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The influence of alkyl substitution in acids on their performance in the buccal absorption test

A. H. BECKETT AND A. C. MOFFAT

A method to determine the passage of acids into the buccal membrane of man is presented. The absorption is shown to be the passive entry into a lipid phase. There is an increase in absorption of the acids due to alterations in partition characteristics with increasing chain length of n-fatty acids from butyric to dodecanoic. Methyl substitution of benzoic acid increases the absorption with mono- and di-substituents, but decreases the absorption with the tri- and tetra-substituents by lowering the pK_a. Predictions of the relative absorption and excretion in man of these acids is included.

KNOWLEDGE of the factors affecting the passive transfer of drugs across biological membranes has enabled predictions to be made about the extent that drugs will be absorbed, distributed and excreted in man (Beckett & Triggs, 1967). The buccal absorption test of these authors is now used to indicate the relative importance, in buccal absorption, of pK_a and alkyl chain length in a closely related series of acids.

Experimental

Buffer solutions in the range pH 3·00–9·09 (at 37°) were prepared using McIlvane's citric acid-phosphate buffer for pH values between 3·00–8·00 (Documenta Geigy, 1962a) and borax (0·05M) for pH 9·09. All pH values were measured at room temperature with a Pye Dynacap pH meter.

Solutions of the acids for buccal absorption tests (2 mg acid/ml) and internal standard solutions for chromatography (0·2 mg acid/ml) were prepared by dissolving a suitable quantity of the acid in a slight excess of sodium hydroxide solution.

BUCCAL ABSORPTION MEASUREMENTS

Men aged 20 to 30 were used.

General method. The method of Beckett & Triggs (1967) was used, with the following refinements. A measuring cylinder was used to measure the volume of the expelled buffer solution after the froth had subsided (or a nominal 0·5 ml was given for the froth) and the pH was also measured. The expelled solutions were combined, the internal standard solution (5 ml) added, the volume adjusted to approximately 200 ml and an aliquot (5 ml) used for analysis. The waiting time between successive tests was 30 min.

Drug mixtures. Mixtures containing 1 mg of two to six different acids were investigated using the general procedure.

Analytical technique. The 5 ml aliquot of the expelled solution was placed in a glass-stoppered centrifuge tube together with 6N hydrochloric acid (0·5 ml). The solution was then extracted with 3 × 2·5 ml freshly distilled Analar diethyl ether using a mechanical tilt-shaker, centrifuged,

From the Department of Pharmacy, Chelsea College of Science and Technology (University of London), London, S.W.3, England.

and the ether extracts transferred to a 15 ml Quickfit test tube with a finely tapered base (see Beckett, 1966). The extract was then concentrated to about 50 μl on a water bath at 40°. After cooling in ice, a few drops of an ice-cold ethereal solution of diazomethane was added and the tube was shaken. After 1 min 1–2 μl was injected into the gas chromatograph.

A Perkin-Elmer F11 gas chromatograph, with a flame-ionization detector, was used with the following conditions: a 2 m, $\frac{1}{4}$ inch o.d. glass tube packed with Chromosorb G (acid washed, DMCS treated, 80–100 mesh) coated with 2·5% SE-30; nitrogen pressure 20 lb/inch^2, hydrogen pressure 24 lb/inch^2, and air pressure 30 lb/inch^2; injection block temperature approximately 50° above the oven temperature. The oven temperature and internal standard used for each acid are summarized in Table 1.

