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Buccal absorption and other properties of pharmacokinetic importance of imipramine and its metabolites

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Imipramine and its major unconjugated metabolites have been characterized by their buccal absorption, partition between water and organic solvents—both as a function of pH—and their ionization constants. Furthermore new data are presented on the selective passage of these compounds across the blood brain barrier and on renal excretion as a function of urinary pH in the rat. The shape of the curves of buccal absorption against pH is characteristic for each one of the ten compounds tested and reflects both lipophility of the unionized compound and the pK value. The value of the buccal absorption test for interpreting or predicting pharmacokinetic behaviour of a drug is emphasized.

Overwhelming evidence has been accumulated in past years that absorption, distribution, and renal excretion of most drugs are governed by simple diffusion through lipoid membranes (Brodie & Hogben, 1957; Schanker, 1961, 1962; Vogt, 1967; Parke, 1968). The pH-partition hypothesis for weak electrolyte drugs is based on the observation that only the un-ionized molecules can easily permeate membranes. Diffusion rate therefore is governed by two key properties: degree of ionization (which is dependent on pK of the drug and pH of the media separated by the membrane), and lipid solubility of the un-ionized form of the drug.

Traditionally the partition of a drug between an organic and aqueous phase has been used to obtain gross information on the lipid solubility of a drug at different pH values. Beckett & Triggs (1967) and Beckett, Boyes & Triggs (1968) have used buccal absorption in man to obtain the same information. This test seems to be superior to partition. Its chief advantage is the use of a biological membrane instead of an organic solvent. The buccal absorption test thus has provided us with a better means of characterizing drugs by their behaviour at the membranes of the gastrointestinal tract, renal tubules, blood brain barrier, tissue and cell boundaries.

The metabolism of a drug frequently leads to the formation of a multitude of metabolites. They occur simultaneously and are redistributed, localized and excreted according to their specific diffusion rates. In a recent study (Bickel & Weder, 1968) on the total fate of imipramine in the rat, the formation of 14 metabolites was detected. They cover a wide range of polarity which obviously governs the patterns of their individual distribution, localization and renal excretion.

The present paper is a comparative study of some properties of a family of derivatives occurring together in the body after administration of imipramine. Results are given on buccal absorption, on partition values as a function of pH, and on ionization constants. In addition data on the passage through the blood brain barrier and on the influence of urinary pH upon renal excretion are given. The data are discussed in terms of interpretation and prediction of pharmacokinetic processes.

EXPERIMENTAL

Materials

Imipramine (IP) and its following metabolites were used: Desipramine (DMI), desdimethylimipramine (DDMI), imipramine-*N*-oxide (IPNO), iminodibenzyl (IDB) and the hydroxylated metabolites 2-OH-IP, 2-OH-DMI, 10-OH-IP, 10-OH-DMI, 2-OH-IDB. With the exception of 2-OH-DMI-fumarate, all compounds used were the hydrochloride salts.

Buccal absorption test

General method. In analogy with the indications given by Beckett & Triggs (1967), 0·5 ml drug solution equivalent to 1 mg base was mixed with 24·5 ml buffer solution and agitated in the subject's mouth for 5 min. After expelling the solution and combining with 10 ml of rinsing water (10 s) the solution was adjusted to 50 ml (2-OH-IP, 2-OH-DMI), 100 ml (IDB, 10-OH-IP, 10-OH-DMI) or 200 ml (others) respectively. The following buffer solutions were used (Documenta Geigy, 1960): Sörensen's phosphate buffer pH 5·5, 6·5, 7·4, 8·2, citrate I pH 4·5, borax-phosphate pH 9·0. Three male and three female subjects, aged 17 to 40 were used.

Analytical techniques. 2-OH-IP, 2-OH-DMI: 10 ml aliquots were titrated to pH 10·0 and extracted with three 5 ml portions of peroxide-free diethyl ether. 5 ml of organic phase was extracted with 5 ml HCl (0·1 N). Extinction was measured at 249 nm.

