>
<td>164</td>
</tr>
<tr>
<td>22</td>
<td>Mean Clearance Values in Intact and Nephrectomized Dogs.</td>
<td>169</td>
</tr>
<tr>
<td>23</td>
<td>Plasma Amphetamine Concentration versus Time Profile in Swine.</td>
<td>173</td>
</tr>
<tr>
<td>24</td>
<td>Mean Cumulative Appearance of Amphetamine in Urine and Bile of Swine.</td>
<td>178</td>
</tr>
<tr>
<td>25</td>
<td>Mean Cumulative Appearances of Amphetamine, p-Hydroxyamphetamine and the Glucuronide Conjugate in Urine of Swine.</td>
<td>180</td>
</tr>
</tbody>
</table>
Amphetamine is a unique drug with respect to the simplicity of its structure and the multiplicity of its biological effects. The pKa value of this organic base is 9.90 (Lewis, 1954). This implies that at the physiological pH only a small fraction is in the neutral or nonionized form. The neutral form is reasonably soluble in various organic solvents whereas the ionized form is water-soluble. The rate of elimination is frequently expressed as the biological half-life which, in turn, is determined by the apparent specific volume of distribution and the sum of the metabolic and the excretion clearance constants:

\[
t_\frac{1}{2} = \frac{0.693 \times V'd}{C'_\text{metabolism} + C'_\text{excretion}}
\]

The clearance constants have the dimensions of millilitre per minute or litre per hour and are dependent on the relative extent of biotransformation and excretion, principally renal, and the physicochemical properties (pKa, partition coefficient) of the drug. The hypothetical volume is also dependent on the properties of the drug as well as those of the body (tissue binding, compartments, that is, kinetically distinguishable pools in terms of the drug concentration-time profile). Curves relating the time-course of plasma amphetamine concentration were not available.
so it was unknown whether elimination was a simple first order process or multiphasic process.

Davson and Danielli (1954) called attention to the hypothesis that certain compounds may penetrate membranes in their non-ionized lipid-soluble state. Somewhat later, a number of workers adduced evidence with specific compounds in support of this concept. It was shown, for example, that certain weak organic acids were excreted in sweat (Thaysen and Schwartz, 1953), saliva (Killman and Thaysen, 1955; Rasmussen 1964), other secretions of the alimentary tract (Shore, Brodie and Hogben, 1957), ocular fluid (Davson and Matchett, 1953), cerebrospinal fluid (Rall, Stabenau and Zubrod, 1959), the mammary gland (Rasmussen, 1958; Stowe and Sisodia, 1963), the renal tubule (Milne, Scribner and Crawford, 1958) and the capillary wall (Renkin, 1952) in accord with their lipid solubility.

The physicochemical factors which govern the passage of drugs across biological membranes have been well delineated (Brodie and Hogben, 1957; Brodie, 1964), and the roles which these intrinsic properties play in the diverse ways in which solutes move across membranes have been extensively reviewed by Schanker (1962, 1964).

Urinary pH was an important factor in determining the rate of excretion of amphetamine in man (Beckett, Rowland and Turner, 1965; Asatoor, Galman, Johnson and Milne, 1965; Beckett and Rowland, 1965; Smart and Turner, 1966). Acidification of the urine increased the rate of excretion of unchanged
drug by decreasing the amount of the undissociated form available for tubular reabsorption.

Drug metabolism is a direct reflection of the multiple enzyme systems which characterise different animal species and is usually the most important single factor in the regulation of plasma drug levels. The liver is the principal organ involved in the metabolism of drugs, although other organs may participate in varying degrees. The drug metabolising enzyme systems are associated mainly with the smooth microsomes of the endoplasmic reticulum (Fouts, 1961). The hepatic microsomal enzyme system metabolises only lipid-soluble drugs. Qualitatively, the metabolic degradation of drugs is similar in man and animals. Despite some exceptions, the main difference among species probably lies in the rates of drug transformation.

Amphetamine undergoes a complex metabolic fate, it markedly alters the physiologic disposition of norepinephrine and it causes pronounced pharmacological and behavioural effects.

In order to obtain valid data, one must be able to measure accurately drug concentrations in biological fluids. The method of determination must be specific, that is, capable of distinguishing the drug from any of its metabolic products. The extent of binding to plasma proteins must be ascertained, because the rate of diffusion across a biological membrane is proportional to the free drug concentration in plasma.

It will be at once admitted that the medical practitioner ought to be acquainted with the strength of the various compounds which he applies as remedial agents, and that he ought, if possible, be able to regulate their potency. John Snow, 1847.
The objective of this work was to describe the pharmacokinetics of amphetamine in various species of animals and to elucidate the principal factors which govern the rate of elimination of this drug from the body. An attempt was made to divide the different species of animals into groups on the basis of biotransformation patterns and pharmacokinetic parameters. Above all it was hoped that this basic work will provide a stimulus towards the advancement of the comparative pharmacokinetic approach in describing more accurately the complex elimination processes of our domesticated animals.
CHAPTER II

REVIEW OF THE LITERATURE

A. THE GENERAL STRUCTURE AND FUNCTION
OF CELLULAR MEMBRANES

The Structure and Function of the Cell Membrane

Cell membranes constitute the most prominent ultrastructural features of the animal cell; they may comprise up to eighty per cent of its dry mass (O'Brien, 1967), with turnover rates as high as one hundred percent each hour (Gross, 1967).

The function of the plasma (external) membrane is to interpose a boundary between the cell and its environment, and to create and maintain the interior environment of the cell by the active transport of ions and nutrients. In certain tissues, for example, of the intestinal mucosa and renal tubules, this function is adapted to transport substances across cellular barriers in the interests of maintaining the internal environment of the organism as a whole. Recent reviews and discussions of the current concepts of cell membrane structure have been presented by Danielli (1958), Sjostrand (1963), Robertson (1964, 1966), Korn (1966), Schoffeniels (1967), Stein (1967), Wallach and Gordon (1968), Dowben (1969), and others.

A great deal was known about the structure and functions of cell membranes before the advent of electron microscopy (for
review see Hofer, 1945). It was known from the work of Overton in Zurich at the end of the last century that lipid-soluble substances penetrated readily into cells, suggesting that the limiting membrane was itself lipid in character. Gorter and Grendel (1925) estimated the thickness of the erythrocyte membrane by extracting lipids from erythrocyte ghosts and spreading them on water. The area of a coherent unimolecular film proved to be twice as large as the total surface area of the erythrocyte from which it was derived; this suggested that the lipid barrier consisted essentially of a lipid bilayer about 40 Å thick. The study of unimolecular films at air-water interfaces, largely initiated by Langmuir (1917) and developed by Adam (1938) provided a clear picture of the structure of such films, and it was realised that the phospholipids and cholesterol which are the principal lipids of erythrocyte ghosts would arrange themselves in a compact film with their polar groups submerged in the water and the non-polar hydrophobic chain projecting upward into the air. A doubling of such a layer, in which the non-polar portions of the lipid molecules were directed inwards and the polar groups were directed outwards, with the addition of adsorbed monolayers of hydrophilic protein, led to the well-known lipid-bilayer membrane model of Danielli and Davson (1935). The inclusion of protein was required to account for the mechanical strength of the membrane and for its very low interfacial tension compared with that of artificial lipid surfaces.
Although the plasma membrane was too thin to be observed directly in the light microscope, visual evidence for its existence was obtained. In the elegant experiments of Chambers (1922), naked star-fish eggs stained with neutral red, were placed in isotonic ammonium chloride solution. The cytoplasm rapidly gave an alkaline reaction due to the selective penetration of non-ionized ammonia formed by the hydrolysis of part of the ammonium chloride. If, on the other hand, ammonium chloride solution was injected into the cell by means of a micropipette, the whole cytoplasm rapidly became acidic as a result of the selective diffusion of ammonia out of the cell. Similar experiments were carried out using sodium bicarbonate solutions, except that the migrating species is now carbon dioxide, and the colour changes are reversed.

The universality of the membrane concept depends to a considerable extent upon direct observations made with the electron microscope. The cell membrane appears as a thin band approximately 75-100 Å thick, consisting of two dark lines, each about 25-30 Å thick on either side of a light zone. This ubiquitous trilaminar structure, commonly referred to as the "unit membrane", has been taken as strong support for the Davson-Danielli model. Unit membranes occur not only at the cell surface but also in the interior of the cells as part of the endoplasmic reticulum and at the boundaries of intracellular organelles.

Robertson (1957) proposed that the membrane had structural polarity, that is, that the inside and outside surfaces were different.
It was conjectured that the outer surface is covered with mucoprotein while unconjugated protein covers the internal surface of the membrane.

The paucimolecular concept of membrane structure envisions the protein layers as existing in the extended or $\beta$-conformation. This arrangement tends to minimize nonpolar or hydrophobic interactions between the protein and lipid. Recent studies using infrared absorption (Maddy and Malcolm, 1966) and optical rotatory dispersion (Wallach and Zahler, 1966) indicated that very little membrane protein was in the extended $\beta$-configuration but that it was largely randomly coiled with a significant percentage in the $\alpha$-helical form.

Until about 1962, the bimolecular leaflet structure and the Danielli model were considered to be virtually universal for biological membranes. We know quite definitely that with certain small but vital modifications the lipid bilayer can account for several, including some quite specific properties of cell membranes. At the present time, however, the general applicability of this concept is regarded as unacceptable by some workers.

In the simplest terms, a micelle is a colloidal aggregate of molecules: a bimolecular leaflet may therefore be regarded as a laminar micelle that is of indefinite extent in two dimensions (Alexander and Johnson, 1949). Micellar organization in relation to membranes, however, is usually understood to mean the presence of an organisation that is different from the bimolecular leaflet structure. In the micellar model for natural membranes proposed
by Lucy (1964), the lipids were arranged in a manner resembling
the organisation of molecules of a colloidal electrolyte in a
spherical micelle. An essential feature of the theoretical
micellar model for the lipids of biological membrane (Lucy, 1964)
was that, within any membrane, globular micelles of lipid can be
in dynamic equilibrium with the bimolecular leaflet structure.
The mechanical stability of micellar areas of membrane may
depend on a number of different factors, such as hydrogen bonds
and electrostatic interactions between adjacent micelles, and also
on interactions of various kinds with the protein, or with glyco-
protein molecules, on the surface of the lipid layer. Aqueous
pores are an inherent feature of the micellar organisation al-
though such a micellar membrane is nevertheless essentially lipid
in character. One or more lipid micelles in an area of micellar
membrane may be replaced by globular protein molecules, and
it has been suggested that in this way enzymes may be an integral
part of the structure of a membrane.

Several electron microscopists have observed repeating
subunits in various membrane preparations, particularly in mito-
chondrial membranes and in chloroplast membranes (Fernandez-
Moran et al., 1964; Fernandez-Moran, 1962; Robertson, 1963;
Sjostrand, 1963; Stoeckenius, 1963). The subunits appear as
regular arrays of beads or transverse densities in the trilaminar
structure with a unit repeat of approximately 40-60 Å (Robertson,
1966).
Recently, Benedetti and Emmelot (1965) examined negatively stained preparations of rat liver membranes in which the staining procedure was carried out at $37^\circ$ and observed an array of hexagons with occasional pentagons about 80-90 Å apart, in addition to the 50-60 Å globular knobs scattered over the membrane surface. The hexagonal patterns were not observed in membranes prepared at low temperatures. Electron micrographs of mixed lipid films also show a similar hexagonal pattern of ultrastructure (Bangham and Horne, 1962; Lucy and Glauert, 1964). Evidence for a phase transition from a lamellar structure to inverted cylindrical micelles was obtained with model phospholipid systems examined by X-ray diffraction (Luzatti and Husson, 1962) or electron microscopy (Stoeckenius, 1962). These observations bring to mind the suggestion by Hechter (1965) that the membrane is composed of structural units in the form of hexagons with a few pentagons to permit three-dimensional flexibility, the arrangement showing icosahedral symmetry.

The observation of subunits prompted Green (1966) to put forward the proposal that biological membranes are always formed by the association of repeating proteolipid structural units. According to this view, each membrane has a specific repeating unit which is characteristic in form, size, and composition. The repeating units always contain a membrane-forming portion; at times there may also be a detachable portion. The detachable portions are an intrinsic part of the membrane, but they are not essential for the integrity of the membrane structure.
An emerging view of membranes regards the structural arrangement of the constituents as dynamic rather than static. The membranes are conceived to be planar aggregates of micellar subunits which are neither constant in their physical dimensions nor collectively arranged in a fixed array (Kavanau, 1965). Instead, the subunits are thought to undergo reversible structural changes possibly corresponding to phase transitions. The rapid turnover of membrane constituents which has been observed can be better reconciled with this view of the membrane than with earlier theories of its structure (Omura, Siekevitz and Palade, 1967). It may be that the membrane proteins are synthesized by polyribosomes which first form a lipid-free membrane, and that the phospholipids are subsequently synthesized in situ.

B. THE MOVEMENT OF MOLECULES ACROSS CELLULAR MEMBRANES

Modes of Transport Across Biological Membranes

The diverse phenomena responsible for the transfer of solutes across membranes and the factors which influence their passage have been reviewed in recent years by a number of investigators with different basic interests. Included amongst these are the following: Brodie and Hogben (1957), Berliner (1959), Wilbrandt and Rosenberg (1961), Wilson (1962), Schanker (1962, 1964), Ussing (1963), Brodie (1964), Levine and Pelikan (1964), Doluisio and Swintosky (1965), Stein (1967), Schoffeniels (1967), Curran and Schultz (1968), Schultz (1969), and others.
A membrane can play any of several roles during the process of transfer of a solute. These, however, may be grouped into general categories, namely,

(A) Passive transfer—in which the membrane behaves as an inert, lipoid-pore boundary and solutes traverse this barrier either by diffusing through the lipoprotein regions or by passing through the aqueous pores.

(B) Specialized transfer—in which the membrane makes an active contribution to the transfer of the solute and this transport occurs in a manner which cannot be simply ascribed to the structure or physical properties of the membrane.

**Passive Transfer Processes**

1. **Simple Diffusion.** In passive diffusion the force moving a solute across a membrane is the concentration gradient of the solute and the rate of passage is a linear function of this concentration difference within the limits of the integrity of the membrane. The rate is determined by physicochemical properties of both the solute and the membrane. During passive diffusion cellular energy is utilized only to maintain membrane structure. The linear relationship between rate of transfer and concentration gradient, while consistent with diffusion, does not exclude transport by enzymatic mechanisms or by a system involving an adsorption reaction (Wilson, 1962). It is worthy of note at this
point that simple diffusion does not occur against a concentration gradient, is not competitively inhibited, is not directly dependent on metabolic processes and occurs in both directions across a membrane.

Some substances penetrate the membrane as though it were a layer of lipoid material, the speed of penetration being determined by the lipid-to-water partition coefficient of the substance (Collander, 1947; Hober, 1945; Schanker, 1960). The best correlations have been obtained with compounds belonging to a homologous series (Schanker, 1962). The partition coefficient is the ratio of concentration in lipid phase to concentration in aqueous phase when a substance is allowed to come to equilibrium in a two-phase system. The conditions of measurement (e.g., temperature, pH) must be specified. Most agents of pharmacological interest are weak organic electrolytes. When a nonionized substance attains a steady-state distribution across a membrane, its concentration is the same on both sides. In contrast, a partly ionized substance may distribute itself unequally, either because of a Donnan type of ionic distribution or because of a difference in hydrogen-ion concentration on the two sides of the membrane. A Donnan distribution occurs whenever the solution on one side contains some ions that cannot cross to the other side. A difference in pH on the two sides of a membrane affects the distribution of a partly ionized substance because of the preferential permeability of membranes to the lipid-soluble nonionized form of the compound. Thus
under such conditions, at the steady-state, the concentration of the nonionized solute is the same on both sides of the membrane but the concentrations of the ionized form are unequal. Accordingly, the total concentration of the solute (ionized plus non-ionized) on both sides of the membrane is a function of the pH of the two fluids and the dissociation constant of the solute. This steady-state distribution is expressed in the following equations (Jacobs, 1940):

for a weak acid,

\[
\frac{C_1}{C_2} = \frac{1 + 10^{(\text{pH}_1 - \text{pKa})}}{1 + 10^{(\text{pH}_2 - \text{pKa})}}
\]

and for a weak base,

\[
\frac{C_1}{C_2} = \frac{1 + 10^{(\text{pKa} - \text{pH}_1)}}{1 + 10^{(\text{pKa} - \text{pH}_2)}}
\]

where \( C_1 \) and \( C_2 \) are the concentrations of solute in the two fluids, and \( \text{pKa} \) is the negative logarithm of the acidic dissociation constant of the weak acid or base. It is important to note that a pH differential as small as 0.1 unit, such as that existing between plasma and cerebrospinal fluid, can significantly affect the distribution of drugs between the two fluids (Albert, 1960). Relatively small pH differences on the two sides of a membrane may appreciably influence the distribution ratio through an ion-trapping effect. There is some hydrogen-ion gradient across most cell membranes, the intracellular pH tending to be lower than that of the extracellular phase. It is,
however, easier to investigate systems where the cells separate two fluid phases of widely different pH. This occurs in the gastric and abomasal mucosae, pancreatic acini, mammary and salivary glands and the renal tubules.

For weak acids and bases the ionized and nonionized forms have completely different lipid/water partition coefficients. The ionized groupings interact strongly with water dipoles and consequently penetrate only poorly or not at all into the lipoidal cell membranes. Thus, drugs that are partially ionized at body pH enter cells at rates that are strongly pH dependent. For all practical purposes the diffusion rate can usually be ascribed to the concentration gradient for the nonionized form alone.

2. **Filtration.** When water flows, in bulk, across a porous membrane, any solute that is small enough to pass through the pores flows with it. For example, the water that filters across the glomerular membrane is accompanied by all of the solutes of plasma except the large protein molecules. Since filtration (hydrodynamic flow) occurs as a result of a hydrostatic or osmotic pressure difference across a membrane, the flow of the water and its solutes is passive in nature.

**Specialised Transport Processes**

1. **Carrier Transport.** The concept of membrane carriers has arisen as a tentative explanation for the peculiar permeability of cell membranes to certain lipid-insoluble solutes. Carriers
are pictured as membrane components capable of forming a
complex with the solute (substrate) at one surface of the mem-
brane; the complex moves across the membrane, the substrate
is released, and the carrier then returns to the original sur-
face. Diffusion can provide the means for cycling (or shuttling)
the carrier across the membrane. There are at least three
types of carrier transport:

(a) active transport

(b) facilitated diffusion (Danielli, 1954)

(c) exchange diffusion (Ussing, 1947)

(a) **Active transport.** The term "active transport" has often
been used to designate any process that appeared to be inconsis-
tent with the laws of simple diffusion. However, in recent
years an attempt was made to restrict the term to those
transfer processes, mediated by a carrier, in which a solute
is moved from a phase of lower to a phase of higher electro-
chemical potential and cellular metabolism supplies the
necessary chemical energy for the work to be done. Moreover
there must be a direct quantitative relationship between the
energy supplied and the transport work performed (Wilson,
1962).

This form of membrane transport is used to designate
processes having the following characteristics.

(i) The solute moves across the membrane against a
concentration gradient or if the solute is an ion against
an electrochemical potential gradient. For this
transport metabolic energy has to be provided. During the active transport process the free energy ($G$) of the transported substrate increases at the expense of metabolic energy produced by the cell. The free energy, or the energy capable of doing work, is related to the enthalpy of the system and the entropy in the following manner:

$$G = H - T \cdot S$$

The change in free energy is expressed as

$$dG = dH - T \cdot dS$$

where $H$ is the enthalpy (heat content) of the system, $S$ the entropy and $T$ is the absolute temperature ($T = ^\circ C + 273$).

(ii) The transport mechanism becomes saturated when the concentration of solute is raised high enough.

(iii) The process shows specificity for a particular type of chemical structure.

(iv) If two substances are transported by the same mechanism, one will competitively inhibit the transport of the other.

(v) The transport mechanism is inhibited noncompetitively by substances which interfere with cell metabolism.

Transport processes with these characteristics have been demonstrated most convincingly in vitro using cellular membranes like frog skin or intestinal wall bathed on both sides by simple aqueous solutions. A more difficult task is demonstration of
active transport in the intact animal. Since the bloodstream and interstitial fluid usually represent the solution on one side of a body membrane, the substrate concentration is difficult to control because of the variables of metabolism, renal excretion, and binding to blood and tissue proteins. Moreover, if intracellular fluid represents the solution on the other side of the membrane, the difficulties are even greater because of the problem of distinguishing freely diffusible substrate from bound substrate inside a cell.

(b) Facilitated diffusion. The term "facilitated diffusion" was coined to describe a carrier-mediated transport system in which the rate of attainment of diffusion equilibrium is greatly accelerated although no direct expenditure of energy is required (Stein, 1967). The fundamental distinction between this process and active transport is the lack of any movement against a concentration or electrochemical gradient. Facilitated diffusion, however, does have some characteristics similar to those of active transport such as specificity, saturability, competitive inhibition by related compounds and a sensitivity to certain metabolic inhibitors (Le Fevre, 1961).

The rate of transfer in this case is, therefore, determined by the properties of the solutes and carrier, and the rate limiting step will be the availability of the carrier. Energy is utilised only to maintain cellular organisation.

(c) Exchange diffusion. In this process a carrier is thought to transport the substrate from one surface of the membrane to
the opposite surface, where it releases the substrate, picks up another molecule of substrate, and transports it to the original surface (Ussing, 1947). Exchange diffusion is thought to occur in carrier mediated transport processes which are near saturation (Wilbrandt and Rosenberg, 1961). There is experimental evidence that chloride ions may cross the isolated frog large intestine in this manner (Cooperstein and Hogben, 1959).

2. Facilitated diffusion not associated with membrane carriers.
Transport processes which apparently are not dependent upon carrier mediated transfer systems but which do result in accelerated passage of certain lipid-insoluble substances across membranes have been suggested. Stein and Danielli (1956) proposed that "polar pores" might exist in lipid membranes. They envisioned these pores as being constituted by hydrogen-bonding structures which would allow the penetration of a polar compound provided that there was stereochemical correspondence. Such an arrangement would produce a transfer process which would have the characteristics of specificity, saturability and could be competitively inhibited.

Augmented diffusion. Detailed kinetic studies involving intestinal absorption of several therapeutic agents have produced results which are not entirely consistent with the concept of drug transfer by only passive diffusion (Levine and Pelikan, 1964). The compounds involved in these investigations were mostly quaternary ammonium compounds. The dose-effect and
time-effect curves were incompatible with transfer only by passive diffusion.

Augmentation of absorption by adjuvants, involving a transfer mechanism other than only passive diffusion, has also been demonstrated for some non-quaterrinary drugs. In the studies of Ragozzino and Malone (1964) the addition of adjuvants seemed to demonstrate that more than one mechanism of absorption of quinine and quinidine was available under some conditions. They found that the addition of thiourea or some thiourea derivatives increased the degree of absorption of quinine or quinidine at higher dose ranges.

Passive diffusion must play a role in the membrane transfer of all drugs in view of the properties of biological membranes but there seems to be evidence that somewhat obscure mechanisms could possibly modify such transfer processes.

3. Pinocytosis. Cells growing in tissue culture take up small droplets of the external medium by an engulfing or sucking-in process known as pinocytosis (Lewis, 1931, 1937; Holter, 1959). This process has been recognized in intestinal epithelial cells (Clark, 1959; Barrnett, 1959). Drugs with high molecular weights, or which exist in solution in molecular aggregates, may possibly be taken up by pinocytosis.

C. THE PASSAGE OF DRUGS ACROSS VARIOUS BODY MEMBRANES

Drug molecules are distributed throughout the body by means of the circulation of blood. Following intravenous
administration a drug is rapidly diluted into the total blood volume as a result of turbulent mixing and of unequal flow rates through the various vascular beds. The rate of transfer of drug molecules from the blood stream into the tissues, by diffusion across capillary membranes, depends upon the concentration gradient of free drug. Thus, plasma protein binding can slow the disappearance of drug from the circulation and also provide a reservoir of bound drug, which will replenish (by dissociation) some of the drug that is lost by metabolism and excretion. The apparent volume of distribution may be defined as that volume of body water which would be required to contain the amount of drug in the body if it were uniformly present in the same concentration in which it is in the blood. It is called "apparent" since it is recognized that all compartments in which the drug is distributed may not have the same concentration. A drug which is distributed beyond the bloodstream and throughout the tissues may have a blood level curve that is characteristic of either a one-compartment or a two-compartment open model.

1. Blood Capillary And Blood

The rate of entry of a drug into the various tissues of the body depends upon the relative rates of blood flow through the respective capillary beds and the permeability of the capillaries for the particular drug molecules. Numerous investigations of capillary permeability suggest that solutes traverse the capillary wall by a combination of two processes, diffusion and filtration
(see reviews Pappenheimer, 1953; Renkin and Pappenheimer, 1957; Schou, 1961). Diffusion is the predominant mode of transfer for lipid-soluble molecules as well as for small, lipid-insoluble molecules and ions. Filtration (hydrodynamic flow), on the other hand, predominates for large, lipid-insoluble molecules whose rates of diffusion across the capillary endothelium are relatively slow.

Studies with a variety of organic compounds indicate that the capillary wall behaves as a lipoid-pore membrane, lipid-soluble substances penetrating readily at rates roughly parallel to their oil-to-water partition coefficients, and lipid-insoluble substances penetrating less readily at rates inversely related to their molecular sizes. While the latter substances appear to pass through aqueous pores whose total cross sectional area comprises less than 0.2 per cent of the capillary surface, lipid-soluble substances appear to penetrate through the entire surface (Pappenheimer et al., 1951; Renkin, 1952, 1953). Curiously, however, no pores have thus far been observed in electron micrographs of mammalian capillaries, although the resolving power of the electron microscope is much better than the postulated pore size (30 Å in radius). But despite the faster rates of transfer of lipid-soluble substances, it should be emphasized that all substances, whether lipid-soluble or not, cross the capillary wall at rates which are extremely rapid in comparison with their rates of passage across other body membranes. In fact, the supply of most drugs to the various tissues is limited by the rate of blood flow rather than by the restraint imposed by the capillary wall. It follows from all of
the above that the rate at which a drug leaves the blood stream will depend upon its lipid solubility, its molecular weight, and its physical state of aggregation. If it is bound to macromolecules, then its rate of passage out of the capillary will be determined by that of the protein or other substance to which it is bound.

Capillaries differ widely in their permeability characteristics; those of the glomeruli, for example, are very much more permeable to molecules of all sizes than are those of skeletal muscles. The sinusoidal capillaries of the liver appear to lack any endothelial wall and therefore permit the passage of large molecules quite readily. In the central nervous system the cerebral capillaries appear to impede markedly the passage of many solutes into the extracellular fluid of the brain. However, it is possibly not the capillary wall itself, but rather the surrounding layer of tightly packed glial cells which forms the so-called 'blood-brain barrier.'

The capillaries are not rigid tubes with invariant properties. They too are subject to the actions of drugs as well as to effects of tissue metabolites and hormones.

**Erythrocyte.** Most drugs are carried in the blood-stream in three forms: (i) as freely diffusible molecules dissolved in the plasma water; (ii) as molecules reversibly bound to proteins and other constituents of the plasma; and (iii) as free or bound molecules contained within the erythrocyte and other formed elements.

A variety of organic bases have been shown to penetrate the human red cell at rates related to their lipid-to-water partition
coefficients at pH 7.4 (Schanker et al., 1961). Moreover, the cell membrane exhibits a preferential permeability to the lipid-soluble, nonionized form of the compounds. Organic acids have been shown to penetrate the red cell at rates roughly parallel to their lipid solubilities; however, highly ionized acids of very low lipid solubility such as phenol red, sulphanilic acid and hippuric acid diffuse into the cell much more rapidly than do highly ionized basic compounds of a similar low lipid solubility (Schanker et al., 1964). Thus the entry of organic anions resembles that of inorganic anions, which also penetrate the erythrocytes at rates greatly exceeding those of cations. To explain the unusual permeability of the erythrocyte to anions, a number of workers have suggested that the cell is bounded by a lipid-like membrane which is perforated with positively charged aqueous channels of various diameters.

2. Central Nervous System

The question of how drugs exchange between the brain, blood and cerebrospinal fluid (CSF) has received a great deal of attention, especially in recent years with the increasing interest in drugs that affect behavior. The brain constitutes less than 2 per cent of the body weight, yet receives about 16 per cent of the cardiac output. One might expect that drugs would equilibrate very rapidly between blood and brain. And indeed some do, but many substances enter brain tissue only very slowly, and some practically not at all.
A drug may gain access to the tissues of the central nervous system by two distinct routes: the capillary circulation and the cerebrospinal fluid. The internal carotid and vertebral arteries come together at the base of the brain to form the circle of Willis, from which major vessels issue to supply each side of the brain, including the choroid plexuses of the lateral and third ventricles where the CSF is formed. Blood flow rates to various parts of the brain have been estimated by measuring the rate of transfer of radioactive krypton \(^{79}\text{Kr}\) from blood to tissues.

**Penetration of Drugs Into Brain and Cerebrospinal Fluid**

Under the title "blood-brain barrier" one is concerned with the exchanges of material between the blood plasma, on the one hand, and the parenchyma of the central nervous system on the other, that is, between the blood plasma and the white and grey matter of the brain and spinal cord. The cerebrospinal fluid is contained within the ventricles of the brain and in the subarachnoid space; thus one must also be concerned with the blood-cerebrospinal fluid barrier. So the "blood-brain barrier" implies the whole complex of diffusional relationships between blood, brain and cerebrospinal fluid, combined with secretory activity which may be limited to the choroid plexuses. The CSF is formed at the choroid plexus by unknown mechanisms that are thought to involve, at least in part, active transport. It flows through the ventriculocisternal system, bathes the surfaces of the brain.
and spinal cord, and then flows into the venous blood sinuses through a system of large channels and valves in the arachnoid villi (Welch and Friedman, 1960).

Studies with a wide array of drugs and other foreign compounds indicate that the blood-brain and blood-CSF barriers, like many other body membranes, exhibit the characteristics of a lipid-like boundary. For example, Mayer et al. (1959) have shown that a variety of compounds penetrate the brain and CSF of the rabbit at rates roughly parallel to their lipid-to-water partition coefficients as determined at pH 7.4. Similarly, Mark et al. (1957, 1958) have demonstrated a relation between the rate of entry into brain and the oil-to-water partition ratio at pH 7.4 of a number of barbiturates.

To evaluate the factors of lipid solubility and degree of ionization in the passage of drugs into cerebrospinal fluid, Brodie and co-workers (1960) investigated a number of compounds of diverse structures and physical properties. The plasma drug levels were held constant by means of a continuous intravenous infusion. The data were plotted on logarithmic ordinates as the difference between the plasma level and the CSF level, divided by the plasma levels at each sampling. The free drug concentrations in plasma water were measured. A penetration process that was truly exponential (as expected from the Fick equation) yielded a family of straight lines with slopes representing the penetration (permeability) rate constant for each drug. A comparison of the penetration rates with the lipid solubilities and degrees of
ionization of the drugs, has led to the following conclusions:

(i) Lipid solubility is the rate limiting factor with drugs that are mainly nonionized in plasma; these compounds penetrate the blood-CSF boundary at rates related to the lipid-to-water partition coefficient of the nonionized molecules.

(ii) The degree of ionization is the rate limiting factor with compounds that are highly ionized in plasma; these drugs enter the CSF at rates roughly parallel to the proportions of drug nonionized at pH 7.4.

(iii) Although both lipid solubility and the degree of ionization are important in governing the passage of drugs into CSF, lipid solubility is probably the dominant characteristic, since the relevance of the degree of ionization is probably a consequence of the poor lipid solubility of organic ions.

Direct evidence that the blood-brain and blood-CSF barriers are highly resistant to the entrance of foreign organic ions has been provided by studies of completely ionized substances such as quaternary ammonium compounds and sulphonic acids. These cations and anions penetrate the brain and CSF much more slowly than do lipid-soluble, nonionized drug molecules (Brodie et al., 1960; Hansson and Schmiterlow, 1961a, 1961b; Levine, 1959; Mayer and Bain, 1956; Rall and Zubrod, 1960).