TABLE 1. GAS-LIQUID CHROMATOGRAPHY CONDITIONS FOR THE ANALYSIS OF SOME ACIDS

Methyl ester of acid	Retention time (min)	Oven temp (° C)	Methyl ester of internal standard	Retention time (min)
n-Butyric	2·6	40	Hexanoic	18·0
n-Valeric	7·0	40	Hexanoic	18·0
n-Hexanoic	3·7	65	Benzoic	13·0
n-Heptanoic	5·6	75	Benzoic	8·7
n-Octanoic	4·3	100	*p*-Toluic	7·6
n-Nonanoic	10·2	95	*p*-Toluic	8·6
n-Decanoic	6·6	125	*p*-Chlorobenzoic	3·9
n-Undecanoic	12·6	125	*p*-Chlorobenzoic	3·9
n-Dodecanoic	23·5	125	*p*-Chlorobenzoic	3·9
Benzoic	4·0	95	*p*-Toluic	8·6
o-Toluic	6·6	100	*p*-Chlorobenzoic	9·6
m-Toluic	7·3	100	*p*-Chlorobenzoic	9·6
p-Toluic	7·6	100	*p*-Chlorobenzoic	9·6
2,4-Dimethylbenzoic	11·8	100	*p*-Toluic	7·6
2,4,6-Trimethylbenzoic	8·2	115	*p*-Toluic	3·9
2,3,5,6-Tetramethylbenzoic	19·4	115	*p*-Toluic	3·9
Phenylacetic	6·1	100	*p*-Chlorobenzoic	9·6
Ibufenac	20·3	125	*p*-Chlorobenzoic	3·9
p-Chlorobenzoic	9·6	100	*p*-Toluic	7·6

The amount of acid remaining in the mouthwash was calculated from the peak height ratio of the acid/internal standard and a calibration curve. The calibration curve was constructed by plotting the peak height ratio against amount of acid, using results obtained from a series of McIlvane buffer solutions containing from 0·1–1·0 mg of acid. All absorption curves were plotted as percentage of acid absorbed against the mean pH of the buffer solution before and after the test. The pH of borax buffer at 37° was calculated from the room temperature measurement and standard correction tables.

Results and discussion

ANALYSIS

The use of gas-liquid chromatography allowed acids in mixtures to be separated (as their methyl esters) and analysed individually. The peaks were almost symmetrical (Fig. 1) and the column had an efficiency equivalent to over 2,500 theoretical plates. One interfering peak was observed

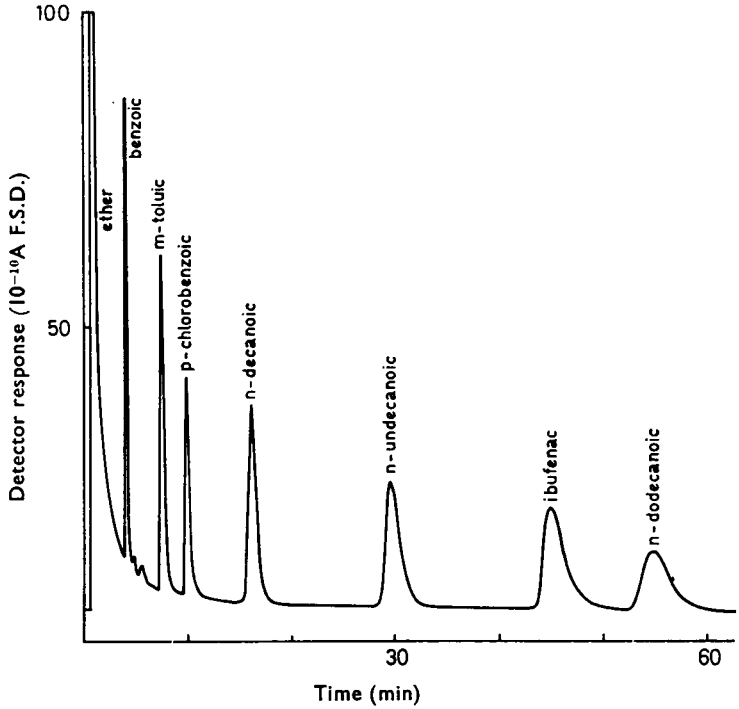

FIG. 1. Chromatogram of some acids (as their methyl esters) on the 2·5% SE-30 column at 100°.

due to methyl citrate (retention time 18·5 min at 125°): this became larger with buffers of low pH.

All calibration graphs were linear over the range 0·1 to 1·0 mg acid in buffer solutions or buffer solutions containing saliva, and the curves were identical for both solutions. Since n-valeric and n-butyric acids were volatile, the error of the analysis of these two acids was high, necessitating duplicate analyses. With all the other acids studied, the standard deviation, obtained from 12 replicate assays, was not greater than 2·5%.