All compounds except the non-fluorescent 2-OH-IP and 2-OH-DMI: 2 ml aliquots were mixed with 0·5 ml NaOH (2 N) (except for 2-OH-IDB). Fluorescence was measured directly with an Aminco-Bowman spectrophotofluorometer at 295/415 nm. With both methods linear standard curves were obtained. Only 2-OH-IDB was pH-dependent. Blank fluorescence due to saliva contamination was almost negligible. Specific fluorescence in relative units per 1 μg/ml was as follows: 2-OH-IDB (3·6–4·7, pH dependent), DMI (4·44), IP (4·14), DDMI (3·78), IPNO (3·72), IDB (2·31), 10-OH-IP (1·35), 10-OH-DMI (1·23). Buccal absorption of IP (pH 8·2) after 1, 2, 5, and 10 min was 46, 56, 64, and 67%. The absence of metabolic conversion of the drugs in the mouth has been proved for all substances used, by thin-layer chromatography according to Bickel & Weder (1968).

Partition experiments

Organic solvents and the following isotonic buffer solutions were used (Documenta Geigy, 1960): Sörensen's phosphate buffer (pH 5–7), borax-phosphate (pH 7–8), glycine II (pH 9–12·5). Previous to partition experiments the organic phases were presaturated by shaking with the same volume of the corresponding aqueous phase for 5–10 min. The substances were dissolved in 5 ml of aqueous phase (40 μg/ml) of a given pH and partitioned by shaking this solution with 5 ml of the organic solvent for 30 min at 20 $\pm$ 2°. Drug concentrations were determined by ultraviolet spectrophotometry of the aqueous phase before partition and of both phases after partition. The extinctions were read at 288 nm for IDB and 2-OH-IDB, and at 249–251 nm for all other compounds. The concentrations were evaluated with linear standard curves. Eight experiments were run per substance and pH value.

Ionization constants

Solutions of the substances (5×10^{-3} M) were prepared with double distilled water. 10 ml of the solutions were titrated with NaOH ($5 \cdot 10^{-2}$ M) in a Metrohm Combititrator 3 D, continuously recording the titration curve. 2–6 experiments were made per

substance and the pK_a values were evaluated from the curves. Controls with dibenzepine, amphetamine, ephedrine, and hydrazine yielded pK_a values in agreement with the literature.

Passage through the blood brain barrier

Male Wistar rats (220–260 g) and guinea-pigs were given high doses of IP or its metabolites by different routes of administration. At different times, brain and extracerebral tissues were homogenized, extracted and submitted to thin-layer chromatography as described by Bickel & Weder (1968). The exclusion from brain of certain metabolites was detected by comparing metabolite patterns of brain and of extracerebral compartments, such as tissues, plasma, excreta.

Renal excretion and urinary pH

Male Wistar rats (about 250 g) were given orally 2·5—5·0 m-equiv NH_4Cl, $NaHCO_3$, or 5 ml H_2O alone and simultaneously 50 mg/kg of IP intraperitoneally. The animals were placed in metabolic cages; urine was collected for 24 h and subjected to periodic pH measurements. Unconjugated and conjugated metabolites in the 24 h urine were determined by means of thin-layer chromatography (Bickel & Weder, 1968).

RESULTS

Buccal absorption

Buccal absorption measurements were made using IP and nine of its unconjugated metabolites. Each substance was tested at pH 5·5, 6·5, 7·4, 8·2 (IP also 4·5 and 9·0) by 3 (IP 4) subjects. The number of tests made per subject and pH value were: 4 (IP), 3 (DMI, DDMI, IPNO, IDB, 2-OH-IP, 2-OH-IDB), 2 (2-OH-DMI), 1 (10-OH-IP, 10-OH-DMI). The mean values of all measurements for a substance for a given pH were calculated.

