THE IMPORTANCE OF STERIC, STEREOCHEMICAL AND PHYSICO-ORGANIC FEATURES IN DRUG METABOLISM AND DRUG ACTION

ARNOLD H. BECKETT

Department of Pharmacy, Chelsea College of Science and Technology (University of London), London, S.W.3, U.K.

There are many examples which show that the introduction of a steric factor near a basic or acidic group in a biologically active molecule can modify greatly the character and duration of action of the parent molecule[1]; similar changes can result from the introduction of lipophilic or hydrophilic groups at any position in the molecule. These introduced groups in addition to altering the potential 'fit' at an active site in an enzyme or target organ, can also alter the distribution, metabolism and excretion of the molecules because of the attendant changes in pK_a values and rate of lipid solubility characteristics. In the observed biological response in the whole animal, it is difficult to assign quantitative aspects to the various influences except in the case of differences in activity of those enantiomorphs which have been proved not to be distributed differently; some approaches which may help to clarify this complex situation will be described later.

A few examples, drawn mainly from work in my laboratories, will suffice to show the influence on biological action of the introduction of even small chemically inert groups into a biologically active molecule. Normethadone has analgesic and antitussive action; the introduction of a correctly orientated α-methyl group to give (−)-methadone leads to enhanced analgesic activity[1], whereas the incorrectly orientated group gives (+)-methadone which is virtually devoid of analgesic activity but in which the antitussive activity of the parent is retained (*Figure 1*). Because the distribution and rate of metabolism of these enantiomorphs is very similar, the introduction of the α-methyl group may be regarded as enhancing or inhibiting the interaction of the molecule with analgesic receptor sites depending upon the geometry of the added group. The cough reflex receptors may be regarded as showing little stereo-selectivity for the methadone enantiomorphs. Similar arguments may be used to explain the differences in CNS activity of the isomers produced by introducing a methyl group adjacent to the nitrogen centre of β-phenylethylamine to give the stimulant (+)-amphetamine and non-stimulant (−)-amphetamine, since these enantiomorphs exhibit only very small differences in their distribution and metabolism pattern in animals and man[2].

The introduction of a correctly orientated β-methyl group into the acetylcholine molecule, to give the L-(+)-isomer, has little effect on the observed muscarinic activity but the incorrectly orientated group in the (−)-isomer virtually abolishes activity (*Table 1*); the introduction of the methyl group into the α-position leads to substantial reduction of the activity

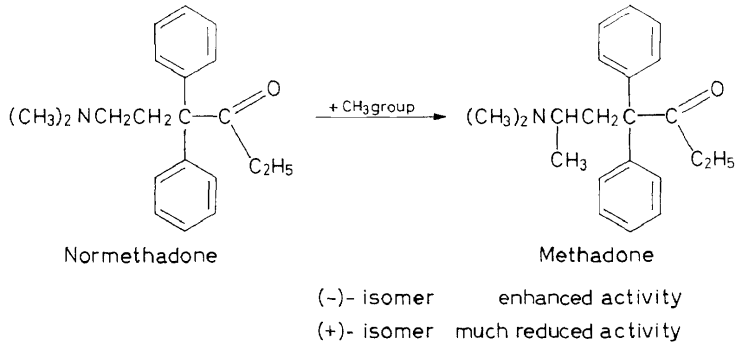


Figure 1. The effect of introduction of a steric factor in an analgesic.

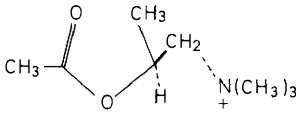
in the (+)-isomer and virtual loss in the (−)-enantiomorph (*Table 1* and *Figure 2*)[3]. The (+)-β-methyl isomer is not quite so good a substrate for acetylcholinesterase as the parent, whereas the enantiomorph is not a substrate; both enantiomorphs of the α-methyl compound are equally good substrates as their parent (*Table 2*). The introduction of the α- or β-methyl group into butyrylcholine has similar effects on the suitability of the compounds as substrates for acylhydrolase[4] with the exception of the greater reduction in the case of the L-β-isomer (*Table 3*). In these acetylcholine-type compounds, steric factors will be the predominant ones because all the molecules have similar lack of lipid solubility; differences in rates of metabolism in the whole body as well as differences in fit at 'muscarinic receptors' may contribute to the observed differences in response. The size of the introduced group in muscarinic compounds can be critical even when distribution is not likely to be affected significantly, e.g. introduction of alkyl groups into 'normuscarine' and 'normuscarone' (*Table 4*)[3].

Steric factors can alter the relative emphasis of alternative metabolic routes, e.g. steric factors in the vicinity of the basic nitrogen in phenothiazines can alter the relative importance of *N*-oxidation by rat liver microsomes

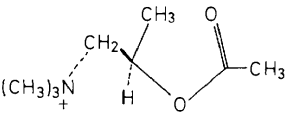
Table 1. Muscarinic activities of acetyl-α- and acetyl-β-methylcholine isomers (Number of molecules equivalent to 1 molecule of acetylcholine)

Compound	*Guinea-pig ileum*	*Ratio* (+)/(−)	*Cat blood pressure*	*Ratio* (+)/(−)
(±)-Acetyl-β-methylcholine iodide	1·6	—	0·97	—
(+)-Acetyl-β-methylcholine iodide	1·0	240	0·75	280
(−)-Acetyl-β-methylcholine iodide	240	—	202	—
(±)-Acetyl-α-methylcholine iodide	50	—	35	—
(+)-Acetyl-α-methylcholine iodide	28	8	13	11
(−)-Acetyl-α-methylcholine iodide	230	—	145	—
(±)-Acetyl-α-methylcholine (ethiodide)	265	—	150	—
(+)-Acetyl-α-methylcholine (ethiodide)	170	12	88	22
(−)-Acetyl-α-methylcholine (ethiodide)	2000	—	1900	—

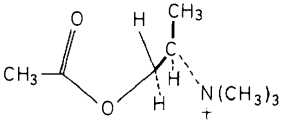
Data from Beckett *et al.*



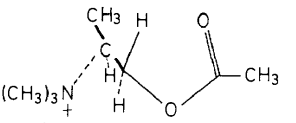
L-(+)-Acetyl-β-methylcholine
(1·0)



D-(−)-Acetyl-β-methylcholine
(240)



D-(+)-Acetyl-α-methylcholine
(28)



L-(−)-Acetyl-α-methylcholine
(230)

Figure 2. Configuration and muscarinic activities of acetyl-α- and acetyl-β-methylcholine isomers.