Ethereal solutions of the methyl esters were stored on ice to prevent evaporation. The standard deviation of the method, for benzoic acid, when the ester solution is held at room temperature, is 8·7% and when stored at 0°, 2·1%.

BUCCAL ABSORPTION METHOD

The error in transferring the buffer solution from the beaker to the subject's mouth was 1·0% and has been neglected. Opalescent solutions were obtained when n-undecanoic and n-dodecanoic acids were placed in buffer solutions of low pH, but their absorptions were not affected.

The percentage buccal absorption of a water-soluble acid, phenylacetic acid, and a water-insoluble acid, ibufenac, at pH 4 and at various time intervals is shown in Fig. 2. The rate of absorption was rapid initially

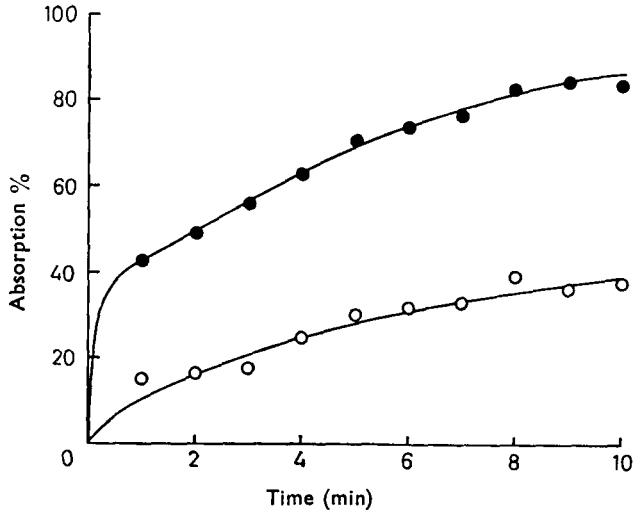

FIG. 2. The effect of solution contact time on the buccal absorption of ibufenac and phenylacetic acid. (Subject PGJ) ●, ibufenac; ○, phenylacetic acid.

but slowed later. A contact time of 5 min was chosen for subsequent experiments with the various acids.

The absorption of *m*-toluic acid at pH 4·0 was determined eight times on one subject, with 30 min wait between each test. The percentage absorption remained constant at about 50% throughout this test and 30 min was therefore allowed to elapse between all tests.

The normal output of saliva from man is 1–2 litres daily, i.e., approximately 3·5–7·0 ml in 5 min, with a mean pH of 6 (Documenta Geigy, 1962b). Thus any solution in the mouth will increase in volume by the amount of saliva and the pH will change towards 6. The buffer solutions must therefore be efficient over a wide pH range and must not interfere with the assay procedure or have a large pH change with temperature. Beckett & Triggs (1967) used Sörensen's phosphate and potassium hydrogen phthalate buffers. However, these are unsuitable for the present studies because the first is not a good enough buffer and the second interferes with the chromatography of the acids. Borax buffer (0·05M) had a large pH-temperature coefficient but standard tables are available for change of pH with temperature. The changes of pH of McIlvane's buffer between room temperature (20°) and 37° did not exceed 0·05 pH units at any pH and its ionic strength remained constant. McIlvane's and the borax buffer were therefore used in the general method.

A pH range of 9·09 to 3·00 was chosen for the general method because solutions of less than pH 3·0 were unpleasant to use and both the volume and pH changes in the mouth were large.

During the early stages of these experiments, many subjects swallowed during the test period and practice was needed before the swallowing reflex could be overcome. The head is best held forwards whilst effecting the test and a second task reduces the temptation to swallow.