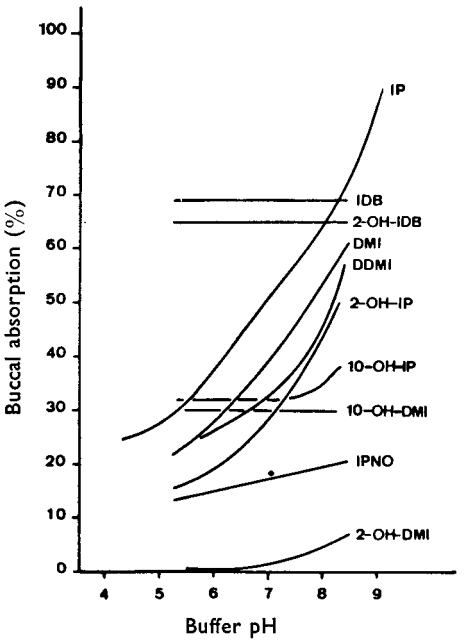

FIG. 1. Buccal absorption of IP and IP metabolites as a function of pH.

The resulting curves of buccal absorption as a function of pH are summarized in Fig. 1. The variations were carefully checked; for IP the mean standard deviation of the inter-subject variation was 8·8% in terms of buccal absorption, and the intra-subject variation was 5·4%. Similar variations were observed for IDB, 2-OH-IDB and 2-OH-IP, whereas the other tested substances showed less variations. Thus the curves are clearly distinct from one another with the reservation of a possible overlap of the pairs IDB and 2-OH-IDB, 10-OH-IP and 10-OH-DMI, and DDMI and 2-OH-IP. Table 1 contains the mean buccal absorption values at pH 7·4 and their total standard deviations.

Table 1. *Partition values of imipramine and imipramine metabolites.* % *in organic phase after equilibration with isotonic phosphate buffer pH* 7·4

Metabolite	Chloroform	n-Hexane	Diethyl-ether	1,2-Dichloro-ethane	Buccal† absorption
IDB	99·6 ± 1·2	99·2 ± 1·2	93·6 ± 1·2		69 ± 13·1
2-OH-IDB ..	98·0 ± 1·2	44·8 ± 2·0	97·5 ± 1·1		67 ± 7·0
IP	99·8 ± 0·8	99·4 ± 1·4	99·3 ± 1·2	98·5 ± 1·4	57 ± 11·1
DMI	98·5 ± 1·6	65·1 ± 2·1	88·4 ± 1·5	97·8 ± 1·8	45 ± 6·7
DDMI	99·1 ± 1·8	70·5 ± 2·3			36 ± 8·0
2-OH-IP	97·4 ± 1·5	25·2 ± 2·0	84·2 ± 1·7	71·0 ± 1·9	34 ± 13·3
10-OH-IP ..	92·5 ± 2·4				32 ± 7·0
10-OH-DMI ..	77·0 ± 2·8				30 ± 2·9
IPNO	97·2 ± 1·5	10·0 ± 2·2	7·0 ± 2·5	71·0 ± 1·8	18 ± 4·5
2-OH-DMI ..	55·2 ± 2·1	6·3 ± 2·0	55·6 ± 2·6	50·2 ± 3·1	2 ± 2·9

* 8 experiments.

† intra- and inter-subject s.d.

Partition experiments

The partitions of IP and nine unconjugated metabolites between phosphate buffer pH 7·4 and several organic solvents have been measured. The results are summarized in Table 1. Mean standard deviation of the values is ± 1·8%. To compare the partition properties of the solvents with biological partion, this Table also contains the buccal absorption values for pH 7·4. Partition values of the above substances were measured with organic solvents against aqueous buffers at four to seven different pH values in the range of pH 5·0–12·5. The partition-pH plots are given in Figs 2 and 3.

Ionization constants

Since the published pK_a values of IP and DMI show wide variation, we made our own measurements. pK_a values were measured with IP and five of its major unconjugated metabolites. The results are summarized in Table 2 which also includes values

Table 2. *pK_a values of imipramine and its metabolites*

	(1)	(2)	(3)	(4)
IP	8·0	7·94	8·6	9·5
DMI	9·4	9·22		10·2
DDMI	9·4	9·32		
IPNO	4·7			
2-OH-IP	8·0	7·93		
2-OH-DMI	9·3	9·10		

(1) This study.

(2) Geigy Inc., Basel, (methyl cellosolve), (unpublished).

(3) Haefliger (1959).

(4) Green (1967).

obtained by other authors. The dissociation of the cyclic amine group is too weak to be measured (IDB). The dissociation of the phenolic groups begins several pH units above the pK_a of the side-chain amine group (cf. Fig. 3).