Drugs become distributed between CSF and plasma according to their pKa value and the pH differential between the two fluids. For example, in the dog p-aminobenzoic acid and a number of sulphonamide compounds (65% ionized in plasma) attain CSF
concentrations that are roughly three fourths of their plasma concentrations (corrected for plasma binding), whereas a nonionized compound like antipyrine distributes evenly between the two fluids (Rall, et al., 1959). These results are exactly what would be predicted for a lipoid membrane separating a fluid of pH 7.3 (CSF) from a fluid of pH 7.4 (plasma). The same principles seem to apply to the distribution of phenobarbital between brain tissue and plasma (Waddell and Butler, 1957).

Although the blood-CSF and blood-brain barriers are qualitatively similar with regard to their permeability to drugs, and other foreign organic compounds, anatomically they are quite different. The blood-CSF barrier seems to consist mainly of the epithelium of the choroid plexuses, whereas the blood-brain barrier appears to be either the brain capillary wall or its surrounding layer of glial cells. The outstanding structural feature underlying the decreased permeability of capillaries in the central nervous system is the close application of the glial connective tissue cells (astrocytes) to the basement membrane of the capillary endothelium. Electron micrographs indicate that this glial sheath is about 85 per cent complete. A drug leaving the capillaries in the central nervous system has therefore to traverse not only the capillary endothelium itself but also the membrane of glial cells in order to gain access to the interstitial fluid. The "blood-brain barrier" really represents a quantitative rather than a qualitative difference in capillary permeability in this region as compared with other tissues. A given drug does not appear to
penetrate all regions of the brain tissue at the same rate. For example, phenobarbital and urea enter the white matter more slowly than the grey matter (Domek et al., 1960; Goldberg et al., 1961; Roth and Barlow, 1961).

Almost nothing is known about the localization of drugs in the brain. A compound that is not bound appreciably to body tissues appears to be distributed evenly throughout the gross areas of the brain (Mayer et al., 1959). On the other hand, most drugs are capable to binding to proteins and lipo-proteins, and would be expected to be distributed unevenly throughout the brain tissue (Roth and Barlow, 1961). From experiments in animals, evidence has accumulated that amphetamine does not distribute uniformly in various tissues. In the rat injected with amphetamine its brain/plasma concentration ratio was 7:8 over a great concentration range (Maickel et al., 1969). After i.p. doses of 0.25 to 4.0 mg/kg in rats, tissue/plasma ratios of d-amphetamine were found to be greater than 1 for all tissues except fat. Maximum levels in plasma and tissues were achieved in less than 30 minutes, and then declined along similar decay curves. Preliminary analysis of the data indicated that the decay curves were biphasic when analysed on semilogarithmic plots. For example, the first phase in plasma extended to about 90 minutes and had a half-life of about 30 minutes; the second phase extended to over 8 hours and had a half-life of about 74 minutes. Axelrod (1954) found the concentrations of amphetamine in plasma and cerebrospinal fluid of dogs to be the same, indicating that there was no hindrance to its
Passage of Drugs Out of the Cerebrospinal Fluid

Although drugs pass from blood into CSF at rates which parallel roughly their lipid solubility, they pass in the reverse direction at rates only partly dependent on this property. All drugs, regardless of molecular size or lipid solubility, would be expected to leave the CSF by a nonspecific process of filtration across the arachnoid villi. If a drug is lipid-soluble, it can also leave the CSF by diffusing across lipoid portions of the blood-CSF boundary, and by diffusing across the ependyma into the brain and thence across the blood-brain barrier. In addition, certain drugs may escape from the CSF by specialized, active transport processes. For example, phenol red and iodopyracet are actively transferred from CSF to blood in the vicinity of the fourth ventricle of the goat. The compounds are transferred against a concentration gradient, they compete with one another for transfer, and the process is saturable (Pappenheimer et al., 1961). A similar transport of phenol red occurs in the rabbit, but transport does not appear to be limited to the region of the fourth ventricle (Prockop et al., 1962). In addition to the above-mentioned anions, certain organic cations appear to be actively transported out of the CSF. For instance, N'-methylnicotinamide, hexamethonium and decamethonium all leave the CSF of rabbits much more rapidly than does inulin; furthermore, a large dose of N'-methylnicotinamide depresses markedly the rates of exit of the other two cations without
affecting the rate of exit of inulin (Shanker et al., 1962). The choroid plexus appears to be the site of cation transport (Lanman et al., 1963).

3. The Eye

Ocular Fluid. The detailed investigations of Davson (1955, 1956, 1960, 1953), Friedenwald (1949), and Kinsey (1950), concerning the transfer of solutes between the aqueous humor and blood, indicate that there are two main routes by which organic compounds penetrate into the ocular fluid:

1) through the epithelium of the ciliary body;

and

2) across the capillary walls and surrounding connective tissue of the iris.

Drug entrance by way of the ciliary body is thought to involve both diffusion and secretion. Thus, drugs diffuse from the blood capillaries into the epithelial cells of the ciliary body; since these cells secrete fluid and solutes (the primary aqueous humor) into the posterior chamber, the more rapidly a solute diffuses into the cells, the greater will be its concentration in the secretion. Of course the rate of fluid secretion becomes a rate-limiting factor with solutes that diffuse into the cells very rapidly.

Drug entrance by way of the iris is primarily a diffusion process. Solute diffuse out of the iris capillaries, pass either through or between the connective tissue cells of the iris, and enter the fluid of the anterior chamber. The intercellular route is probably unimportant except for lipid-insoluble solutes, which
cannot readily penetrate through the cells.

It is apparent from the relation between the lipid-solubility of drugs and their rate of passage into the ocular fluid that diffusion across all membranes is an important part of the transfer process. For example, a number of organic compounds, including ethanol, glycerol, sulphonamides, and thiourea derivatives, enter the aqueous humor of rabbits at rates roughly parallel to their lipid-to-water partition coefficients (Ross, 1951; Davson, 1955; Davson and Matchett, 1953). Moreover in the dog, thiopental, with its high lipid solubility, penetrates the ocular fluid much more rapidly than does the moderately lipid-soluble barbital (Dayton et al., 1961).

Drugs escape from the ocular fluid in several different ways. All solutes can leave via the drainage route of the ocular fluid, passing through the spaces of Fontana and the canal of Schlemm into the blood-stream (Davson, 1956, 1960). If a solute is lipid soluble, it can also leave the eye by diffusing across the lipoid boundary separating ocular fluid from blood. Active transport is a third mechanism of exit (Becker, 1960; Forbes and Becker, 1960). Evidence that the ciliary body is the site of transport has been provided by studies in vitro which show that this structure accumulates iodopyracet (Diodrast) by a process exhibiting all of the characteristics of active transfer (Becker, 1960).

It is interesting to compare the ocular fluid and the cerebrospinal fluid with regard to the entrance and exit of organic
substances. Both fluids are separated from blood by similar boundaries, which allow the ready passage of lipid-soluble compounds and impede the passage of lipid-insoluble compounds. In addition, the drainage structures for both fluids are permeable to virtually all solutes, whether lipid soluble or not, ocular fluid and its solutes flowing through the spaces of Fontana and canal of Schlemm into the blood-stream, and cerebrospinal fluid flowing across the arachnoid villi to reach the blood-stream. Finally there are specialized processes in both systems which actively transport certain organic anions into the blood.

Cornea. Many drugs traverse the cornea at rates related to their degree of ionization and lipid solubility. Cogan and Hirsch (1944) showed that an organic acid like salicylic acid penetrated the excised cornea of rabbits most readily from solutions of low pH value, while organic bases like aniline, atropine, ephedrine, and pilocarpine penetrated most readily from solutions of high pH value. Moreover, in the living animal, aniline passed from the corneal surface into the aqueous humor at rates directly related to the proportion of drug present as nonionized molecules.

The importance of lipid-solubility in determining the speed of penetration of drugs into the cornea has been demonstrated by Swan and White (1942) in experiments with a number of naphthalene derivatives. The lipoidal barrier of the cornea appeared to be located within the anterior epithelial layer since, in isolated corneas from which the epithelium had been removed, lipid-soluble
and lipid-insoluble compounds penetrated at almost identical rates (Coogan and Hirsch, 1944).

4. Salivary Glands

The transfer of drugs from the blood-stream into saliva seems to be determined mainly by three characteristics of the compounds: their molecular size, lipid solubility and degree of ionization. The importance of the size and lipid solubility of molecules is apparent from the pattern of salivary excretion of nonelectrolytes (Amberson and Hober, 1932; Burgen, 1956). Evidence that the parotid gland epithelium is almost impermeable to the ionized form of drugs has been provided by an investigation by Killmann and Thaysen (1955) of the salivary excretion of acidic compounds. The compounds were administered orally or by continuous intravenous infusion to human subjects, saliva was collected from the parotid duct, and plasma samples were obtained at various times. An almost completely ionized compound like p-aminohippuric acid appeared in saliva in very low concentration, a saliva-to-plasma concentration ratio of 0.015 was obtained. In contrast, a number of sulphonamide compounds, partly to completely nonionized in plasma, appeared in the acid saliva in relatively high concentrations; the saliva-to-plasma ratios, which ranged from 0.31 to 0.94, paralleled roughly the pKa values of the drugs so that the less ionized the compound, the more of it was found in the saliva. Rasmussen (1964) demonstrated that the concentration of various sulphonamides in the alkaline saliva of
cows and goats was higher than or equal to the plasma concentration. Alexander and Nicholson (1968) found that the saliva-barbiturate concentration in the horse was dependent on the concentration of the non-protein bound barbiturate in the blood and not on the concentration of the nonionized drug. This conclusion conflicts with the theories of other investigators (Thaysen and Schwartz, 1953; Killmann and Thaysen, 1955; Rasmussen, 1964), who found that it is the nonionized drug concentration in the plasma which governs the saliva concentration, but is readily explained by the equality in pH between horse plasma and saliva (Alexander, 1966).

5. Gastrointestinal Tract

The transfer of drugs and other foreign compounds across the gastrointestinal epithelium is for the most part explainable in terms of simple diffusion across a lipid-pore membrane. The transfer of certain inorganic ions, sugars, amino acids, pyrimidines, and other natural substrates, on the other hand, seems to involve specialized transport processes (Cooperstein and Brockman, 1959; Crane, 1960; Hogben, 1960; Schanker, 1961; Schanker and Tocco, 1960; Wilson et al., 1960).

Since amphetamine was administered intravenously in all experiments in this study to avoid the variable absorption rate, I shall review only the passage of drugs across the gastric mucosa and reticulo-ruminal epithelium.

Gastric Absorption of Organic Acids and Bases. Bradley et al.
found that salicylic acid and related salicylates were most readily absorbed from the dog's stomach when pH values were lowest. On the other hand, strychnine, an organic base, was shown by Travell (1940) to be more readily absorbed from alkaline solutions. This finding resulted in the formulation of the idea of the gastric mucosa as a barrier selectively permeable to the undissociated form of a drug. Studies of the gastric absorption of drugs in the rat (Schanker et al., 1957) and the human (Hogben et al., 1957) have shown that the epithelial lining of the stomach is permeable to the lipid-soluble, nonionized form of drugs and relatively impermeable to the ionized form.

Reticulo-Ruminal Absorption and Secretion of Drugs. When a drug is administered orally to a ruminant it passes initially into the rumen. The capacities of the adult reticulo-rumen are generally presented in standard texts (Martin and Schauder, 1938; Sisson and Grossman, 1953; Dukes, 1955, 1970) and representative figures given are 100-225 litres for cattle and 8-25 litres for sheep.

The reticulo-rumen biological barrier may be described as follows: A papillated non-glandular keratinized stratified squamous epithelium with a rich blood and lymphatic supply, histologically somewhat similar to mammalian skin in some respects but with a number of important differences especially the structural arrangement which would facilitate absorption from the rumen. Being a multilayered epithelium as compared with normal intestinal epithelium, this barrier then represents a composite series of cellular membranes with an additional proteinaceous layer of
keratin in the stratum corneum. However, these barriers may possibly be bypassed via intercellular spaces down to the basal layer. Furthermore the development, function and integrity of ruminal epithelium is very intimately associated with the physical form, quantity and quality of the feed ingested.

Despite the stratified squamous nature of its epithelial lining the rumen has been shown to be capable of a considerable absorptive capacity (Trautmann, 1933; Phillipson and McAnally, 1942; Rankin, 1942; McAnally and Phillipson, 1942, 1944; Barcroft, et al., 1944; Danielli et al., 1945; Gray, 1948; Masson and Phillipson, 1951; Sperber and Hyden, 1952; Phillipson, 1946, 1955; Tsuda, 1956; Austin, 1962, 1967; Jenkins, 1969). Ruminal contents are of a semisolid consistency and have an acidic reaction, pH 5.5-7.0 (Annison and Lewis, 1959). Weak organic acids with pKa values about the pH of rumen liquor would exist predominantly in the nonionized form under these conditions and should thus be available for absorption providing other factors such as binding onto protein material does not interfere with their free diffusion. Austin (1962, 1967), Corker (1966), Stowe (1967) and Jenkins (1969) have demonstrated that drug absorption from the rumen is indeed dependent on the pKa of the compounds studied and the pH of the fluids concerned, and have confirmed that it does appear to be the lipid-soluble, nonionized form of a drug which penetrates the ruminal epithelium by a process of simple diffusion. An additional important aspect of drug distribution in ruminant animals about which we still have very limited knowledge is the extent of passive
diffusion of drugs from plasma into the reticulo-rumen. Corker (1966) has observed the appearance of sulphanilamide, sulphamethazine, sulphapyridine, antipyrine and ephedrine in the rumen fluid following intravenous infusion of these compounds. Tetracycline and oxytetracycline were not detected in the ruminal fluid in these studies. Furthermore, Corker (1966) found that ephedrine attained concentrations which were several times greater than the corresponding plasma levels and this was highly suggestive of the ion-trapping mechanism which occurs when a pH difference exists between two body compartments (Schanker, 1962). Stowe (1967) has also recorded the sequestration of salicylate and benzoate in rumen fluid which was buffered to pH 7.6-8.0, and this author then noted the therapeutic significance of these findings, namely, (1) the dilution of the drug into an additional volume of water which would lower the blood and tissue levels more rapidly, (2) distribution into rumen water may result in a potential reservoir of drug which could be absorbed at a later time, and (3) the passage of antimicrobial agents from the blood into the ruminal contents may have a deleterious effect on the rumen microflora. Following a detailed study of the passage of some acids, bases and quaternary ammonium compounds across the ruminal epithelium Jenkins (1969) concluded that posology in ruminants presents great difficulties. The possible contribution of the reticulo-ruminal weight and volume to dosage rate and drug distribution respectively would have to be considered for each individual compound to achieve accuracy in establishing therapeutic plasma levels.
Gastric Secretion of Drugs. Cooke et al. (1941) determined the gastric juice/plasma concentration ratios for five sulphonamide compounds in dogs. Davenport (1942) concluded that the gastric secretion of sulphonamide drugs depends only upon their physico-chemical characteristics, such as their activities and diffusion constants, and not upon the nature of the specific secretory processes.

Several groups of workers (Dawson and Ivy, 1925; Kobayashi, 1926; Ingraham and Visscher, 1935; Visscher, 1942; Cambel et al., 1954) have studied by means of gastric pouches in dogs, the diffusion of intravenously injected dyes into gastric juice, and are in general agreement as to which are concentrated in the stomach. Only basic dyes diffused into the juice, whilst acidic dyes often could not be detected therein. Maximum concentration ratios of basic dyes between gastric juice and arterial plasma were of the order of forty times (Kobayashi, 1926; Ingraham and Visscher, 1935). In contrast, only acidic dyes diffused into alkaline pancreatic juice. Similar observations have been made with various drugs. The weak bases α-acetylmethadol (Sung and Way, 1954) and dromoran (Shore et al., 1955) were found to behave similarly to the basic dyes. The accumulation of weak bases in the stomach by ion trapping mimics a secretory process; if the drug is administered systemically it accumulates in the stomach. Dogs were given various drugs intravenously by continuous infusion to maintain a constant drug level in the plasma, and the gastric contents were sampled by means of an indwelling catheter (Shore et al.,
1957). The stronger bases (pKa > 5), e.g. quinine, levorphanol, tolazoline, accumulated in stomach contents to many times their plasma concentrations; the weak bases appeared in about equal concentrations in gastric juice and in plasma. Only the weakest acids appeared in detectable amounts in the stomach. The strong bases, which are completely ionized in gastric juice, and whose theoretical concentration ratios (gastric juice/plasma) are very large, nevertheless attained only about 40-fold excess over plasma. Direct measurements of arterial and venous blood showed that essentially all the blood flowing through the gastric mucosa was cleared of these drugs; obviously, no more drug could enter the gastric juice in a given time than was brought there by the circulation. Another limitation came into play when the base pKa exceeded 7.4; a major fraction of the circulating base was cationic and a decreasing fraction was nonionized, so that the effective concentration gradient for diffusion across the stomach wall was reduced.

D. COMPARATIVE DRUG METABOLISM

Evidence that species differences occur during the metabolism of drugs and other foreign compounds is to be found here and there in the older literature. For example, Jaffe in 1877 reported that, unlike other species, hens excreted benzoic acid as di-benzoylornithine (ornithuric acid) and not as hippuric acid, while nearly 40 years later Thierfelder and Sherwin in 1914 showed that in man phenylacetic acid was excreted as phenacetylglutamine and
not as phenaceturic acid. The importance of species differences in the mode and extent of the conjugation of foreign compounds was emphasized by Williams in 1938 who also listed a number of other factors which could be involved.

One of the most important factors regulating the response of an organism to a drug is the rate at which the drug is eliminated from the body. With respect to differences between species, Williams (1963) pointed out that many of the reactions which foreign compounds undergo in the common laboratory animals were found to occur also in man. There were exceptions to this, however, and they must be taken into account. 'The main difference between man and common laboratory animals probably lies in the rates of the reactions which transform drugs. These differences in rates may have profound effects on drug action' (Williams, 1963).

The most common way in which elimination of a drug is estimated is to measure its rate of disappearance from the circulating plasma. Drug plasma levels are the product of several interrelated mechanisms, the more important of which are:
(1) absorption from the site of administration, (2) distribution and storage of the drug in tissues, (3) excretion of the drug, and (4) metabolism of the drug by enzymes.

In some instances excretion of a drug by the kidneys may significantly influence the rate of drug elimination. Butler (1958) has estimated the half-lives of substances eliminated by the kidney based on their volume of distribution and clearance rates.
Drug metabolism is a direct reflection of the multiple enzyme systems which characterize different animal species and for many drugs is the most important single factor in the regulation of their concentrations in plasma. The liver is the principal organ involved in the metabolism of drugs, although other organs (intestines, kidneys) may participate in this function in varying degrees. The oxidative metabolism of many drugs is mediated by enzymes located in the microsomal fraction of mammalian liver. The hepatic microsomal fraction consists of subcellular components that are derived from the endoplasmic reticulum. The latter comprises two major components: a rough-surfaced form consisting of lipid tubules studded with small dense particles called ribosomes and a smooth-surfaced form devoid of ribosomes. The role of the drug metabolising enzymes has been studied intensively in recent years (Brodie et al., 1955, 1958; Fouts, 1962; Conney and Burns, 1962; Remmer and Merker, 1965; Fouts and Hart, 1965) and only some general comments on their significance will be made in the present discussion. The liver microsomal enzyme system metabolises only lipid-soluble compounds, the metabolites are almost invariably less lipid-soluble than the parent compound. These findings suggest that the microsomal oxidative systems are surrounded by a lipid barrier, penetrated only by fat-soluble substances.

Foreign compounds also may be metabolised by non-microsomal enzyme systems. These reactions include deamination of amines, oxidation of alcohols and aldehydes, reduction of aldehydes and ketones, and hydrolysis of some esters and amides.
These processes may occur in the mitochondria, lysosomes, the cell supernatant fraction, or in the circulating plasma. A thorough discussion of these non-microsomal mechanisms has been presented by Parke (1968). These reactions are confined to Phase I: oxidations, reductions, and hydrolyses.

**Qualitative Aspects of Comparative Drug Metabolism.** The important metabolic conversions of drugs fall into four reaction classes: oxidations, reductions, hydrolyses, and syntheses or conjugations. Gillette (1963) has presented a very thorough discussion of the individual mechanisms and their application to various classes of drugs. Williams (1959) has developed a convenient and useful way of looking at the problems of drug metabolism. This scheme is shown in diagrammatic form in Figure 1.

**Figure 1:** Routes of drug metabolism.
In this scheme, a foreign compound, A, may follow one of four main routes of metabolism to produce the product, C. In the first route (i), A, an inactivate compound, could be converted into an active compound, B, by a Phase I reaction (oxidation, reduction, or hydrolysis). Examples of this type of reaction are hydroxylation of acetalid, reduction of prontosil, or hydrolysis of cyclophosphamide. The compound, B, may be further metabolised by a Phase II reaction (conjugation) to form the inactive material, C, which is then excreted. In certain cases A may be a biologically active compound which is converted to B, which has a similar or different activity from A (Route ii). An example of this is the metabolic conversion of codeine to morphine. A can be regarded as a biologically active compound which is converted in a third route (iii) to an inactive metabolite, B'. B', in turn may be converted to C by conjugation mechanisms. In the fourth route (iv), A is an active or inactive compound which is directly conjugated by Phase II reactions to form the inactive C. There is a fifth possibility in which a lethal metabolite, C', could be derived from A, B, or B'. An example of this is the conversion of fluoroacetic acid to fluorocitric acid (Albert, 1960).

Some important generalizations may be derived from the scheme proposed by Williams: (a) phase I reactions may either activate or deactivate a foreign compound, (b) phase II reactions almost always deactivate, (c) phase I reactions modify the chemical structure of a foreign compound so that it can undergo phase II reactions, i.e., phase I reactions provide a functional group for
attack by phase II reactions. The latter are the classical de-
toxication mechanisms, and they involve a biosynthetic reaction
between the drug (or a metabolite of it) and an endogenous metabo-
lite which is usually a carbohydrate or an amino acid, or is
derived from these.

Although it has not yet proved possible to solubilize the
enzyme system located in the liver microsomal fractions (Imai and
Sato, 1960; Krisch, 1962), it is known that the enzyme activity is
associated with the so-called smooth-surfaced endoplasmic
reticulum (Fouts, 1961). The microsomal enzyme systems required
reduced nicotinamide-adenine dinucleotide phosphate (NADPH) and
molecular oxygen, and contained a haemoprotein, cytochrome
P-450, which was inhibited by carbon monoxide. Cytochrome P-450
functions as an oxygen-activating enzyme for drug oxidations
(Cooper et al., 1961; Omura et al., 1965). The relationship be-
tween NADPH oxidation and drug oxidation may be summarized
as follows:

1. \[
\text{NADPH} + P_{450} + H^+ \rightarrow \text{reduced P}_{450} + \text{NADP}^+ \\
\text{NADPH oxidase system}
\]

2. \[
\text{Reduced P}_{450} + O_2 \rightarrow P_{450}^O + H_2O \\
\text{(atmospheric)} \quad \text{("active oxygen")}
\]

3. \[
\text{Drug substrate} + P_{450} \rightarrow \text{oxidized drug} + P_{450} \\
\text{enzymes (microsomes)}
\]
The "active oxygen" produced in the reaction of reduced P-450 cytochrome with molecular oxygen, was thought (Brodie, Gillette and La Du, 1958; Gillette, 1963) to be a hydroxylating intermediate of some kind, possibly a peroxide.

The parallel rise and fall of cytochrome P-450 with drug metabolism observed by Remmer and Merker (1965) suggested that phenobarbital increased drug metabolism by increasing the amount of cytochrome P-450 in the microsomes. Perhaps the drug-induced synthesis of cytochrome P-450 in hepatic microsomes may be related to an effect of the drug on delta-aminolevulinic acid synthetase in the mitochondria.

Many other enzymes concerned with the metabolism of foreign compounds are also located in the hepatic endoplasmic reticulum, as, for example, the glucuronyl transferases, enzymes that catalyse the synthesis of glucuronic-acid conjugates. A phase II reaction (Parke and Williams, 1968, 1969).

The enzyme UDP transglucuronylase occurred in liver of man, pig, sheep, dog, rabbit, guinea pig, rat, mouse, pigeon, hen and frog (Dutton and Greig, 1957; Dutton, 1961), a sex difference was reported in the rat (Axelrod and Inscoe, 1959). The system may be present to a very limited extent in brown trout liver (Dutton and Montgomery, 1958), but was apparently absent from other fish and from tadpoles (Maickel, Jondorf and Brodie, 1958). Among mammals, the cat was an exception. Hartiala (1955) pointed out that cat liver slices form very little glucuronide, and this has been confirmed for the intact animal by others.
(Robinson and Williams, 1958; Borrell, 1959), the reason was not lack of uridine diphosphate glucuronic acid but of UDP trans-glucuronylase (Dutton and Greig, 1957).

Nature of Metabolites. When a foreign compound is metabolized in the body, it is converted into more polar compounds. Phase I products of metabolism are usually more polar than the parent compound, and phase II products are usually acidic compounds, such as glucuronic acid conjugates, ethereal sulphates, and glycine conjugates. These are water soluble and highly ionized at the pH of the blood and tend to be readily excreted by the kidney. Many of these conjugates are excreted into urine and bile by special secretory mechanisms which allow foreign compounds to be cleared very rapidly from plasma.

Factors Influencing Rates of Metabolism. Qualitatively, the metabolic degradation of drugs is similar in man and animals. Parke (1968) concluded that the quantitative differences in the metabolism of foreign compounds are dependent on the reaction velocities of alternative metabolic pathways. These are determined principally by:

(i) Characteristics of the animal species.

(a) The relative concentrations of different detoxication enzymes.

(b) The concentration of coenzymes and cosubstrates (e.g., UDPGA, glycine, etc.)
(ii) Characteristics of the foreign compound.

(a) Its physical and chemical nature that determines which enzyme reactions are possible.

(b) The amount of drug which determines tissue concentrations. The reaction velocities of enzyme reactions are affected differently by substrate concentration and are dependent on the reaction kinetics. Initially, the metabolic elimination of drugs may be zero order or first order, depending upon the drug concentration and the affinity of the particular drug for the metabolizing enzymes. Eventually, as the drug concentration in plasma falls, the biotransformation process will become first order.

On the basis of kinetic studies (Bray et al., 1952; Knoefel, Haung and Despopoulos, 1959; Bray et al., 1955), Smith suggested that the major factors governing the choice of two alternative metabolic routes are the first- and zero-order rate constants of the two reactions. The first-order rate constant in conjugation reactions may not be subject to serious species variations, but the zero order rate constant depends not only upon the mobilization rate of glycine, glucuronic acid, etc., which is different in different species, but also on the compound metabolized. The compound may affect the zero-order rate constant either by virtue of its tissue level, which depends on dose, or by its effect on the maximum velocity of the enzymatic reaction (Smith, 1964).

Gillette and others (Brodie and Reid, 1967; Gillette, 1967; Shannon, 1945-1946) have suggested that pharmacologic, toxic and
therapeutic effects of drugs should be related to the plasma or tissue concentration of the drug, that is, that pharmacologic responses of various species were similar for equal plasma or tissue levels of drug. This suggestion was based on evidence that, in many instances, the receptors for a given type of drug were quite similar in various mammalian species. Koppanyi and Avery (1966) have stated:

Strains and different species, including Homo, may differ in the rate of turnover and metabolic degradation of drugs. These differences can be measured by the methods of essential elimination as well as by chemical or biochemical techniques. Such studies may permit a limited extrapolation from experiments on lower animals to drug effects in the human species.

E. THE ELIMINATION OF DRUGS

The biological activity of drugs in the body is usually terminated by a combination of the processes of metabolism and excretion. The metabolic changes undergone by drugs in vivo are in general directed to the formation of metabolites which have physicochemical properties favourable for their rapid elimination from the body. The final products of metabolism are usually highly polar and these behave as efficient substrates for the transfer mechanisms functioning in the excretory organs. Furthermore, the polarity of the metabolites tends to confine them to the extra-cellular fluid, another factor conducive to their rapid elimination.

The drug and its metabolites are cleared from the systemic circulation by transfer into the urine and the various digestive
secretions and perhaps by elimination in the sweat and expired air. In ruminant animals some drugs may diffuse into the rumen in large amounts.

1. The Renal Excretion of Drugs

Our present concepts of renal tubular function are surprisingly recent in their origins. The eighty years (1842-1922) that followed the publication of Bowman's classical monograph on the structure of the glomerulus were more productive of controversy than of new information which could be considered helpful in appraising the relative contributions of glomerular filtration, tubular reabsorption and tubular secretion in the elaboration of urine. Throughout this period, two principal schools of thought contended for supremacy. One, representing the views of Bowman, Heidenhain and their adherents, held that tubular secretory activity accounted for the addition of the urinary solutes to water separated from the blood by glomerular filtration, the water serving only to sweep out the secretory products. Ludwig and his followers, in contrast, proposed the formation of a protein-free ultrafiltrate of plasma at the glomeruli, with subsequent concentration of the urinary solutes by water reabsorption in the tubules. It is noteworthy that as late as 1917, Cushny's "modern theory" of kidney function envisioned little more than the ultrafiltration of plasma and the tubular reabsorption of an ideal solution approximating Locke's solution in composition. The application of micro-puncture techniques in 1923 by Richards, Walker and their
colleagues permitted the sampling and analysis of capsular and tubular fluids which provided the first unequivocal evidence for glomerular ultrafiltration, together with information concerning the sites and nature of certain of the tubular processes. The subsequent development by Smith (1937) and his associates of suitable methods for estimating the volume of the glomerular filtrate by clearance techniques enabled the renal physiologist to attempt precise appraisals of tubular reabsorption and secretion. During the last forty-five years the growth of our knowledge of several aspects of renal function was based largely on studies of the mechanisms by which foreign organic acids were excreted. Starting with the first conclusive demonstration of tubular secretion of phenol red (Marshall, 1923, 1924, 1931), subsequent work was focused on foreign compounds such as dyes, iodinated aromatic derivatives and synthetic drugs. Indeed, the apparent limitation of tubular secretion to the handling of foreign compounds raised serious questions as to whether this mechanism had any physiological significance for the normal economy of the body. Despite these early doubts about its normal or physiological function, the study of tubular secretion was actively pursued and, by the time of Smith's monograph (1951), a well defined picture of the secretion of organic acids had emerged. Inherent in this evaluation of the secretory process was the tacit assumption that transport occurred in one direction only. For actively reabsorbed substances, particularly glucose, this concept was based on ample experimental evidence and for secretion it was supported by high
values for the renal extraction of foreign organic acids in mammals
(White, 1940; Corcoran et al., 1941) and by studies on the agglomeru-
lar kidney (Shannon, 1948). Furthermore, in the analysis of phenol
red, diodrast and para-aminohippurate (PAH) secretion, the
concept of unidirectional movement was fundamental to the cal-
culation of the maximal rate of tubular transport (Shannon, 1939).
This was interpreted as evidence for a rate-limiting cellular re-
action, a finding consistent with other biological systems, and this,
in turn, appeared to provide further validity to the underlying
concept of unidirectional transport.