The inter-subject variations, determined for ten subjects, in volume and pH changes of buffer solutions of pH values 9·09, 7·0, 5·0 and 3·0 during the 5 min buccal absorption test were mean (with range): 4·7 (1·5 to 10·5); 4·1 (0·0 to 8·5); 8·2 (3·0 to 14·0); 18·3 (8·0 to 31·0) ml and −0·08 (−0·02 to −0·12); −0·01 (0·01 to 0·18); 0·17 (0·07 to 0·44); 0·46 (0·16 to 0·71) respectively. Intrasubject variations, determined for a single subject on ten different occasions were 2·0 (1·0 to 3·0); 2·3 (1·0 to 4·5); 3·5 (2·5 to 5·0); 10·0 (7·0 to 13·0) ml respectively and −0·15 (−0·10 to −0·22); −0·04 (0·00 to −0·09); 0·12 (0·07 to 0·23); 0·24 (0·18 to 0·31) respectively. The absorption of 1 mg of *m*-toluic acid for the same ten subjects at pH 5·0 was 32·1 (22·6 to 40·6) % absorption and at pH 3·0, 61·1 (47·2 to 74·5) % absorption, compared with 49·0 (45·6 to 56·2) % absorption at pH 4·0 for a single subject. Since intra-subject variations in volume and pH changes of the buffer solutions and absorption of *m*-toluic acid were less than the inter-subject variations, a single subject was used for measurements of the buccal absorption of the series of acids below.

Since the concentration of unionized molecules was reduced by increased pH and volume of the test solution, the absorption-pH curves were drawn using the mean pH value before and after the buccal absorption test.

Fig. 3A shows the absorption of *p*-chlorobenzoic acid on three separate occasions using the same individual. Nearly all the experimental points

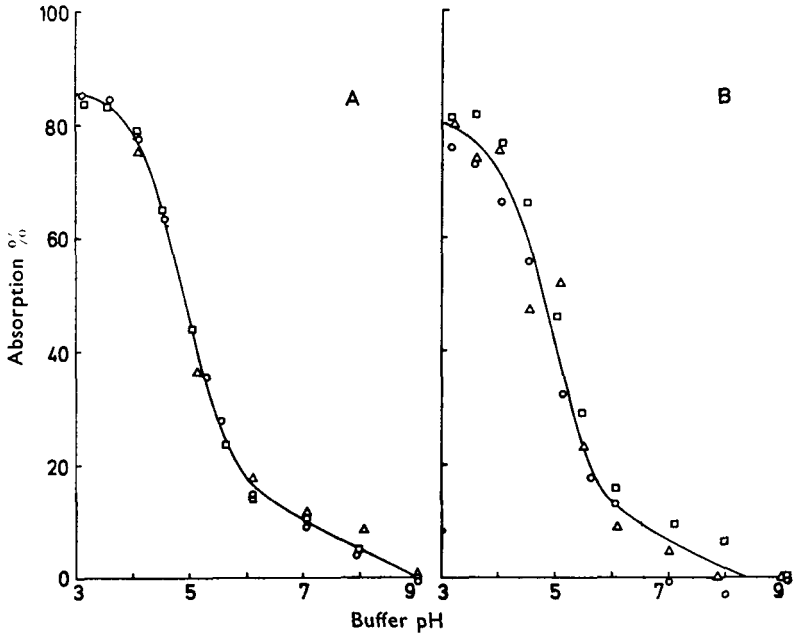

FIG. 3A. The buccal absorption of *p*-chlorobenzoic acid on three separate occasions. (Subject ACM) △, trial 1; ○, trial 2; □, trial 3.
B. The effect of acid concentration on the buccal absorption of *m*-toluic acid (Subject ACM). △, 0·01 mg/25 ml; □, 0·1 mg/25 ml; ○, 1·0 mg/25 ml.

lie on the drawn line showing the reproducibility of absorption of an acid using the buccal absorption test over the whole pH range studied.

The use of 0·01 or 0·1 or 1 mg of acid in the buccal absorption test does not affect its percentage absorption (Fig. 3B), although the analytical error increases with small quantities of acids. Therefore with acids that have not previously been given to man only 0·1 or 0·01 mg quantities of acids need be used, and a borax buffer mouthwash afterwards will return some of the acid to the mouth. Also the number of tests at low pH values should be reduced. For instance, with n-heptanoic acid, at pH 3·15, 91% was absorbed after 5 min, but the use of a borax buffer mouthwash afterwards returned 8%. Thus if 0·01 mg had been used—only 8·3 μg would have remained in the body.