Table 2. Rates of hydrolysis of acetyl-α- and acetyl-β-methylcholine isomers by acetylcholinesterase at 37° at optimum concentrations

Compound	[S] opt. × 10^3 M	*Rates of hydrolysis* (acetylcholine = 100)
Acetylcholine	4·9	100
(±)-Acetyl-β-methylcholine iodide	18·0	46
L-(+)-Acetyl-β-methylcholine iodide	10·0	54
D-(−)-Acetyl-β-methylcholine	—	weak inhibition
(±)-Acetyl-α-methylcholine iodide	6·3	92
L-(−)-Acetyl-α-methylcholine iodide	6·7	97
D-(+)-Acetyl-α-methylcholine iodide	6·7	78

Data from Beckett *et al.*

Table 3. Rates of hydrolysis of acylcholines by acylcholine acylhydrolase

Acylcholine	*Rate of hydrolysis* (butyrylcholine-100) using		
	Purified horse enzyme	*Horse serum*	*Human serum*
Acetyl	44	46	47
Propionyl	71	—	69
Butyryl	100	100	100
Pentanoyl	—	—	80†
Hexanoyl	—	—	46†
Heptanoyl	—	—	44†
L-Butyryl-α-methyl	81	93	93
D-Butyryl-α-methyl	66	78	79
L-Butyryl-β-methyl	4·5	5·5	5·0
D-Butyryl-β-methyl	0	0	0

Data from Beckett *et al.* except where marked with a dagger.
† Data from Davies *et al.*

Table 4. Influence of size of alkyl group (= 2-position in muscarine) on muscarinic activity
(Cat blood pressure; number of molecules equivalent to 1 molecule of acetylcholine)

2-*Substituent*	*Muscarine*	*Muscarone*	*Dioxolane*	*Furan*
H	79	48	60	20
CH_3	0·3	0·23	1·6	2
C_2H_5	—	—	—	120
	Acetylcholine (R of acyl group ≡ 2-position)			
CH_3		1·0		
C_3H_7		370·0		

Data from Cohen *et al.* 1956; Fourneau *et al.* 1945; Waser, 1958, 1962.
( ) = number of molecules equivalent to 1 molecule of acetylcholine.

while leaving ring hydroxylation and sulphoxidation virtually unchanged[5] (*Figure 3*).

Although the quantitative importance of added steric and stereochemical features can be established in isolated enzyme systems, and with somewhat less precision in isolated preparations, the attendant changes which may be produced in distribution and metabolism make it hazardous to rely exclusively on observed relative biological efficacies of the molecules after oral doses as a measure of relative molecule–drug receptor interactions. Even consideration of observed changes in the metabolism in conjunction with observed changes in biological response is fraught with difficulties in many cases. For instance, the pH of the urine in man and animals can alter the reabsorption through the lipid tubule walls of drugs in urine after glomerular filtration (see *Figure 4*)[5]. Thus, molecular changes which alter the pK_a values of molecules or their rate of partitioning into lipids can result in changes in biological half-lives and differences in their ratio of metabolite to parent

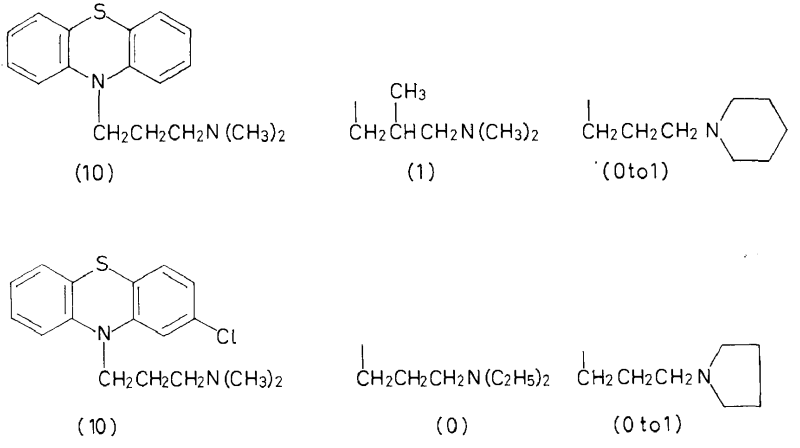


Figure 3. Influence of steric factors on the *N*-oxidation of phenothiazines by rat liver microsomes [all compounds underwent ring hydroxylation and sulphoxidation].

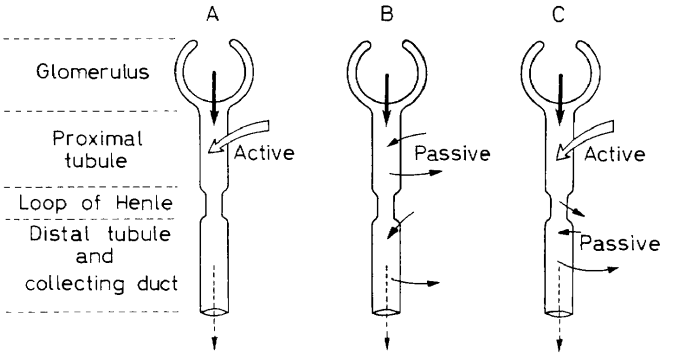


Figure 4. Schematic presentation of renal tubular mechanisms of organic base transport.

drug which are influenced differently by pH changes in the urine. The following examples serve to illustrate this point.

