J. Pharm. Pharmac., 1971, **23**, *Suppl.*, 73S–76S *Received February* 26, 1971

Buccal absorption as a parameter of analgesic activity of some *p*-substituted acetanilides

J. C. DEARDEN AND ERIC TOMLINSON

School of Pharmacy, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF, U.K.

The extent of buccal absorption of 16 *p*-substituted acetanilides is related parabolically to analgesic activity. The correlation is slightly better than that between log (partition coefficient) and analgesic activity. Thus, for this series of compounds at least, an *in vivo* partition test provides a slightly better parameter of biological response than does an *in vitro* test.

The effectiveness of sublingual administration of a drug was implied by Walton (1944) to be a function of the ability of the drug to penetrate the buccal mucosa. Recently, following the work of Beckett & Triggs (1967) in devising a quantitative assessment of buccal absorption, biological response has been discussed in this context (Flanagan, Broad & others, 1969; Tucker, Boyes & others, 1970). However, so far as we are aware, no attempt has been made directly to correlate a biological response with buccal absorption. The present study is concerned with such a relation, for a series of *p*-substituted acetanilides.

EXPERIMENTAL AND RESULTS

Buccal absorption and protein binding

Buccal absorption was determined as described by Dearden & Tomlinson (1971a), and protein-binding as described by Dearden & Tomlinson (1970).

Analgesic activity

Analgesic activities were measured by the abdominal constriction response method, using groups of 10 female albino mice (Tuck strain T.S.1), each of ~18 g, for each of four dose levels and for controls. Drugs were administered orally in 0·2% gum tragacanth suspension, and the challenge was by intraperitoneal acetylcholine. None of the drugs used showed any anti-acetylcholine activity, as indicated by a physostigmine lethality test (Collier, Dinneen & others, 1968). One compound, *N*-methylacetanilide, gave rise to some motor disco-ordination, and was therefore not included in regression analyses.

Partition coefficient (P)

The determination of partition coefficient was made using 1-octanol and Clark and Lubs 0·2M phosphate buffer, pH 7·2, as the solvent pair. Results of the partition measurements are expressed as Hansch's hydrophobic substituent constant, π (Fujita, Iwasa & Hansch, 1964).

Table 1 lists the results obtained for acetanilide and 15 *p*-substituted acetanilides. Buccal absorption values are uncorrected for dilution by saliva (Dearden & Tomlinson, 1971b), since the correction is very small over the duration of a normal 5 min test (e.g. 3% for *p*-chloroacetanilide).

Table 1. *The buccal absorptions, analgesic activities, π values and protein-binding constants of a series of* p-*substituted acetanilides.*

p-Substituent	% Buccal absorption in 5 min	ED50 (mmol kg^{-1}) (with 95% confidence limits)	π	ΔG of protein-binding at 19°* (kJ mol^{-1})
-H	26·9	0·390 (0·364–0·417)	0	−24·2
-Me	34·3	0·318 (0·273–0·369)	+0·239	−25·0
-OH	14·6	0·608 (0·578–0·640)	−0·360	−23·6
-OMe	23·3	0·404 (0·383–0·427)	−0·133	−23·5
-OEt	25·2	0·450 (0·425–0·477)	−0·098	−24·0
$-NH_2$	3·6	2·054 (1·912–2·205)	−1·076	−21·5
-F	33·6	0·234 (0·219–0·248)	+0·309	−25·0
-Cl	44·2	0·462 (0·430–0·496)	+0·714	−26·8
-Br	52·5	0·975 (0·929–1·024)	+1·130	−28·1
-I	57·5	1·392 (1·194–1·498)	+1·303	−28·8
-COOH	34·9	2·600 (2·194–3·081)	−0·605	−23·1
-CHO	7·6	0·457 (0·412–0·507)	+0·091	−24·7
$-NO_2$	42·4	0·195 (0·184–0·208)	+0·499	−26·2
>N-Me,-H	20·9	0·319 (0·300–0·339)	−0·187	−23·9
>N-Me,-OH	16·9	1·479 (1·296–1·687)	−0·549	−23·5
>N-Me,-OMe	19·4	0·977 (0·820–1·163)	−0·436	−23·6

* ΔG = Gibbs free energy of binding.