The absorption of individual acids in mixtures containing up to six acids, was the same as the absorption of the acids determined singly. Thus the measured absorption is a true passive transfer into the lipid membrane of the mouth and no specialized transport system for these acids exists.

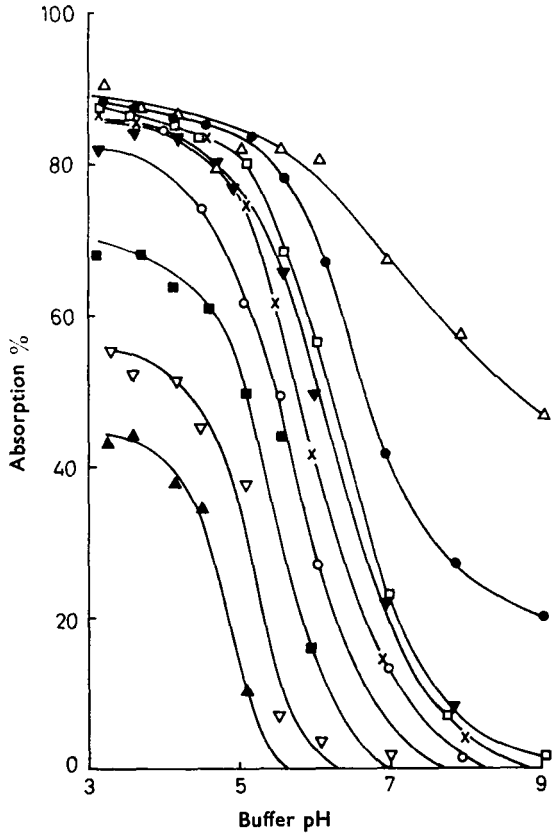

FIG. 4. The buccal absorption of straight chain fatty acids (Subject ACM) △, dodecanoic; ●, undecanoic; □, decanoic; ▼, nonanoic; ×, octanoic; ○, heptanoic; ■, hexanoic; ▽, valeric; ▲, butyric.

BUCCAL ABSORPTION MEASUREMENTS

Three groups of acids have been examined: (*a*) those with the same pK_a value and different lipid solubilities; (*b*) those with different pK_a values and the same lipid solubility; (*c*) those with different pK_a values and lipid solubilities.

(*a*) The absorption of all the long-chain fatty acids studied increased as the pH decreased and the concentration of unionized acid increased (Fig. 4). Since all the acids have approximately the same pK_a value (n-butyric acid, 4·82; n-octanoic acid, 4·85; at 25°, Fieser & Fieser, 1956) the different absorptions of the acids at each pH is due to the different partition characteristics of the unionized forms between the aqueous buffer solution and the cells constituting the epithelium of the buccal cavity. The absorption-pH curves for acids from butyric to decanoic show a gradation of increased absorption of the unionized form with chain length. n-Butyric acid has the lowest lipid-water partition coefficient and the smallest absorption at each pH value. Increasing the chain length by one methylene group greatly increases the rate of entry into the biological membrane. The addition of another one or two methylene groups increases this effect by approximately the same amount. The differences in absorption of n-dodecanoic and n-undecanoic acids from that of n-decanoic acid at pH 9·0 are due to the increased lipid solubility of the ionized forms of the C_{11} and C_{12} acids, so that they penetrate the membrane. On the other hand, surface-active properties would concentrate the molecules at the buffer-membrane interface and also organize some of the ions into micelles.*

(*b*) The curves for the buccal absorption of the three toluic acids are the same (Fig. 5A) displaced from each other by approximately the differences in their pK_a values. This would be expected since the partition characteristics of the unionized forms of the three acids are the same (Beckett & Moffat, unpublished observation).