Amphetamine excretion in urine in man shows fluctuations when rate is plotted against time and these are a reflection of changes in urinary pH (*Figure 5*); less reabsorption occurs in the tubules when the drug is substantially protonated and thus not lipid soluble. When the urine is kept acidic (pH 4·8 to 5·0) by the oral administration of ammonium chloride, an increased excretion of unchanged drug occurs and there is a smooth curve when the rate of excretion is plotted against time (*Figure 6*); normal changes in rate of urinary flow do not affect this smooth curve[6,7]. Similar pH dependent fluctuations are seen in the urinary excretion of methylamphetamine (and its metabolite amphetamine) (*Figure 7*) and these fluctuations are likewise abolished when the urine is kept acidic[6]. Big differences occur under different pH conditions in the cumulative plot of drug excreted against time, e.g. see *Figure 8* for methylamphetamine. The reabsorption of many drugs which are partially ionized at physiological pH, and thus the duration of their exposure to metabolising enzymes, is dependent upon urinary pH which is itself influenced substantially by diet, exercise, time of day, etc. Hence

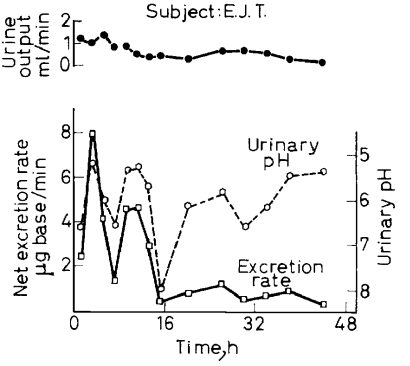


Figure 5. The influence of urinary pH and urine output on the urinary excretion of amphetamine in man, after oral administration of 15 mg (+)-amphetamine sulphate (similar patterns were observed in other subjects).

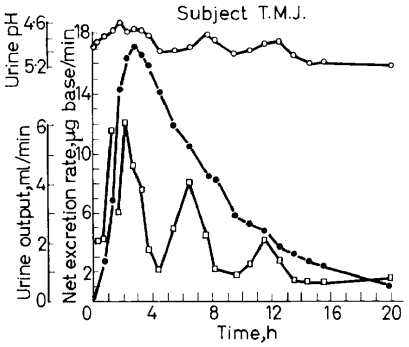


Figure 6. Urinary excretion of amphetamine after oral administration of 15 mg dexamphetamine sulphate (capsule) while under acidic urinary control [●—●, rate of excretion of amphetamine; □—□, urine output].

the significance of how changes in structure can influence metabolism of drugs in the body can be completely obscured by changes in urinary pH values.

Methylephedrine is metabolised to ephedrine, which is less lipid soluble in the unionized state than the parent drug, and also to norephedrine which is water soluble in the unionized state; the pH dependence of excretion is therefore substantially different in these compounds, despite the similarity of their pK_a values (*Table 5*). The ratio of the amount of unchanged drug to metabolite in the urine therefore varies greatly with the pH of the urine when methylephedrine and ephedrine are given (see *Table 5*)[7].

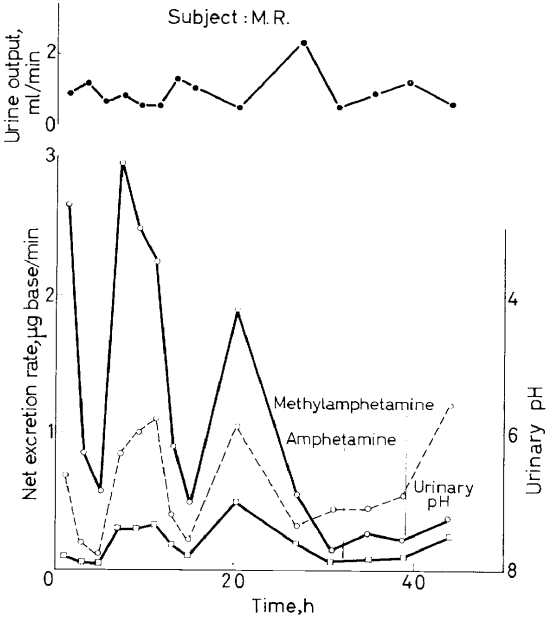


Figure 7. Effect of urinary pH and urine output on the urinary excretion of methylamphetamine (and its metabolite) in man, after oral administration of 11·0 mg (+)-methylamphetamine (similar patterns were obtained in other subjects).

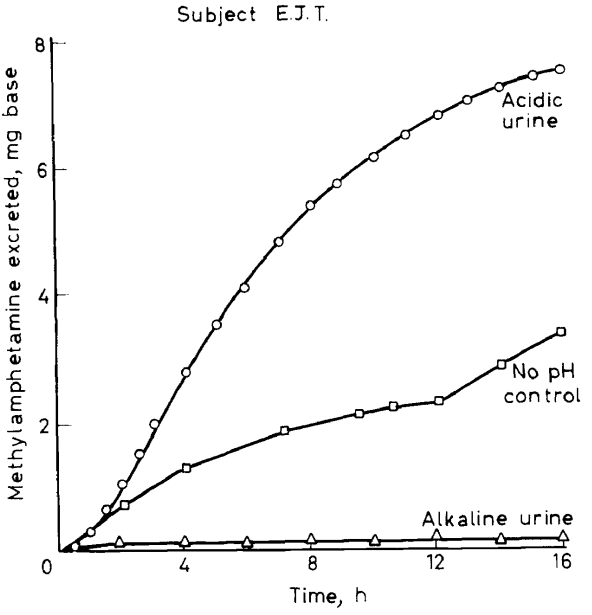


Figure 8. Cumulative urinary excretion of methylamphetamine in man under varying conditions of urinary pH after oral administration of 11·0 mg (+)-methylamphetamine.

The differences[8] in the effect of pH of the urine in man upon changing the excretion of amphetamine and the less lipid soluble norpseudoephedrine, possessing an additional alcoholic hydroxyl group, are shown in *Figure 9*. The analogous relationship[8] between the excretion of methylamphetamine and its hydroxy derivative, pseudo-ephedrine, is shown in *Figure 10*.

Mephentermine is excreted primarily in man unchanged but there is some demethylation to form phentermine. The ratio of metabolite to unchanged drug excreted and the pattern of excretion[8] depends on the pH of the urine (*Figure 11*). When a drug is more extensively metabolised, the ratio of drug to metabolite can be very sensitive to pH changes in the urine, e.g. fenfluramine, which is de-ethylated to norfenfluramine[8] (see *Figure 12*).