In 1947 the renal secretion of strong organic bases was
discovered (Rennick et al., 1947; Sperber, 1947) and the excretory
pattern in the mammalian kidney was shown to be similar to that
outlined for organic acids (Rennick et al., 1954). Not all in-
vestigators subscribed to this rather rigid scheme of unidirec-
tional transport. In some studies the possibility was raised
that secreted organic compounds might also be subject to re-
absorption (Eggleton and Habib, 1949; Eggleton, 1956). A parallel
and somewhat independent development was the demonstration
that certain weak organic acids and bases were excreted at rates
dependent on urinary pH and that, as determined by clearance
ratios, such compounds underwent either net secretion or net
reabsorption (Jailer, Rosenfeld and Shannon, 1947; Orloff and

The kidney is admirably suited to the task of drug elimi-
nation. This organ receives a very large blood supply (25 per
cent of the cardiac output) through wide, short renal arteries that permit blood to enter the tissue with but little drop in hydrostatic pressure. The afferent arterioles bring blood to the glomeruli for filtration; the efferent arterioles carry about four-fifths of the same blood (one-fifth having been filtered) to the tubules, and thence to the venous collecting system. A drug will be filtered if its molecular size is not excessively large; since even some plasma albumin appears in the filtrate, most drugs, being smaller, will encounter no difficulty. The glomerular capillaries contain large pores readily visible in electron micrographs, and filtration constants derived experimentally reveal the glomerulus to be far more permeable to solutes than are the capillaries of muscle. Only free drug in plasma water (not drug that is bound to plasma proteins) can be filtered.

The renal tubular epithelium has a dual character with regard to the transfer of foreign organic compounds: it behaves as a lipoid boundary allowing the ready passage of nonionized, lipid-soluble molecules, and it has specialized processes for transporting many organic ions from plasma to urine.

Compounds of high lipid solubility do not appear in the urine in large proportions, because most of the molecules filtered at the glomerulus return to the blood-stream by diffusing across the lipid-like boundary of the tubular cells (see reviews, Brodie and Hogben, 1957; Butler, 1958). Conversely compounds of low lipid solubility are readily excreted in the urine, because they are only partly reabsorbed in the tubule.
When the pH of tubular fluid was altered, the changes in the rates of urinary excretion of weak acids and bases were consistent with the review that the tubular epithelium was selectively permeable to the lipid soluble, nonionized form of drugs. For example, when the tubular urine was made alkaline, weak bases became less concentrated in urine than in plasma, and as a result were excreted more slowly; when the urine was made acidic, the bases became concentrated in the urine and were excreted more rapidly. Conversely, weak acids were excreted more readily in an alkaline urine, and more slowly in an acidic urine (Milne et al., 1958; Orloff and Berliner, 1956; Waddell and Butler, 1957a, b; Baggot, 1968, 1970).

The tubular epithelium has at least two transport mechanisms for moving substances from plasma to urine; one for organic anions such as phenol red, iodopyracet, penicillin, chlorothiazide, probenecid and para-aminohippurate, and one for organic cations such as tetraethylammonium and N'-methylnicotinamide (see reviews, Orloff and Berliner, 1961; Peters, 1960; Sperber, 1959; Taggart, 1958). Numerous studies indicated that these substances were transported against a concentration gradient, that there was competition for transport among various anionic compounds, and similarly competition for transport among various cationic compounds. Both of these transport-secretion systems evidently depended upon high-energy phosphate compounds, for they were equally sensitive to the "uncoupling" agent 2,4-dinitrophenol. It could thus be concluded that many strong acids and bases were
secreted into the urine by active transport processes of rather low structural specificity. The normal function of the anion secretion system is apparently to eliminate from the body metabolites that have been conjugated with glycine, with sulphate, or with glucuronic acid. Certain weak acids and bases are also excreted in part by these active transport processes. For example, the ionized form of salicylic acid was secreted into urine by the mechanism that secreted p-aminobipirurate; and the ionized form of quinine was secreted by the mechanism that secreted N'-methylnicotinamide (Orloff and Berliner, 1961; Peters, 1960; Torretti et al., 1960; Volle et al., 1960; Weiner et al., 1959, 1960). Thus, the renal excretion of certain weak electrolytes involves three processes:

(i) glomerular filtration
(ii) active tubular secretion of the ionized moiety, and
(iii) passive tubular reabsorption of the nonionized moiety.

Stop-flow studies with pH-sensitive acids and bases have shown that they were secreted in the same portion of the nephron as the strong acids and bases (Weiner et al., 1959; Huang et al., 1960; Malvin et al., 1958; Pilkington and Keyl, 1963). The reabsorption site was distal to the secretory site; stop-flow analysis indicated that when conditions favoured reabsorption the lowest urine:plasma ratios were found distally (Weiner et al., 1959; Huang et al., 1960; Edwards et al., 1961; Pilkington and Keyl, 1963). Moreover, this did not exclude reabsorption in the proximal segments of the tubule.
For any compound excreted by these mechanisms, renal clearance is a complicated function of a number of determinants. The structure of the compound undoubtedly determines the amount secreted, and its pKa value and solubility in the lipid membrane determine the extent of nonionic diffusion. In addition, at least three physiologic variables contribute to the determination of net clearance, namely, renal blood flow rate, urinary pH and urine flow rate. Butler (1958) has estimated the half-lives of substances eliminated by the kidneys based on their volume of distribution and clearance rates.

Renal Excretion in the Neonate. In newborn infants, especially premature infants, the renal tubular secretory mechanisms are incompletely developed and inefficient. The elimination half-time for inulin (glomerular filtration) was about three times longer in the infant, suggesting some partial impermeability of the glomerular membrane, or more likely a smaller renal blood flow relative to the body water volume. Even more striking was the deficiency in PAH excretion (tubular secretion); here the elimination half-time was nearly four times longer than in the adult.

For the calf the clearance values of inulin and PAH which are indicative of glomerular filtration rate and renal plasma flow respectively were comparable to those of the adult human. Dalton (1968) has shown that the neonatal calf had not the features of "renal immaturity" evident in the human baby, kitten, puppy, piglet and neonatal rat and rabbit.
2. **Mechanisms Involved in the Elimination of Drugs in the Bile.**

Substances which make their appearance in the bile following their administration have been divided by Brauer (1959) into three classes according to their concentration in the bile compared to that in the blood. Substances of class A were those whose concentration in the bile was close to that in the plasma, that is, those for which the ratio bile concentration/blood concentration was close to unity. Such compounds included glucose and the ions, Na\(^+\), K\(^+\) and Cl\(^-\). Class B included substances for which the bile/blood ratio was greater than 1, usually from 10 to 1000, such as the bile salts, bromsulphthalein and bilirubin glucuronide whilst class C comprised compounds for which the bile/blood ratio was less than 1, such as insulin, phosphates and proteins.

Substances of class A appeared to have little affinity for the hepatic transfer mechanism and probably appeared in the bile as a result of diffusive processes. Thus, the bile in running down the biliary tree did come into restricted contact with the blood plasma and this afforded the opportunity for equilibration of various ions and compounds to occur. The actual amount of compound excreted in the bile by this process was small and was probably never much more than a few percent of the dose.

Compounds of class B appeared in the bile in such concentrations that there could be little doubt that some kind of active secretory process must have been involved in the transfer of these substances from blood to bile. First, they appeared in the
bile in concentrations far in excess of those found in the plasma. Second, many substances which were actively transferred by the liver cells competed for transport across the hepatic membrane into the bile; presumably all compounds in the group were handled by the same carrier. A third characteristic of the transport mechanism was that it could be saturated by an excess of substrate.

The main importance of the biliary route of excretion was for the elimination of certain organic anions and cations that cannot be reabsorbed from the intestine because they are ionized at the intestinal pH. In addition to some organic acids and bases, many polar metabolites of drugs, including glucuronides and other conjugates appeared in bile in high concentrations, suggesting that specific transport processes might have been involved. On the other hand, these acidic substances might have simply shared the relatively non specific acid transport process of the liver. Compounds were excreted in the bile provided they commanded an affinity for the carrier mechanism which was normally concerned with the hepatic transfer of physiological metabolites. In some respects the hepatic mechanism was similar to that present in the kidney. However, many single compounds known to be efficiently secreted by one mechanism were transferred only inefficiently or not at all by the other mechanism, suggesting that the systems involved were not completely identical. Metabolic transformation of many compounds provided a molecular species suitable as a substrate for the transfer mechanism into bile (Smith, 1966).
The biliary excretion of a compound involves the transfer of the substance across the cell wall separating the hepatic parenchymal cell from the plasma, through the cell and then via the active transport system across the membrane separating the liver cell from the bile canal. The passage of drugs and other foreign substances into the parenchymal cells involves few problems since compounds of widely different lipid-solubility penetrate liver slices with apparent ease. Schanker (1962) considered that the endothelium of the blood sinusoids, like that of blood capillaries in general, behaved as an extremely porous membrane permitting the ready equilibration between plasma and the extracellular fluid of liver of virtually all molecules and ions whose size was less than that of protein molecules. The membrane of the hepatic parenchymal cell acted as a lipoid membrane containing fairly large aqueous pores; the pores were large enough to admit a number of lipid-insoluble substances that did not penetrate other body cells, but were smaller than the pores of the sinusoidal endothelium. Finally, the bile duct epithelium must have the properties of a lipoid membrane that is relatively impermeable to large lipid-insoluble molecules and ions; otherwise glucuronides, bile acids and certain other organic anions and cations would not remain in bile in concentrations so much greater than that in plasma.

One important consequence of the biliary excretion of a compound is that it may establish enterohepatic cycling of the drug. This term implies the passage of a compound from the liver into
the bile and then into the gut, from which it is absorbed and re-circulated. The factors which are involved in the entero-hepatic cycling of a compound are numerous and complex but some of the most important are as follows:

(i) Rate of excretion of compound by liver,

(ii) Rate of metabolism of drug in gut; particularly the kinetics of the breakdown of conjugates in the intestinal tract.

(iii) Rate of absorption from the enteric tract.

(iv) Rate of loss of compound in faeces.

(v) Efficiency of liver in re-excreting the compound. Here the efficiency of excretory activity of the liver must be considered in relation to the efficacy of competing excretory mechanisms such as the kidney. Extra-hepatic elimination will decrease the total amount of drug available for recycling.

(vi) Effect of gall-bladder. The entero-hepatic cycling of a drug may be complicated in those species, such as man, dog and the cat, by the presence of a gall-bladder which may intermittently release large amounts of drug into the enteric tract.

One obvious consequence of the entero-hepatic cycling of a compound is that it may persist in the body for unduly long periods. This has been pointed out for morphine whose urinary excretion in dogs persisted for several days after its administration (Woods, 1954). Similar findings have been reported for
glutethimide in rats (Keberle et al., 1962) and for digitoxin in man (Okita et al., 1954). The persistence of a drug in vivo by its entero-hepatic cycling may be reflected in its plasma half-life.

F. AMPHETAMINE

(+)-2-amino-1-phenylpropane

Amphetamine, racemic β-phenylisopropylamine, is a unique drug with respect to the simplicity of its structure and the multiplicity of its biological effects (see Goodman and Gilman, 1970). Pharmacologically, amphetamine displays potent cardiovascular, central stimulant, hyperthermic and anorexigenic properties. Tolerance or tachyphylaxis develop to most of these effects upon repeated administration.

Biochemically, amphetamine releases catecholamines from their neuronal storage sites but does not affect neuronal serotonin concentrations. It is a potent inhibitor of membranal uptake of norepinephrine (NE) and a moderately active inhibitor of monoamine oxidase (MAO). The intimate mechanism by which amphetamine releases norepinephrine remains unsolved. This liposoluble molecule was not selectively concentrated by nerve endings (Ross and Renyi, 1966) as were tyramine, metaraminol and norepinephrine, whose concentration gradients were as high as 10,000:1 between the sympathetic fibres and the external media (Iverson, 1965; Lindmar and Muscholl, 1964). This consideration suggests that amphetamine is much more active than tyramine and
metaraminol in releasing norepinephrine and that amphetamine, unlike other sympathomimetic releasers, does not release norepinephrine by supplanting the natural mediator in the storage granules but by some other mechanism. The persistent depletion of norepinephrine after a single large does of d-amphetamine was due to the presence of p-hydroxynorephedrine in the nerve endings (Brodie, Cho and Gessa, 1970). This metabolite of d-amphetamine was found in the heart of rats (Goldstein and Anagnoste, 1965) and in the spleen of cats (Thoenen et al., 1966) injected with the parent compound. Thoenen et al. (1966) reported that the spleen concentrations of p-hydroxynorephedrine were never greater than those of amphetamine; Goldstein and Anagnoste (1965) could not detect p-hydroxynorephedrine in the brain of rats injected with d-amphetamine. Groppetti and Costa (1969) showed that p-hydroxynorephedrine accumulated in brain and heart of rats given d-amphetamine. The tissue content of p-hydroxynorephedrine declined slowly while that of the parent compound had a half-life of about 1 hour. The presence of this metabolite in tissues may be of value to explain the persistent depletion of NE stores induced by d-amphetamine. A pretreatment with desipramine, which reduces the hydroxylation of d-amphetamine (Sulser et al., 1966), blocked the accumulation of p-hydroxynorephedrine and prevented the depletion of cardiac NE elicited by the parent compound. Neither depletion of peripheral NE nor the accumulation of p-hydroxynorephedrine are absolute requirements of hyperthermia, piloerection and increased motor activity caused by d-amphetamine.
Clinically, in the human d-amphetamine is an effective appetite depressant, as well as an antifatigue, euphoric and mild antidepressant drug. On chronic administration of increasingly higher doses it precipitates paranoid psychoses.

All of the structural elements of amphetamine are critical to its pharmacological and biochemical activity spectrum. Any structural modifications, additions or subtractions will accentuate some of the actions, abolish or attenuate others, or uncover latent ones not previously demonstrable with the parent structure (see reviews by Biel, 1970; Beckett and Brookes, 1970).

Physico-Chemical Properties. For a variety of drugs, positive correlations have been noted between partition coefficients and (a) in vivo absorption (Walton, 1935; Mayer, Maickel and Brodie, 1959; Schanker, 1959; and others), (b) renal tubular reabsorption (Knoefel, Huang and Jarboe, 1961, 1962; Weiner and Mudge, 1964; Wilkinson, 1966; and others) and (c) hypnotic activity (Hansch, Maloney and others, 1968).

For many organic solvents there is a direct relation between the logarithms of partition coefficients (organic solvent-water) of compounds in an homologous series in one solvent-water system to the logarithms of the partition coefficients in another solvent-water system (Collander, 1947). However, the relative magnitudes of partition coefficients for compounds of widely differing structures change when different solvents are used, especially when solvent-solute interaction takes place (Wilkinson, 1966). The use of partition coefficients in predicting absorptions of drugs may
therefore give erroneous results if an unsuitable solvent is used as the lipid substitute. Even when partition experiments were performed using lipid extracted from animal buccal mucosa, the K values were not necessarily valid for the living tissue (Brandstrom, 1964). This is because the lipid layers bind to protein and this modifies their solvent properties. The passage of amphetamines across a biological membrane is governed by their lipid-solubility. The \textit{n-heptane-aqueous system} (or \textit{n-hexane-aqueous system}) partition coefficient gives a good indication of the degree of \textit{in vivo} absorption of the nonionized form of amphetamines (Beckett and Moffat, 1969) and imipramine and its metabolites (Bickel and Weder, 1969). The amount of amine in the nonionized form is calculated from the pH of the buffer solution and the pKa of the compound as follows:

\[
\text{pH} - \text{pKa} = \log \left( \frac{[\text{nonionized}]}{[\text{ionized}]} \right)
\]

The pKa value of dexamphetamine is 9.90 (Leffler \textit{et al.}, 1951; Lewis, 1954; Kisbye, 1958). This implies that at the physiological pH (7.4) only a small fraction (0.31 per cent) is in the neutral or nonionized form. The neutral moiety is reasonably soluble in various organic solvents whereas the ionized form is water-soluble. The N-alkyl substituted amphetamines are in general more lipid-soluble than the parent compound (Vree, Muskens and Rossum, 1969). The lipid solubility of the neutral form is reflected in the so-called true partition coefficient (Table 1).
TABLE 1

PARTITION COEFFICIENTS OF
_\textit{d-AMPHETDAMINE}_

<table>
<thead>
<tr>
<th>Apparent PC</th>
<th>True Partition Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.4</td>
<td></td>
</tr>
<tr>
<td>CHCl$_3$/H$_2$O</td>
<td>Heptane/H$_2$O</td>
</tr>
<tr>
<td>0.48</td>
<td>1.88</td>
</tr>
</tbody>
</table>

Tissue Distribution of _\textit{d-Amphetamine}_. The distribution of _\textit{d-amphte}

The distribution of _\textit{d-amphte}

The animal was sacrificed one hour 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. The concentrations of the drug in plasma and cerebrospinal fluid were the same (3.8 μg/ml) indicating that there was no hindrance to the passage of the drug through the blood-cerebrospinal fluid barrier. At plasma concentrations of 2 to 10 mg per litre approximately 15 per cent of the compound was found to be bound to the nondiffusible constituents of the plasma.

The relative tissue distribution of _\textit{d-amphetamine} in the mouse one hour after intraperitoneal administration of the drug was: kidney > lung > liver, spleen, brain > heart > blood (Fuller and Hines, 1967). This distribution resembled that in the dog as reported by Axelrod. All the tissues studied contained a higher concentration of amphetamine than did blood, indicating an ability
of the organs to concentrate and/or bind amphetamine. Similar findings were reported in cats (Siegel et al., 1968) and rats (Brzezinski, 1968). Most of the drug was shunted toward tissues of high blood perfusion, that is, the kidney, brain, liver and lungs. Throughout the brain no sites of high concentration were apparent. At all times the radioactivity appeared to be distributed evenly.

The chromatographic determinations of Brzezinski (1968) proved the presence of p-hydroxyamphetamine in the liver and kidney in small amounts as compared to those detected in urine. These findings were in agreement with Richter (1938) and Axelrod's (1954, 1955) hypothesis, that the liver was the main organ for the biochemical transformation of amphetamine and that p-hydroxyamphetamine was quickly excreted from the body.

The Comparative Metabolism of Amphetamine. The initial pharmacologic investigations on the actions of amphetamines in dogs and man were reported 41 years ago (Piness et al., 1930). The present knowledge of the metabolic fate of this important amine is considerable but still incomplete. Excretion studies on d-amphetamine in the rat have been reported by several investigators (Alleva, 1963; Cartoni and Di Stefano, 1963; Axelrod, 1954; Ellison, Gutzait and Van Loon, 1966; Rosso, Dolfini and Franchi, 1968; Gunne and Galland, 1967; Creaven and Barbee, 1969; and Dring, Smith and Williams, 1970). The comparative metabolism of amphetamine was studied by Axelrod (1954), Ellison, Gutzait and Van Loon (1966) and very extensively by Dring, Smith and Williams, (1966, 1970). Winters
et al. (1966) studied the fate of this drug in the human and Ellison et al. (1968) reported the comparative metabolism of radioactive amphetamine in tolerant and nontolerant cats. Zalis et al. (1967) suggested that factors which prevent either the central excitatory effects of amphetamine or the skeletal muscle response significantly reduce the toxicity of amphetamine by preventing hyperactivity. In tolerant cats apparently less drug was metabolised than in nontolerant cats indicating that the development of tolerance to amphetamine was not due to hepatic microsomal stimulation (Ellison et al., 1968).

Amphetamine (2-amino-1-phenylpropane) can be metabolised along two pathways, either by hydroxylation of the aromatic ring to p-hydroxyamphetamine 4-(2'-aminopropyl)phenol or by deamination of the side chain to 1-phenylpropan-2-one (benzyl methyl ketone), which is then degraded to benzoic acid. These two pathways have been shown to occur and their relative extent appears to vary with species (Axelrod, 1954a, b, 1955; Alleva, 1963; Ellison, Gutzait and Van Loon, 1966; Dring, Smith and Williams, 1966, 1970). The known metabolites of amphetamine in urine, apart from the unchanged drug itself, were p-hydroxyamphetamine and benzoic acid and their conjugates. The extent to which amphetamine was excreted unchanged depended upon urinary pH, more was excreted in an acid than in alkaline urine (Asatoor, Galman, Johnson and Milne, 1965; Beckett, Rowland and Turner, 1965; Beckett and Rowland, 1965) and it appeared that one optical isomer was metabolised more readily than the other (Gunne, 1967; Gunne and Galland,
The quantitative differences in the metabolism of the optical forms were not very marked within a species (Dring, Smith and Williams, 1970). The major metabolic route of d-amphetamine in the rat was through ring hydroxylation followed by glucuronidation of the hydroxylated derivative. A secondary route for amphetamine metabolism by this species involved oxidative deamination of the aliphatic side chain to form benzoic acid, which in turn was conjugated with glycine to form hippuric acid (Alleva, 1963; Ellison, Gutzait and Van Loon, 1966; Dring, Smith and Williams, 1970). Rats also excreted some unchanged amphetamine (11%) together with what appeared to be N-acetyl-amphetamine. The rat produced no 1-phenyl-propan-2-one precursor.

In the guinea pig the metabolism of amphetamine appeared to be relatively simple. The major route of metabolism led to benzoic acid and its conjugates. About 22% of the drug was excreted unchanged. Hydroxylation did not occur to any appreciable extent. There appeared to be no 1-phenylpropan-2-one precursor.

The metabolism of amphetamine in the mouse was dissimilar to that in the rat. There was a high excretion of unchanged amphetamine (33%) and of benzoic acid, both of which were excreted in low amounts in the rat. Although the mouse hydroxylated amphetamine better than the other species studied except the rat, the extent of hydroxylation was only about one-fourth of that in the rat.
Man, squirrel and rhesus monkeys and the dog appeared to metabolize the drug in an approximately similar manner. In these species, about half of the excreted drug was unchanged. The major transformation product in these species was benzoic acid and its conjugate (Dring, Smith and Williams, 1970). A precursor of 1-phenyl-propan-2-one was found in small amounts in human and dog urine, but not in monkey urine. Amphetamine was 4-hydroxylated to a minor extent. Axelrod (1954) and Ellison, Gutzait and Van Loon (1966) indicated that hydroxylation was an important metabolic pathway in the rat and dog. In the dog an oral dose of drug resulted in the excretion of 17.1% of the dose as hydroxylated metabolites and 19% as hippuric acid. Therefore, the dog appeared unusual among the animals studied in that dl-amphetamine was metabolised equally well through two different pathways. Ellison, Siegel, Silverman and Okun (1968) showed that the cat metabolized dl-amphetamine primarily by ring hydroxylation and secondarily by oxidative deamination similar to the biotransformation pattern of the dog. The major portion of the dose administered was excreted unchanged (61%). No significant differences in total drug excretion was observed between tolerant and nontolerant cats.

The rabbit appeared to be unique among the species studied, since it excreted little unchanged amphetamine or 4-hydroxyamphetamine. The major route of metabolism was by oxidative deamination (Axelrod, 1954; Dring, Smith and Williams, 1966, 1968, 1970), leading to 1-phenylpropan-2-one (benzyl methyl
ketone, phenylacetone) and the corresponding carbinol, and benzoic acid. The ketone was not found as such in the urine, but as an acid-labile precursor, compound X. The latter was not isolated in a pure form, but it was shown to contain 1-phenylpropan-2-one and sulphate.

The optical isomers of amphetamine show different pharmacological properties. The acute toxicity of \( d \)-amphetamine in mice was about ten times that of the \( l \)-isomer (Fairchild and Alles, 1967) and it is possible that a difference in the metabolic fate of the isomers might be involved in the difference in pharmacological activity. It has been reported that in man and the rat more of the \( l \)-isomer was excreted unchanged than the \( d \)-isomer when the \( dl \)-isomer was administered (Gunne, 1967; Gunne and Galland, 1967).

The administration of \( d \)-amphetamine to rats and cats resulted in the accumulation of \( p \)-hydroxynorephedrine in the heart and spleen (Goldstein and Anagnost, 1965; Thoenen et al., 1966). After giving \( d \)-p-hydroxyamphetamine to man, about 5% was excreted as the \( \beta \)-hydroxylated metabolite, \( p \)-hydroxynorephedrine (Sjoersma and von Studnitz, 1963). These findings indicated that a small quantity of \( d \)-p-hydroxyamphetamine was \( \beta \)-hydroxylated. The \( l \)-isomer of amphetamine was not a substrate for dopamine-\( \beta \)-hydroxylase, hence it was not converted to \( p \)-hydroxynorephedrine \textit{in vivo} (Goldstein and Anagnost, 1965). There was also a marked stereo-specificity in the deamination of amphetamine by the microsomal enzyme (Axelrod, 1955).
There are wide species variations in the metabolism of amphetamine, but these variations cannot be correlated with any possible species variations in the activity of the drug, since no reliable information on this point is available, except that the drug appeared to be more toxic to the rat and mouse than to the rabbit (see Spector, 1956).

Factors Affecting the Rate of Disappearance of Amphetamine in Rats. Groppetti and Costa (1969) showed that sex, age, and pre-treatment with estradiol changed the rate of amphetamine disappearance, but these changes were not always in a direction predictable from the available data for other substrates of microsomal aromatic hydroxylase. Reports in the literature have shown that the action of many lipid-soluble drugs endured longer in female than in male rats and this difference was frequently related to the rate of drug metabolism (Quinn, Axelrod and Brodie, 1958). The rate of disappearance of amphetamine was faster in male adult Sprague-Dawley rats than in adult females. Chronic administration of estradiol to male rats decreased the rate of amphetamine disappearance. Jondorf, Maickel and Brodie (1959) and Fouts and Adamson (1959) have reported that the liver of newborn animals lacked the microsomal systems for the metabolism of many foreign compounds. The enzymatic activity appeared within the first week after birth and reached a maximum when the animal was sexually mature. The half-life of d-amphetamine in young male rats was shown to be longer than in adult male or female rats. Pre-treatment of rats with either phenobarbital or 3-methyl-chlor-
anthrene in doses sufficient to decrease the half-life of hexobarbital and zoxazolamine did not change the rate of amphetamine disappearance. Conney et al. (1965) reported that a pretreatment with diphenylhydantoin induced the liver microsomal cortisol-6-β-hydroxylase. Groppetti and Costa (1969) found that diphenylhydantoin did not increase but actually decreased the rate of disappearance of amphetamine from rat tissues. These workers suggested that amphetamine hydroxylase of rat liver had some peculiar properties and indicated that either the molecular organization of the enzyme or its localization differed from that of the microsomal aromatic hydroxylase of rat liver.

Imipramine and other tricyclic antidepressant agents prolonged pharmacological and behavioral effects of amphetamine (Carlton, 1961; Stein, 1964; 1967; Lapin and Schelkunov, 1965) and in particular potentiated the amphetamine induced increase in body temperature (Morpurgo and Theobald, 1965; Jori and Garattini, 1965). Imipramine and desipramine given before amphetamine may have potentiated its pharmacological activity by allowing an increase in brain and other tissue levels of amphetamine (Valzelli, Consolo and Morpurgo, 1967) as a result of inhibition of its metabolism to p-hydroxyamphetamine (Consolo, Dolfini, Garattini and Valzelli, 1967). Lewander (1969) demonstrated that desmethyliimipramine inhibited the p-hydroxylation of amphetamine in a dose-dependent manner. The concentrations of amphetamine in tissues were considerably higher in rats pretreated with DMI than in controls and the half-life of amphetamine in tissues was prolonged (Sulser, Owens and
Dingell, 1966; Valzelli, Consolo and Morpurgo, 1967). Other drugs found to inhibit p-hydroxylation of amphetamine were: imipramine, nortriptyline, protriptyline, chlorpromazine, promethazine, chlor-diazepoxide (Rushton and Steinberg, 1966), diazepam, nialamide and cocaine (Lewander, 1969). The following drugs were without effect on amphetamine hydroxylation: haloperidol, meprobamate, secobarbital, phenobarbital, reserpine and atropine. Reserpine (Quinton and Halliwell, 1963; Rech, 1964; Stolk and Reck, 1968; Morpurgo and Theobald, 1966, 1968) and atropine (Carlton and Didamo, 1961; Morpurgo and Theobald, 1968) have been reported to potentiate the pharmacological effects of amphetamine. Lewander found that neither of these drugs interfered with the metabolism of amphetamine in rats. The concentration of amphetamine in the rat brain was not changed by reserpine pretreatment (Valzelli, Dolfini, Tansella and Garattini, 1968).

Chemically the drugs that caused inhibition of amphetamine metabolism were bases like amphetamine, while the barbiturates and meprobamate were acids. The existence of two or more hydroxylation enzymes in the liver microsomes (Conney, 1967 and Kuntzman et al., 1968), that metabolise bases and acids respectively, may therefore be possible.

The Determination of Amphetamine. Various methods have been employed for the estimation of amphetamine in biologic fluids but they lacked specificity or sensitivity. McNally, Bergman and Polli (1947) used a combined method of purification, distillation and diazotization with paranitrobenzene diazonium chloride for the
determination of dl-amphetamine in blood, urine and tissues. This method was claimed to estimate an amount of dl-amphetamine as low as 30 microgram in 25 gm. of tissue. Considerably more work has been reported on the estimation of dl-amphetamine in urine than in blood. Richter (1938) reported a picric acid method for the determination of dl-amphetamine in urine. Jacobsen and Gad (1940) extracted dl-amphetamine from a steam distillate of urine and developed a yellow colour with picric acid. Beyer and Skinner (1940) coupled dl-amphetamine with paranitrobenzene diazonium chloride and measured the red complex spectrophotometrically. Harris, Searle and Joy (1946) modified the Jacobsen and Gad method by replacing the distillation by one in which the interfering urine substances were absorbed on magnesium oxide in a buffer medium. Keller and Ellenbogen (1952) introduced the methyl orange method for the determination of dl-amphetamine in blood and urine. This method was based on one introduced by Brodie and Udenfriend (1945), for the colorimetric estimation of cinchona alkaloids and other organic bases in plasma and urine. This method was satisfactory for the estimation of dl-amphetamine down to 1 microgram/5 ml. of dog plasma. The method, however, was not specific for dl-amphetamine. The methyl orange when accompanied by a buffer wash was applicable to urine level determinations of dl-amphetamine. Baeumler, Brault and Im Obersted (1964) employed thin-layer chromatography and Erlich's Reagent for separation and identification of the drug. Debackere and Massart-Leen (1965) studied plasma levels of amphetamine in the horse by thin-layer chromo-
tography with detection by diazotized p-nitroaniline. They were able to detect amphetamine in 20 ml of plasma only if the samples were hydrolysed. The limit of sensitivity for their method was 5 to 10 μg. Schubert (1967) and Heaton and Blumberg (1969) employed thin-layer chromatography for the detection of amphetamine in urine of medicated subjects. Karawya et al. (1967, 1968) described the chromatographic separation of amphetamine, methylamphetamine and ephedrine from horse urine on alkaline Silica Gel G plates developed with acetone-methanol (1:3). After elution, the bases were determined colorimetrically.

G. THE PHILOSOPHICAL BASIS OF PHARMACOKINETICS

The word pharmacokinetics means the application of kinetics to pharmakon, the Greek word for drugs and poisons. The purpose of pharmacokinetics is to study the time course of drug and metabolite concentrations and amounts in different tissues and excreta, and to construct suitable models to interpret such data. The birth of modern pharmacokinetics, both from practical and theoretical standpoints, occurred during the years 1937-1948 (Marshall, Emerson and Cutting, 1937; Marshall, Cutting and Emerson, 1938; Terell, 1937; Stewart, Rourke and Allen, 1938; Boxer et al., 1948). With some exceptions, the review of Nelson (1961) and the book by Rescigno and Segre (1966) are considered to cover the literature to 1961. Since that time reviews of the basic concept of pharmacokinetics have been written by Doluisio and Swintosky (1965), Wagner (1968), Garrett (1969) and Notari (1971).

The molecules of a drug must reach the sites of action in a complex organism to be effective as a pharmacological agent. The primary proof of availability at these receptor sites is the quantification of biological response as a function of the drug concentration in the biophase compartment that surrounds these receptor sites. It may be and usually is difficult to assess this quantification and to analyze the drug concentration in the biophase. All too frequently one does not know the site of action. A secondary proof of availability of the drug in the biophase that surrounds the receptor sites is the appearance of the drug and its metabolites.
in the blood tissues and excreta of the organism. This is based on the reasonable assumptions that the drug will exercise its action in the body when it does appear therein, and that biological activity is related to the amounts that do. Thus, the appropriate dosage and its regimen must be formulated in the light of knowledge of rates and amounts of distribution, metabolic and excretory processes involving the drug in the individual organism where the necessary amounts are maintained in the body to give the desired pharmacological response for the desired time interval. These patterns are best understood by analyzing the time courses in vivo of drug release from the dosage form, that is absorption, distribution, metabolism, and excretion as functions of time and the amount administered.