(*c*) Fig. 5B shows the increased absorption of mono- and di-methyl substituted benzoic acids, over that of benzoic acid. An increase of pK_a by only 0·03 and 0·07 unit respectively (Wilson, Gore & others, 1967) would not account for such large differences between the curves (Fig. 5A). The increased absorption of these two acids is therefore primarily due to the expected increase in the lipid-water partition characteristics of the unionized forms. Absorption of the tri- and tetra-methyl substituted acids were less than that of benzoic acid. This anomolous behaviour cannot be explained simply by their pK_a differences from benzoic acid of −0·65 and −0·72 unit respectively (Wilson & others, 1967) unless the pH of the buffer solution is not that at the buffer-buccal membrane interface where absorption takes place. Were this true, changes of pH of the buffer solution would be greater than those at the interface and the above behaviour of the tri- and tetra-methyl benzoic acids is explained.

Beckett & Triggs (1967) showed that results from buccal absorption

* McBain & Hutchinson (1955) state the critical micelle concentration of potassium laurate to be 0·0234M, whilst in these studies the equivalent of a 0·000168M solution was used.

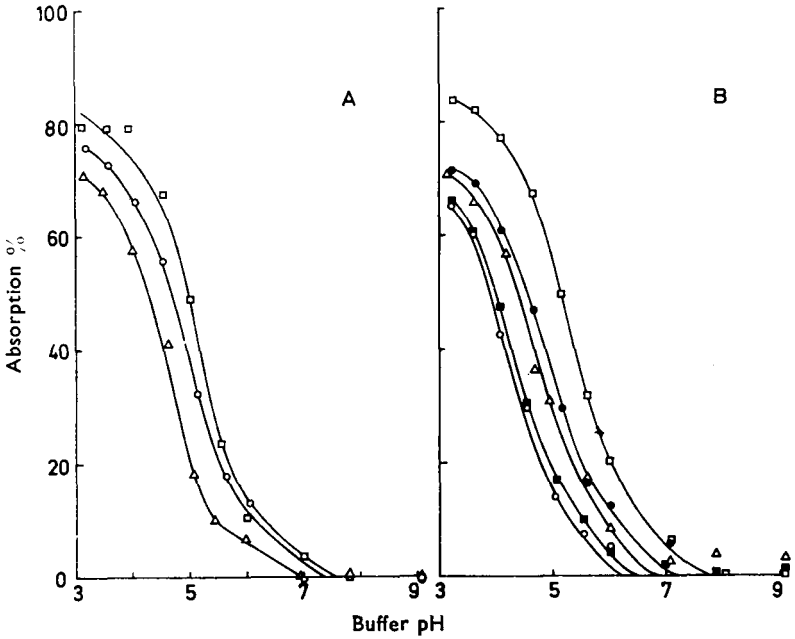

FIG. 5A. The buccal absorption of toluic acids (Subject ACM). □, *para* (pK_a 4·33); ○, *meta* (pK_a 4·24); △, *ortho* (pK_a 3·92).
B. The buccal absorption of methyl substituted benzoic acids (Subject ACM.) □, 2,4-dimethylbenzoic acid (pK_a 4·28); ●, *m*-toluic acid (pK_a 4·24); △, benzoic acid (pK_a 4·21); ■ 2,4,6-trimethylbenzoic acid (pK_a 3·56); ○, 2,3,5,6-tetramethylbenzoic acid (pK_a 3·49).

measurements may be used to predict the excretion of drugs by man. Thus, assuming that there is no binding of the acids nor active transport mechanisms and that the nature of all the cell membranes in the body is the same, predictions may be made of the fate of the long-chain acids in man.

They would be rapidly absorbed from both the stomach (pH 1) and the small intestine (pH 5), (Hogben, Tocco & others, 1959), and since the average pH of human urine is 6·3, all the acids would be reabsorbed from the glomerular filtrate. However, if the urine became alkaline, the excretion of the shorter-chain acids would increase until, at pH 8·0–8·5, both n-butyric and n-valeric acids would be excreted without reabsorption.

The differences in the passage into a biological membrane of acidic molecules differing by even a single methylene group, or having small differences in pK_a values, are clearly shown by the buccal absorption test. The test, whether for acidic or basic substances, is thus a powerful tool for distinguishing between the partition characteristics of members of a closely related group of compounds and has advantage over classical partition coefficient experiments where organic solvents are used which may bear little resemblance to biological membranes.

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