Table 5. pH dependence of excretion and the ratio of unchanged drug to metabolite in the urine in the case of methylephedrine, ephedrine and norephedrine

Drug	pK_a	*Dose* (mg)	*Average % dose recovered in* 16 h (*3 subjects*)					
			Acidic urine			*Alkaline urine*		
			M	E	N	M	E	N
Methylephedrine	9·20	27·12	65·03	10·65	≃1–2	3·06	4·39	1·47
Ephedrine	9·47	25·00		83·41	6·72		17·01	10·30
Norephedrine	9·44	22·87			91·82			84·86

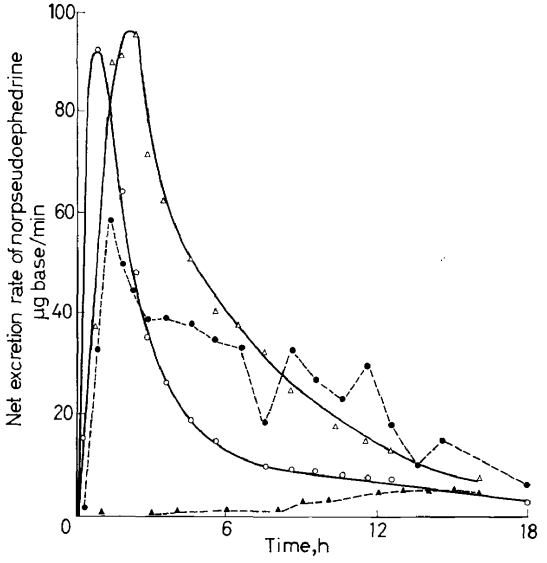


Figure 9. Urinary excretion of norpseudoephedrine and amphetamine from a subject following oral administration of (±)-norpseudoephedrine. HCl (≡ 25 mg base) and (+)-amphetamine. HCl (≡ 11·05 mg base) in aqueous solution with (a) acidic urine control, and (b) alkaline urine control [Norpseudoephedrine: ○—○, acidic urine control (pH range 4·69–5·08); ●– – –●, alkaline urine control (pH range 7·05–8·12). Amphetamine: △– – – △, acidic urine control (pH range 4·60–4·81); ▲—▲, alkaline urine control (pH range 7·18–8·25)].

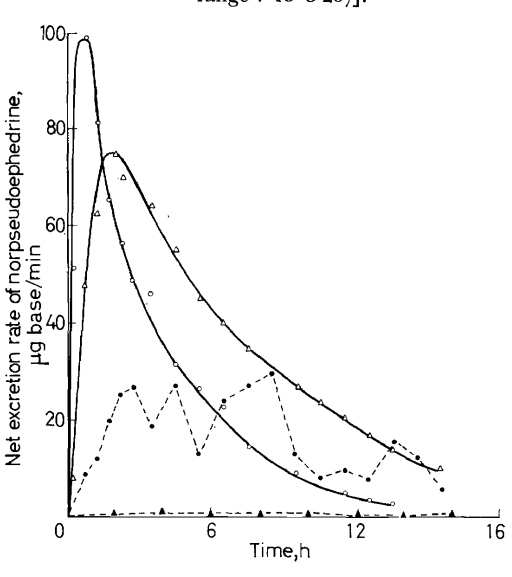


Figure 10. Urinary excretion of pseudoephedrine and methylamphetamine from a subject following oral administration of (±)-pseudoephedrine. HCl (≡ 25 mg base) and (+)-methylamphetamine. HCl (≡ 12·01 mg base) in aqueous solution with (a) acidic urine control and (b) alkaline urine control [Pseudoephedrine: ○—○, acidic urine control (pH range 4·65–4·98); ●– – –●, alkaline urine control (pH range 7·45–8·05). Methylamphetamine: △—△, acidic urine control (pH range 4·59–4·82); ▲– – –▲, alkaline urine control (pH range 7·18–8·25)].

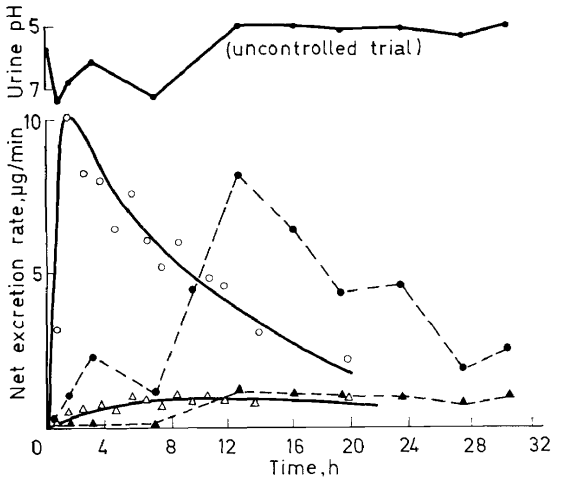


Figure 11. Urinary excretion of mephentermine and its metabolite phentermine after oral administration of 14·11 mg mephentermine sulphate with no urinary pH control (a) and acidic urine control (b) [Acidic urine control: ○—○, mephentermine; △—△ phentermine. No urinary pH control: ● - - - ●, mephentermine; ▲ - - - ▲ phentermine].

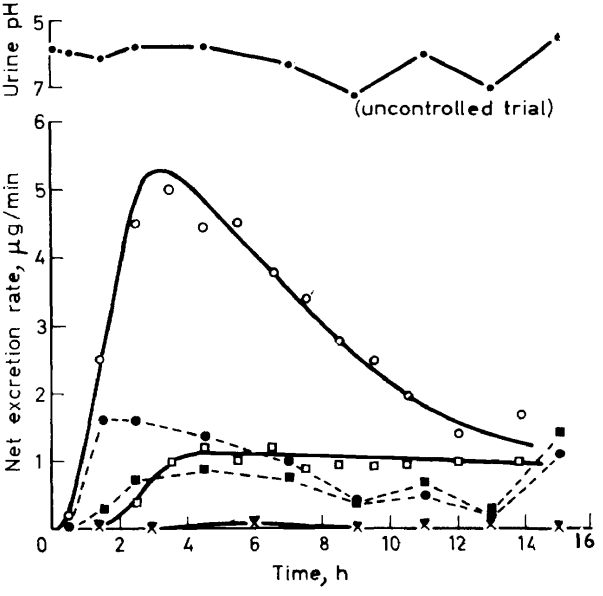


Figure 12. Urinary excretion of fenfluramine and its metabolite norfenfluramine after an oral dose of fenfluramine hydrochloride (20 mg) to a subject (L.B.) under (a) acidic urine control, (b) alkaline urine control and (c) pH of the urine not controlled [Acidic urine: ○—○, fenfluramine; □—□, norfenfluramine. Alkaline urine: x—x, fenfluramine; ▼—▼, norfenfluramine. Uncontrolled urine: ● - - - ●, fenfluramine; ■ - - - ■, norfenfluramine].