The quantifications of these processes are based on the assumptions that the body, although a complex of many organs, tissues and processes, can be conceived of as a multicompartmental organism. It is presumed that the drug and/or its metabolites may be equitably dispersed in one or several tissues of the body so that the drug in these tissues may act as a kinetically homogeneous pool. Such a tissue, which acts as an isotropic fluid, in which any molecules of drug that many enter are homogeneously dispersed, where the kinetic dependencies of pharmacokinetic processes can be formulated as functions of amounts or concentrations in that tissue, is called a compartment. These compartments are separated by barriers that inhibit free diffusion from one compartment to another. The barriers are kinetically definable in that the rate
of transport of drug or metabolite across this membrane barrier between compartments is a function of the amounts or concentrations in these compartments. The simplest postulate is that the rates of transport in either direction are proportional to the amounts or concentrations in the separated hypothetical compartments. If experimental studies as functions of increased dosage indicate that this simple postulate is not entirely valid, then saturable transport or metabolic processes, rate-limited by the capacity of the membrane-barrier or enzyme may be postulated.
CHAPTER III

MATERIALS AND METHODS

A. EXPERIMENTAL PROCEDURE

The experiments performed in this study may be divided into six sections as follows:

Section 1: The objective of the experimental work in this section was to determine the biologic half-life of amphetamine in various species of animals. The experimental subjects were comprised of randomly selected groups of 'healthy' animals of both sexes from each of six domesticated mammalian species and chickens. The equine (ponies), caprine, porcine, canine, feline and lapine species of animals were selected. A similar dose (0.66 mg/kg body weight, calculated as free base) of dl-amphetamine sulphate (2 or 5 per cent solution) was administered intravenously to each animal in all of the experiments. Blood samples were collected at regular, precisely timed intervals; disodium or dipotassium EDTA was the anticoagulant employed.

Section 2: The aim of the experiments in this section was to determine quantitatively the relative amounts of unchanged amphetamine, p-hydroxyamphetamine and its conjugates in cumulative (24-hour) urine samples. The animals were retained in metabolism cages for the duration of this experiment. A urine collection
period which exceeded five half-lives for the drug was employed (24 hours for all species except cats, for these animals the urine was collected for 48 hours). The amounts of unchanged amphetamine and some of its metabolites were determined in five hourly cumulative urine samples from catheterised mares.

To obtain urine devoid of faecal contamination from chickens, the rectum was tied off with a ligature, a ring was inserted into the cloaca and a toy balloon secured to the ring. This method, designed and performed by Walter Swanson, proved satisfactory.

Section 3: To determine the extent of plasma protein binding in vitro. Blood was obtained from animals of each species under investigation and in addition from monkeys, rats and opossum. Disodium and dipotassium EDTA was the anticoagulant used.

Section 4: The objective of the experimental work in this section was to define some of the pharmacokinetic constants describing the elimination of amphetamine from the body in a single species of animal. To achieve the objective of this section the following experiments were performed, the specific aims of which may be summarized as follows:

Experiment (i): To determine the biologic half-life of amphetamine, the elimination rate constant and the apparent specific volume of distribution in 'healthy' randomly selected mongrel dogs of both sexes performed as described in Section 1.
Experiment (ii): To determine simultaneously the relative amounts of amphetamine excreted in urine and bile and the influence, if any, of the pH gradient between urine and plasma. Under pentobarbital anaesthesia the ureters and common bile duct were cannulated and the neck of the gallbladder was occluded by a ligature. Urine and bile were collected continuously, the sample tubes were changed each half-hour during the experiment. The volumes and pH values (pH Meter 26, Radiometer Copenhagen) of urine and bile were quickly measured and the tubes were then carefully capped. Blood samples were also collected at half-hourly intervals.

Experiment (iii): To establish the rate of elimination of the drug from plasma by processes other than renal excretion, namely, biotransformation, biliary excretion and perhaps other minor routes. For this purpose a randomly selected group of dogs was nephrectomized and the drug was administered on the second day after the operation. In these animals an attempt was made to establish the rate constant for penetration of amphetamine into CSF by collecting cerebrospinal fluid and blood samples simultaneously at regular intervals.

Experiment (iv): To determine the influence of nephrectomy upon the amount of unchanged amphetamine excreted in bile. In other words, to see if the rate of biliary excretion increased when renal excretion was absent.
Nephrectomized dogs with the common bile ducts cannulated were employed.

Samples of cerebrospinal and ocular fluids were collected simultaneously with the final blood sample from each dog in experiments (ii, iii) and (iv).

Section 5: To determine the influence of urinary pH changes upon the half-life of amphetamine in horses. Clinically healthy, adult, nonpregnant, standard-bred mares were used as experimental subjects. The same three animals were employed on four occasions, there being an intervening period of one month between each experiment. Urinary pH values were adjusted mainly by alteration of dietary regimens but sodium bicarbonate (15 gm) or ammonium chloride (2 gm enteric coated tablets) was administered on each of the two days prior to amphetamine administration when alkaline or acid urine was desired. All urine samples were collected by catheterisation and the pH values quickly determined (pH Metre Beckman, Model 76A). Urine and blood samples were collected from each animal immediately prior to drug administration. A total dose of 250 mg amphetamine sulphate was then injected intravenously. This represented a dosage rate of approximately 0.66 mg/kg body weight. Blood samples were collected at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hours following drug administration and urine samples were obtained at three hour intervals.

Section 6: The experiments in this section are diverse in nature and are best classified as miscellaneous.
Experiment (i): The concentrations of amphetamine in plasma and rumen liquor collected at regular intervals following intravenous administration of the drug were determined in four goats. This experiment was performed as an attempt to elucidate the short biologic half-life and large apparent specific volume of distribution of amphetamine in the caprine species of animal.

Experiment (ii): Samples of gastric fluid were collected from dogs at varying times following intravenous administration of amphetamine. The aim of this experiment was to test for the presence of the drug in the stomach of a monogastric animal.

B. ANALYTICAL TECHNIQUES

The procedure employed for the determination of amphetamine in this study was a modification of the method described by Noonan, Murdick and Ray (1969). Amphetamine was extracted from alkaline biologic fluid into cyclohexane. Trichloroacetyl chloride was added to an aliquot of the organic phase. The amphetamine in the aliquot reacted to form 1-phenyl-2-(trichloroacetamide) propane, \( C_6H_5CH_2\cdot CH(NHCOCCl_3)CH_3(TCAA) \). Excess trichloroacetyl chloride was removed with aqueous sodium hydroxide. Four microlitres of the organic phase were injected into the gas chromatography apparatus.

The concentration of amphetamine in the biologic fluids of medicated animals was determined by comparing sample peak
heights with standard peak heights. Standards were prepared by making 0, 25, 50, 75, 100, 150, 200, 250, 300, 400, 500, 600, 800, 1000 ng amphetamine up to 1 ml with blank biologic fluid of similar nature from the same species of animal under investigation. Fisher, micro Sahli pipettes (0.05 ml ± 1%, 0.1 ml ± 1% and 0.2 ml ± 1% at 20° C) and an Adams suction apparatus were used to measure the required volumes of the amphetamine standard solutions. Standards were analysed in the same manner as the unknowns to compensate for losses occurring in the procedure. They were analysed immediately before and after the analysis of each set of samples. Response to the electron capture detector was linear in the 25 to 250 ng/ml concentration range. Quantitation was satisfactory only in this concentration range. (See Figure 2).

Derivatized samples whose concentration did not exceed 1 microgram/ml were diluted with an appropriate volume of cyclohexane and then quantitated. Samples whose concentration exceeded 0.5 microgram/ml were diluted with distilled deionized water prior to extraction. The concentration of amphetamine was measured in the following biological fluids: plasma, cerebrospinal fluid, ocular fluid, bile, gastric fluid, rumen liquor and urine.

Reagents. The following reagents were used.

(i) Standard solution of amphetamine (100 mg/100 ml):amphetamine sulphate, 136 mg, Sumner Chemical Co., N. Y. 17 N. Y. corresponding to 100 mg of free base, was diluted to 100 ml with distilled deionized water. Working standards were prepared by dilution of stock standard solution with distilled
AMPHE TAMINE CONC. (ng ml$^{-1}$)

Figure 2. The response of the electron capture detector to standard solutions of amphetamine.
water.

TABLE 2
AMPHETAMINE STANDARD SOLUTIONS

<table>
<thead>
<tr>
<th>Stock</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>1 mg/ml</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>10 μg/ml</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1 μg/ml</td>
<td></td>
</tr>
<tr>
<td>Working</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.5 μg/ml</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>2 μg/ml</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>5 μg/ml</td>
<td></td>
</tr>
</tbody>
</table>

(ii) 1 normal sodium hydroxide solution. Prepared from sodium hydroxide pellets.

(iii) Cyclohexane, reagent, A.C.S. (Matheson, Coleman and Bell, Norwood, Ohio).

(iv) Trichloroacetyl chloride (K&K Laboratories, Inc., Plainview, N.Y.).

Analytical procedure. One millilitre of the biologic fluid was pipetted (volumetric pipette) into a glass screw-cap culture tube (15 ml) 4 ml of cyclohexane (Matheson, Coleman and Bell, Norwood, Ohio) and 1 ml 1 N NaOH were added. The tubes were capped and contents mixed on a rotating rack (Roto-Rack, Fisher Scientific Company, Pittsburg, Pa.) at approximately 100 inversions per minute for 5 minutes. The clamps on the rack were positioned at a 45° angle to impart a rolling action to the mixture. This
provided efficient extraction and reduced emulsion formation. The aqueous and organic phases were separated by centrifugation. Emulsions were broken by removing the tube from the centrifuge, tapping it sharply against the hand and recentrifuging. Two millilitres (volumetric pipette) of the clear organic phase were transferred to a clean screw-cap tube (15 ml). Trichloroacetyl chloride, 50 µl, was added, using 50 µl (+1%) disposable capillary pipettes (Corning Glass Works, N.Y., 14830) and an adapted Adams suction apparatus.

The tubes were immediately capped, then gently shaken to insure mixing and were allowed to stand at room temperature for 20 minutes. Two millilitres of 1 N NaOH were then added. The tubes were capped tightly, placed on the rotating rack for five minutes and then centrifuged. The organic phase was transferred by means of a disposable pipette to a clean tube. Four microlitres of this solution were injected into the gas chromatograph using a 10-µl syringe (The Hamilton Co., Inc., Whittier, Calif.). The TCAA was detected and quantitated with the electron capture detector. In the drug determinations performed upon horse plasma samples the trichloroacetamide derivative of amphetamine was prepared following alkaline (0.5 ml of 10 N NaOH) extraction of the drug from plasma (4 ml) into cyclohexane (4 ml). All gas chromatograms were obtained on a gas chromatography apparatus (model GC-5, Beckman Instruments, Inc., Fullerton, Calif.) equipped with electron capture detectors. Typical temperatures, gas flow rates and electrical settings are
# TABLE 3
## CONDITIONS FOR CHROMATOGRAPHY

<table>
<thead>
<tr>
<th>Flow Rates:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carrier gas</strong></td>
<td>40 ml/min, helium</td>
</tr>
<tr>
<td><strong>Auxiliary flow</strong></td>
<td>60 ml/min, helium</td>
</tr>
<tr>
<td><strong>CO₂ flow</strong></td>
<td>2 ml/min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperatures:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inlet flash evaporator</strong></td>
<td>225° C – 240° C</td>
</tr>
<tr>
<td><strong>Column oven</strong></td>
<td>180° C – 180° C</td>
</tr>
<tr>
<td><strong>Detector compartment</strong></td>
<td>302° C – 302° C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Electrical settings:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polarizing voltage</strong></td>
<td>840 (128 V)</td>
</tr>
<tr>
<td><strong>Bias voltage</strong></td>
<td>0</td>
</tr>
<tr>
<td><strong>Source current</strong></td>
<td>4 mA</td>
</tr>
<tr>
<td><strong>Electrometer current</strong></td>
<td>1.28x10⁻⁸ A full scale</td>
</tr>
<tr>
<td><strong>Suppression</strong></td>
<td>3.7x10⁻¹⁰ - 4.6x10⁻⁸ A.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Column</th>
<th>4 feet x 1/8 inch outside diameter, 1/16 inch inside diameter, glass (silanized with dichlorodimethyl-silane),</th>
</tr>
</thead>
</table>

| Packing                     | 3% OV-1 on 80/100 mesh Chromosorb GHP* |

described in Table 3.

The column was glass tubing, 1/8th-inch outside diameter, 1/16th-inch inside diameter, 4 feet long. The column was cleaned and silane treated as described by Fales and Pisano (1964). The glass wool used for the end plugs was treated in the same way except that the chromic acid cleaning procedure was omitted. The column was packed with 3 per cent OV-1 on 80/100 mesh Chromosorb GHP (Supelco Inc., Bellefonte, Pa.).

To establish the exact retention time for TCAA, a sample of the pure amphetamine derivative whose structure had been confirmed by mass spectrometry at Battelle Memorial Institute, was dissolved in cyclohexane and injected into the gas chromatograph. The heptafluorobutyramide derivative was prepared on randomly selected samples of the various biological fluids to confirm that authentic amphetamine was being detected.

The use of derivative formation in the gas chromatographic analysis of amines is desirable for several reasons. Firstly, the free amines are subject to adsorption phenomena in the chromatographic system due to the hydrogen bonding characteristic of the nitrogen. Secondly, the use of sensitive detectors such as electron capture detectors makes it necessary to convert the amines into derivatives which possess both good chromatographic properties and strong affinity for free electrons. Thirdly, the use of mass spectrometry in combination with gas chromatography requires derivatives which will give mass spectra suitable for identification and for structural analysis.
The determination of para-hydroxyamphetamine in biological fluids.

The procedure employed for the determination of p-hydroxyamphetamine was that of Axelrod (1954). An aliquot of the biological fluid was saturated with sodium chloride. The free p-hydroxyamphetamine was then extracted at pH 9-10 into diethyl ether. The compound was then returned to 0.1 N HCl, treated with 1 nitroso-2-naphthol reagent (Gerngross et al., 1933) and assayed spectrophotometrically at 520 nm.

Procedure. 2.5 ml of biologic fluid was pipetted (volumetric pipette) into a 50 ml centrifuge tube fitted with linear high density polyethylene stopper (Will Scientific). A sufficient amount of sodium chloride was added to saturate the sample. The pH was adjusted to 9 to 10 (pH Metre 26, Radiometer, Copenhagen) by the addition of solid sodium carbonate. Then 25 ml of anhydrous ether (Baker Chemicals) was added, the tubes were stoppered tightly and the contents mixed thoroughly for 30 minutes (Burrell, wrist action shaker, Pittsburg, Pa.). A 20 ml aliquot of the ether layer (upper) was transferred to another 50 ml centrifuge tube (Baker Chemical Co., Phillipsburg, N.J.) which contained 3 ml of 0.1 N hydrochloric acid. The tubes were stoppered and placed on the shaker for 10 minutes. After this period 2 ml of the acid layer was pipetted into a 15 ml glass culture tube; 0.25 ml of 0.1 per cent 1-nitroso-2-naphthol (recrystallized) in ethanol and 0.5 ml of freshly prepared nitric acid reagent (1 ml 2.5% sodium nitrite plus 50 ml of 2.5 N nitric acid) were added. The
tubes were placed in the dark for thirty minutes. The optical density was read at 520 nm in a spectrophotometer (Gilford 240). A blank containing all of the reagents was used to set the spectrophotometer to read 100 per cent transmission.

Hydroxyamphetamine standards were prepared and analyzed in the same manner as the samples to compensate for losses occurring in the procedure. The stock standard solution of hydroxyamphetamine containing 1 mg/ml (hydroxyamphetamine hydrobromide, Smith, Kline and French) 153 mg was diluted to 100 ml with distilled deionized water. The working hydroxyamphetamine standard had a concentration of 100 μg/ml. A linear relationship existed between absorbance and concentration of hydroxyamphetamine in standard solutions.

Estimation of p-hydroxyamphetamine conjugates. The procedure used for the estimation of p-hydroxyamphetamine conjugates (glucuronide and sulphate) was similar to that for the free metabolite but was preceded by incubation of sample with appropriate enzyme in a suitable buffer for 1 hour. Hydroxyamphetamine glucuronide: To 3 ml 0.2 molar acetate buffer (pH 4.6) was added 3 ml biologic fluid and 50 mg β-glucuronidase (beef liver, salt free, 70,000-100,000 units/10 grm; Nutritional Biochemicals Corporation, Cleveland, Ohio 44128). Hydroxyamphetamine sulphate: To 3 ml 0.2 M acetate buffer (pH 5.0) was added 3 ml biologic fluid and 1 unit sulphatase (Sulfatase, Type III: from Limpets; Sigma Chemical Co., St. Louis, Mo., 63178). The mixtures were incubated in a Dubnoff metabolic shaking incubator.
for 1 hour at 37° C. Following this period the hydroxyamphetamine concentration was determined in 2.5 ml of the incubated sample. The concentration of conjugate was estimated by difference between total and unconjugated hydroxyamphetamine concentrations.

Extent of plasma protein binding.

The effect of varying drug concentration upon the extent of protein binding was established at the following concentrations of $H^3$-d-amphetamine sulphate (2.5, 5, 10, 20, 25, 30 and 40) $\times 10^{-7}$ Molar in canine, feline and caprine plasma. Since the extent of plasma protein binding was found to be independent of amphetamine concentration, it was decided to determine the extent of protein binding in all species under investigation at an amphetamine concentration of $10^{-6}$ Molar, that is, 100 ng/ml. The extent of protein binding of amphetamine was determined by the equilibrium dialysis technique for each of the following species: caprine, porcine, equine, lapine, avian (chicken), canine, feline, Rhesus monkey, rat and opossum.

The total plasma protein concentrations were determined by refractometry with a serum protein metre (Bausch and Lomb, Rochester, N.Y.). The dialysis system was based on double equilibrium dialysis cells (Chemical Rubber Company, Cleveland, Ohio) used in conjunction with a CRC Dialysis Cell Shaker placed on a Laboratory Warming Plate with an Acrylic Plastic Hood (Will "Gentle-Therm", Will Scientific Inc., Rochester, N.Y.), maintained at 37° C. A premoistened cellophane membrane strip
was placed between the half-cells and clamped in place. Plasma (1 ml) was introduced into one-half cell and in the opposing cell 1 ml isotonic dialysis buffer (pH 7.4) containing 100 ng H\textsuperscript{3}-d-amphetamine sulphate with activity 2.5 \(\mu\)Ci (New England Nuclear). The dialysis buffer consisted of 0.16 molar \(\text{KH}_2\text{PO}_4\)+0.16 molar \(\text{Na}_2\text{HPO}_4\). Following a 24 hour incubation period 100 microlitre aliquots were removed from each side, added to 0.5 ml Soluene (Packard Instrument Company) and 15 ml of scintillation medium and the radioactivity was assayed. The per cent of plasma protein binding was calculated according to the formula:

\[
\text{per cent protein binding} = \frac{\text{dpm/ml plasma} - \text{dpm/ml buffer}}{\text{dpm/ml plasma}} \times 100
\]

Radioassay Reagent. Bray's solution (1960) was prepared as follows: 4 gm Permablend 1 and 60 gm naphthalene were placed in a volumetric flask, 100 ml absolute methanol was added followed by 20 ml ethylene glycol and made up to 1 liter mark with 1,4-dioxane. The prepared solution was stored at 2-5\(^\circ\) C.

The premixed scintillator Permablend 1 (Packard Instrument Company, Downers Grove, Illinois 60515) contained 91 per cent, 2,5-diphenyloxazole (PPO) and 9 per cent 1-4-bis-2-(4-methyl-5-phenyloxazole)-benzene (dimethyl POPOP) as primary and secondary scintillators respectively.

General Radioassay Procedure. The use of \(^3\)H-amphetamine sulphate required that liquid scintillation counting techniques be employed. The standard procedures for quantitation were found to be suitable (Bell and Hayes, 1958; Chase and Rabinowitz, 1962;
Rapkin, 1964). Standard 20 ml low-potassium borosilicate glass counting vials, with 24 mm screw caps lined with polyethylene discs, were used to contain the samples (Packard Instrument Company, Downers Grove, Illinois). A three-channel liquid scintillation spectrometer (Packard 3380 Tri Carb Liquid Scintillation Spectrometer) with absolute activity analyzer, automatic external standardization and automatic printout was used for the radioassay procedure. The sample compartment was refrigerated, each sample was counted for ten minutes and changed automatically.

C. PHARMACOKINETIC COMPUTATIONS AND STATISTICAL ANALYSES

All the pharmacokinetic parameters were determined for each individual animal. Calculations were performed by hand and on a Wang electronic calculator with punch-card programmer (Model 360K, Wang Laboratories Incorporated, Tewksbury, Mass.). The mean value of each parameter, the standard deviation and standard error about the mean were estimated for each species of animal studied. The plasma amphetamine (free + protein bound) versus time profiles following intravenous injection of the drug were plotted on semilogarithmic paper.

The biological half-life, $t_{1/2}$, may be defined as the time required for the body to eliminate one half of the drug which it contains. The half-life is associated with the rate constant for elimination, $\beta$, by the expression
\[ t_{\frac{1}{2}} = \frac{0.693}{\beta} \]

where \(-\beta\) is the slope of the first order plot for a one compartment model \((P = Be^{-\beta t})\) and the final slope of the biphasic plot for a two-compartment model \((P = Ae^{-\alpha t} + Be^{-\beta t})\). The assumption made in using the value \(\beta\) is that the tissue:plasma ratio remained constant during the elimination process. Since amphetamine was distributed into extravascular body fluids the overall elimination was reflected by \(\beta\) rather than by \(k_2\), the specific rate constant for elimination from the central compartment (Notari, 1971).

The biological half-life was estimated from the decline in amphetamine concentration in plasma after diffusion equilibrium had been attained. The body behaved as a one compartment open model in describing the pharmacokinetics of this drug.

The value \(B\) (ng/ml) may be defined as the concentration of drug which would be found in the plasma at time zero if the distribution of drug to tissue fluids occurred instantaneously. The value of this term was calculated using the least square regression line of the logarithm of plasma concentration versus time as the best-fit of the data (Snedecor and Cochran, 1967). The least square regression calculations were programmed for the Wang calculator, so that the normal equations for the least square line were solved simultaneously:

\[ X = a_0 N - a_1 \sum X \]
\[ \sum XY = a_0 \sum X - a_1 \sum X^2 \]

where \[ a_1 = \frac{N \sum XY - (\sum X)(\sum Y)}{N \sum X^2 - (\sum X)^2} \]

and \[ a_0 = \frac{\sum Y - a_1 \sum X}{N} \]

The least square line of \( Y \) on \( X \) has the equation:

\[ Y = a_0 - a_1 X \]

The kinetic constants were determined as follows:

concentration at time zero \( (B) = \) antilog of \( a_0 \)

half-life in hours \( (t_{1/2}) = -\frac{0.301}{a_1} \)

where \( a_1 \) was the slope of the least square regression line.

Apparent first order elimination rate constant in hour\(^{-1}\)

\[ \beta = \frac{0.693}{t_{1/2}} \]

or

\[ \beta = -2.303 \times a_1 \]

The apparent volume of distribution may be defined as that volume of body water which would be required to contain the amount of drug in the body if it were uniformly present in the same concentration in which it is in the blood. The value of \( V_d \) was calculated from the equation

\[ V_d = \frac{D}{B} \]

where \( D \) was the dose administered (\( \mu g \)) and \( B \) was the drug concentration at time zero (\( \mu g/litre \)), that is the intercept value obtained by extrapolation of the first-order plot for mono-
exponential loss of drug. It is obvious that the lower the value of B the greater the apparent volume of distribution. 

The apparent specific volume of distribution, \( V'd \), expressed in litres per kg = \( \frac{Vd (\text{litres})}{\text{Body Weight (kg)}} \). The assay procedure measured both free and bound amphetamine in plasma samples so the apparent specific volume of distribution had to be corrected for the extent of plasma protein binding.

The mean percent bound amphetamine was used to correct the B values which were employed in computing the corrected apparent specific volumes of distribution (\( V'd^* \)). The corrected B value (\( B^* \)) was calculated as follows

\[
B^* = B - (B \times \text{bound amphetamine } \%)
\]

\[
Vd^* = \frac{D}{B^*}
\]

and

\[
V'd^* = \frac{Vd^*}{\text{Body Weight}}
\]

A clearance value may be defined as the volume of plasma cleared of drug by an elimination process during a given time interval. Typically, a clearance value is based upon the assumption that the rate of elimination from the plasma is proportional to the plasma concentration or

\[
\text{Rate of elimination} = -\frac{dP}{dt} = k_2P
\]

The clearance value, C, may be calculated from

\[
C(\text{ml min}^{-1}) = \left(\frac{\text{amount eliminated per unit time}}{P_t}\right)
\]
where the rate of elimination and the plasma concentration are both determined at time, t. This equation was employed in calculating Renal clearance values in normal dogs and biliary clearance values in normal and nephrectomized dogs.

\[ C_{\text{renal}}(\text{ml min}^{-1}) = \frac{\text{amphetamine conc. in urine per minute}}{\text{amphetamine conc. in plasma}} \times \text{urine volume} \]

The rate appearance of amphetamine in urine was obtained by cannulating both ureters and collecting all the urine at half-hourly periods. A blood sample was collected mid-way through each urine collection period and the plasma amphetamine concentration was determined. The renal clearance (ml min\(^{-1}\)) was calculated by dividing the amount of amphetamine in urine per minute (ng min\(^{-1}\)) by the plasma amphetamine concentration (ng ml\(^{-1}\)). A similar procedure was employed in calculating the rate of appearance of amphetamine in bile. The biliary clearance (ml min\(^{-1}\)) was calculated by dividing the amount of amphetamine in bile per minute (ng min\(^{-1}\)) by the plasma amphetamine concentration (ng ml\(^{-1}\)). For convenience of comparison all clearance values were expressed on a unit weight bases (ml min\(^{-1}\) kg\(^{-1}\)).

To calculate the overall clearance in units of ml per min per kg the following equation was employed

\[ C'_{\text{total}}(\text{ml min}^{-1} \text{ kg}^{-1}) = \frac{0.693 \times V'd^* (\text{ml kg}^{-1})}{t_{1/2}(\text{min})} \]

Since elimination takes place only from the central compartment, the rate constant of elimination from this central compartment, \(k_2\),
may be derived by dividing the overall clearance value by 50 ml $k_e^{-1}$. The rate constant $k_2$ represents the elimination constant for loss of drug from the central compartment (blood) by all routes, that is,

$$k_2 = k_{\text{metabolism}} + k_{\text{excretion}}$$

The fraction of the dose recovered as metabolite or intact drug can be used to calculate the individual rate constants provided elimination is shown to be first-order. When the amount of drug in the body has become zero then

$$k_{\text{ex}} = k_2 \cdot C_{\infty}/D$$

and

$$k_m = k_2 \cdot M_{\infty}/D$$

where $C_{\infty}$ is the total amount excreted intact, $M_{\infty}$ is the total amount metabolized and $D$ is the dose.

The rate constant of excretion, $k_{\text{ex}}$, is the sum of $k_{\text{urine}} + k_{\text{bile}}$. The values of these microconstants were derived from the respective clearance values per kilogram by dividing the clearance values by fifty. The volume of plasma per kilogram body weight was assumed to be 50 ml.

Standard statistical methods were used to interpret the results of the studies (Snedecor and Cockran, 1967; Goldstein, 1964; Sokal and Rohlf, 1969). The standard error of the mean was calculated and reflected the reproducibility of an observed value. The standard error of a statistic such as the mean is the standard deviation of a distribution of means for samples of a given sample
size n (S.E.M. = S.D./√n). Fixed model one-way analyses of variance were performed to test for differences between means of various pharmacokinetic parameters for different species of animals. The F-test was employed to determine the significance of observed differences between mean values. The Student's "t" test was used to evaluate the significance of differences between mean values of two independent sets of observations. A probability of 0.05 or less was required before the null hypothesis was rejected.
CHAPTER IV

RESULTS

The experimental results for each section (Chapter III, part A) will be presented separately. The principles of pharmacokinetics were employed in interpreting the plasma amphetamine concentration-time profile for each experimental subject. The individual animals within each species were considered as a group to constitute a comparative study.

The range of sensitivity of the method for quantitation of amphetamine was 25-600 ng/ml of biological fluid. Linear relations between detector responses and the added standards were routinely obtained. Figure 3 shows the amphetamine peaks produced by a range of standards. The linear range of the EC-detector was from 25 ng/ml to 250 ng/ml. The response of the EC-detector was consistent when a single sample was injected repeatedly into the gas chromatograph (Figure 4). The detector response to hourly plasma samples collected from two dogs following the intravenous injection of dl-amphetamine sulphate (0.66 mg/kg, calculated as free base) are illustrated in Figures 5 and 6.

A systematic study on derivatives suitable for analysis of amphetamine, using gas chromatography with the electron capture detector, showed that the trichloroacetyl (TCA) derivative had excellent properties in terms of high EC-response, good
AMPHETAMINE CONC. (ng/ml⁻¹)
(LINEAR RESPONSE OF ECD)

Figure 3. The linear response of the electron capture detector to standard solutions of amphetamine.
AMPHETAMINE CONC. IN A PLASMA SAMPLE

Figure 4. Consistent response of the electron capture detector to a number of similar TCAA injections.
Figure 5. Amphetamine peaks showing the response of the electron capture detector to TCAA prepared from plasma extracts. The blood samples were collected from a dog at hourly intervals following the intravenous injection of dl-amphetamine sulphate (0.66 mg/kg, calculated as free base).

1 2 3 4 5
HOURS

AMPHETAMINE, PLASMA
Figure 6. Amphetamine peaks showing the response of the electron capture detector to TCAA prepared from dog plasma extracts.
chromatographic properties in the subnanogram range, and facile formation (Anonymous, 1968). Similar results were independently reported by Noonan, Murdick and Ray (1969), Anggard and Hankey (1969) and Anggard, Gunne and Niklasson (1970).

Sections 1 and 3: Curves relating logarithm of the amphetamine concentration in plasma (ng/ml) versus time (hour) following the intravenous injection of 0.66 mg/kg d, l-amphetamine sulphate (calculated as free base) are shown in Figures (7-13 ) for goats, swine, ponies, dogs, cats, rabbits and chickens respectively. These figures show typical first-order elimination curves after intravenous injection of the drug. The disappearance from the body of a constant fraction of the drug in each equal interval of time is characteristic of a first-order elimination process. For first-order (exponential) elimination the familiar equations apply, where P denotes total drug in the body at time t, B the drug present at time zero, and β the rate constant for elimination:

\[ P = Be^{-\beta t} \]

\[ \log P = \log B - \beta t/2.303 \]

\[ t_{\frac{1}{2}} = \frac{0.693}{\beta} \]

The half-time for elimination is called the biologic half-life. The value of B, which is the antilog of a in the equation of the least square line of Y (plasma amphetamine concentration) on X (time) is an estimate of the concentration that would have been present initially if all the injected dose had been distributed in the volume
Figure 7. Disappearance of amphetamine from the blood plasma of goats following the intravenous injection of amphetamine sulphate (0.66 mg/kg, calculated as free base). Each point represents the mean concentration (10 goats) and each vertical bar is ± 1 S.D.
Figure 8. Disappearance of amphetamine from the blood plasma of swine following the intravenous injection of amphetamine sulphate (0.66 mg/kg, calculated as free base). Each point represents the mean concentration (9 pigs) and each vertical bar = ±1 S.D. $t_{1/2} : 1.05 \text{ hr.}$
Figure 9. Disappearance of amphetamine from the blood plasma of ponies following the intravenous injection of amphetamine sulphate (0.66 mg/kg, calculated as free base). Each point represents the mean concentration (5 ponies) and each vertical bar = ± 1 S.D.