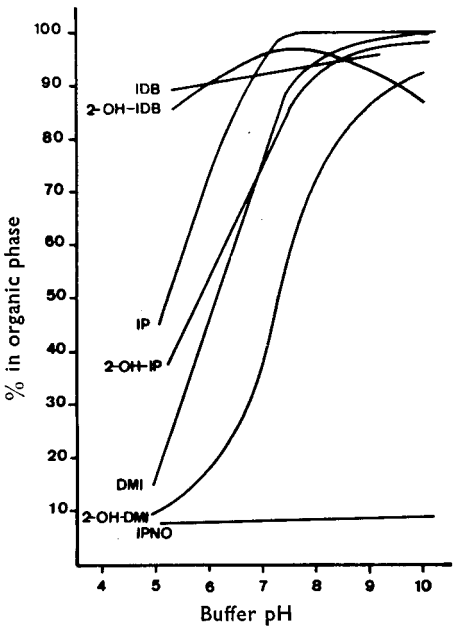

FIG. 2. Partition of IP and IP metabolites between aqueous buffer solutions and diethyl ether.

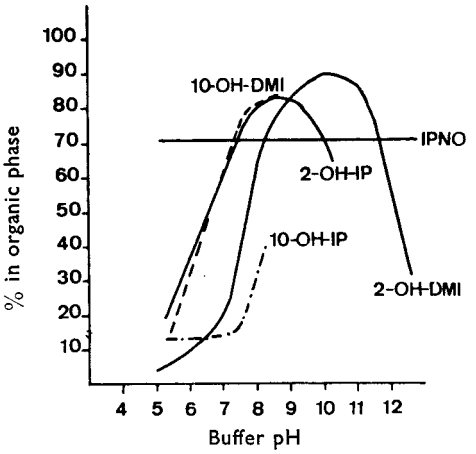

FIG. 3. Partition of IP metabolites between aqueous buffer solutions and 1,2-dichloroethane (———), chloroform (- - - - -), and n-hexane (-·-·-).

Passage through the blood brain barrier

Metabolite patterns in brain and extracerebral compartments were compared after administration of IP and its metabolites at different dosage and time schedules . The results are summarized in Table 3. Some experiments were aimed specifically at the study of blood brain barrier passage whilst others were part of pharmacokinetic studies (Bickel, Weder & Baggiolini, 1966; Bickel & Weder, 1968). One to 28 animals

were used for each type of experiment in Table 3. In most cases hydroxylated and conjugated metabolites could be detected in extracerebral compartments (plasma, excreta, liver, other organs) but not in brain. Glucuronides and minor metabolites (DDMI, 2-OH-IDB, 10-OH-metabolites) were not administered.

Table 3. *Appearance of imipramine and its metabolites in brain after administration to rats*

Species	Drug	Dose (mg/kg)	Route	Time	Detected in brain*	
					IP	DMI
Rat	IP	10	i.v.	5 min	+	—
	IP	50	s.c.	1; 6 h	+	+
	IP	50	i.m.	6 h	+	+
	IP	50	oral	1; 6 h	—	+
	IP	50	i.p.	15–120 min	+	+
	IP	chron.	i.p.	30 min	+	+
	IP	chron.	i.p.	6 h	—	+
	IDB†	50	i.p.	15 min	—	—
	DMI	50	i.p.	15 min	—	+
	IPNO	50	i.p.	1 h	—	+
	IPNO	50	i.p.	2 h	—	+
	IPNO	15	i.v.	5 min	—	—
	2-OH-IP‡	20	i.v.	5 min	—	—
	2-OH-DMI	40	i.p.	15 min	—	—
Guinea-pig	IP	50	i.p.	1 h	+	+
	DMI	50	i.p.	1 h	—	+
	IPNO	50	i.p.	1 h	+	—
	2-OH-DMI	50	oral	1 h	—	—

* Hydroxylated metabolites present in extracerebral compartments

† IDB detected only.

‡ 2-OH-IP detected only.

No IPNO or 2-OH-DMI were detected after imipramine and the other metabolites.