DISCUSSION

The work of Beckett and co-workers (e.g. Beckett & Moffat, 1969) and the work reported here shows that there exists a good rectilinear correlation of buccal absorption and log P or Hansch's hydrophobic substituent constant π (see Fig. 1), even when the compounds concerned cover a wide range of partition coefficients. Thus buccal absorption gives a measure of partition from the oral cavity into and on to the buccal membrane. We emphasize this point because the term "absorption" is usually taken to mean "passage through a membrane" (Wagner, 1968).

However, the good correlation of buccal absorption and π does not necessarily imply that passive partition between aqueous and lipid phases is the only factor governing buccal absorption. Dearden & Tomlinson (1971a) have shown that protein-binding probably plays a significant part in buccal absorption. This does not greatly perturb the relation between buccal absorption and π, however, since very good correlation also holds between π and log k, where k is the equilibrium constant of binding to bovine serum albumin ($F_{1,14} = 452{\cdot}8$; $F_{1,14}\ \alpha,\ 0{\cdot}001 = 17{\cdot}14$). Similar correlations have been observed for other series of drugs (Penniston, Beckett & others, 1969), and indeed are to be expected, since non-specific protein-binding is essentially a partition between a hydrophobic surface and an aqueous solution. It follows that there should also be a rectilinear correlation of buccal absorption and log k, and this is indeed observed for the present results ($F_{1,14} = 215{\cdot}5$; $F_{1,14}\ \alpha$, $0{\cdot}001 = 17{\cdot}14$).

It has recently been demonstrated (Penniston & others, 1969) that the penetration of a drug *through* a series of lipid membranes is a parabolic function of log P. Thus compounds with very low partition coefficients are too hydrophilic to enter the lipid

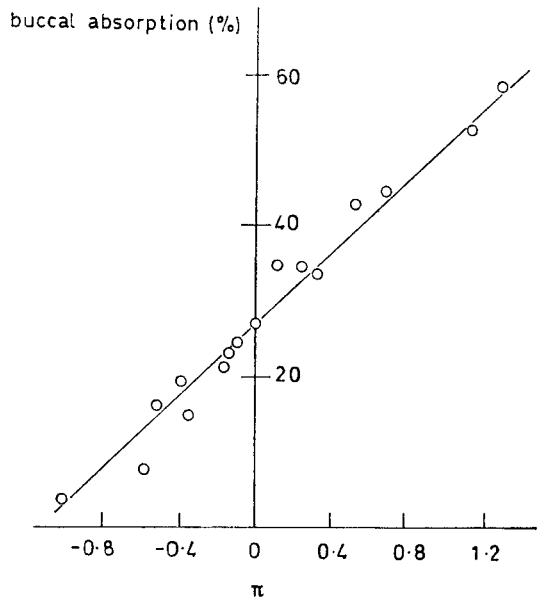

FIG. 1. Correlation of buccal absorption of *p*-substituted acetanilides with Hansch's hydrophobic substituent constant, π. The regression equation is: % absorption in 5 min = 23·73 (1·154) π + 27·37 (0·713). Figures in brackets are standard errors of the regression coefficients. $F_{1,14}$ = 427·6; $F_{1,14}$ α, 0·001 = 17·14.