\( t_{\frac{1}{2}} : 1.39 \text{ hr.} \)
Figure 10. Disappearance of amphetamine from the blood plasma of intact and nephrectomized dogs following the intravenous injection of amphetamine sulphate (0.66 mg/kg, calculated as free base).
Figure 11. Disappearance of amphetamine from the blood plasma of cats following the intravenous injection of amphetamine sulphate (0.66 mg/kg, calculated as free base). Each point represents the mean concentration (6 cats) and each vertical bar = ±1 S.D.

$\frac{t_1}{2} = 6.53$ hr.
Mean concentration (4 rabbits) ± 1 S.D.

Figure 12. Disappearance of amphetamine from the blood plasma of rabbits following the intravenous injection of amphetamine sulphate (0.66 mg/kg, calculated as free base).
Figure 13. Disappearance of amphetamine from the blood plasma of chickens following the intravenous injection of amphetamine sulphate (0.66 mg/kg, calculated as free base). Each point represents the mean concentration (8 chickens) and each vertical bar = ± 1 S.D.

[t_1/2 : 2.27 hr.]
of distribution ultimately attained. The B value, which is expressed in units of concentration, is reciprocally related to the apparent volume of distribution of the drug. A similar dose of amphetamine per kilogram body weight was administered to each experimental subject so the B values indirectly provide a basis for comparing the extent of drug distribution per kg between different species. The lower the B value the greater the apparent specific volume of distribution of the drug. The lines describing the disappearance of amphetamine from plasma of the seven species of animals studied are shown in Figure 14. The slopes of these regression lines appear different and the B values appear to vary between species. This figure indicates that the distribution phase for this drug was rapid, less than 1 hour for all species, so that the body which is best described as a two compartment open model (Figure 15) reduces to a one compartment open model in describing the pharmacokinetics of amphetamine. The one compartment open model implies that the sum of the microconstants describing the rate of attainment of the apparent volume of distribution is considerably greater than the rate constant for elimination from the blood, that is,

\[ k_{12} + k_{21} \gg k_2 \]

The one compartment open model is an approximation or simplification used to describe the two compartment open model when \( \alpha \gg \beta \). The data are described by the equation \( P = Be^{-\beta t} \), the ratio of \( P/T \) remains constant and equal to the equilibrium value or \( k_{12}/k_{21} \).
Figure 14. Disappearance of amphetamine from the blood plasma of the feline, canine, avian, lapine, equine (pony), porcine and caprine species following the intravenous injection of amphetamine sulphate (0.66 mg/kg, calculated as free base).
Figure 15. The body: a two compartment open model. B represents the central compartment, blood plasma; T represents tissues and C excreta, principally urine. The rate constant for elimination from the central compartment, $k_2$, is the sum of the rate constants for metabolism, $k_m$, and excretion, $k_{ex}$. 

$$k_2 = k_m + k_{ex}$$
The mean value of the biological half-life, one standard deviation and the standard error about the mean for each species of animal investigated are tabulated in Table 4. The mean biologic half-life under normal conditions of fluctuating urinary pH reaction in these randomly selected groups of animals varied between species (F test, \( p < 0.01 \)). The shortest half-life was in goats; ponies, rabbits, chickens and swine had half-lives for this drug of intermediate duration while longer half-lives were in dogs and cats. The standard deviation was particularly wide in dogs and cats. No sex difference in persistence of the drug was observed in any species studied.

Table 5 contains the values of the pharmacokinetic constants describing the extent of distribution and the rate of elimination of this drug. The relative values of \( B \) and \( V'd \) are reciprocally related. The concentration of amphetamine which would be found in the plasma at time zero if the distribution of drug to tissue fluids occurred instantaneously was low (less than 0.50 microgram per ml) in all species. This explains the previous lack of information upon the plasma decay and biological half-life of this drug. Since amphetamine was distributed into body fluids the overall elimination is reflected by \( \beta \) rather than by \( k_2 \), the specific rate constant for elimination from the central compartment. The half-life is associated with \( \beta \).

The assay procedure measured both free and bound amphetamine in plasma samples so the apparent specific volume of distribution had to be corrected for the extent of plasma protein
TABLE 4

THE BIOLOGICAL HALF-LIFE OF AMPHETAMINE IN VARIOUS SPECIES OF ANIMALS

<table>
<thead>
<tr>
<th>Species (number)</th>
<th>Mean</th>
<th>S.D.</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caprine (10)</td>
<td>0.62</td>
<td>0.13</td>
<td>0.04</td>
</tr>
<tr>
<td>Porcine (9)</td>
<td>1.05</td>
<td>0.14</td>
<td>0.05</td>
</tr>
<tr>
<td>Equine a (5)</td>
<td>1.39</td>
<td>0.18</td>
<td>0.08</td>
</tr>
<tr>
<td>Lapine (4)</td>
<td>1.40</td>
<td>0.15</td>
<td>0.08</td>
</tr>
<tr>
<td>Avian b (8)</td>
<td>2.27</td>
<td>0.48</td>
<td>0.17</td>
</tr>
<tr>
<td>Canine (11)</td>
<td>4.49</td>
<td>0.80</td>
<td>0.24</td>
</tr>
<tr>
<td>Feline (6)</td>
<td>6.53</td>
<td>0.94</td>
<td>0.39</td>
</tr>
</tbody>
</table>

a Ponies
b Chickens
<table>
<thead>
<tr>
<th>Species (number)</th>
<th>B (ng/ml)</th>
<th>T 1/2 (hr)</th>
<th>$\beta$ (hr$^{-1}$)</th>
<th>V'd (litre/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caprine (10)</td>
<td>235</td>
<td>0.62</td>
<td>1.118</td>
<td>3.08</td>
</tr>
<tr>
<td>Porcine (9)</td>
<td>330</td>
<td>1.05</td>
<td>0.674</td>
<td>2.23</td>
</tr>
<tr>
<td>Equine (5)</td>
<td>281</td>
<td>1.39</td>
<td>0.500</td>
<td>2.61</td>
</tr>
<tr>
<td>Lapine (4)</td>
<td>333</td>
<td>1.40</td>
<td>0.498</td>
<td>2.29</td>
</tr>
<tr>
<td>Avian (8)</td>
<td>438</td>
<td>2.27</td>
<td>0.305</td>
<td>1.81</td>
</tr>
<tr>
<td>Canine (11)</td>
<td>263</td>
<td>4.50</td>
<td>0.159</td>
<td>2.67</td>
</tr>
<tr>
<td>Feline (6)</td>
<td>401</td>
<td>6.53</td>
<td>0.108</td>
<td>1.83</td>
</tr>
</tbody>
</table>
binding. The percent amphetamine bound to plasma protein was independent of drug concentration over the concentration range observed in vivo (25-400 ng ml\(^{-1}\), Table 6). The extent of plasma protein binding of amphetamine for various species of animals at a drug concentration of 100 ng ml\(^{-1}\) (i.e., \(10^{-6}\) Molar) is tabulated in Table 7. The mean percent bound amphetamine and standard error are shown for each species. The mean and S.E.M. of the plasma protein concentration, expressed in gm/100 ml for the group of animals representing each species are also tabulated. The extent of plasma protein binding varied significantly between species (F test, \(p < 0.01\)). The species of animals may be divided into two groups on the basis of the extent of plasma protein binding of amphetamine. The first group comprises goats, swine, monkeys, and rats in which the extent of binding was about forty percent. Dogs, cats, horses, ponies, rabbits and opossum comprise the second group and bound approximately twenty-five percent of the drug. The chickens did not belong to either group, the percent bound amphetamine was low (15\%) and the total plasma protein concentration was approximately half the value observed in mammalian species.

The mean percent bound amphetamine for each species was used to correct the B values for the individual animals within the species. The corrected B values were then employed to compute the corrected apparent specific volumes of distribution (\(V'd^{*}\)). The corrected B values and thus the corrected apparent specific volumes of distribution varied significantly between
TABLE 6
THE EXTENT OF PLASMA PROTEIN BINDING OF AMPHETAMINE UNDER CONDITIONS OF VARYING DRUG CONCENTRATIONS.

<table>
<thead>
<tr>
<th>Species</th>
<th>Amphetamine conc. (10^{-7} Molar)</th>
<th>% bound amphetamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog A</td>
<td>5</td>
<td>26.4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>24.8</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>25.4</td>
</tr>
<tr>
<td>Dog B</td>
<td>2.5</td>
<td>21.8</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>26.3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>24.7</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>26.9</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>24.0</td>
</tr>
<tr>
<td>Cat</td>
<td>5</td>
<td>25.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>25.2</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>26.8</td>
</tr>
<tr>
<td>Goat</td>
<td>5</td>
<td>42.3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>42.8</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>38.3</td>
</tr>
</tbody>
</table>
TABLE 7
TOTAL PLASMA PROTEIN CONCENTRATION AND EXTENT OF PROTEIN BINDING OF AMPHETAMINE IN VARIOUS SPECIES OF ANIMALS.

Amphetamine concentration: $10^{-6}$ Molar

<table>
<thead>
<tr>
<th>Species</th>
<th>% bound amphetamine Mean S.E.M.</th>
<th>Plasma protein conc. (g%) Mean S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equine (9)</td>
<td>25.3 2.6</td>
<td>7.5 0.3</td>
</tr>
<tr>
<td>Caprine (12)</td>
<td>40.7 1.7</td>
<td>6.6 0.3</td>
</tr>
<tr>
<td>Porcine (6)</td>
<td>39.6 3.0</td>
<td>6.4 0.6</td>
</tr>
<tr>
<td>Canine (17)</td>
<td>27.1 1.5</td>
<td>6.7 0.3</td>
</tr>
<tr>
<td>Feline (12)</td>
<td>26.4 1.2</td>
<td>7.3 0.2</td>
</tr>
<tr>
<td>Lapine (4)</td>
<td>31.0 2.9</td>
<td>5.5 0.3</td>
</tr>
<tr>
<td>Avian (6)</td>
<td>14.5 0.9</td>
<td>3.0 0.3</td>
</tr>
<tr>
<td>Monkey (11)</td>
<td>40.2 0.7</td>
<td>7.8 0.2</td>
</tr>
<tr>
<td>Opossum (4)</td>
<td>26.0 4.0</td>
<td>6.8 0.4</td>
</tr>
<tr>
<td>Rat (4)</td>
<td>40.5 3.0</td>
<td>6.1 0.8</td>
</tr>
</tbody>
</table>
species (F-test, p < 0.01). The corrected volumes of distribution were large in all species (Table 8), the values obtained for the porcine, equine, lapine and canine species were similar. The ruminant animals had a particularly large apparent volume of distribution (5.19 litre/kg). Cats and chickens, on the other hand, had smaller volumes of distribution for this drug than those estimated for the other animals.

The values of $k_2$, the specific rate constant for elimination from the central compartment, and $\beta$, the overall elimination rate constant, both expressed in reciprocal minutes are also tabulated in Table 8. The value of $k_2$ relative to $\beta$ is directly related to the apparent specific volume of distribution of the drug. The value of $k_2$ was one hundred times the value of $\beta$ in goats, $k_2$ was seventy-four times $\beta$ in swine and fifty times the value of $\beta$ in cats. The following equation relates the values of $k_2$ and for a one compartment model:

$$\beta = \frac{k_{12}}{k_{12} + k_{21}} \cdot k_2$$

The wider the ratio of $k_2: \beta$ the larger the apparent specific volume of distribution and the shorter the biological half-life.

Section 2: The quantities of unchanged amphetamine, p-hydroxyamphetamine and its glucuronide and sulphate conjugates in cumulative urine samples were measured. The time period for collection of urine exceeded five half-lives of amphetamine in each species. The amounts of each fraction recovered for the
### TABLE 8

**PHARMACOKINETIC CONSTANTS DESCRIBING THE DISTRIBUTION AND ELIMINATION OF AMPHETAMINE (2)**

<table>
<thead>
<tr>
<th>Species (number)</th>
<th>% bound Amphetamine $10^{-6}$ M</th>
<th>$V'd^*$ (litre/kg)</th>
<th>$\beta \times 10^{-2}$ (min$^{-1}$)</th>
<th>$k_2$ (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caprine (10)</td>
<td>40.7</td>
<td>5.19</td>
<td>1.9</td>
<td>1.934</td>
</tr>
<tr>
<td>Porcine (9)</td>
<td>39.6</td>
<td>3.69</td>
<td>1.1</td>
<td>0.812</td>
</tr>
<tr>
<td>Equine (5)</td>
<td>25.3</td>
<td>3.43</td>
<td>0.8</td>
<td>0.582</td>
</tr>
<tr>
<td>Lapine (4)</td>
<td>31.0</td>
<td>3.32</td>
<td>0.8</td>
<td>0.548</td>
</tr>
<tr>
<td>Avian (8)</td>
<td>14.5</td>
<td>2.12</td>
<td>0.5</td>
<td>0.292</td>
</tr>
<tr>
<td>Canine (11)</td>
<td>27.1</td>
<td>3.66</td>
<td>0.3</td>
<td>0.188</td>
</tr>
<tr>
<td>Feline (6)</td>
<td>26.4</td>
<td>2.49</td>
<td>0.18</td>
<td>0.088</td>
</tr>
</tbody>
</table>
individual subjects within each species are tabulated in Tables 9 to 16. The urinary pH reactions of the cumulative samples are also tabulated. There is no apparent relationship between the urinary pH reaction of the cumulative urine sample and the amount of any fraction recovered. The percent dose, mean and standard error, of unchanged amphetamine, 4-hydroxyamphetamine and its conjugates recovered in cumulative urine of the various species are tabulated in Table 17. The amount of each fraction recovered, expressed as percent of dose administered, is shown in histogram form (Figure 16) and the total recovery for each species in Figure 17. About 60 percent of the dose administered was recovered in the urine of goats, swine, chickens and cats. The urine collection time exceeded seven half-lives of drug in these species. Approximately 50 percent of the dose injected was recovered in the urine of dogs, the duration of urine collection just exceeded five half-lives of amphetamine in this species. This factor may partly explain the lower total recovery in dogs, however, the pattern of biotransformation may be more important. The total recoveries in rabbits and the equine species were low, about 30 percent of the dose. The time period for collection of urine in these species was about eight half-lives of the drug.

The concentrations of unchanged amphetamine in urine were measured by the gas chromatographic method following appropriate dilution of the urine samples. The concentrations of p-hydroxyamphetamine and its conjugates, determined as p-hydroxyamphetamine following enzymatic hydrolysis, were
TABLE 9

THE DOSE ADMINISTERED AND THE AMOUNTS OF AMPHETAMINE AND SOME METABOLITES RECOVERED, EXPRESSED IN MILLIGRAM, IN 24-HOUR URINE OF GOATS (10).

<table>
<thead>
<tr>
<th>Goat</th>
<th>Dose (1 mg/kg)</th>
<th>Urine pH</th>
<th>Amphetamine</th>
<th>p-Hydroxyamphetamine + conjugates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p-OH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>glucur.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sulphate</td>
</tr>
<tr>
<td>A</td>
<td>65</td>
<td>6.55</td>
<td>10.5</td>
<td>11.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16.3</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>6.98</td>
<td>5.6</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.8</td>
</tr>
<tr>
<td>C</td>
<td>40</td>
<td>7.25</td>
<td>4.0</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15.0</td>
</tr>
<tr>
<td>D</td>
<td>35</td>
<td>8.12</td>
<td>5.1</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.5</td>
</tr>
<tr>
<td>E</td>
<td>35</td>
<td>8.00</td>
<td>4.5</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.1</td>
</tr>
<tr>
<td>F</td>
<td>45</td>
<td>8.40</td>
<td>2.0</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.0</td>
</tr>
<tr>
<td>G</td>
<td>16</td>
<td>6.07</td>
<td>2.4</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>H</td>
<td>18</td>
<td>6.80</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td>J</td>
<td>15</td>
<td>6.52</td>
<td>1.9</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.1</td>
</tr>
<tr>
<td>K</td>
<td>15</td>
<td>6.60</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.7</td>
</tr>
</tbody>
</table>
TABLE 10

THE DOSE ADMINISTERED AND THE AMOUNTS OF AMPHETAMINE AND SOME METABOLITES RECOVERED, EXPRESSED IN MILLIGRAM, IN 24-HOUR URINE OF PIGS (6).

<table>
<thead>
<tr>
<th>Pig</th>
<th>Dose (0.66 mg/kg)</th>
<th>Urine pH</th>
<th>Amphetamine</th>
<th>p-Hydroxyamphetamine + conjugates</th>
<th>p-OH glucur.</th>
<th>Sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.5</td>
<td>8.50</td>
<td>0.86</td>
<td>2.16</td>
<td>2.31</td>
<td>0.36</td>
</tr>
<tr>
<td>B</td>
<td>8.5</td>
<td>7.63</td>
<td>1.60</td>
<td>1.66</td>
<td>2.32</td>
<td>0.28</td>
</tr>
<tr>
<td>C</td>
<td>9.0</td>
<td>6.67</td>
<td>1.00</td>
<td>1.85</td>
<td>2.77</td>
<td>0.16</td>
</tr>
<tr>
<td>D</td>
<td>6.5</td>
<td>5.58</td>
<td>1.30</td>
<td>0.75</td>
<td>1.05</td>
<td>0.08</td>
</tr>
<tr>
<td>E</td>
<td>9.0</td>
<td>6.45</td>
<td>1.25</td>
<td>1.35</td>
<td>1.65</td>
<td>0.20</td>
</tr>
<tr>
<td>F</td>
<td>10.2</td>
<td>6.26</td>
<td>1.40</td>
<td>2.40</td>
<td>3.60</td>
<td>0.40</td>
</tr>
</tbody>
</table>
TABLE II

THE DOSE ADMINISTERED AND THE AMOUNTS OF AMPHETAMINE AND SOME METABOLITES RECOVERED, EXPRESSED IN MILLIGRAM, IN 24-HOUR URINE OF RABBITS (9).

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Dose (1 mg/kg)</th>
<th>Urine pH</th>
<th>Amphetamine</th>
<th>p-Hydroxyamphetamine + conjugates</th>
<th>p-OH glucur.</th>
<th>Sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1</td>
<td>5</td>
<td>7.84</td>
<td>0.19</td>
<td>0.34</td>
<td>0.56</td>
<td>0.31</td>
</tr>
<tr>
<td>R-2</td>
<td>5</td>
<td>7.56</td>
<td>0.26</td>
<td>0.23</td>
<td>0.65</td>
<td>0.34</td>
</tr>
<tr>
<td>R-3</td>
<td>5</td>
<td>7.98</td>
<td>0.17</td>
<td>0.28</td>
<td>0.58</td>
<td>0.32</td>
</tr>
<tr>
<td>R-4</td>
<td>5</td>
<td>8.01</td>
<td>0.24</td>
<td>0.60</td>
<td>0.39</td>
<td>0.22</td>
</tr>
<tr>
<td>R-5</td>
<td>5</td>
<td>7.82</td>
<td>0.20</td>
<td>0.60</td>
<td>0.33</td>
<td>0.23</td>
</tr>
<tr>
<td>R-6</td>
<td>5</td>
<td>8.49</td>
<td>0.15</td>
<td>0.56</td>
<td>0.36</td>
<td>0.25</td>
</tr>
<tr>
<td>R-X</td>
<td>5</td>
<td>8.29</td>
<td>0.09</td>
<td>0.60</td>
<td>0.16</td>
<td>0.37</td>
</tr>
<tr>
<td>R-Y</td>
<td>5</td>
<td>7.89</td>
<td>0.10</td>
<td>0.45</td>
<td>0.36</td>
<td>0.40</td>
</tr>
<tr>
<td>R-Z</td>
<td>5</td>
<td>8.06</td>
<td>0.09</td>
<td>0.67</td>
<td>0.28</td>
<td>0.33</td>
</tr>
<tr>
<td>Subject</td>
<td>Time (hr)</td>
<td>Urine pH</td>
<td>Amphetamine</td>
<td>p-Hydroxyamph. + conj.</td>
<td>p-OH glucur.</td>
<td>Sulphate</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
<td>----------</td>
<td>--------------</td>
<td>-----------------------</td>
<td>--------------</td>
<td>----------</td>
</tr>
<tr>
<td>Lassie</td>
<td>5</td>
<td>7.91</td>
<td>0.48</td>
<td>4.3</td>
<td>5.8</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>7.83</td>
<td>0.42</td>
<td>4.1</td>
<td>5.4</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>8.31</td>
<td>0.20</td>
<td>4.2</td>
<td>6.5</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>7.92</td>
<td>0.08</td>
<td>4.0</td>
<td>7.1</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>8.06</td>
<td>0.03</td>
<td>3.0</td>
<td>5.9</td>
<td>3.2</td>
</tr>
<tr>
<td>Filly Byrd</td>
<td>5</td>
<td>7.85</td>
<td>0.48</td>
<td>5.2</td>
<td>4.9</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>7.25</td>
<td>0.40</td>
<td>5.4</td>
<td>5.0</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>7.16</td>
<td>0.24</td>
<td>5.5</td>
<td>6.1</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>7.93</td>
<td>0.08</td>
<td>5.1</td>
<td>6.5</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>8.31</td>
<td>0.02</td>
<td>4.7</td>
<td>5.8</td>
<td>2.0</td>
</tr>
</tbody>
</table>
### TABLE 13

THE DOSE ADMINISTERED AND THE AMOUNTS OF AMPHETAMINE AND SOME METABOLITES RECOVERED, EXPRESSED IN MILLIGRAM, IN 24-HOUR URINE OF PONIES (2)

<table>
<thead>
<tr>
<th>Pony</th>
<th>Dose (mg)</th>
<th>Urine pH</th>
<th>Amphetamine</th>
<th>p-Hydroxyamph. + conjugates</th>
<th>glucur.</th>
<th>sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>30</td>
<td>8.32</td>
<td>1.25</td>
<td>3.0</td>
<td>2.5</td>
<td>1.3</td>
</tr>
<tr>
<td>B</td>
<td>30</td>
<td>8.21</td>
<td>1.00</td>
<td>4.0</td>
<td>3.0</td>
<td>1.8</td>
</tr>
</tbody>
</table>
TABLE 14
THE DOSE ADMINISTERED AND THE AMOUNTS OF AMPHETAMINE
AND SOME METABOLITES RECOVERED, EXPRESSED IN
MILLIGRAM, IN 24-HOUR URINE OF CHICKENS (4).

<table>
<thead>
<tr>
<th>Chicken</th>
<th>Dose (0.66 mg/kg)</th>
<th>Urine pH</th>
<th>Amphetamine</th>
<th>p-Hydroxyamph. + conjugates</th>
<th>p-OH</th>
<th>glucur.</th>
<th>sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>3</td>
<td>6.92</td>
<td>0.80</td>
<td>0.16</td>
<td>0.40</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>2</td>
<td>6.62</td>
<td>0.50</td>
<td>0.18</td>
<td>0.14</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>2</td>
<td>6.23</td>
<td>0.75</td>
<td>0.16</td>
<td>0.08</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Q</td>
<td>2</td>
<td>6.45</td>
<td>0.80</td>
<td>0.12</td>
<td>0.08</td>
<td>0.22</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 15
THE DOSE ADMINISTERED AND THE AMOUNTS OF AMPHETAMINE AND SOME METABOLITES RECOVERED, EXPRESSED IN MILLIGRAM, IN 24- HOUR URINE OF DOGS (6).

<table>
<thead>
<tr>
<th>Dog</th>
<th>Dose (1 mg/kg)</th>
<th>Urine pH</th>
<th>Amphetamine</th>
<th>p-Hydroxyamphetamine + conjugates</th>
<th>p-OH glucur.</th>
<th>Sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>15</td>
<td>7.67</td>
<td>4.74</td>
<td>2.96</td>
<td>0.72</td>
<td>0.49</td>
</tr>
<tr>
<td>D</td>
<td>15</td>
<td>7.48</td>
<td>4.26</td>
<td>0.70</td>
<td>0.21</td>
<td>0.33</td>
</tr>
<tr>
<td>E</td>
<td>25</td>
<td>6.65</td>
<td>7.44</td>
<td>2.79</td>
<td>0.93</td>
<td>0.62</td>
</tr>
<tr>
<td>F</td>
<td>30</td>
<td>7.56</td>
<td>10.32</td>
<td>3.20</td>
<td>1.00</td>
<td>0.70</td>
</tr>
<tr>
<td>N</td>
<td>15</td>
<td>7.75</td>
<td>4.49</td>
<td>1.04</td>
<td>0.46</td>
<td>0.61</td>
</tr>
<tr>
<td>K</td>
<td>20</td>
<td>7.60</td>
<td>5.13</td>
<td>2.85</td>
<td>1.20</td>
<td>0.88</td>
</tr>
</tbody>
</table>
TABLE 16

THE DOSE ADMINISTERED AND THE AMOUNTS OF AMPHETAMINE AND SOME METABOLITES RECOVERED, EXPRESSED IN MILLIGRAM, IN 48-HOUR URINE OF CATS (8).

<table>
<thead>
<tr>
<th>Cat</th>
<th>Dose (1 mg/kg)</th>
<th>Urine pH</th>
<th>Amphetamine</th>
<th>pHydroxyamph. + conjugates glucur.</th>
<th>sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5</td>
<td>7.74</td>
<td>2.17</td>
<td>0.29</td>
<td>0.31</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>7.55</td>
<td>2.10</td>
<td>0.25</td>
<td>0.48</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>6.86</td>
<td>1.23</td>
<td>0.18</td>
<td>0.35</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>7.42</td>
<td>1.55</td>
<td>0.28</td>
<td>0.40</td>
</tr>
<tr>
<td>E</td>
<td>6</td>
<td>8.00</td>
<td>2.08</td>
<td>0.40</td>
<td>0.70</td>
</tr>
<tr>
<td>F</td>
<td>6</td>
<td>6.45</td>
<td>2.07</td>
<td>0.35</td>
<td>0.55</td>
</tr>
<tr>
<td>G</td>
<td>6</td>
<td>7.58</td>
<td>2.36</td>
<td>0.32</td>
<td>0.79</td>
</tr>
<tr>
<td>H</td>
<td>6</td>
<td>7.23</td>
<td>1.88</td>
<td>0.30</td>
<td>0.64</td>
</tr>
</tbody>
</table>
TABLE 17

PERCENT DOSE, MEAN (S.E.M.), OF AMPHETAMINE AND SOME OF ITS METABOLITES IN 24 HOUR URINE OF DIFFERENT SPECIES.

<table>
<thead>
<tr>
<th>Species (number)</th>
<th>Amphetamine</th>
<th>4-Hydroxy-amphetamine</th>
<th>Conjugates</th>
<th>Sulphate</th>
<th>Percent dose recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caprine (10)</td>
<td>12 (1.5)</td>
<td>11 (1.0)</td>
<td>13 (2.3)</td>
<td>21 (2.0)</td>
<td>57</td>
</tr>
<tr>
<td>Porcine (6)</td>
<td>15 (1.5)</td>
<td>20 (2.5)</td>
<td>27 (3.1)</td>
<td>3 (0.4)</td>
<td>65</td>
</tr>
<tr>
<td>Equine (4)</td>
<td>2</td>
<td>12</td>
<td>13</td>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td>Lapine (9)</td>
<td>3 (0.4)</td>
<td>10 (0.8)</td>
<td>8 (0.8)</td>
<td>6 (0.4)</td>
<td>27</td>
</tr>
<tr>
<td>Avian (4)</td>
<td>32 (3.8)</td>
<td>7 (1.0)</td>
<td>7 (2.2)</td>
<td>12 (1.0)</td>
<td>58</td>
</tr>
<tr>
<td>Canine (6)</td>
<td>30 (1.2)</td>
<td>11 (2.2)</td>
<td>4 (0.6)</td>
<td>3 (0.4)</td>
<td>48</td>
</tr>
<tr>
<td>Feline (8) a</td>
<td>35 (2.2)</td>
<td>5 (0.3)</td>
<td>9 (0.8)</td>
<td>10 (0.4)</td>
<td>59</td>
</tr>
</tbody>
</table>

a. 48 hour urine
Figure 16. The mean percent of dose of amphetamine (unshaded), p-hydroxyamphetamine and its conjugates in cumulative urine (24 hours except cats, 48 hours) of different species. The key is provided on Figure 17. The vertical bar = ±1 S.E.M.
Figure 17.

The percent of dose of amphetamine, p-hydroxyamphetamine and its conjugates in cumulative urine (24 hours except cats, 48 hours) of different species. The numbers in brackets are the number of animals studied in the species.

Species:
- CP. (10)
- Pr. (6)
- Eq. (4)
- Lp. (9)
- Avian (4)
- Cn. (6)
- Fl. (8)

% of Dose Recovered in 24 Hour Urine

- Sulphate
- Glucuronide
- p-Hydroxyamphetamine
- Amphetamine

The numbers in brackets are the number of animals studied in the species.
measured by the colorimetric method of Axelrod (1954). The proportion of the dose recovered as unchanged amphetamine varied between species. About one-third of the dose administered was recovered unchanged in the urine of cats, dogs and chickens, one-eighth of the dose unchanged in the urine of swine and goats and only one-fortieth of the dose was excreted as amphetamine in the urine of rabbits, horses and ponies. Since $k_{ex} = k_2 \cdot C_\infty / D$ the magnitude of $k_{ex}$ varied between different species. The rate constant for elimination from the blood, $k_2$, is equal to the sum of $k_{metabolism}$ and $k_{excretion}$. Therefore $k_{ex}$ constituted approximately one-third of $k_2$ in cats, dogs and chickens, one-eighth of $k_2$ in swine and goats and contributed negligibly to $k_2$ in rabbits, horses, and ponies. Biotransformation is thus the major route of elimination of this drug, particularly in herbivorous animals and swine. The amount of amphetamine which is eliminated by renal excretion, that is the magnitude of the contribution of $k_{ex}$ to $k_2$, may be of importance in the influence of urinary pH change upon the biological half-life of this drug. The urinary pH determines the proportion of nonionized drug in the renal tubular fluid and thus the amount of this lipid-soluble form which is available for reabsorption by passive nonionic diffusion.

Section 4: The objective of the experimental work in this section was to determine the pharmacokinetic constants describing the extent of distribution and the rate of elimination of amphetamine in normal and nephrectomized dogs. The amphetamine concentration
in plasma versus time profile in the normal and nephrectomized
dogs is shown in Figure 18.

Experiment (i): The mean biologic half-life under normal
conditions of fluctuating urinary pH reaction in this ran-
domly selected group of 'normal' dogs was 4.56 hours.
Extravascular distribution of this drug attained equilibrium
in less than one half-hour (Figure 18), and the corrected
apparent specific volume of distribution was large (3.63
litres/kg, Table 18).

The extent of plasma protein binding was determined
in vivo by measuring the amphetamine concentrations by
the gas chromatographic method in cerebrospinal fluid
and plasma samples collected simultaneously after
diffusion equilibrium was attained. After diffusion
equilibrium was attained the concentrations of amphet-
amine in cerebrospinal and ocular fluid collected simul-
taneously were similar (Figure 19). The equilibrium
dialysis technique using $^3$H-d-amphetamine sulphate and
liquid scintillation counting was employed to determine
the extent of plasma protein binding in vitro. The extent
of protein binding of amphetamine in the dog determined
by the two methods compared favourably (Table 19).

Experiment (ii): The total dose of amphetamine adminis-
tered, the biologic half-life of the drug, the cumulative
amounts of unchanged amphetamine excreted in urine and
bile and the range of urinary pH values in individual dogs
Figure 18. Disappearance of amphetamine from the blood plasma of intact and nephrectomized dogs following the intravenous injection of amphetamine sulphate (0.66 mg/kg, calculated as free base).
**TABLE 18**

PHARMACOKINETIC CONSTANTS DESCRIBING DISTRIBUTION AND ELIMINATION OF AMPHETAMINE IN DOGS.