Renal excretion and urinary pH

IP and its metabolites were determined in 24 h urine specimens from normal, aciduric and alkaluric rats dosed with IP. The molecular species determined were summarized as three groups: low, medium, and high polarity metabolites. The results of these experiments are listed in Table 4.

Table 4. *Influence of urinary pH on the excretion of metabolites after administration of imipramine to rats* (50 mg/kg i.p.)*

Pretreatment	ml urine mean	pH ± s.d.	Metabolites		
			Low polarity (1)	medium polarity (2)	high polarity (3)
(a) 24 h excretion (% of dose):					
NH_4Cl	9·7	5·8 ± 0·6	1·7 — 2·6	0·8 — 1·5	8·5 — 34·5
H_2O	9·0	6·3 ± 0·3	0·3 — 1·5	0·1 — 4·7	8·3 — 25·3
$NaHCO_3$ ..	9·5	8·5 ± 0·4	0·03 — 0·2	0·2 — 1·1	7·6 — 13·2
(b) Metabolite patterns (%, mean):					
NH_4Cl			17·7	10·0	72·3
H_2O			11·2	31·3	57·5
$NaHCO_3$ ..			1·0	5·1	93·9

* 3 rats per group.

(1) IP, DMI. (2) IPNO, 2-OH-IP, 2-OH-DMI. (3) Glucuronides.

DISCUSSION

Our testing of the buccal absorption test fully confirms the findings of Beckett & Triggs (1967) and Beckett & others (1968a) about reliability, inter- and intra-patient variations, kinetics and other parameters. All buccal absorption-pH curves of IP and its metabolites depicted in Fig. 1 have a distinct and characteristic position. Buccal absorption of IP, DMI, DDMI, and 2-OH-IP strongly increases in the tested pH range 5–9. A second group containing IDB, 2-OH-IDB, 10-OH-IP, 10-OH-DMI, IPNO and 2-OH-DMI shows little or no pH dependence of buccal absorption. The members of the first group are medium strength bases with pK_a values between 7 and 10 (Table 2) and thus are partly ionized at the tested pH range. Therefore with increasing pH there is a decrease in ionization and an increase in diffusion through the (buccal) membrane. On the other hand the members of the second group, IDB, 2-OH-IDB, and IPNO are weak bases with pK_a values far below neutral (IPNO = 4·7). The basicity is decreased by the partial sharing of the terminal-N free electron pair by oxygen in the case of IPNO and of the cyclic-N free electron pair by resonance mesomers in the case of IDB. These substances are practically un-ionized over the tested pH range, and their lipid solubilities and diffusion rates are therefore not pH dependent. With 2-OH-DMI it must be assumed that even the un-ionized base (pK_a 9·3, equalling DMI) is very hydrophilic because of the amino- and phenolic-groups and therefore does not easily penetrate the buccal membranes. The lipid solubility of all compounds at a given pH value is reflected by the (vertical) order in the pH buccal absorption plot. For reasons discussed below it must be inferred that the 10-OH metabolites are relatively hydrophilic medium strength bases.

The partition of a substance between a polar and a non-polar solvent is dependent on the lipid solubility of the substance, the polarity (dielectric constant) of the organic solvent, the pH of the polar solvent and the temperature. Furthermore the solubility of the substance in the non-polar solvent can limit partition. The partition values for a constant pH of 7·4 (Table 1) are in accordance with these considerations. The differences of partition values of one substance in the four solvents must be ascribed to differences of dielectric constants (chloroform 5·2, n-hexane 1·9) which also determine the discrimination of the substances tested. Except for a few inversions, the (vertical) order of lipid solubility of the substances is similar with all solvents. The substances can be divided into a group of low polarity metabolites (IDB, 2-OH-IDB, IP, DMI, DDMI) and a group of medium polarity metabolites (phenolic, alcoholic and *N*-oxide metabolites), although there seems to be no absolute division between the members at the boundary. The conjugates, not included in this study, could be labelled as high polarity metabolites. Preliminary partition experiments with the glucuronides of 2-OH-IP and 2-OH-DMI did not show detectable amounts in the chloroform phase. Inversed phase paper chromatography (Herrmann, 1963) leads to an Rf sequence of unconjugated IP metabolites comparable to the lipid solubility order in Table 1.