It is thus obvious that meaningful investigations of the importance of steric, stereochemical and physico-organic features in the metabolism of molecules *in vivo* and on biological half-lives and on enzyme induction, etc. in man and animals and biochemical differences between species, require the reabsorption of the unionized drug species in kidney tubules be minimised by suitable pH control (and by diuresis in some cases).

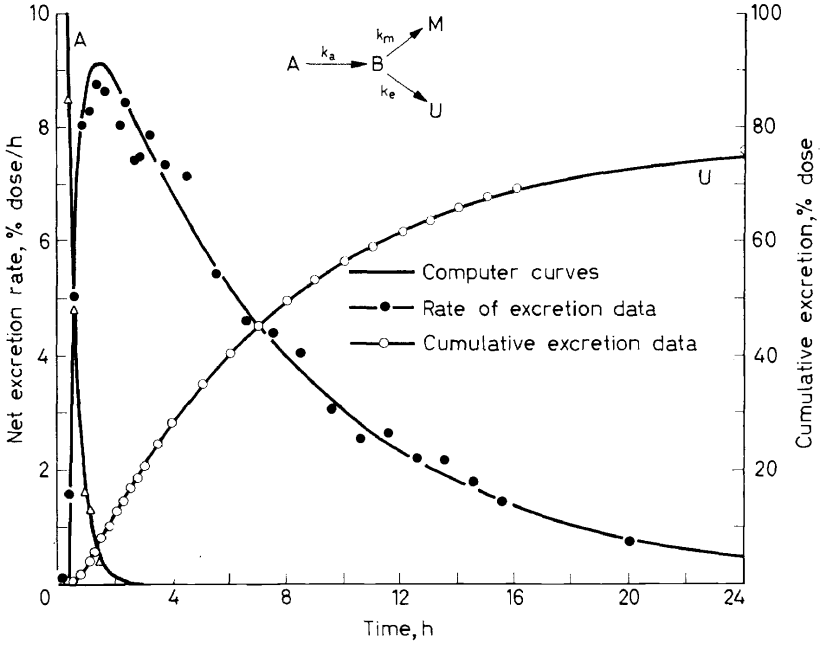


Figure 13. Agreement between computer calculated and experimental excretion data for amphetamine in man (constant acidic urinary pH).

Under these *controlled* conditions, it is not only possible to see the effects of changes in the molecules or the formulation of the drugs but to consider 'models' for drug absorption, metabolism and excretion in the body. Since intra-subject variations in results are thus very small, and inter-subject variations are also small unless genetically controlled metabolism or diseased states are involved[6], analogue computer techniques can be utilised[9], e.g. see *Figure 13*, for amphetamine for single doses, and for repeated doses see *Figure 14*. Thus the effect of changes in structure of the drug on the relative importance of various metabolic routes can be established. From computer calculations under conditions of acidic urine and then under normal conditions of varying urinary pH (*Figure 15*) it is possible to establish the relationship between pH and excretion (or reabsorption) of a particular drug[10] (see *Figure 16*) and then use an analogue computer to predict the excretion profile of a drug when only the time and pH of the urine are measured after the administration of the drug[11]; agreement of predicted values and experimental results are good (see *Figure 17*).

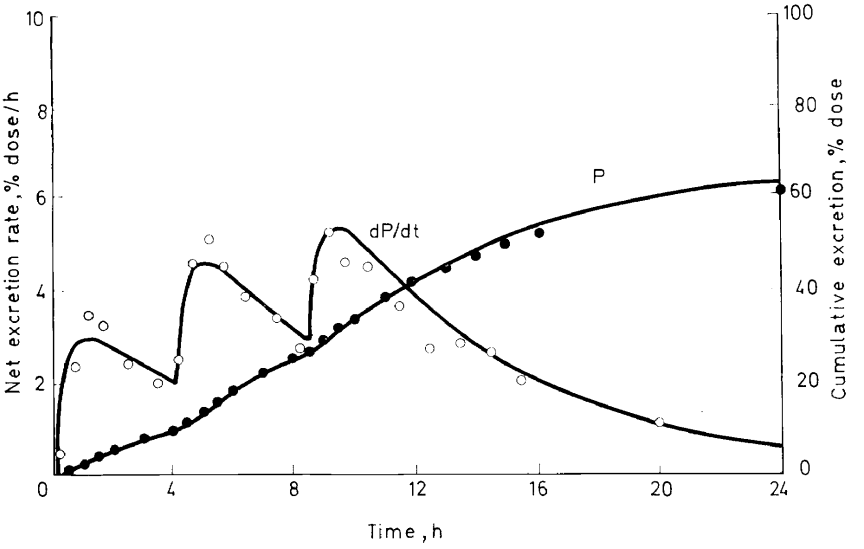


Figure 14. Computer curves and experimental data points for the urinary excretion of amphetamine, after oral administration, of divided doses of (+)-amphetamine sulphate in aqueous solution (subject, A.C.M.).