<table>
<thead>
<tr>
<th>Dogs (number)</th>
<th>B (ng/ml) (S.D.)</th>
<th>T₁/₂ (hour) (S.E.M.)</th>
<th>β (hour⁻¹) (S.E.M.)</th>
<th>V'd (litre/kg) (S.E.M.)</th>
<th>% bound in vivo</th>
<th>V'd* a (litre/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1 A to F (6)</td>
<td>275 (18)</td>
<td>4.56 (0.29)</td>
<td>0.156 (0.012)</td>
<td>2.65 (0.06)</td>
<td>27.1 b</td>
<td>3.63</td>
</tr>
<tr>
<td>Exp. 2 R to Z (5)</td>
<td>248 (30)</td>
<td>4.42 (0.44)</td>
<td>0.162 (0.013)</td>
<td>2.69 (0.15)</td>
<td>23.6</td>
<td>3.51</td>
</tr>
<tr>
<td>Exp. 3 NA to NE (5)</td>
<td>262 (10)</td>
<td>5.81 (0.28)</td>
<td>0.120 (0.005)</td>
<td>2.53 (0.04)</td>
<td>25.0</td>
<td>3.36</td>
</tr>
<tr>
<td>Exp. 4 NF to NL (3)</td>
<td>251 (12)</td>
<td>5.47 (0.30)</td>
<td>0.127 (0.007)</td>
<td>2.63 (0.07)</td>
<td>23.0</td>
<td>3.42</td>
</tr>
</tbody>
</table>

a V'd* is the apparent specific volume of distribution corrected for protein binding.

b *in vitro* (mean of 17 dogs).
Figure 19. Amphetamine peaks show the response of the electron capture detector to extracts of plasma, cerebrospinal and ocular fluids. The samples were collected simultaneously from a dog 4 hours after the intravenous injection of dl-amphetamine sulphate (0.66 mg/kg, calculated as free base).
TABLE 19

PLASMA PROTEIN BINDING OF AMPHETAMINE IN DOGS.

<table>
<thead>
<tr>
<th>Variable</th>
<th>% bound amphetamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in vitro</td>
</tr>
<tr>
<td>Amphet. conc.</td>
<td>100 ng. ml⁻¹</td>
</tr>
<tr>
<td>Mean</td>
<td>27.1</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>1.5</td>
</tr>
</tbody>
</table>
are tabulated in Table 20. No attempt was made to control the urinary pH reaction, the observations were within the range of normal values for the canine species. The biological half-lives varied somewhat due to natural biological, or individual variation. However, there was a direct relationship between the urinary pH reaction and the biologic half-life. The higher the pH of the urine the longer the biologic half-life.

The cumulative amounts of amphetamine in urine and bile varied between individuals, the amount of unchanged drug in urine was about five hundred times the amount in bile in the same individual.

The detector response showing the concentration of amphetamine per millilitre of urine, diluted to 1 in 400, of a dog are shown in Figure 20.

The volume and pH reaction of each half-hour urine sample and the concentration and amount of amphetamine in these urine samples are tabulated for individual dogs in Table 21 to 25. The amounts of unchanged amphetamine and p-hydroxyamphetamine excreted in urine each half-hour by each experimental subject are contained in Tables 26 to 30. The volume of bile excreted each half-hour and the concentration and amount of unchanged amphetamine in the bile samples are tabulated for individual dogs in Tables 31 to 35. The mean cumulative amounts of amphetamine excreted in urine and bile, and the mean
<table>
<thead>
<tr>
<th>Subject: dog</th>
<th>Total dose (mg)</th>
<th>Urine pH a mean value (range)</th>
<th>$T_{1/2}$ (hr.)</th>
<th>Cumulative amt. of amphetamine (4 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>13</td>
<td>7.50 (7.38-7.76)</td>
<td>6.13</td>
<td>1.25 3.79</td>
</tr>
<tr>
<td>T</td>
<td>10</td>
<td>5.96 (5.92-6.00)</td>
<td>3.67</td>
<td>2.10 5.00b</td>
</tr>
<tr>
<td>V</td>
<td>10</td>
<td>6.55 (6.48-6.61)</td>
<td>4.25</td>
<td>1.77 5.50</td>
</tr>
<tr>
<td>X</td>
<td>10</td>
<td>6.51 (6.48-6.54)</td>
<td>4.18</td>
<td>1.80 6.87</td>
</tr>
<tr>
<td>Z</td>
<td>13</td>
<td>6.28 (6.20-6.38)</td>
<td>3.85</td>
<td>3.25 6.50</td>
</tr>
</tbody>
</table>

a  Correlation coefficient ($r$) = 0.982, of urinary pH and $T_{1/2}$.

b  3 hours
Figure 20. Amphetamine peaks showing the response of the electron capture detector to TCAA prepared from dog urine extracts. The detector response to a standard solution containing 100 ng/ml is also shown.
TABLE 21

AMOUNT OF AMPHETAMINE EXCRETED
IN THE URINE OF DOG R.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Volume (ml)</th>
<th>pH</th>
<th>Conc. (ug/ml)</th>
<th>Amount (ug)</th>
<th>Cumulative amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>6</td>
<td>7.38</td>
<td>90</td>
<td>540</td>
<td>0.54</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>7.43</td>
<td>60</td>
<td>360</td>
<td>0.90</td>
</tr>
<tr>
<td>1 1/2</td>
<td>5</td>
<td>7.40</td>
<td>32</td>
<td>160</td>
<td>1.06</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>7.46</td>
<td>12</td>
<td>72</td>
<td>1.13</td>
</tr>
<tr>
<td>2 1/2</td>
<td>6</td>
<td>7.52</td>
<td>8</td>
<td>48</td>
<td>1.18</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>7.50</td>
<td>5</td>
<td>30</td>
<td>1.21</td>
</tr>
<tr>
<td>3 1/2</td>
<td>4</td>
<td>7.56</td>
<td>6</td>
<td>24</td>
<td>1.23</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>7.76</td>
<td>4</td>
<td>20</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Total dose administered: 13 mg amphetamine I.V.
TABLE 22
AMOUNT OF AMPHETAMINE EXCRETED IN THE URINE OF DOG T.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Urine Volume (ml)</th>
<th>pH</th>
<th>Concentration (ug/ml)</th>
<th>Amount (ug)</th>
<th>Cumulative Amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>6</td>
<td>6.00</td>
<td>80</td>
<td>480</td>
<td>0.48</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>5.94</td>
<td>85</td>
<td>425</td>
<td>0.91</td>
</tr>
<tr>
<td>1 1/2</td>
<td>5</td>
<td>5.96</td>
<td>64</td>
<td>320</td>
<td>1.23</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>5.92</td>
<td>56</td>
<td>280</td>
<td>1.51</td>
</tr>
<tr>
<td>2 1/2</td>
<td>5</td>
<td>5.94</td>
<td>40</td>
<td>200</td>
<td>1.71</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>5.96</td>
<td>37</td>
<td>160</td>
<td>1.87</td>
</tr>
<tr>
<td>3 1/2</td>
<td>5</td>
<td>5.96</td>
<td>25</td>
<td>125</td>
<td>2.00</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>6.00</td>
<td>20</td>
<td>100</td>
<td>2.10</td>
</tr>
</tbody>
</table>

Total dose administered: 10 mg amphetamine I. V.
<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Volume (ml)</th>
<th>pH</th>
<th>Conc. (ug/ml)</th>
<th>Amount (ug)</th>
<th>Cumulative amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\frac{1}{2}$</td>
<td>6</td>
<td>6.61</td>
<td>80</td>
<td>480</td>
<td>0.48</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>6.58</td>
<td>94</td>
<td>376</td>
<td>0.86</td>
</tr>
<tr>
<td>$1\frac{1}{2}$</td>
<td>3</td>
<td>6.58</td>
<td>94</td>
<td>282</td>
<td>1.14</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>6.55</td>
<td>64</td>
<td>192</td>
<td>1.33</td>
</tr>
<tr>
<td>$2\frac{1}{2}$</td>
<td>3</td>
<td>6.49</td>
<td>54</td>
<td>162</td>
<td>1.49</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>6.48</td>
<td>30</td>
<td>120</td>
<td>1.61</td>
</tr>
<tr>
<td>$3\frac{1}{2}$</td>
<td>4</td>
<td>6.55</td>
<td>22</td>
<td>88</td>
<td>1.70</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>6.53</td>
<td>24</td>
<td>72</td>
<td>1.77</td>
</tr>
</tbody>
</table>

Total dose administered: 10 mg amphetamine I. V.
### TABLE 24

**AMOUNT OF AMPHETAMINE EXCRETED IN THE URINE OF DOG X.**

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Volume (ml)</th>
<th>pH</th>
<th>Conc. (ug/ml)</th>
<th>Amount (ug)</th>
<th>Cumulative amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>5</td>
<td>6.53</td>
<td>90</td>
<td>450</td>
<td>0.45</td>
</tr>
<tr>
<td>1</td>
<td>3.5</td>
<td>6.51</td>
<td>100</td>
<td>350</td>
<td>0.80</td>
</tr>
<tr>
<td>1½</td>
<td>3.5</td>
<td>6.48</td>
<td>70</td>
<td>245</td>
<td>1.05</td>
</tr>
<tr>
<td>2</td>
<td>3.5</td>
<td>6.49</td>
<td>55</td>
<td>193</td>
<td>1.24</td>
</tr>
<tr>
<td>2½</td>
<td>3.5</td>
<td>6.52</td>
<td>47</td>
<td>165</td>
<td>1.41</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>6.54</td>
<td>50</td>
<td>150</td>
<td>1.56</td>
</tr>
<tr>
<td>3½</td>
<td>3</td>
<td>6.49</td>
<td>42</td>
<td>126</td>
<td>1.69</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>6.51</td>
<td>35</td>
<td>105</td>
<td>1.80</td>
</tr>
</tbody>
</table>

*Total dose administered: 10 mg amphetamine I.V.*
TABLE 25
AMOUNT OF AMPHETAMINE EXCRETED IN THE URINE OF DOG Z.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Volume (ml)</th>
<th>pH</th>
<th>Conc. (ug/ml)</th>
<th>Amount (ug)</th>
<th>Cumulative amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>4</td>
<td>6.30</td>
<td>170</td>
<td>680</td>
<td>0.68</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>6.34</td>
<td>180</td>
<td>540</td>
<td>1.22</td>
</tr>
<tr>
<td>1.5</td>
<td>3</td>
<td>6.38</td>
<td>168</td>
<td>504</td>
<td>1.73</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>6.32</td>
<td>153</td>
<td>459</td>
<td>2.19</td>
</tr>
<tr>
<td>2.5</td>
<td>3</td>
<td>6.26</td>
<td>132</td>
<td>396</td>
<td>2.59</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>6.22</td>
<td>87.6</td>
<td>262</td>
<td>2.85</td>
</tr>
<tr>
<td>3.5</td>
<td>3</td>
<td>6.22</td>
<td>70</td>
<td>210</td>
<td>3.06</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>6.20</td>
<td>62</td>
<td>186</td>
<td>3.25</td>
</tr>
</tbody>
</table>

Total dose administered: 13 mg amphetamine I. V.
TABLE 26
THE AMOUNTS OF AMPHETAMINE AND p-HYDROXYAMPHETAMINE EXCRETED IN URINE OF DOG R.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>AMPHETAMINE</th>
<th>p-HYDROXYAMPHETAMINE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount (ug)</td>
<td>Cumulative amount (mg)</td>
</tr>
<tr>
<td>1/2</td>
<td>540</td>
<td>0.54</td>
</tr>
<tr>
<td>1</td>
<td>360</td>
<td>0.90</td>
</tr>
<tr>
<td>1 1/2</td>
<td>160</td>
<td>1.06</td>
</tr>
<tr>
<td>2</td>
<td>72</td>
<td>1.13</td>
</tr>
<tr>
<td>2 1/2</td>
<td>48</td>
<td>1.18</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>1.21</td>
</tr>
<tr>
<td>3 1/2</td>
<td>24</td>
<td>1.23</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Total dose administered: 13 mg amphetamine I. V.
TABLE 27
THE AMOUNTS OF AMPHETAMINE AND P-HYDROXYAMPHETAMINE EXCRETED IN URINE OF DOG T

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>AMPHETAMINE Amount (ug)</th>
<th>Cumulative amount (mg)</th>
<th>p-HYDROXYAMPHETAMINE Amount (ug)</th>
<th>Cumulative amount (ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>480</td>
<td>0.48</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>425</td>
<td>0.91</td>
<td>17</td>
<td>27</td>
</tr>
<tr>
<td>1½</td>
<td>320</td>
<td>1.23</td>
<td>16</td>
<td>43</td>
</tr>
<tr>
<td>2</td>
<td>280</td>
<td>1.51</td>
<td>17</td>
<td>60</td>
</tr>
<tr>
<td>2½</td>
<td>200</td>
<td>1.71</td>
<td>18</td>
<td>78</td>
</tr>
<tr>
<td>3</td>
<td>160</td>
<td>1.87</td>
<td>14</td>
<td>92</td>
</tr>
<tr>
<td>3½</td>
<td>125</td>
<td>2.00</td>
<td>15</td>
<td>107</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>2.10</td>
<td>12</td>
<td>119</td>
</tr>
</tbody>
</table>

Total dose administered: 10 mg amphetamine I.V.
## TABLE 28

THE AMOUNTS OF AMPHETAMINE AND p-HYDROXYAMPHETAMINE EXCRETED IN URINE OF DOG V.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>AMPHETAMINE</th>
<th>p-HYDROXYAMPHETAMINE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount (ug)</td>
<td>Cumulative amount (mg)</td>
</tr>
<tr>
<td>½</td>
<td>480</td>
<td>0.48</td>
</tr>
<tr>
<td>1</td>
<td>376</td>
<td>0.86</td>
</tr>
<tr>
<td>1½</td>
<td>282</td>
<td>1.14</td>
</tr>
<tr>
<td>2</td>
<td>192</td>
<td>1.33</td>
</tr>
<tr>
<td>2½</td>
<td>162</td>
<td>1.49</td>
</tr>
<tr>
<td>3</td>
<td>120</td>
<td>1.61</td>
</tr>
<tr>
<td>3½</td>
<td>88</td>
<td>1.70</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>1.77</td>
</tr>
</tbody>
</table>

Total dose administered: 10 mg amphetamine I.V.
## Table 29

The amounts of amphetamine and p-hydroxyamphetamine excreted in urine of dog X.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>AMPHETAMINE</th>
<th>p-HYDROXYAMPHETAMINE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount (ug)</td>
<td>Cumulative amount (mg)</td>
</tr>
<tr>
<td>1/2</td>
<td>450</td>
<td>0.45</td>
</tr>
<tr>
<td>1</td>
<td>350</td>
<td>0.80</td>
</tr>
<tr>
<td>1 1/2</td>
<td>245</td>
<td>1.05</td>
</tr>
<tr>
<td>2</td>
<td>193</td>
<td>1.24</td>
</tr>
<tr>
<td>2 1/2</td>
<td>165</td>
<td>1.41</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>1.56</td>
</tr>
<tr>
<td>3 1/2</td>
<td>126</td>
<td>1.69</td>
</tr>
<tr>
<td>4</td>
<td>105</td>
<td>1.80</td>
</tr>
</tbody>
</table>

Total dose administered: 10 mg amphetamine I. V.
TABLE 30

THE AMOUNTS OF AMPHETAMINE AND p-HYDROXYAMPHETAMINE EXCRETED IN URINE OF DOG Z.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>AMPHETAMINE</th>
<th>p-HYDROXYAMPHETAMINE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount (ug)</td>
<td>Cumulative amount (mg)</td>
</tr>
<tr>
<td>1/2</td>
<td>680</td>
<td>0.68</td>
</tr>
<tr>
<td>1</td>
<td>540</td>
<td>1.22</td>
</tr>
<tr>
<td>1 1/2</td>
<td>504</td>
<td>1.73</td>
</tr>
<tr>
<td>2</td>
<td>459</td>
<td>2.19</td>
</tr>
<tr>
<td>2 1/2</td>
<td>396</td>
<td>2.59</td>
</tr>
<tr>
<td>3</td>
<td>262</td>
<td>2.85</td>
</tr>
<tr>
<td>3 1/2</td>
<td>210</td>
<td>3.06</td>
</tr>
<tr>
<td>4</td>
<td>186</td>
<td>3.25</td>
</tr>
</tbody>
</table>

Total dose administered: 13 mg amphetamine I. V.
TABLE 31

AMOUNT OF AMPHETAMINE EXCRETED
IN THE BILE OF DOG R.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Volume of bile (ml)</th>
<th>Conc. (ng/ml)</th>
<th>Amount (ug)</th>
<th>Cumulative amount (ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>½</td>
<td>6</td>
<td>226</td>
<td>1.36</td>
<td>1.36</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>200</td>
<td>0.80</td>
<td>2.16</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>145</td>
<td>0.44</td>
<td>2.60</td>
</tr>
<tr>
<td>2½</td>
<td>3</td>
<td>105</td>
<td>0.32</td>
<td>2.92</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>100</td>
<td>0.30</td>
<td>3.22</td>
</tr>
<tr>
<td>3½</td>
<td>3</td>
<td>100</td>
<td>0.30</td>
<td>3.52</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>90</td>
<td>0.27</td>
<td>3.79</td>
</tr>
</tbody>
</table>

Total dose administered: 13 mg amphetamine I. V.
TABLE 32
AMOUNT OF AMPHETAMINE EXCRETED IN THE BILE OF DOG T.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Volume of bile (ml)</th>
<th>Conc. (ng/ml)</th>
<th>Amount (ug)</th>
<th>Cumulative amount (ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>½</td>
<td>3.5</td>
<td>600</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>350</td>
<td>1.05</td>
<td>3.15</td>
</tr>
<tr>
<td>1½</td>
<td>3</td>
<td>220</td>
<td>0.66</td>
<td>3.81</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>150</td>
<td>0.45</td>
<td>4.26</td>
</tr>
<tr>
<td>2½</td>
<td>3</td>
<td>135</td>
<td>0.40</td>
<td>4.66</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>110</td>
<td>0.34</td>
<td>5.00</td>
</tr>
</tbody>
</table>

Total dose administered: 10 mg amphetamine I. V.
TABLE 33
AMOUNT OF AMPHETAMINE EXCRETED IN THE BILE OF DOG V.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Volume of bile (ml)</th>
<th>Conc. (ng/ml)</th>
<th>Amount (ug)</th>
<th>Cumulative amount (ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\frac{1}{2}$</td>
<td>3</td>
<td>500</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>380</td>
<td>1.14</td>
<td>2.64</td>
</tr>
<tr>
<td>1$\frac{1}{2}$</td>
<td>3</td>
<td>215</td>
<td>0.65</td>
<td>3.29</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>150</td>
<td>0.60</td>
<td>3.89</td>
</tr>
<tr>
<td>2$\frac{1}{2}$</td>
<td>4</td>
<td>140</td>
<td>0.56</td>
<td>4.45</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>100</td>
<td>0.40</td>
<td>4.85</td>
</tr>
<tr>
<td>3$\frac{1}{2}$</td>
<td>3</td>
<td>115</td>
<td>0.35</td>
<td>5.20</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>100</td>
<td>0.30</td>
<td>5.50</td>
</tr>
</tbody>
</table>

Total dose administered: 10 mg amphetamine I, V.
TABLE 34
AMOUNT OF AMPHETAMINE EXCRETED IN THE BILE OF DOG X.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Volume of bile (ml)</th>
<th>Conc. (ng/ml)</th>
<th>Amount (ug)</th>
<th>Cumulative amount (ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>4</td>
<td>560</td>
<td>2.24</td>
<td>2.24</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>345</td>
<td>1.38</td>
<td>3.62</td>
</tr>
<tr>
<td>1 1/2</td>
<td>3</td>
<td>300</td>
<td>0.90</td>
<td>4.52</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>150</td>
<td>0.60</td>
<td>5.12</td>
</tr>
<tr>
<td>2 1/2</td>
<td>4.5</td>
<td>110</td>
<td>0.50</td>
<td>5.62</td>
</tr>
<tr>
<td>3</td>
<td>3.5</td>
<td>130</td>
<td>0.46</td>
<td>6.08</td>
</tr>
<tr>
<td>3 1/2</td>
<td>4</td>
<td>100</td>
<td>0.40</td>
<td>6.48</td>
</tr>
<tr>
<td>4</td>
<td>3.5</td>
<td>110</td>
<td>0.39</td>
<td>6.87</td>
</tr>
</tbody>
</table>

Total dose administered: 10 mg amphetamine I. V.
TABLE 35
AMOUNT OF AMPHETAMINE EXCRETED IN THE BILE OF DOG Z.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Volume of bile (ml)</th>
<th>Conc. (ng/ml)</th>
<th>Amount (ug)</th>
<th>Cumulative amount (ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>3</td>
<td>670</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>1</td>
<td>3.5</td>
<td>400</td>
<td>1.40</td>
<td>3.40</td>
</tr>
<tr>
<td>1 1/2</td>
<td>4</td>
<td>200</td>
<td>0.80</td>
<td>4.20</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>150</td>
<td>0.60</td>
<td>4.80</td>
</tr>
<tr>
<td>2 1/2</td>
<td>4</td>
<td>135</td>
<td>0.54</td>
<td>5.34</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>115</td>
<td>0.46</td>
<td>5.80</td>
</tr>
<tr>
<td>3 1/2</td>
<td>4</td>
<td>100</td>
<td>0.40</td>
<td>6.20</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>100</td>
<td>0.30</td>
<td>6.50</td>
</tr>
</tbody>
</table>

Total dose administered: 13 mg amphetamine I. V.
### TABLE 36

**AMOUNT OF AMPHETAMINE EXCRETED IN THE BILE OF NEPHRECTOMIZED DOG NF.**

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Volume of bile (ml)</th>
<th>Conc. (ng/ml)</th>
<th>Amount (ug)</th>
<th>Cumulative amount (ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\frac{1}{2}$</td>
<td>2.5</td>
<td>300</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>285</td>
<td>0.57</td>
<td>1.32</td>
</tr>
<tr>
<td>$1\frac{1}{2}$</td>
<td>2</td>
<td>225</td>
<td>0.45</td>
<td>1.77</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>165</td>
<td>0.33</td>
<td>2.10</td>
</tr>
<tr>
<td>$2\frac{1}{2}$</td>
<td>2</td>
<td>125</td>
<td>0.25</td>
<td>2.35</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>115</td>
<td>0.23</td>
<td>2.58</td>
</tr>
<tr>
<td>$3\frac{1}{2}$</td>
<td>2</td>
<td>105</td>
<td>0.21</td>
<td>2.79</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>85</td>
<td>0.17</td>
<td>2.96</td>
</tr>
</tbody>
</table>

Total dose administered: 5 mg amphetamine I.V.
TABLE 37
AMOUNT OF AMPHETAMINE EXCRETED IN THE BILE OF NEPHRECTOMIZED DOG NG.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Volume of bile (ml)</th>
<th>Conc. (ng/ml)</th>
<th>Amount (ug)</th>
<th>Cumulative amount (ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>3</td>
<td>460</td>
<td>1.38</td>
<td>1.38</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>320</td>
<td>0.96</td>
<td>2.34</td>
</tr>
<tr>
<td>1 1/2</td>
<td>3</td>
<td>225</td>
<td>0.68</td>
<td>3.02</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>160</td>
<td>0.48</td>
<td>3.50</td>
</tr>
<tr>
<td>2 1/2</td>
<td>3</td>
<td>150</td>
<td>0.45</td>
<td>3.95</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>135</td>
<td>0.41</td>
<td>4.36</td>
</tr>
<tr>
<td>3 1/2</td>
<td>3</td>
<td>125</td>
<td>0.38</td>
<td>4.74</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>120</td>
<td>0.36</td>
<td>5.10</td>
</tr>
</tbody>
</table>

Total dose administered: 15 mg amphetamine I. V.
# Table 38

**Amount of Amphetamine Excreted in the Bile of Nephrectomized Dog NL.**

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Volume of bile (ml)</th>
<th>Conc. (ng/ml)</th>
<th>Amount (ug)</th>
<th>Cumulative amount (ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>7</td>
<td>320</td>
<td>2.24</td>
<td>2.24</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>185</td>
<td>1.30</td>
<td>3.54</td>
</tr>
<tr>
<td>1 1/2</td>
<td>6</td>
<td>153</td>
<td>0.92</td>
<td>4.46</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>95</td>
<td>0.67</td>
<td>5.13</td>
</tr>
<tr>
<td>2 1/2</td>
<td>6</td>
<td>105</td>
<td>0.63</td>
<td>5.76</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>95</td>
<td>0.57</td>
<td>6.33</td>
</tr>
<tr>
<td>3 1/2</td>
<td>7</td>
<td>80</td>
<td>0.56</td>
<td>6.89</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>85</td>
<td>0.51</td>
<td>7.40</td>
</tr>
</tbody>
</table>

Total dose administered: 8 mg amphetamine I. V.
Figure 21. The mean cumulative appearances of amphetamine in urine and bile, and p-hydroxyamphetamine in urine of five dogs.
cumulative amount of p-hydroxyamphetamine in urine, are presented graphically in Figure 21. The appearance of amphetamine in urine and bile were first-order processes while the appearance of p-hydroxyamphetamine in urine seemed to be a zero-order process. This latter phenomenon might be explained by a zero order metabolic reaction or the low availability of amphetamine to the hydroxylating enzymes due to the wide extravascular distribution of this drug.

Experiments (iii) and (iv): The amphetamine concentration in plasma versus time profile in nephrectomized and intact dogs is shown in Figure 18. The biological half-life was extended by almost 25 percent in nephrectomized dogs and the difference between the mean half-lives in intact and nephrectomized dogs was significant (Student's t-test, p < 0.001).

Nephrectomy did not change the B value (ng ml⁻¹) or the apparent specific volume of distribution of the drug (litres kg⁻¹). The pharmacokinetic parameters describing the distribution and elimination of amphetamine in intact and nephrectomized dogs are compared in Tables 18 and 39. The percent bound amphetamine in plasma was estimated from the concentrations of amphetamine, measured by gas chromatography, in plasma and cerebrospinal fluid collected at the same time.
TABLE 39

THE APPARENT SPECIFIC VOLUMES OF DISTRIBUTION AND THE RATE OF ELIMINATION OF AMPHETAMINE IN NORMAL AND NEPHRECTOMIZED DOGS.

<table>
<thead>
<tr>
<th>Subject: dog</th>
<th>$V'd$ (litre/kg)</th>
<th>% bound amphetamine</th>
<th>$V'd^*$ (litre/kg)</th>
<th>$\beta$ (hour$^{-1}$)</th>
<th>$T_{1/2}$ (hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>3.14</td>
<td>18</td>
<td>3.83</td>
<td>0.113</td>
<td>6.13</td>
</tr>
<tr>
<td>T</td>
<td>2.29</td>
<td>30</td>
<td>3.27</td>
<td>0.189</td>
<td>3.67</td>
</tr>
<tr>
<td>V</td>
<td>2.83</td>
<td>20</td>
<td>3.53</td>
<td>0.163</td>
<td>4.25</td>
</tr>
<tr>
<td>X</td>
<td>2.70</td>
<td>25</td>
<td>3.60</td>
<td>0.166</td>
<td>4.18</td>
</tr>
<tr>
<td>Z</td>
<td>2.49</td>
<td>25</td>
<td>3.32</td>
<td>0.180</td>
<td>3.85</td>
</tr>
<tr>
<td>NF</td>
<td>2.67</td>
<td>20</td>
<td>3.34</td>
<td>0.115</td>
<td>6.08</td>
</tr>
<tr>
<td>NG</td>
<td>2.50</td>
<td>25</td>
<td>3.66</td>
<td>0.129</td>
<td>5.37</td>
</tr>
<tr>
<td>NL</td>
<td>2.74</td>
<td>23</td>
<td>3.25</td>
<td>0.138</td>
<td>5.02</td>
</tr>
</tbody>
</table>

$\% \text{ bound amphetamine} = (1 - \frac{C_{CSF}}{C_{pl}}) \times 100\%$

$V'd^*$ is the apparent specific volume of distribution corrected for extent of plasma protein binding.
A clearance value may be defined as the volume of plasma cleared of drug by an elimination process during a given time interval. The renal, biliary and total clearance values, expressed in ml min\(^{-1}\) kg\(^{-1}\), for individual intact and nephrectomized dogs are tabulated in Table 40 and illustrated in Figure 22. The renal clearance values were similar, 4-5 ml min\(^{-1}\) kg\(^{-1}\), in dogs producing urine of pH reaction 6.0-6.5, but a renal clearance value of 2.8 ml min\(^{-1}\) kg\(^{-1}\) was observed in dog R, the mean urinary pH reaction of this dog was 7.50. This lower renal clearance value supported the earlier observation that the biological half-life increased with increasing urinary pH reaction. The variation in urinary pH of dogs may explain the wide individual variation in biological half-life observed in this species. The renal clearance values provide evidence that amphetamine probably undergoes glomerular filtration and tubular reabsorption, the extent of reabsorption increasing with increasing urinary pH values. The mean biliary clearance values were similar, 0.012 ml min\(^{-1}\) kg\(^{-1}\), in intact and nephrectomized dogs. The low biliary clearance values (Table 40) and the cumulative amount of unchanged amphetamine in bile (Table 20) clearly indicate that this pathway played only a minor role in the elimination of amphetamine from the body. In agreement with this hypothesis was the observation that interruption of the potential enterohepatic cycle
TABLE 40

CLEARANCE VALUES OF AMPHETAMINE IN NORMAL AND NEPHRECTOMIZED DOGS.

<table>
<thead>
<tr>
<th>Subject: Dog</th>
<th>$t_{1/2}$ (hour)</th>
<th>$V'd^*$ (litre/kg)</th>
<th>$C'_{renal}$ (ml/min/kg)</th>
<th>$C'_{biliary}$ (ml/min/kg)</th>
<th>$C'_{total}$ (ml/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>6.13</td>
<td>3.83</td>
<td>2.80</td>
<td>0.006</td>
<td>7.21</td>
</tr>
<tr>
<td>T</td>
<td>3.67</td>
<td>3.27</td>
<td>4.27</td>
<td>0.012</td>
<td>10.30</td>
</tr>
<tr>
<td>V</td>
<td>4.25</td>
<td>3.53</td>
<td>4.38</td>
<td>0.013</td>
<td>9.59</td>
</tr>
<tr>
<td>X</td>
<td>4.18</td>
<td>3.60</td>
<td>4.03</td>
<td>0.017</td>
<td>9.94</td>
</tr>
<tr>
<td>Z</td>
<td>3.85</td>
<td>3.32</td>
<td>5.07</td>
<td>0.011</td>
<td>9.96</td>
</tr>
<tr>
<td>NF</td>
<td>6.03</td>
<td>3.34</td>
<td>-</td>
<td>0.012</td>
<td>6.40</td>
</tr>
<tr>
<td>NG</td>
<td>5.37</td>
<td>3.66</td>
<td>-</td>
<td>0.013</td>
<td>7.87</td>
</tr>
<tr>
<td>NL</td>
<td>5.02</td>
<td>3.25</td>
<td>-</td>
<td>0.010</td>
<td>7.48</td>
</tr>
</tbody>
</table>
Figure 22. The mean total clearance values in intact (11) and nephrectomized (8) dogs. The clearance values were determined each half-hour following the intravenous injection of amphetamine sulphate (0.66 mg/kg, calculated as free base).
by cannulation of the common bile ducts in intact and nephrectomized dogs had no significant effect upon the biological half-life of the drug (Table 18). The total clearance value is a measure of the sum of the metabolic and excretory clearances. The mean total clearance values in intact and nephrectomized dogs (Table 40) were not significantly different (Student's t-test, p > 0.05). This was surprising and may have been due to the following factors. First the equation employed in estimating that total clearance was

\[
C'_{\text{total}}(\text{ml min}^{-1} \text{kg}^{-1}) = \frac{0.693 \times V'd^* (\text{ml kg}^{-1})}{t_{1/2}(\text{min})}
\]

The corrected apparent specific volumes of distribution, \(V'd^*\), were similar in the two groups of dogs. Second, the wide individual variation in biological half-life of individual dogs and third, the small number of animals in the two groups (Table 18, experiments ii and iv).