The pH dependence of the partition values (Figs 2 and 3) are obvious with IP, DMI, 2-OH-IP, and 2-OH-DMI (pK_a 8·0–9·4). 10-OH-IP and 10-OH-DMI are also pH-dependent suggesting that their pK_a values are in a similar range. Partition values of the 2-OH-metabolites decrease again in the strongly alkaline range due to dissociation of the phenolic group. The slope of the pH-partition curve is comparable for all these metabolites. On the other hand the metabolites with pK_a values far below neutral show practically identical partition values over the tested pH range 5–12·5 (IDB, 2-OH-IDB, IPNO).

It is of interest to compare the partition values with the buccal absorption data. At pH 7·4 the gross order of buccal absorption corresponds to the order of lipid solubility obtained with all solvents. Again, the same groups of low polarity and medium polarity metabolites can be distinguished with the reservation of a possible overlap of the members at the boundary. Comparison of discrimination by the solvents shows that the highly discriminating and most lipophilic of these, n-hexane, displays partition properties closer to buccal absorption than do the less lipophilic ether, dichloroethane, and chloroform. However, the search for a solvent that truly imitates (buccal) absorption remains as an important goal of further research. Both partition in different solvents and buccal absorption distinguish identical groups of pH-dependent and pH-independent metabolites. The relative similarity of n-hexane and the buccal membrane is also reflected in the pH dependence of 10-OH-IP (Figs 1 and 3). The poor buccal absorption of 2-OH-DMI—sharing the same pK_a value with DMI—can be explained by the fact that the highly lipophilic membrane strongly discriminates against this metabolite unless it is highly un-ionized which would only occur at unphysiological pH values (compare partition behaviour of 2-OH-DMI and the 10-OH-metabolites in Fig. 3).

For the purpose of predicting the behaviour of drugs at biological membranes the buccal absorption test is no doubt superior to the partition test using organic solvents. However, pH-partition values will keep their importance as a basis for selective extraction procedures. In the case of IP metabolites an example of this has been described by Weder & Bickel (1968).

The experiments aimed at a detection of selective passage through the blood brain barrier demonstrate a physiological application of the above findings. The results summarized in Table 3 show that only IP, DMI, and IDB easily cross from plasma into brain. Crossing of the more polar 2-OH-IP was detected only once under extreme conditions. 2-OH-DMI and IPNO did not cross the blood brain barrier, and other phenolic, alcoholic, and conjugated metabolites were formed from precursors and detected in extracerebral but not cerebral tissue (see also Bickel & Weder, 1968). Further information supporting these results is given by Quinn, Marano & Greengard (1964) who found 2-OH-IP in the brain but no 2-OH-DMI, the major plasma metabolite. In a human suicide case with IP, Faragó (1965) found no phenolic metabolites except 2-OH-IDB, and in a similar DMI case (Bickel, Brochon & others, 1967) the major metabolite, 2-OH-DMI, was present in body fluids and tissues except brain. The only indication of 2-OH-DMI in brain was reported after an extreme overdosage of IP (4500 mg) (Herrmann, 1963). Despite the crude experimental approach which does not take into account the complex interrelation between blood, brain and cerebrospinal fluid (Davson & Oldendorf, 1967) the results are in full agreement with the general observations that the blood brain barrier behaves like a pure lipid membrane (Vogt, 1967). The fact that several medium polarity metabolites, which do not enter the brain, can appear in liver and other cellular tissues (Bickel & Weder, 1968) agrees with the suggestion of a greater discrimination in favour of more lipid soluble substances by the blood brain barrier than by the (liver) cell barrier (Kurz, 1964). All imipramine metabolites which easily cross the blood brain barrier belong to the low polarity group emerging from buccal absorption and partition experiments at pH 7·4.