From the foregoing, it is obvious that the rate of partitioning of drugs across membranes at different pH values can have great effects on the extent of metabolism and the distribution and excretion of drugs, and the interpretation of observations on the biological effect of changes in drug molecules. The use of partition coefficient experiments using buffered solutions or the rate of passage of drugs through an organic phase between two

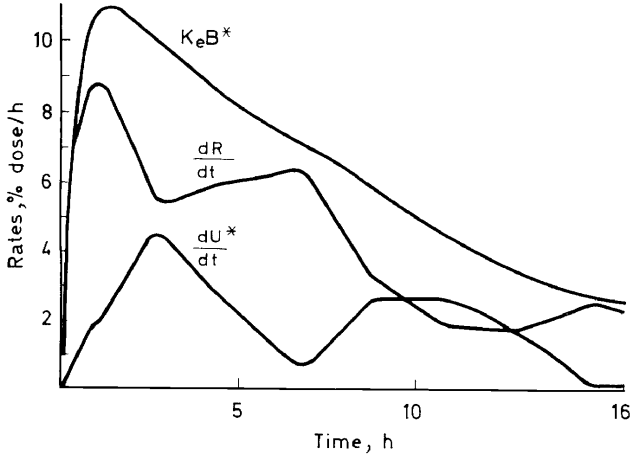


Figure 15. Computer calculations of the rates of amphetamine as functions of time under uncontrolled urinary pH conditions (subject 1) [presentation to the kidney, *keB**; kidney reabsorption, d*R*/d*t*; urinary excretion, d*U*/d*t*].

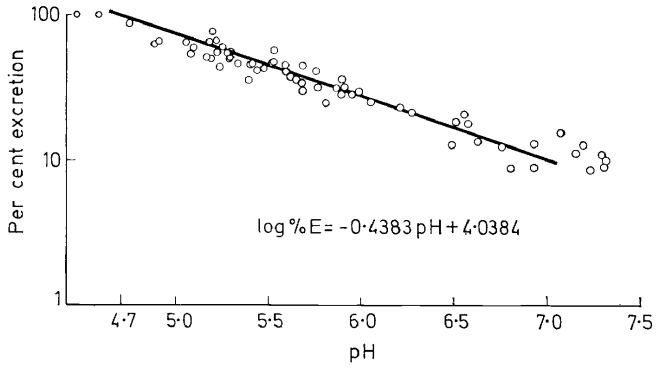


Figure 16. Relationship between log percentage urinary excretion of amphetamine and measured urinary pH (data from 4 trials in 2 subjects).

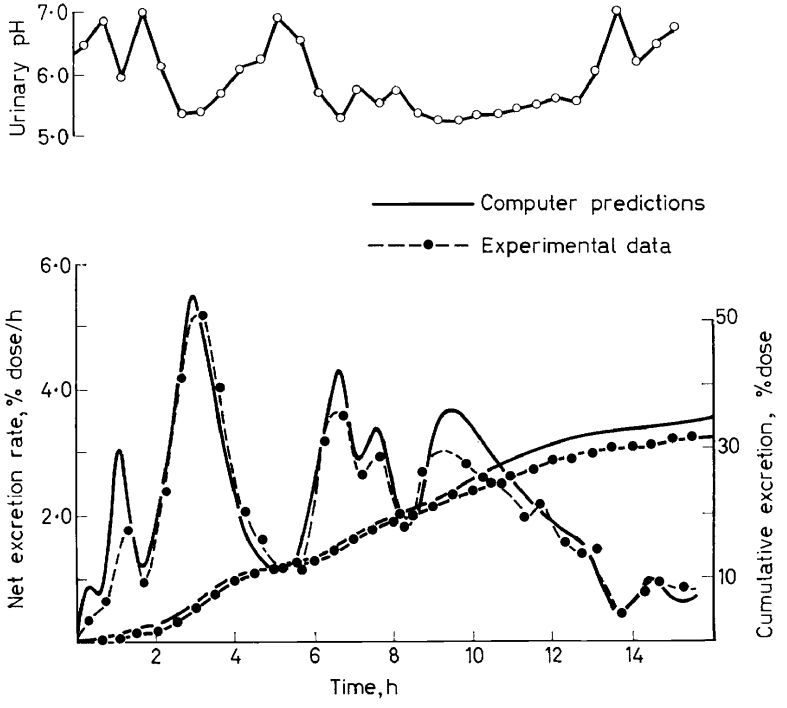


Figure 17. A comparison of computer predicted and actual rates of excretion and cumulative excretion of amphetamine after oral administration of 15 mg of D(+)-amphetamine sulphate in solution.

buffered solutions is not completely satisfactory as a basis of consideration of passage of drugs through biological membrane, since the relative order in which members of a series of drugs are placed frequently depends on the type of organic phase used. We therefore now use a human membrane for these studies—the buccal one—and have introduced the 'Buccal Absorption Test' as a means of classifying the relative partitioning of drugs from solutions of differing pH values. Basic drugs were classified into four main types on the result of this test[12] (see *Figure 18*). Amphetamine gave a different shaped

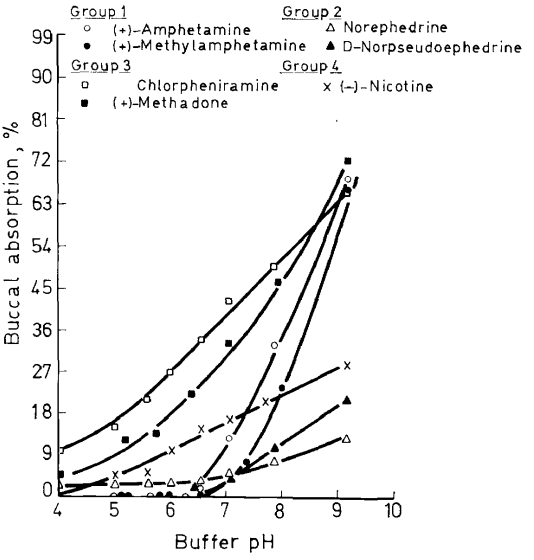


Figure 18. Buccal absorption of some basic drugs representing different classes in the Buccal absorption test.

curve from that of norephedrine, which is in accord with the pH dependence between pH 5 and 8 of the tubular reabsorption of the former but not of the latter (see *Table 5*); correlation between results in the buccal absorption test and kidney reabsorption of many basic drugs has been established. The relative order of partitioning of basic drugs is independent of the subject used and many drugs can be placed in the mouth without mutual interference with the results in the test. The effect on partitioning of the introduction of a lipophilic or hydrophilic group into a molecule can be readily seen, e.g. introduction of the trifluoromethyl group into ethylamphetamine to give fenfluramine and introduction of the alcoholic hydroxyl group into fenfluramine[8] (see *Figure 19*). Similarly, the effect of altering the chain length in *N*-substituted amphetamines can be seen readily[8] (see *Figure 20*).