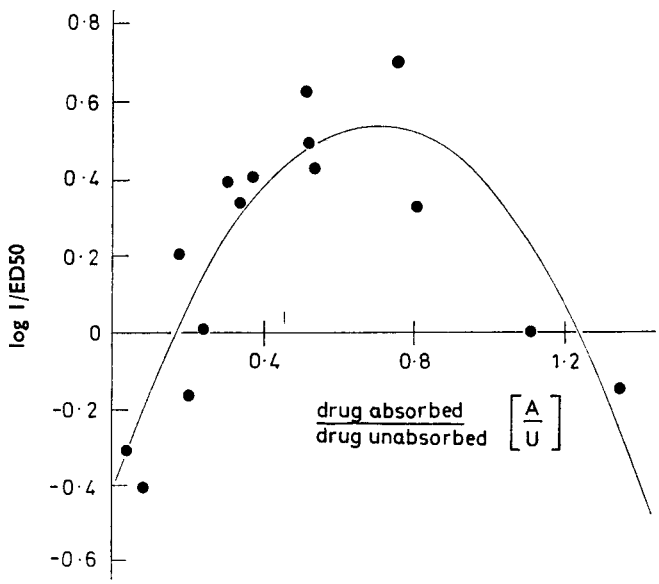

FIG. 2. Correlation of analgesic activtiy of *p*-substituted acetanilides with their buccal absorption. The regression equation is: log (1/ED50) = − 1·989 (0·318) $(A/U)^2$ + 2·800 (0·440) A/U − 0·439 (0·116), where A/U is the ratio of drug absorbed to drug unabsorbed in 5 min. Figures in brackets are standard errors of the regression coefficients. $F_{2,12}$ = 4·86; $F_{2,12}$ α, 0·05 = 3·89.

phase readily, those with very high lipid solubility tend to remain in the membrane rather than to pass through it.

We have found (unpublished observations) that, for the acetanilides studied here, a parabolic relation exists between analgesic activity and π; this is to be expected, since the biological activity of a compound must be a function of its ability to penetrate lipid membranes. Similar parabolic relations have been shown to exist with many different biological responses and types of compound (Hansch, 1969).

From the above correlations of buccal absorption and π, and between π and analgesic activity, it follows that there should be a parabolic relation between buccal absorption and analgesic activity. Fig. 2 shows that this is indeed the case. The correlation is slightly better than that between π and analgesic activity (for which $F_{2,12} = 3{\cdot}97$), and is similarly significant at the 95% level. It is perhaps surprising that an *in vivo* test does not correlate much better with biological activity than does an *in vitro* test. There are a number of possible reasons for this. Firstly, the reproducibility of buccal absorption measurements (mean % standard deviation = 3·7%) is not as good as that of the partition measurements (mean % standard deviation = 2·0%). Secondly, the enzymes present in saliva may cause degradation of a drug during its time in the mouth. Thirdly, after buccal absorption, the mouth is rinsed out to remove any drug solution still remaining; this could also remove some protein-bound drug, although in the present work the rinse-time was kept as short as possible (10 s) to minimize this. Finally, absorption can occur not only into the buccal mucosa, but also into the tongue, which has different, and probably specific, binding characteristics (Dastoli, Lopiekes & Price, 1968) and may thus absorb some members of a series of drugs more readily than others.

Acknowledgements

One of us (E.T.) gratefully acknowledges the receipt of a research assistantship from Liverpool Corporation.

REFERENCES

BECKETT, A. H. & MOFFAT, A. C. (1969). *J. Pharm. Pharmac.*, **21**, *Suppl.*, 144*S*–150*S*.

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

COLLIER, H. O. J., DINNEEN, L. C., JOHNSON, C. A. & SCHNEIDER, C. (1968). *Br. J. Pharmac. Chemother.*, **32**, 295–310.

DASTOLI, F. R., LOPIEKES, D. V. & PRICE, S. (1968). *Biochem.*, **7**, 1160–1164.

DEARDEN, J. C. & TOMLINSON, E. (1970). *J. Pharm. Pharmac.*, **22**, *Suppl.*, 53S—59S.

DEARDEN, J. C. & TOMLINSON, E. (1971a). Ibid., **23**, *Suppl.*, 68S—72S.

DEARDEN, J. C. & TOMLINSON, E. (1971b). *J. pharm. Sci.* **60**, 1278—1279.

FLANAGAN, T. H., BROAD, R. D., RUBINSTEIN, M. H. & LONGWORTH, A. R. (1969). *J. Pharm. Pharmac.*, **21**, *Suppl.*, 129*S*–134*S*.

FUJITA, T., IWASA, J. & HANSCH, C. (1964). *J. Am. chem. Soc.*, **86**, 5175–5180.

HANSCH, C. (1969). *Accts chem. Res.*, **2**, 232–239.

PENNISTON, J. T., BECKETT, L., BENTLEY, D. L., & HANSCH, C. (1969). *Molec. Pharmac.*, **5**, 333–341.

TUCKER, G. T., BOYES, R. N., BRIDENBAUGH, P. O. & MOORE, D. C. (1970). *Anesthesiology*, **33**, 287–303.

WAGNER, J. G. (1968). *Drug intelligence*, **2**, 294–301.

WALTON, R. P. (1944). *J. Am. Med. Ass.*, **124**, 138–143.