The overall elimination rate constant, \(\beta\), the rate constant for elimination from the blood, \(k_2\), and the microconstants \(k_{\text{metabolism}}\), \(k_{\text{urine}}\) and \(k_{\text{bile}}\), all expressed in reciprocal minutes, are tabulated in Table 41. The value of \(k_2\) was equal to the sum of the microconstants \(k_m\), \(k_u\), and \(k_b\). The microconstant describing the rate of biotransformation per minute, \(k_{\text{metabolism}}\,\text{constituted about sixty percent of } k_2\). The rate constant describing the rate of elimination of amphetamine in urine per minute,
### TABLE 41

THE PHARMACOKINETIC CONSTANTS DESCRIBING THE ELIMINATION OF AMPHETAMINE IN NORMAL AND NEPHRECTOMIZED DOGS.

<table>
<thead>
<tr>
<th>Subject: Dog</th>
<th>$K_e(\beta) \times 10^{-3}$ (min⁻¹)</th>
<th>$k_m \times 10^{-1}$ (min⁻¹)</th>
<th>$k_u \times 10^{-1}$ (min⁻¹)</th>
<th>$k_b \times 10^{-4}$ (min⁻¹)</th>
<th>$k_2 \times 10^{-1}$ (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>1.9</td>
<td>0.88</td>
<td>0.56</td>
<td>1.2</td>
<td>1.44</td>
</tr>
<tr>
<td>T</td>
<td>3.1</td>
<td>1.21</td>
<td>0.85</td>
<td>2.4</td>
<td>2.06</td>
</tr>
<tr>
<td>V</td>
<td>2.7</td>
<td>1.04</td>
<td>0.88</td>
<td>2.6</td>
<td>1.92</td>
</tr>
<tr>
<td>X</td>
<td>2.8</td>
<td>1.18</td>
<td>0.81</td>
<td>3.4</td>
<td>1.99</td>
</tr>
<tr>
<td>Z</td>
<td>3.0</td>
<td>0.99</td>
<td>1.01</td>
<td>2.2</td>
<td>2.00</td>
</tr>
<tr>
<td>NF</td>
<td>1.9</td>
<td>1.27</td>
<td>-</td>
<td>2.4</td>
<td>1.28</td>
</tr>
<tr>
<td>NG</td>
<td>2.2</td>
<td>1.56</td>
<td>-</td>
<td>2.6</td>
<td>1.57</td>
</tr>
<tr>
<td>NL</td>
<td>2.3</td>
<td>1.49</td>
<td>-</td>
<td>2.0</td>
<td>1.50</td>
</tr>
</tbody>
</table>
kurine, constituted about forty percent of $k_2$. The portion of $k_2$ represented by the rate constant for elimination of amphetamine in bile per minute, $k_{\text{bile}}$, was negligible. In the nephrectomized dogs $k_{\text{metabolism}}$ was almost equal to $k_2$, the value of $k_{\text{bile}}$ was similar to that in intact dogs. The ratio of $k_2$ to $\beta$ was 70:1 in intact and nephrectomized dogs. The wide ratio reflected the large apparent specific volume of distribution of the drug. The value of $k_2$ was lower and the biological half-life longer in nephrectomized dogs than in intact dogs.

**Experimental subjects:** swine.

The logarithm of the amphetamine concentration in plasma versus time profile is shown in Figure 23. This is a typical first-order decay describing the elimination of a drug following intravenous administration. The extravascular distribution of the drug was rapid, less than one half-hour, so that the body behaved as a one-compartment open model. The mean value of B, which is the antilog of $a_0$ in the equation of the least square regression line of $Y$ (plasma amphetamine concentration) on $X$(time), was 350 ng ml$^{-1}$. The biological half-life was calculated for each animal from the overall elimination rate constants, $k_2$. The B values were estimated for each animal and were corrected for the extent of plasma protein binding of the drug in this species. The corrected apparent specific volumes of distribution were then computed (Table 42).
Figure 23. Disappearance of amphetamine from the blood plasma of swine following the intravenous injection of amphetamine sulphate (0.66 mg/kg, calculated as free base). Each point represents the mean concentration (3 pigs) and each vertical bar = ± 1 S.D.
TABLE 42

PHARMACOKINETIC CONSTANTS DESCRIBING THE DISTRIBUTION AND ELIMINATION OF AMPHETAMINE IN SWINE.

<table>
<thead>
<tr>
<th>Subject</th>
<th>( B ) (ng ml(^{-1} ))</th>
<th>( \beta ) (hour(^{-1} ))</th>
<th>( t_{\frac{1}{2}} ) (hour)</th>
<th>( V'd ) (litre kg(^{-1} ))</th>
<th>( V'd^* ) (litre kg(^{-1} ))</th>
<th>( k_2 ) (hour(^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>OB</td>
<td>370</td>
<td>0.745</td>
<td>0.93</td>
<td>1.79</td>
<td>2.96</td>
<td>44.12</td>
</tr>
<tr>
<td>OC</td>
<td>295</td>
<td>0.608</td>
<td>1.14</td>
<td>2.26</td>
<td>3.74</td>
<td>45.48</td>
</tr>
<tr>
<td>OX</td>
<td>380</td>
<td>0.754</td>
<td>0.92</td>
<td>1.74</td>
<td>2.88</td>
<td>43.48</td>
</tr>
</tbody>
</table>

The mean extent of plasma protein binding, determined in vitro, was 39.6 percent.

\( V'd^* \) is the apparent specific volume of distribution corrected for the extent of plasma protein binding.
The values of $\beta$ and $k_2$, expressed in reciprocal hours, for each animal are also tabulated. The ratio of $k_2$ to $\beta$ was approximately 60:1. The corrected apparent specific volumes of distribution were similar in the two species (Student's t-test, $p > 0.05$) but the biologic half-life was significantly shorter in swine (Student's t-test, $p < 0.001$).

The total dose of amphetamine administered, the biologic half-life of the drug, the cumulative amounts of unchanged amphetamine excreted in urine and bile and the range of urinary pH values in individual pigs are tabulated in Table 43. No attempt was made to control the urinary pH reaction but the observed values had a similar range in the individual animals. The time period of this experiment was almost three half-lives of the drug in this species. The mean cumulative amount of unchanged amphetamine excreted in urine was 8.18 percent of the dose administered. A considerably smaller amount of unchanged amphetamine was excreted in bile, 0.12 percent of the dose. The mean cumulative amounts of amphetamine excreted in urine and bile each half-hour are tabulated in Table 44 and are illustrated in Figure 24. The appearance of the drug in urine and bile were first-order processes. The mean cumulative amounts of amphetamine, p-hydroxyamphetamine and the glucuronide conjugate in urine each half-hour are tabulated in Table
TABLE 43

THE 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 PIGS.

<table>
<thead>
<tr>
<th>Subject Pig</th>
<th>Total dose (mg)</th>
<th>Urine pH mean (range)</th>
<th>$t_{1/2}$ (hour)</th>
<th>Cumulative amount of Amphetamine (3 hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OB</td>
<td>13.2 (5.71-6.05)</td>
<td>5.87</td>
<td>0.93</td>
<td>1.08 Urine (mg) 14.4 Bile (ug)</td>
</tr>
<tr>
<td>OC</td>
<td>16.2 (5.61-5.76)</td>
<td>5.82</td>
<td>1.14</td>
<td>1.36 Urine (mg) 20.3 Bile (ug)</td>
</tr>
<tr>
<td>OX</td>
<td>11.8 (5.88-6.34)</td>
<td>6.07</td>
<td>0.92</td>
<td>0.93 Urine (mg) 14.9 Bile (ug)</td>
</tr>
</tbody>
</table>
TABLE 44
MEAN CUMULATIVE AMOUNT OF AMPHETAMINE IN BIOLOGICAL FLUIDS OF 3 PIGS.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Bile (ug)</th>
<th>Urine (ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>4.17</td>
<td>250.0</td>
</tr>
<tr>
<td>1</td>
<td>7.56</td>
<td>486.3</td>
</tr>
<tr>
<td>1½</td>
<td>10.62</td>
<td>694.0</td>
</tr>
<tr>
<td>2</td>
<td>13.02</td>
<td>872.2</td>
</tr>
<tr>
<td>2½</td>
<td>14.86</td>
<td>1125.4</td>
</tr>
<tr>
<td>3</td>
<td>16.51</td>
<td>1125.4</td>
</tr>
</tbody>
</table>

Mean dose administered: 13.7 mg amphetamine I. V.
Figure 24. The cumulative appearance of amphetamine in urine and bile of swine following the intravenous injection of amphetamine sulphate (0.66 mg/kg, calculated as free base). Each point represents the mean amount of amphetamine in the biological fluid of three animals.
TABLE 45
MEAN CUMULATIVE AMOUNTS (UG) OF AMPHETAMINE AND SOME METABOLITES IN URINE OF 3 PIGS.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Amphetamine</th>
<th>p-Hydroxy-amphetamine</th>
<th>Glucuronide conjugate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>250.0</td>
<td>98.4</td>
<td>149.8</td>
</tr>
<tr>
<td>1</td>
<td>486.3</td>
<td>324.0</td>
<td>351.4</td>
</tr>
<tr>
<td>1 1/2</td>
<td>694.0</td>
<td>610.2</td>
<td>535.4</td>
</tr>
<tr>
<td>2</td>
<td>872.2</td>
<td>821.6</td>
<td>792.2</td>
</tr>
<tr>
<td>2 1/2</td>
<td>1015.4</td>
<td>1039.8</td>
<td>1004.0</td>
</tr>
<tr>
<td>3</td>
<td>1125.4</td>
<td>1223.0</td>
<td>1212.0</td>
</tr>
</tbody>
</table>

Mean dose administered: 13.7 mg amphetamine I. V.
The cumulative appearance of amphetamine, p-hydroxyamphetamine and the glucuronide conjugate in urine of swine following the intravenous injection of amphetamine sulphate (0.66 mg/kg, calculated as free base). Each point represents the mean amount of the drug or metabolite in the urine of three animals.
45 and illustrated in Figure 25. The total cumulative amounts of amphetamine, p-hydroxyamphetamine and the glucuronide conjugates in urine were 8.18, 8.91 and 8.83 percent of the dose respectively. The appearance of amphetamine, p-hydroxyamphetamine and the glucuronide conjugate in urine are shown in Figure 25. The appearances of the p-hydroxy derivative and the conjugate were zero order, this may be a reflection of a zero order p-hydroxylation process, or the low availability of the amphetamine to the hydroxylating enzymes due to the wide extravascular distribution of this drug. In support of the hypothesis that elimination took place by two simultaneous routes where one, biotransformation, was capacity limited was the observation that the ratio of metabolite to unchanged drug increased with time (Table 45). The ratio of the p-hydroxy derivative to amphetamine in the 24-hour cumulative urine sample was 10:3 (Table 17).

No unconjugated p-hydroxyamphetamine was detected in bile, the glucuronide conjugate was present in bile. No methamphetamine was detected in any biological fluid collected from swine.

Section 5: The objective of the experiments in this section was to determine the influence of urinary pH change upon the half-life of amphetamine in horses. The urine pH values were adjusted mainly by alteration of dietary regimens but sodium bicarbonate or ammonium chloride supplementation was provided on each of the
two days prior to amphetamine administration when alkaline or acid urine was required. The urinary pH values at regular intervals in each experiment of this study are tabulated in Table 46. The mean urine pH reaction in experiment A (control) was 8.00, experiment B (alkaline) 8.50, experiment C (acid) 6.00 and experiment D (control) 8.10. The biological half-life was not significantly altered (Student's t-test, p > 0.05) by any dietary regimen or urinary pH reaction (Table 47). The apparent specific volumes of distribution corrected for the extent of plasma protein binding were not significantly affected by alteration of urine pH. The apparent volumes of distribution were large (ca. 2.8 litres/kg) indicating that the drug had a wide extravascular distribution. The mean values of $k_2$ and $\beta$ for each experiment are also tabulated in Table 47.

The cumulative amounts, expressed in milligrams, of amphetamine, p-hydroxyamphetamine and the glucuronide and sulphate conjugates in urine of mares collected over five hour periods are tabulated in Table 12. The mean total amount of unchanged amphetamine excreted in the urine of two mares and two ponies over a twenty-four hour period was 2 percent of the dose administered (Table 17). The small amount of unchanged amphetamine excreted in urine would explain the observed lack of effect of urine pH change upon the biological half-life of the drug in the equine species.

Section 6: Experiment (i): The concentrations of amphetamine in plasma and rumen liquor of goats at 0.5, 1.0 and 1.5
### TABLE 46

**THE pH REACTION OF HORSE URINE.**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>L.</th>
<th>S.</th>
<th>J.</th>
<th>L.</th>
<th>S.</th>
<th>J.</th>
<th>L.</th>
<th>S.</th>
<th>J.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Control)</td>
<td>8.36</td>
<td>8.01</td>
<td>8.60</td>
<td>7.70</td>
<td>8.52</td>
<td>7.91</td>
<td>7.24</td>
<td>7.91</td>
<td>7.15</td>
</tr>
<tr>
<td>B (Alkaline)</td>
<td>8.48</td>
<td>8.54</td>
<td>8.32</td>
<td>8.84</td>
<td>8.70</td>
<td>8.22</td>
<td>8.30</td>
<td>8.80</td>
<td>8.62</td>
</tr>
<tr>
<td>C (Acid)</td>
<td>5.43</td>
<td>5.71</td>
<td>5.95</td>
<td>5.65</td>
<td>6.78</td>
<td>5.79</td>
<td>5.58</td>
<td>6.43</td>
<td>6.60</td>
</tr>
<tr>
<td>D (Control)</td>
<td>8.34</td>
<td>8.38</td>
<td>7.86</td>
<td>8.15</td>
<td>8.15</td>
<td>8.00</td>
<td>7.98</td>
<td>8.05</td>
<td>7.95</td>
</tr>
</tbody>
</table>

L = Lassie  
S = Sorrell  
J = Jane
TABLE 47

PHARMACOKINETIC CONSTANTS DESCRIBING THE DISTRIBUTION AND ELIMINATION OF AMPHETAMINE IN HORSES (3) PRODUCING URINE OF DIFFERENT pH VALUES

<table>
<thead>
<tr>
<th>Experiment</th>
<th>B (ng ml⁻¹)</th>
<th>β (hour⁻¹)</th>
<th>( t_{\frac{1}{2}} ) (hour)</th>
<th>( V'd ) (litre kg⁻¹)</th>
<th>( V'd^* ) (litre kg⁻¹)</th>
<th>( k_2 ) (hour⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-Control</td>
<td>317</td>
<td>0.352</td>
<td>1.97</td>
<td>2.08</td>
<td>2.77</td>
<td>19.5</td>
</tr>
<tr>
<td>B-Alk.</td>
<td>312</td>
<td>0.298</td>
<td>2.32</td>
<td>2.11</td>
<td>2.82</td>
<td>17.0</td>
</tr>
<tr>
<td>C-Acid</td>
<td>302</td>
<td>0.378</td>
<td>1.87</td>
<td>2.18</td>
<td>2.90</td>
<td>21.5</td>
</tr>
<tr>
<td>D-Control</td>
<td>330</td>
<td>0.357</td>
<td>1.97</td>
<td>1.99</td>
<td>2.65</td>
<td>18.7</td>
</tr>
</tbody>
</table>

The mean extent of plasma protein binding, determined in vitro, was 25 percent.

\( V'd^* \) is the apparent specific volume of distribution corrected for the extent of plasma protein binding.
hours following intravenous injection of the drug are tabulated in Table 48. The pH range of the rumen fluid was 6.0-6.5. The concentration of amphetamine was higher (ca. 50%) in rumen fluid than in plasma one half-hour following intravenous administration of drug. This was probably due to passive diffusion from plasma into the rumen of the nonionized lipid-soluble moiety of the circulating drug. The pH of the rumen liquor was approximately 1 unit below the normal physiological pH of plasma. The drug was ionized and thus water soluble at the lower pH reaction and consequently became localized in the rumen. The decline of amphetamine concentration in the plasma was a typical first-order exponential process, the decrease in drug concentration in the rumen fluid was very slow. The amphetamine concentration ratio in rumen liquor to plasma was about 5:1 at 1.5 hours following drug administration. Since the volume of rumen liquor at the time of sampling was unknown it was not possible to estimate the amount of drug which had diffused into the rumen.

Experiment (ii): The bile was aspirated from the gallbladder and the gastric fluid collected from the stomach about 4.5 hours after intravenous injection of amphetamine in six randomly selected dogs. The concentrations of unchanged drug in these fluids were determined and the amounts of amphetamine estimated. The quantities (mean ± S.E.M.) of amphetamine in bile and gastric fluid
TABLE 48

THE CONCENTRATIONS OF AMPHETAMINE IN PLASMA AND RUMEN LIQUOR OF GOATS FOLLOWING I.V. ADMINISTRATION OF THE DRUG.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Goat</th>
<th>pH of rumen fluid</th>
<th>Amphetamine plasma (ng ml(^{-1})) rumen liquor</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>140</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>(\frac{1}{2})</td>
<td>B</td>
<td>125</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>110</td>
<td>190</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>120</td>
<td>200</td>
</tr>
<tr>
<td>A</td>
<td>6.1</td>
<td>75</td>
<td>210</td>
</tr>
<tr>
<td>B</td>
<td>6.0</td>
<td>85</td>
<td>180</td>
</tr>
<tr>
<td>1</td>
<td>C</td>
<td>6.4</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>6.5</td>
<td>55</td>
</tr>
<tr>
<td>A</td>
<td>30</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>45</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>(\frac{3}{2})</td>
<td>C</td>
<td>30</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>25</td>
<td>150</td>
</tr>
</tbody>
</table>
were 5.2 ± 1.0 and 13.5 ± 5 microgram respectively.

Owing to the lack of sensitivity and absolute specificity of the colorimetric assay for p-hydroxyamphetamine no conclusions could be drawn about the presence of this metabolite in gastric contents, rumen liquor, bile, cerebrospinal and ocular fluids. The results indicated that the unconjugated p-hydroxy derivative was absent from gastric fluid, bile CSF and ocular fluid but that the glucuronide conjugate was present in bile (30-100 microgram in gallbladder bile collected 4.5 hours after intravenous injection of ca. 8 milligram amphetamine).

The gas chromatographic method employed to detect amphetamine was also sensitive to the presence of methylamphetamine. The retention times of the drug and the derivative were distinctly different. No methylamphetamine was detected in any biological fluid examined.

The Statistical Analysis of Experimental Data.

The Student's "t" test was used to evaluate the significance of differences between the mean values of two independent sets of observations. The biological half-lives of amphetamine, the extent of plasma protein binding at a drug concentration of 10^{-6} Molar and the B values, corrected for the extent of plasma protein binding, are compared for the various species of animals taken two at a time in Tables 49, 50 and 51 respectively. The observed "t" value and the level of significance are tabulated for each "t" test.
### TABLE 49

**STUDENT'S "t" TEST COMPARING MEAN VALUES OF THE BIOLOGICAL HALF-LIFE OF AMPHETAMINE IN THE VARIOUS SPECIES OF ANIMALS TAKEN TWO AT A TIME.**

<table>
<thead>
<tr>
<th></th>
<th>Pig</th>
<th>Pony</th>
<th>Rabbit</th>
<th>Chicken</th>
<th>Dog</th>
<th>Cat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m:9</td>
<td>m:5</td>
<td>m:4</td>
<td>m:8</td>
<td>m:11</td>
<td>m:6</td>
</tr>
<tr>
<td>Goat</td>
<td>7.2</td>
<td>10.0</td>
<td>10.4</td>
<td>11.2</td>
<td>15.1</td>
<td>23.2</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Pig</td>
<td>0</td>
<td>3.9</td>
<td>4.1</td>
<td>7.7</td>
<td>12.7</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.005</td>
<td>p&lt;0.005</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Pony</td>
<td>---</td>
<td>0</td>
<td>0.03</td>
<td>4.0</td>
<td>8.4</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td></td>
<td>p&lt;0.005</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>---</td>
<td>---</td>
<td>0</td>
<td>3.7</td>
<td>7.5</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.005</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0</td>
<td>7.0</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

**Degrees of freedom = n + m - 2**

**Not Significant (n.s.) = p > 0.05.**
TABLE 50

STUDENT'S "t" TEST COMPARING MEAN VALUES OF EXTENT OF PLASMA PROTEIN BINDING OF AMPHETAMINE AT A DRUG CONCENTRATION OF $10^{-6}$ MOLAR IN VARIOUS SPECIES OF ANIMALS TAKEN TWO AT A TIME.

<table>
<thead>
<tr>
<th></th>
<th>Pig m:6</th>
<th>Pony m:9</th>
<th>Chicken m:6</th>
<th>Dog m:17</th>
<th>Cat m:12</th>
<th>Monkey m:11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat n:12</td>
<td>0.34 p&lt;0.001</td>
<td>6.27 n.s.</td>
<td>10.27 p&lt;0.001</td>
<td>6.00 p&lt;0.001</td>
<td>6.80 p&lt;0.001</td>
<td>0.44 n.s.</td>
</tr>
<tr>
<td>Pig n:6</td>
<td>0 p&lt;0.001</td>
<td>4.57 p&lt;0.001</td>
<td>8.15 p&lt;0.001</td>
<td>4.08 p&lt;0.001</td>
<td>4.98 p&lt;0.001</td>
<td>0.42 n.s.</td>
</tr>
<tr>
<td>Pony n:9</td>
<td>--- 0 p&lt;0.001</td>
<td>4.93 n.s.</td>
<td>0.80 n.s.</td>
<td>0.60 n.s.</td>
<td>5.95 p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Chicken n:6</td>
<td>--- ---</td>
<td>0 4.81 p&lt;0.001</td>
<td>6.65 p&lt;0.001</td>
<td>19.89 p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog n:17</td>
<td>--- --- ---</td>
<td>0 0.34 n.s.</td>
<td>6.46 p&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat n:12</td>
<td>--- --- ---</td>
<td>0 9.76 p&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Degrees of freedom: $n + m - 2$

Not Significant (n.s.): $p > 0.05$. 
<table>
<thead>
<tr>
<th>Animal</th>
<th>Pig m:9</th>
<th>Pony m:5</th>
<th>Rabbit m:4</th>
<th>Chicken m:8</th>
<th>Dog m:11</th>
<th>Cat m:6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat n:10</td>
<td>5.1</td>
<td>3.26</td>
<td>6.0</td>
<td>15.4</td>
<td>4.8</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.010</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Pig n:9</td>
<td>0</td>
<td>0.18</td>
<td>1.65</td>
<td>10.9</td>
<td>1.41</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n. s.</td>
<td>n. s.</td>
<td>p&lt;0.001</td>
<td>n. s.</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Pony n:5</td>
<td>---</td>
<td>0</td>
<td>0.7</td>
<td>6.5</td>
<td>1.0</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>n. s.</td>
<td>p&lt;0.001</td>
<td>n. s.</td>
<td>p&lt;0.005</td>
</tr>
<tr>
<td>Rabbit n:4</td>
<td>---</td>
<td>---</td>
<td>0</td>
<td>7.7</td>
<td>3.8</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.005</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Chicken n:8</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0</td>
<td>14.3</td>
<td>4.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.005</td>
</tr>
<tr>
<td>Dog n:11</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0</td>
<td>12.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Degrees of freedom = n + m - 2

Not Significant (n. s.) = p > 0.05
Fixed model one-way analyses of variance tables (Tables 52, 53 and 54) show that differences between means of the various pharmacokinetic parameters exist among the different species. The significance of the observed differences between mean values were evaluated by the F-test.
Null hypothesis: The extent of plasma protein binding of amphetamine at a drug concentration of 100 ng ml$^{-1}$ (10$^{-6}$ Molar) is similar in goats, swine, ponies, chickens, dogs and cats.

Alternative: The extent of plasma protein binding of amphetamine at a drug concentration of 100 ng ml$^{-1}$ is not similar in goats, swine, ponies, chickens, dogs and cats.

**TABLE 52**

ANOVA TABLE

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>$F_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between species</td>
<td>5</td>
<td>3852.89</td>
<td>770.58</td>
<td>21.76</td>
</tr>
<tr>
<td>Within species</td>
<td>56</td>
<td>1983.42</td>
<td>35.42</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>5836.31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Let the type I error ($\alpha$) = 0.01

\[
F_{0.01(5, 56)} = 3.40
\]

Conclusion: Since $F_s >> F_{0.01(5, 56)}$, reject the null hypothesis. On the basis of the results I reject the hypothesis that the extent of plasma protein binding of amphetamine at a concentration of 100 ng ml$^{-1}$ is similar in these species of animals.
Null hypothesis: The B values corrected for the extent of plasma protein binding (B*) were similar in goats, swine, ponies, rabbits, chickens, dogs and cats following I. V. injection of amphetamine (0.66 mg kg⁻¹).

Alternative: The B values corrected for the extent of plasma protein binding (B*) were not similar in goats, swine, ponies, rabbits, chickens, dogs and cats following I. V. injection of amphetamine (0.66 mg kg⁻¹).

**TABLE 53**

**ANOVA TABLE**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>Fₛ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between species</td>
<td>6</td>
<td>309,717</td>
<td>51,567</td>
<td>47.1</td>
</tr>
<tr>
<td>Within species</td>
<td>46</td>
<td>50,402</td>
<td>1,096</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>360,119</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Let the type I error (α) = 0.01

\[ F_{0.01(6,46)} = 3.25 \]

**Conclusion:** Since \( Fₛ > F_{0.01(6,46)} \), reject the null hypothesis. On the basis of the results I reject the hypothesis that the B values corrected for the extent of plasma protein binding (B*) were similar in goats, swine, ponies, rabbits, chickens, dogs and cats following intravenous injection of amphetamine (0.66 mg/kg). An extension of this conclusion is the conclusion that the apparent specific volumes of distribution corrected for the extent of plasma protein binding of this drug are significantly different (p < 0.01) in goats, swine, ponies, rabbits, chickens, dogs and cats.
Null hypothesis: The biological half-lives of amphetamine are similar in goats, swine, ponies, rabbits, chickens, dogs and cats.

Alternative: The biological half-lives of amphetamine are not similar in goats, swine, ponies, rabbits, chickens, dogs and cats.

TABLE 54
ANOVA TABLE

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>$F_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between species</td>
<td>6</td>
<td>206.68</td>
<td>34.45</td>
<td>121.80</td>
</tr>
<tr>
<td>(elimination)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within species</td>
<td>46</td>
<td>13.01</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>219.69</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Let the type I error ($\alpha$) = 0.01
$F_{0.01(6,46)} = 3.25$

Conclusion: Since $F_s >> F_{0.01(6,46)}$, reject the null hypothesis. On the basis of the results I reject the hypothesis that the half-lives of amphetamine in these species of animals are similar.
CHAPTER V

DISCUSSION

Upon closer examination, it is usually found that the black boxes of biology are actually various hues of grey. Grove C. Nooney, 1966.

The results of this investigation show that the distribution and elimination of a drug in different species of animals is satisfactorily compared by pharmacokinetic parameters. The distribution of amphetamine was rapid in all species studied so that the body which is best described as a two compartment open model (Figure 15) reduces to a one compartment open model in describing the pharmacokinetics of this drug. This implies that the sum of the microconstants describing the rate of attainment of the apparent volume of distribution is considerably greater than the rate constant for elimination of the drug from the blood, that is,

\[ k_{12} + k_{21} \gg k_2. \]

The multi-compartmental body model includes "deep" compartments that never equilibrate with the drug in the blood throughout the elimination processes. Such compartments may be bone, fat, embryos, etc. Their existence allows the body to retain drug for longer periods than the arbitrary half-life estimates made on the basis of a major depletion of drug content in the body would permit. The reliability of a first order multicompartamental model...
for the distribution, metabolism and excretion of a drug can only be tested by varying intravenous dosage within the clinical dose range and determining whether the derived intrinsic rate constants and apparent volumes of distribution are invariant with dose. The apparent elimination rate constant will vary with dose when the plasma protein and the enzymic processes are saturable or when the drug has the property of changing the diffusivity, permeability or size of distribution compartments as a function of dose.

The general operating rule in a pharmacokinetic analysis is to postulate the minimum number of compartments consistent with physiological reality. No absolute claim can be made that more compartments than those chosen are not more valid reflections of the true biological processes. An approach such as this should be parsimonious in its postulates unless experimental evidence dictates that parsimony leads to the denial of reality (Garrett, Thomas, Wallach and Alway, 1960).

One of the most important factors regulating the response of an organism to a drug is the rate at which the drug is eliminated from the body. 'The main difference between man and the common laboratory animals probably lies in the rates of the reactions which transform drugs. These differences in rates may have profound effects on drug action' (Williams, 1963). Elimination refers to all the processes that operate to reduce the effective drug concentration in the body fluids.
Prior to this work curves of the decline of plasma amphetamine concentrations were not available, so it was unknown whether elimination was a simple first-order process or a multiphasic process. The plasma amphetamine concentration-time profiles indicate that the disappearance of this drug from plasma was a first order (exponential) process (Figure 14). The half-life of a first order decay is a constant value, independent of the initial concentration or dose.

Intravenous dosing was employed to estimate the rate constants. The biological half-life of a drug is associated with the overall elimination rate constant. The values of this rate constant, expressed in reciprocal hour, the standard deviation and standard error for the different species of animals studied are tabulated in Table 55. The assumption made in using the value \( \beta \) to estimate the biological half-life was that the tissue to plasma ratio remained constant during the elimination process. When the data are described by the equation

\[
P_t = B e^{-\beta t}
\]

the ratio of drug concentration in plasma to tissues remains constant and equal to the equilibrium value or \( k_{12}/k_{21} \).

The rate of elimination is frequently expressed as the biological half-life which, in turn, is determined by the apparent specific volume of distribution and the sum of the metabolic and the excretion clearance constants:
## TABLE 55

**THE OVERALL ELIMINATION RATE CONSTANT OF AMPHETAMINE IN VARIOUS SPECIES OF ANIMALS**

<table>
<thead>
<tr>
<th>Species (number)</th>
<th>Mean</th>
<th>( \beta ) (hour(^{-1}))</th>
<th>S.D.</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caprine (10)</td>
<td>1.118</td>
<td>0.26</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Porcine (9)</td>
<td>0.674</td>
<td>0.09</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Equine(^a) (5)</td>
<td>0.500</td>
<td>0.07</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Lapine (4)</td>
<td>0.498</td>
<td>0.05</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Avian(^b) (8)</td>
<td>0.305</td>
<td>0.08</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Canine (11)</td>
<td>0.159</td>
<td>0.03</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Feline (6)</td>
<td>0.108</td>
<td>0.02</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Ponies  

\(^b\) Chickens
The clearance constants have the dimensions of millilitre per minute or litre per hour and are dependent on the relative extent of bio-
transformation and excretion, principally renal, and the physicochem-
ical properties (pKa, partition coefficient) of the drug. The hypothe-
tical volume is also dependent on the properties of the drug as well as those of the body (tissue binding, compartments, that is, kinetical-
ly distinguishable pools in terms of the drug concentration-time pro-
file).