This test can be applied similarly to acids—the effect on partitioning of the introduction of even one methylene group into a long chain acid is seen even when all the acids have identical pK_a values[13] (*Figure 21*). The effect of altering lipid solubility without altering pK_a values is also seen in a series of *p*-alkyl or halogen substituted phenylacetic acids prepared for anti-inflammatory testing (see *Figures 22* and *23*). The large difference between

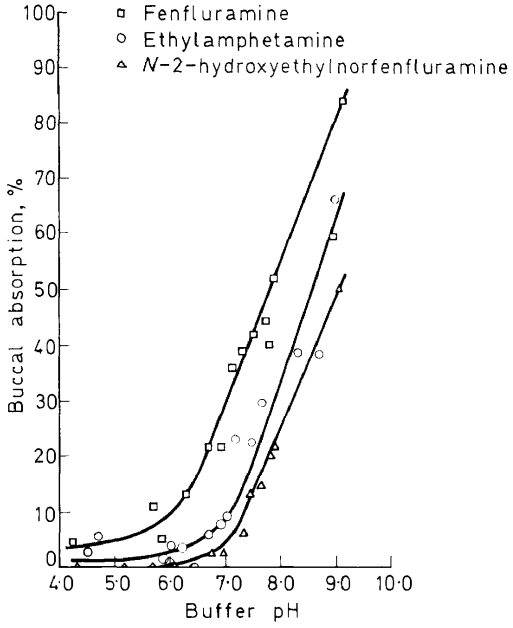


Figure 19. The buccal absorption of fenfluramine, ethylamphetamine and *N*-2-hydroxyethylnorfenfluramine within the pH range 4·0–9·05. Time period for absorption, 5 min. 1 mg (base) of each compound was used.

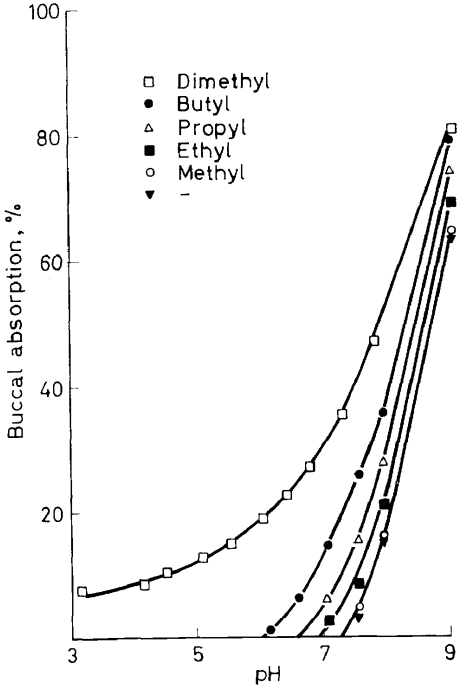


Figure 20. The buccal absorption of *N*-substituted amphetamines (subject A.C.M.).

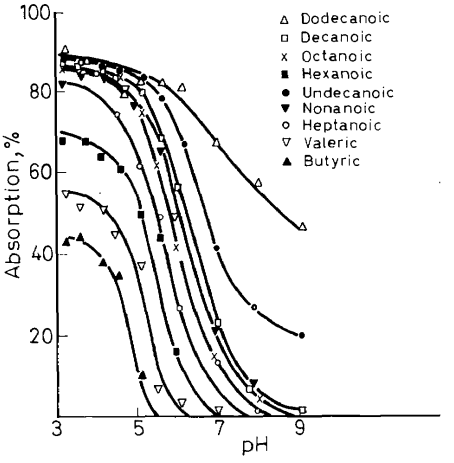


Figure 21. The buccal absorption of straight chain fatty acids (subject A.C.M.).

the *p*-tertiary butyl derivative 'Ibufenac' and phenylacetic acid (*Figure 24*) and the difference between substitution with an alkoxyl group rather than with the corresponding alkyl group (*Figure 25*) illustrates the importance of these changes on the ability of the compound to pass through biological membranes under various conditions[13].

The changes in the likely reabsorption characteristics of a metabolite

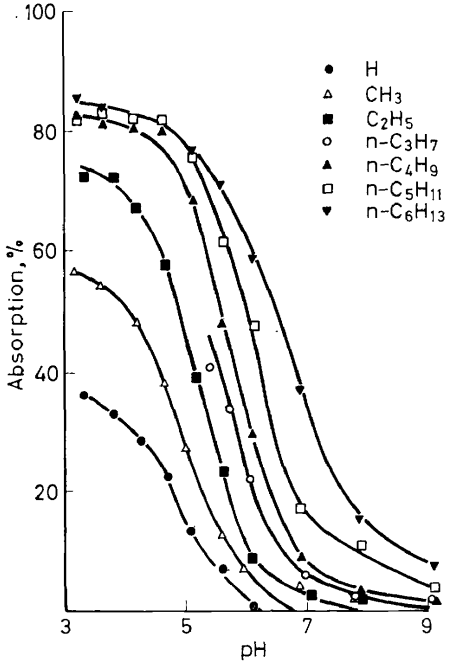


Figure 22. The buccal absorption of *p*-alkyl substituted phenylacetic acids (subject A.C.M.).

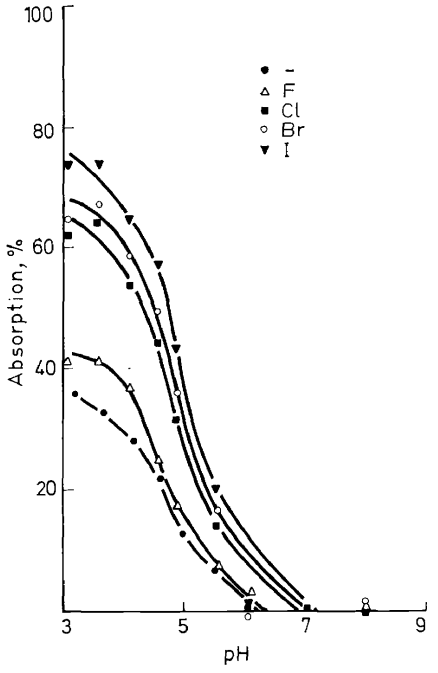


Figure 23. The buccal absorption of *p*-halogenated phenylacetic acids.