The studies of urinary excretion of IP and its metabolites are another physiological application of buccal absorption and partition studies. In urine of normal IP-treated rats the ratio low : medium : high polarity metabolites is 1 : 3 : 6, and the corresponding

ratio in plasma is 5 : 1 : 4 (Bickel & Weder, 1968). The reason for the small amounts of low polarity metabolites (IP, DMI) in urine is found in their high degree of tubular reabsorption by non-ionic diffusion. Due to the pK_a values of these substances, pH changes of the tubular fluid markedly influence the degree of reabsorption and hence of excretion, which significantly decreases in the order of urinary pH 5·8, 6·3, 8·5. On the other hand, the excretion of phenolic and conjugated metabolites does not significantly change as a function of pH. Since the low polarity metabolites are only a small fraction of the urinary metabolites, total excretion is not significantly influenced by urinary pH (Table 4). Unaffected total drug excretion of IP-treated alkalinuric rats was also reported by Crammer, Scott & others (1968). Urinary IP and DMI determinations in patients (Demiaux, Motin & others, 1966; Gaultier, Fournier & others, 1967) point in the same direction and explain the failure of drug-induced aciduria, dialysis, or forced diuresis in the treatment of IP poisoning.

Again the behaviour of IP metabolites at the tubular membrane is in agreement with the characterization of these substances by the buccal absorption test. Relations between buccal absorption and pH-dependent tubular reabsorption have been extensively studied by Beckett & others (1968a, b) using amphetamine.

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REFERENCES

BECKETT, A. H. & TRIGGS, E. J. (1967). *J. Pharm. Pharmac.*, **19**, *Suppl.*, 31–41.

BECKETT, A. H., BOYES, R. N. & TRIGGS, E. J. (1968a). *Ibid.*, **20**, 92–97.

BECKETT, A. H., BOYES, R. N. & TUCKER, G. T. (1968b). *Ibid.*, **20**, 269–276.

BRODIE, B. B. & HOGBEN, C. A. M. (1957). *Ibid.*, **9**, 345–380.

BICKEL, M. H., WEDER, H. J. & BAGGIOLINI, M. (1966). *Helv. physiol. Acta*, C 77–78.

BICKEL, M. H., BROCHON, R., FRIOLET, B., HERRMANN, B. & STOFER, A. R. (1967). *Psychopharmacologia*, **10**, 431–436.

BICKEL, M. H. & WEDER, H. J. (1968). *Archs int. Pharmacodyn. Thér.*, **173**, 433–463.

CRAMMER, J. L., SCOTT, B., WOODS, H. & ROLFE, B. (1968). *Psychopharmacologia*, **12**, 263–277.

DAVSON, H. & OLDENDORF, W. H. (1967). *Proc. Roy. Soc. Med.*, **60**, 326–329.

DEMIAUX, J. P., MOTIN, J., ROCHE, L. & BADINAND, A. (1966). *C.R. Séanc. Soc. Biol.*, **160**, 1470.

Documenta Geigy, Wissenschaftliche Tabellen, 6th edition. (1960).

FARAGÓ, A. (1965). *Arch. f. Toxikol.*, **21**, 30–37.

GAULTIER, M., FOURNIER, E., BOURDON, R., NICAISE, A. M., FREJAVILLE, J. P. & PEBAY-PEYROULE, F. (1967). 2 ième Congr. Europ. Centres Lutte contre Poisons, 25–34. Paris: Masson.

GREEN, A. L. (1967). *J. Pharm. Pharmac.*, **19**, 10–16.

HAEFLIGER, F. (1959). *Canad. Psychiat. Ass. J.*, **4**, S 69.

HERRMANN, B. (1963). *Helv. physiol. Acta*, **21**, 402–408.

KURZ, H. (1964). *Arch. exp. Path. Pharmak.*, **247**, 164–179.

PARKE, D. V. (1968). *The Biochemistry of Foreign Compounds*. London: Pergamon.

QUINN, G. P., MARANO, B. J. & GREENGARD, P. (1964). *Pharmacologist*, **6**, 197.

SCHANKER, L. S. (1961). *Ann. Rev. Pharmac.*, **1**, 29–44.

SCHANKER, L. S. (1962). *Pharmac. Rev.*, **14**, 501–530.

VOGT, W. (1967). In *Symposium on Biophysics and Physiology of Biological Transports*, 120–121, Berlin: Springer.

WEDER, H. J. & BICKEL, M. H. (1968). *J. Chromatog.*, **37**, 181–189.