The mean values of the biological half-lives under normal conditions of fluctuating urinary pH reaction in these randomly selected groups of animals varied between species (Tables 54 and 56). On the basis of the results the null hypothesis, which stated that the half-lives were similar for this drug in different species of animals, was rejected. The shortest half-life was in the rum-
inant animals, the other herbivorous animals, chickens and swine had half-lives for this drug of intermediate duration while the longest half-lives were in the carnivorous animals. A similar pattern of plasma half-lives was observed for salicylate in goats, ponies, swine, dogs and cats (Davis and Short, 1967; Davis and Westfall, 1971). The plasma disappearance of injected phenol occurred most rapidly in the goat, followed in order of decreasing rate by the pig, the dog and the cat (Oehme, 1969). The relative order of plasma disappearance of phenol may be related to the rate of substrate conjugation by each species. Rowland (1969)
TABLE 56

THE BIOLOGICAL HALF-LIFE OF AMPHETAMINE IN VARIOUS SPECIES OF ANIMALS

<table>
<thead>
<tr>
<th>Species (number)</th>
<th>Mean</th>
<th>S.D.</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caprine (10)</td>
<td>0.62</td>
<td>0.13</td>
<td>0.04</td>
</tr>
<tr>
<td>Porcine (9)</td>
<td>1.05</td>
<td>0.14</td>
<td>0.05</td>
</tr>
<tr>
<td>Equine a (5)</td>
<td>1.39</td>
<td>0.18</td>
<td>0.08</td>
</tr>
<tr>
<td>Lapine (4)</td>
<td>1.40</td>
<td>0.15</td>
<td>0.08</td>
</tr>
<tr>
<td>Avian b (8)</td>
<td>2.27</td>
<td>0.48</td>
<td>0.17</td>
</tr>
<tr>
<td>Canine (11)</td>
<td>4.49</td>
<td>0.80</td>
<td>0.24</td>
</tr>
<tr>
<td>Feline (6)</td>
<td>6.53</td>
<td>0.94</td>
<td>0.39</td>
</tr>
</tbody>
</table>

a Ponies
b Chickens
reported a half-life of about 12 hours for amphetamine in man. The half-life of the antirheumatic agent phenylbutazone is 3 hours in the rat and dog, yet in man its half-life is three days.

Both the rate and the mechanism of elimination of amphetamine varied with the species of animal. The rate constant \( k_2 \) is the elimination rate constant for loss of drug from the blood by all routes. For example, if the drug is eliminated by both excretion, renal and/or biliary, and biotransformation then

\[
k_2 = k_{\text{metabolism}} + k_{\text{excretion}}
\]

Since the overall elimination of amphetamine was first order the fraction of the dose recovered as metabolite or unchanged drug could be used to calculate the individual rate constants. The amount of drug excreted in bile was shown to be negligible in the dog (Table 20), small in the pig (Table 43) and absent from human bile (Beckett and Rowland, 1965). The fraction of the dose recovered as unchanged amphetamine in cumulative (24-hour) urine samples varied considerably between different species (Table 17 and Figure 16). Thus one can conclude that the relative contributions of \( k_{\text{metabolism}} \) and \( k_{\text{excretion}} \) to the rate constant for elimination from the blood \( (k_2) \) assumed different magnitudes for each species. On the basis of \( k_{\text{ex}} \) values one might classify the species of animals studied in the following manner:

Class A - feline, canine, avian
Class B - porcine, caprine
Class C - equine, lapine
The per cent of dose of \(^{14}\)C-amphetamine in 24-hour urine of different species, namely, man, Rhesus monkey, rabbit, guinea pig, rat, mouse and greyhound was determined by Dring, Smith and Williams (1970). Including their results in the classification of species on the basis of \(k_{ex}\) the following Table may be formed:

<table>
<thead>
<tr>
<th>Class</th>
<th>Species of animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>cat, dog, chicken, man, Rhesus monkey, mouse, guinea pig</td>
</tr>
<tr>
<td>B</td>
<td>pig, goat, rat</td>
</tr>
<tr>
<td>C</td>
<td>pony, horse, rabbit</td>
</tr>
</tbody>
</table>

One might predict that the biological half-life of amphetamine in animals of class A, that is, those species in which \(k_{excretion}\) constituted a large proportion of \(k_2\), might be subject to variation with alteration of urinary pH. A most important corollary to this prediction is the relationship of \(k_2\) to \(\beta\), as the latter determines the biological half-life.

The plasma half-lives of the drug were longer in dogs, cats and man (Rowland, 1969) than in animals in Classes B and C. The large contribution of \(k_{excretion}\) to \(k_2\) and the longer half-lives in animals in Class A might reflect a slower metabolic rate in these species. Chickens were an exception as 32 percent of the dose administered was excreted unchanged in the 24-hour
cumulative urine sample and the mean biological half-life was only 2.27 hours. Quinn et al. (1958) have correlated the half-life of certain drugs with the activity of drug-metabolizing enzymes in liver microsomes and were able to correlate duration of pharmacodynamic action with enzyme activity. Chemicals which were metabolized by highly active enzyme systems had short plasma half-lives and a short duration of action. The relatively short plasma half-lives in animals of Classes B and C reflect the large contribution of $k_{\text{metabolism}}$ to $k_2$. Among mammalian species there are wide differences in drug metabolism, but no rationale for these differences is apparent. Rates of metabolism may differ, even when the pathways are the same, and different species may also have entirely different metabolic pathways for dealing with the same drug. The half-lives of amphetamine in the goat and the pig were significantly shorter than the half-lives in the rabbit and pony. The magnitude of $k_{\text{excretion}}$ was approximately five times greater for the animals in Class B than for those in Class C; the principal metabolic pathway was $p$-hydroxylation in the goat and pig and oxidative deamination in the rabbit (Axelrod, 1954; Dring, Smith and Williams, 1966, 1968, 1970) and possibly the pony. The hydroxylation reaction might have proceeded at a faster rate than oxidative deamination. The major metabolic route of $d$-amphetamine in the rat was through ring hydroxylation followed by glucuronidation of the hydroxylated derivative (Dring, Smith and Williams, 1970). Groppetti and Costa (1969) reported half-lives of 76 minutes and 130 minutes for amphetamine in the male and female rat.
respectively. No sex difference in persistence of the drug was observed in any of the domesticated species studied.

Species differences have been demonstrated for the plasma protein binding of acidic drug (Anton, 1960; Genazzani and Pagnini, 1963; Sturman and Smith, 1967; Davis, 1970), but few studies have been made on the plasma protein binding of basic drugs. It is generally accepted that only the unbound portion of a drug is available for pharmacological activity. The unbound portion also is available to the liver for biotransformation and appears in the glomerular ultrafiltrate. Hence, the degree of protein binding can be of great quantitative significance in the pharmacological (therapeutic as well as toxic) action of drugs.

In general, an inverse relationship of acidic drug concentration and extent of plasma protein binding has been found by other workers. The percent amphetamine bound to plasma proteins was independent of drug concentration over the range of concentrations observed in vivo (Table 6). This finding was supported by Franksson and Anggard (1970). The extent of protein binding of this drug in a single species varied directly with the total plasma protein concentration. These observations suggest that the mechanism of protein binding of amphetamine might be a simple adsorption process. The protein binding values show that a species difference exists (Tables 7 and 52) for the binding of amphetamine at a concentration of 100 ng ml⁻¹, yet the total plasma protein concentration for the various mammalian species was similar. For all species of animals studied the extent of binding
was low (less than 45 percent) for this drug. On the basis of the extent of plasma protein binding of amphetamine the species of animals studied may be divided into two groups:

(a) goats, swine, monkeys and rats (ca. 40 percent binding of drug)
(b) dogs, cats, ponies, rabbits and opossum (ca. 27 percent bound).

The extent of binding of amphetamine in chickens was lower than in any mammalian species. This feature might be explained by the low concentration of plasma proteins (3 gm/100 ml) in the bird.

Marked species and strain differences have been reported for the pharmacological effects of one group of basic drugs, the tricyclic antidepressants (Brodie, 1965). Borga, Azarnoff and Sjoquist (1968) showed marked species differences in the degree of plasma protein binding of desipramine. It is interesting to speculate whether the reported species differences in pharmacological effect of tricyclic antidepressants could be related to the degree of protein binding.

The plasma protein binding of the catecholamines and their O-methylated metabolites was shown by Franksson and Anggard (1970) to be of a low order, that is, 14 to 23% in physiological concentration. This seems rational since epinephrine at least is released into the bloodstream and acts on the peripheral organs. The low binding of the catecholamines also rules out the possibility that part of the circulatory response to
intravenously injected amphetamine could be due to the displacement of endogenous amines from a plasma protein pool.

Displacement of drugs from binding sites would be pharmacologically important only when a drug is eliminated at an abnormally slow rate and only when more than 90 percent of the drug in plasma is bound (Gillette, 1968). The tricyclic antidepressants, which are also nonpolar amines, were shown to have a high extent of binding—more than 90 percent—(Borga et al., 1969). It is possible that the presence of the large tricyclic ring system interacted more closely with the protein by short range secondary molecular forces, and that the amino group in the basic drugs therefore plays a minor role in the binding.

The fact that plots of the logarithm of the total plasma concentration of amphetamine against time are linear suggest: (a) that the plasma contains a constant proportion of the total drug in the body, and (b) that the instantaneous rate of drug loss is directly proportional to the concentration of total drug. The latter suggest that the protein-bound drug acts as if it were not protein bound with respect to biotransformation. This may account for the observed direct relationship between the extent of plasma protein binding and the rate of formation of p-hydroxyamphetamine in goats, swine, rats and dogs and cats.

The pKa value of dexamphetamine is 9.90 (Leffler et al., 1951; Lewis, 1954; Kisbye, 1958). This implies that at the physiological pH (7.4) only a small fraction, 0.31 per cent, is in the neutral or nonionized form. The neutral moiety is reasonably
soluble in various organic solvents whereas the ionized form is water soluble. These physicochemical properties are important in determining the distribution and renal excretion of this drug.

Domínguez (1950) defined volume of distribution as the volume of body fluids which holds the substance in solution at the same concentration as the plasma. The analytical procedure measured the total amount of unchanged amphetamine in the plasma samples and since only unbound drug was available for diffusion out of the circulation the apparent volumes of distribution had to be corrected for the extent of plasma protein binding. The protein binding values in Table 7 were employed in correcting the B values which were employed in estimating the corrected apparent specific volumes of distribution (Table 8). The B values corrected for the extent of plasma protein binding varied significantly between species (F-test, p < 0.01, Table 53). The corrected volumes of distribution (V'd*) were large in all species, the values obtained for the porcine, equine, lapine and canine species were similar. The ruminant animals had a particularly large apparent volume of distribution which may have been due to passive diffusion into the rumen of some of the unbound nonionized portion of the circulating drug (Table 48). The pH gradient across the reticuloruminal epithelium (ca. 1 pH unit) would favour this passive transfer process. Corker (1966) found that ephedrine attained concentrations in rumen fluid which were several times greater than the corresponding plasma levels following intravenous infusion of this compound. Stowe (1967) has also recorded
the sequestration of salicylate and benzoate in rumen fluid which was buffered to pH 7.6-8.0. Jenkins (1969) showed that quinine diffused from the plasma into the rumen and was trapped there. Apparently quinine became bound in some fashion to some constituent in the ruminal fluid. A great number of potential binding sites exist within rumen liquor. The significant sequestration of ephedrine in rumen fluid observed by Corker (1966) may also have been due in part to binding, in addition to the ion-trapping mechanism. Following a detailed study of the passage of some acids (pentobarbital, salicylic acid), bases (antipyrine and quinine) and an homologous series of tetraalkylammonium compounds across the ruminal epithelium Jenkins (1969) concluded that posology in ruminants presents great difficulties. The possible contribution of the reticuloruminal weight and volume to dosage rate and drug distribution respectively would have to be considered for each individual compound to achieve accuracy in establishing therapeutic plasma levels. Davis and Sturm (1970) found that the apparent specific volume of distribution of pentazocine varied from 5.77 litres/kg in goats to 2.78 litres/kg in cats. Cats and chickens had smaller apparent specific volumes of distribution of amphetamine than the values estimated for the other species of animals (Table 8). The apparent specific volumes of distribution for free phenol in dogs, pigs and goats indicated tissue binding of phenol. Indications of the tissue binding of phenol were not found in the cat (Oehme, 1969). The values of $k_2$, the rate constant for elimination of amphetamine from the blood, and $\beta$, the overall elimination
rate constant, expressed in reciprocal minutes, for the different species of animals, are tabulated in Table 8. For any species of animal the value of $k_2$ relative to $\beta$ is directly related to the apparent specific volume of distribution of the drug. In the goat the value of $k_2$ was one hundred times the value of $\beta$ while in the cat $k_2$ was fifty times $\beta$. It may be concluded from these results that the larger the apparent specific volume of distribution of the drug the wider the ratio of $k_2$ to $\beta$ and the shorter the biological half-life of the drug. The biological half-life is associated with the overall elimination rate constant. This conclusion, derived from the pharmacokinetic analysis of the experimental observations in the various species, shows the importance of the apparent volume of distribution of a drug in determining the biological half-life of the compound. Quantitative differences in disposition or action of drugs between species are often ignored by the comparative pharmacologist who is only seeking dramatic and exploitable qualitative differences (Rall, 1968).

The objective of the experiments in Chapter III, section 4 was to determine in greater detail the relative magnitudes of the components of $k_2$ in dogs and swine. The tissue distribution of amphetamine was examined in the dog (Axelrod, 1954); the drug was found to be localized in most tissues including kidney, liver, lung, spleen and brain. The concentrations of amphetamine in plasma and cerebrospinal fluid were the same (3.8 ug/ml) indicating that there was no hindrance to its passage through the blood-CSF barrier. In the experiments performed in this study
amphetaamine was found to attain similar concentrations in cerebrospinal and ocular fluids (Figure 19). The extents of plasma protein binding in the dog determined by the in vitro method and by measuring the amphetamine concentrations in plasma and cerebrospinal or ocular fluids collected simultaneously were not significantly different (Student's "t" test, p > 0.05, Table 58).

TABLE 58
PROTEIN BINDING OF AMPHETAMINE IN DOGS.

<table>
<thead>
<tr>
<th>Variable</th>
<th>% bound amphetamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in vitro</td>
</tr>
<tr>
<td>Amphetamine conc.</td>
<td>100 ng. ml⁻¹</td>
</tr>
<tr>
<td>Mean % bound</td>
<td>27.1</td>
</tr>
<tr>
<td>Number of animals</td>
<td>17</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>1.5</td>
</tr>
</tbody>
</table>

One can conclude that after diffusion equilibrium was attained similar concentrations of unbound amphetamine were present in cerebrospinal and ocular fluids and plasma.

Amphetamine was shown to be excreted in the urine of man substantially unchanged, the values of recovered drug varied from 12 to 100 per cent (Richter, 1938; Beyer and Skinner, 1940; Jacobsen and Gad, 1940; Keller and Ellenbogen, 1952; Chapman, Shenoy and Campbell, 1959; Alles and Wisegarver, 1961, Cartoni and de Stefano, 1963; Beckett and Rowland, 1965; Rowland, 1969).
The various analytical techniques used may account for the spread of the recoveries. Urinary pH was shown to be an important factor in determining the rate of excretion of amphetamine in man (Beckett, Rowland, and Turner, 1965; Asatoor, Galman, Johnson and Milne, 1965; Smart and Turner, 1966). Following oral administration of 10 mg dextroamphetamine sulphate in 50-100 ml of water to man Rowland (1969) found that the urinary kinetic data gave a monoexponential decay for at least seven half-lives, with a value corresponding to that in the blood (about 12 hours). Proportionality between blood and urine data provided an estimate of 120 ml/min for the apparent renal clearance of amphetamine. The renal clearance value indicated that in man amphetamine was probably excreted by glomerular filtration. Beckett, Salmon and Mitchard (1969) observed that the decline in plasma concentration of the drug was more rapid under controlled acidic conditions than under conditions of fluctuating urinary pH. The apparent rate of urinary excretion of amphetamine was directly proportional to its plasma concentration only under the controlled acidic urinary conditions. 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. Their results indicated that about 75 per cent of the drug was transferred from the plasma into the renal tubules by routes other than glomerular filtration when the urine was acidic. They suggested that 'probably as urine flowed down the renal tubules, the drug passed from the blood into the urine because of the high concentration gradient of unionized
drug across the lipid membrane."

In this study no attempt was made to control urinary pH, the observations were within the range of normal values for the canine species (Table 20). The urinary pH determines the proportion of nonionized drug in the renal tubular fluid and thus the amount of this lipid soluble form which is available for reabsorption by passive nonionic diffusion. The observations indicate that in dogs the urinary pH had a direct effect upon the biological half-life and the renal clearance of this drug. At mean urinary pH values of 6.28 (dog Z) and 7.50 (dog R) the renal clearance values were 5.07 and 2.80 ml/min/kg respectively (Table 40). The renal clearance values provide evidence that in dogs amphetamine probably undergoes glomerular filtration and tubular reabsorption, the extent of reabsorption increasing with increasing urinary pH values. In the classification of species on the basis of the magnitude of $k_{\text{excretion}}$ the dog belonged to class A; horses belong to class C, that is, those species which excrete only a small percentage of the administered dose unchanged in the urine. In support of this grouping of species the biological half-life of amphetamine in the horse was not significantly altered (Student's "t" test, $p > 0.05$) by any dietary regimen or urinary pH reaction.

From the kinetic standpoint enterohepatic circulation of a drug may be very important. If a drug or metabolite or both are excreted in significant amounts in the feces, complete elucidation of the kinetics would require determination of whether the material in the feces was partly or wholly unabsorbed drug,
drug which arose from biliary excretion, or drug which arose from secretion through the intestinal wall. Williams, Milburn and Smith (1965) pointed out that enterohepatic circulation may be responsible for prolonged retention of certain drugs and metabolites in the body. Enterohepatic circulation may cause secondary peaks, shoulders, or oscillations in blood concentration-time curves (Fujii et al., 1963; Turco et al., 1966). These are usually most evident in the initial part of the curve following intravenous administration (Turco et al., 1966), but following oral administration may occur at later times particularly following food ingestion when the gall bladder evacuates (Wagner, Gerard and Kaiser, 1966; Fujii et al., 1963). If enterohepatic circulation is prominent, the compartmental model should have a reversible transfer between the compartments representing blood and the absorption sites. For such models, the true rate constant for elimination cannot be estimated from the terminal blood concentration data (Wagner, Gerard and Kaiser, 1966).

In the dog the microconstant describing the rate of biotransformation per minute, \( k_{\text{metabolism}} \), constituted approximately sixty percent of \( k_2 \) and \( k_{\text{urine}} \) forty percent of \( k_2 \). The portion of \( k_2 \) represented by the rate of excretion of unchanged amphetamine in bile per minute, \( k_{\text{bile}} \), was negligible. Interruption of the enterohepatic pathway for cycling of nonionized lipid-soluble drug by cannulation of the common bile duct had no effect upon the biological half-life or the apparent volume of distribution of the drug in intact and nephrectomized dogs (Table 18).
These results were supported by the small value of the rate constant for elimination of unchanged amphetamine in bile and by the figure obtained for the cumulative amount of amphetamine excreted in bile (Table 20). The mean cumulative amount of unchanged amphetamine excreted in bile was 5 microgram as compared to 2 milligram excreted in urine during the same time period. The biliary clearance values, ca. 0.012 ml/min/kg were similar in intact and nephrectomized dogs. The biliary clearance values indicate that this route of elimination, or perhaps distribution, played a minor role in the overall elimination of amphetamine from the body. The biliary excretion of foreign compounds varies among animal species, but is generally highest in the dog and rat (Parke, 1968). Perel et al. (1964) found that 20 percent of injected phenylbutazone was excreted in the bile as the glucuronide in six hours. Oxyphenylbutazone was excreted very little in bile, and a shorter half-life was found for oxyphenylbutazone than for phenylbutazone. The longer half-life of the phenylbutazone may be related to the enterohepatic cycle. The alkaloids strychnine and quinine appear in bile as conjugates of the bile acids, although their major excretory pathway is the kidney. Chloramphenicol glucuronide was shown to be excreted in the bile, hydrolyzed in the intestine and the active drug reabsorbed.

The mean cumulative amount of unchanged amphetamine excreted in the bile of three pigs was 15 microgram and the amount in urine was 1 milligram. The quantities of unchanged drug excreted in urine and bile were 8.18 and 0.12 percent of the dose.
administered. The appearance of amphetamine in urine and bile were first order processes. The appearance of the p-hydroxy derivative and the glucuronide conjugate in urine seemed to be zero order processes, this might be a reflection of a zero order metabolic reaction or the low availability of the amphetamine to the hydroxylating enzymes due to the wide extravascular distribution of this drug (Figures 24 and 25). An overall exponential elimination of a drug may comprise simultaneously occurring first order and zero order processes. The rates of disappearance of p-hydroxyamphetamine from plasma and the appearance of the glucuronide conjugate in urine following the intravenous injection of the p-hydroxy derivative would provide useful information concerning the order of the conjugation reaction. Metabolite administration concomitant with drug should permit a decision as to whether the enzymic system is product-inhibited. The pharmacokinetics of p-hydroxyamphetamine should be monitored, it being realized that the apparent volume of distribution would be considerably smaller than that of amphetamine. The correlation of pharmacokinetics parameters with structural modifications of the drug molecule may provide insight into those body distributive and elimination factors which affect the intensity and duration of drug action.

Nephrectomy was found to have no effect on the apparent specific volume of distribution of the drug. The ratio of $k_2/\beta$ was similar for intact and nephrectomized dogs, the rate constant for elimination from the blood was approximately seventy times the overall elimination rate constant. The value of $k_2$ was
lower and the biological half-life was significantly longer in the nephrectomized animals than in intact dogs (Student's "t" test, 
p < 0.001).

The influence of biotransformation upon the biological half-life of amphetamine could be determined by measuring the rate of decline of amphetamine in plasma following the administration of a suitable metabolic inhibitor. The choice of an inhibitor would be governed by the species of animal chosen as the metabolic pattern of amphetamine is known to vary between species. Desmethyllumipramine, imipramine, chlorpromazine, diazepam, nialamide and cocaine were shown to inhibit p-hydroxylation of amphetamine (Sulser, Owens and Dingell, 1966; Valzelli, Consolo and Morpurgo, 1967; Consolo, Dolfini, Garattini and Valzelli, 1967; Lewander, 1969).

'The main difference between man and common laboratory animals probably lies in the rates of the reactions which transform drugs' (Williams, 1963). The rates of metabolism may differ, even when the pathways are the same, and different species may also have entirely different metabolic pathways for dealing with the same drug. The pathways which constitute k_{metabolism} of amphetamine have been shown to vary with the animal species (Dring, Smith and Williams, 1966, 1970; Ellison, Gutzait and Van Loon, 1966). In rats p-hydroxylation followed by conjugation of the hydroxylated derivative was the main metabolic pathway. In man, squirrel and Rhesus monkeys, mouse, rabbit and guinea pig oxidative deamination of the aliphatic side-chain to form benzoic acid was the predominant metabolic route. The benzoic acid was, in turn, conjugated with glycine to
form hippuric acid. The dog (Axelrod, 1954; Ellison, Gutzait and Van Loon, 1966; Dring, Smith and Williams, 1966, 1970) and cat (Ellison, Siegel, Silverman and Okun, 1968) metabolized dl-amphetamine primarily by ring hydroxylation and secondarily by oxidative deamination. The dog is unusual in that either pathway may be utilized (Ellison, Gutzait and Van Loon, 1966). Smith and Dring (1970) suggested a relationship between dietary habit and metabolic pattern. Herbivorous species metabolized the phenylisopropylamine group largely by oxidative deamination. Carnivorous and omnivorous species utilized aromatic hydroxylation as well as oxidative deamination for the metabolism of these compounds, and the extent to which these two reactions occurred varied with the structure of the compound and the species of animal.

The amounts, expressed as percent of the dose administered, of amphetamine, p-hydroxyamphetamine and its conjugates recovered in 24-hour urine of goats, swine, ponies, rabbits, chickens, and dogs and 48-hour urine of cats are shown in Table 17 and Figures 16 and 17. The low total recovery in the equine species (33 percent of dose) may have been due to biotransformation of this drug by oxidative deamination to benzoic acid, similar to the rabbit. This metabolite and its glycine conjugate, hippuric acid, were not measured as the latter is an endogenous metabolite in horse urine.

Interspecies differences in drug responses may result from variations in the fate of the drug, that is, its distribution, excretion and biotransformation (pharmacokinetic factors), and/or from variations in the responding systems themselves (pharmacodynamic factors). The former were investigated in this study.
The perturbations of magnitudes of pharmacokinetic parameters among individuals challenged with a drug can be used as a diagnostic tool in evaluating the state of dynamic processes, the presence of metabolic diseases and genetic abnormalities, and the failure of physiological functions. It must be recognized that delineation of pharmacokinetic and pharmacodynamic influences can be difficult. In most cases, concentrations of a drug at its site of action are not known and are not necessarily in direct and simple relation with its blood or even tissue levels. Since receptor systems have been sometimes represented with a surrounding "biophase", some differences attributed to pharmacodynamic variations might, in fact, be pharmacokinetic differences (Jacob, 1968). The variation of the time-course of drug distribution in a complex organism when considered with the concomitant time-course of pharmacological activity can give insight into the properties and nature of the compartment in contact with the receptor sites, that is, the biophase compartment.

In conclusion, a relationship appeared to exist between dietary habit of the various species of animals and the following pharmacokinetic parameters:

(i) magnitude of the overall elimination rate constant and thus biological half-life

(ii) relative contributions of $k_{\text{metabolism}}$ and $k_{\text{urine}}$ to $k_2$, and

(iii) metabolic pattern.

The ratio of $k_2:\beta$ was directly related to the corrected apparent
specific volume of distribution of the drug and the wider the ratio
the shorter the biological half-life.

The following similarities in the fate of amphetamine were
found in the different species of animals:

(i) the low extent of plasma protein binding
(ii) the rapid extravascular distribution of the drug, and
(iii) the large apparent volume of distribution.
CHAPTER VI

SUMMARY AND CONCLUSIONS

1. The disappearance of amphetamine from plasma was a first-order (exponential) process in all species studied. The mean biologic half-life of this drug under normal conditions of fluctuating urinary pH reaction in these randomly selected groups of animals varied significantly between species (F-test, p<0.01). Plasma half-lives of 0.62, 1.05, 1.39, 1.40, 2.27, 4.49 and 6.53 hours were found in goats, swine, ponies, rabbits, chickens, dogs and cats respectively. No sex difference in persistence of the drug was observed in any species studied.

2. The distribution of this drug was rapid so that the body which is best described as a two compartment open model reduces to a one compartment open model in describing the pharmacokinetics of amphetamine.

3. The percent amphetamine bound to plasma proteins was found to be independent of drug concentration over the range of concentrations observed in vivo (25-400 ng/ml). The extent of plasma protein binding of amphetamine differed significantly between species (F-test, p<0.01). At a drug concentration of 10^-6 Molar the mean percent binding of amphetamine was 40.7,
39.6, 25.3, 31.0, 14.5, 27.1, 26.4, 40.5, 40.2 and 26.0 for goats, swine, ponies, rabbits, chickens, dogs, cats, rats, monkeys and opossum respectively.

4. These protein binding values were employed in correcting the apparent specific volumes of distribution. The corrected volumes of distribution were large in all species, the values obtained for the porcine, equine, lapine, and canine species were similar (ca. 3.5 litres/kg). The ruminant animals had a particularly large apparent volume of distribution (5.19 litres/kg) which may have been due to passive diffusion into the rumen of some of the unbound nonionized portion of circulating drug. The pH gradient across the reticulorumenal epithelium (ca. 1 pH unit) would favour this passive transfer process. Cats and chickens, on the other hand, had considerably smaller volumes of distribution for this drug than those estimated for the other animals.

5. The value of $k_2$, the specific rate constant for elimination from the central compartment, relative to $\beta$, the overall elimination rate constant, was directly related to the apparent specific volume of distribution of the drug. In cats the value of $k_2$ was fifty times that of $\beta$ while in goats $k_2$ was one hundred times $\beta$.

6. The components of $k_2$, namely $k_{\text{metabolism}}$ and $k_{\text{excretion}}$ had different relative magnitudes in the various species of animals. The quantities of unchanged amphetamine, p-hydroxyamphetamine and its glucuronide and sulphate
conjugates in cumulative urine samples were measured. The time period for collection of urine exceeded five half-lives of amphetamine in all species. The amount of each fraction recovered, expressed as percent of the dose administered, varied between species. A relationship appeared to exist between dietary habit and metabolic pattern of amphetamine. About one-third of the amphetamine injected was excreted unchanged in the urine of the carnivorous animals (dogs and cats) and chickens while a small fraction of the dose was excreted unchanged in the urine of herbivorous animals. The amount of amphetamine which is eliminated by renal excretion, that is, the magnitude of the contribution of $k_{ex}$ to $k_2$, may be of importance in determining the effectiveness of urinary pH change upon the biological half-life of this drug.

7. The urinary pH reaction had a profound effect upon the renal clearance values and directly influenced the biological half-life of amphetamine in dogs. The renal clearance values provide evidence that amphetamine probably undergoes glomerular filtration and tubular reabsorption, the extent of reabsorption increased with increasing urinary pH values.

8. In horses the biological half-life was not significantly altered by any dietary regimen or urinary pH reaction.

9. The biliary clearance values and the cumulative amount of unchanged amphetamine in bile (dogs and swine) clearly indicate that this pathway plays a very minor role in the elimination of amphetamine from the body.
10. Amphetamine was found to attain similar concentrations in cerebrospinal and ocular fluids.

11. The appearance of p-hydroxyamphetamine in urine of dogs and swine seemed to be a zero order process. This may reflect a zero order metabolic process or the limited availability of amphetamine to the hydroxylating enzymes due to the wide extravascular distribution of this drug.

12. The mean biological half-life of the drug in intact (4.49 hr) and nephrectomized (5.68 hr) dogs was significantly different (Student's "t" test, p < 0.001). Nephrectomy was found to have no effect on the apparent specific volume of distribution of the drug. The biliary clearance values in nephrectomized and normal dogs were similar. Thus, the rate of excretion of amphetamine in bile was constant and independent of the extent of renal excretion of this drug.

13. No unconjugated p-hydroxyamphetamine was detected in bile, gastric fluid, cerebrospinal and ocular fluids. The glucuronide conjugates was present in bile.

14. No methylamphetamine was detected in any biological fluid.

15. There are species differences (i) in the extent of plasma protein binding, (ii) the apparent specific volume of distribution, (iii) the rate of elimination and (iv) the biotransformation pattern of amphetamine.

16. The following similarities in the fate of amphetamine were found in the different species of animals:

(i) the low extent of plasma protein binding
(ii) the rapid extravascular distribution of the drug, and
(iii) the large apparent volume of distribution.
BIBLIOGRAPHY


Baggot, J. D. 1968. Influence of Urinary pH on Persistence of Plasma Levels of Sulphonamides in Dogs. Vet. Rec. 82; 266


Conney, A. H., Jacobsen, M., Schneidman, K. and Sturtzman, R. 1965. Induction of Liver Microsomal Cortisol 6-β-Hydroxylase by Diphenylhydantoin or Phenobarbital; An Explanation of the Increased Excretion of 6-Hydroxy-β-cortisol in Humans Treated with These Drugs. Life Sci. 4:1091-1098.


Davis, L. E. and Short, C. R. 1967. Species Differences in Bio-
transformation and Excretion of Salicylate. Federation
Proc. 26:619.


Davis, L. E. and Sturm, B. L. 1970. Drug Effects and Plasma
Concentrations of Pentazocine in Domesticated Animals.

Davis, L. E. and Westfall, B. A. Species Differences in the Bio-
transformation and Excretion of Salicylate. J. Pharmac.
exp. Ther. (in press).

Davson, H. and Danielli, J. F. 1943. The Permeability of Natural
Press. p. 196.

Davson, H. and Danielli, J. F. 1952. The Permeability of Natural
Membranes. 2nd Edition. London: Cambridge University

Davson, H. and Matchet, P. A. 1953. The Kinetics of Penetration

Davson, H. 1955. A Comparative Study of the Aqueous Humor and

Davson, H. 1956. Physiology of the Ocular and Cerebrospinal

Dawson, A. B. and Ivy, A. C. 1925. Contributions to the Physiolo-
gy of Gastric Section. VII. The Elimination of Dyes

Dayton, P. G., Brand, L., Taller, D. and Mark, L. C. 1961,
Passage of Thiopental and Barbital into Ocular and Cere-
107:180-183.

Dearborn, E. H. 1967. Comparative Toxicity of Drugs. Federati-
on Proc. 26:1075-1077.

Debackere, M. and Massart-Leen, A. M. 1965. Identification and
Metabolism of Amphetamine in Some Domestic Animals.

Di Augustine, R. P. and Fouts, J. R. 1969. The Effects of Un-
saturated Fatty Acids on Hepatic Microsomal Drug Metabo-