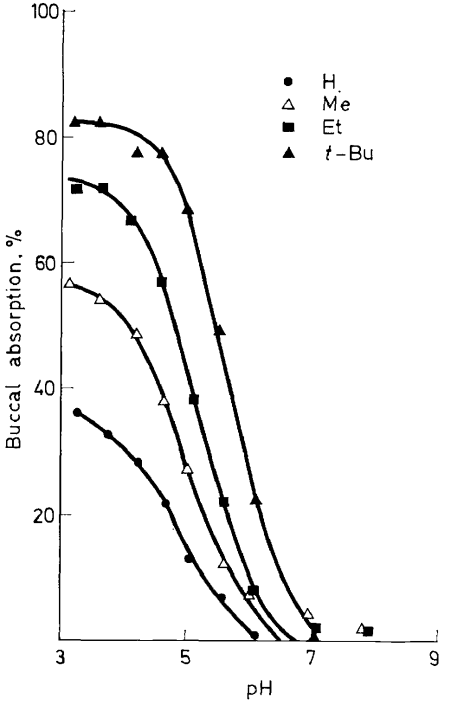


Figure 24. The buccal absorption of *p*-alkyl substituted phenylacetic acids (subject A.C.M.).

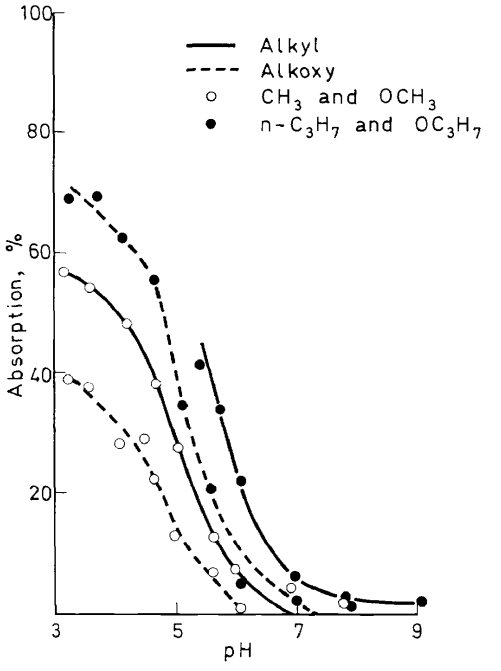


Figure 25. The buccal absorption of *p*-alkyl and *p*-alkoxy phenylacetic acids (subject A.C.M.),

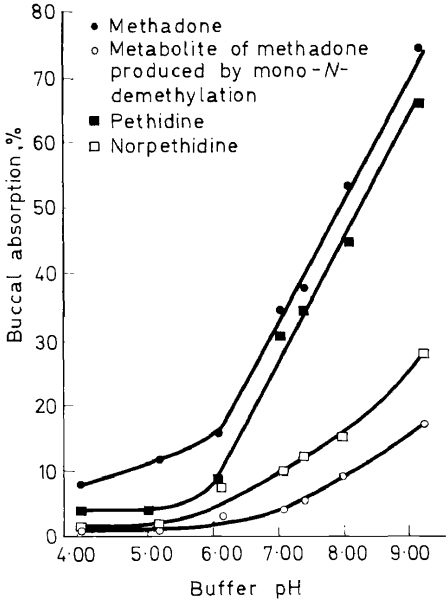


Figure 26. Buccal absorption of some basic compounds.

versus the parent drug in kidney tubules can be readily shown in the buccal absorption test (see *Figure 26*); i.e. the ratio of the metabolites, norpethidine and 'cyclic normethadone', to the unchanged drugs pethedine and methadone in the urine, will increase greatly as the pH of the urine is made less acidic[14], but this will not be the case for the metabolite, amphetamine, from methylamphetamine (see *Figure 16*).

The steric, stereochemical and physico-organic features of drugs can thus influence drug absorption, distribution, metabolism and excretion, and therefore drug action and the duration of action in a complex inter-related fashion. If we are to make further progress in structure–activity relationship of drugs in the whole animal, it is imperative that we attempt to disentangle the separate parts which contribute to the observed quantitative values of a biological response.

References

[1] A. H. Beckett. *Arzneimittel-Forsch.* **1,** 455 (1959), and refs. cited therein.
[2] A. H. Beckett and M. Rowland. *J. Pharm. Pharmac.* **17,** 628 (1965).
[3] A. H. Beckett. *N.Y. Acad. of Sci.* **144,** 675 (1967), and refs. cited therein.
[4] R. O. Davies *et al. Canad. J. Biochem. Physiol.* **38,** 545 (1960).
[5] A. H. Beckett and D. S. Hewick, to be published.
[6] A. H. Beckett and G. T. Tucker. *J. Mond. Pharm.* **3,** 181 (1967), and refs. cited therein.
[7] A. H. Beckett. *Dansk. Tiddskrift. Farm.* **40,** 197 (1966), and refs. cited therein.
[8] A. H. Beckett, to be published.
[9] A. H. Beckett and G. T. Tucker. *J. Pharm. Pharmac.* **20,** 174 (1968).
[10] A. H. Beckett, R. N. Boyes and G. T. Tucker. *J. Pharm. Pharmac.* **20,** 269 (1968).
[11] A. H. Beckett, R. N. Boyes and G. T. Tucker. *J. Pharm. Pharmac.* **20,** 277 (1968).
[12] A. H. Beckett and E. J. Triggs. *J. Pharm. Pharmac.* **19,** 31s (1967).
[13] A. H. Beckett and A. C. Moffat. *J. Pharm. Pharmac.* (1969), in the press.
[14] A. H. Beckett and J. F. Taylor, to be published.