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A possible relation between pKa_1 and lipid solubility and the amounts excreted in urine of some tobacco alkaloids given to man

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The amount of unchanged (−)-nicotine, (−)-nornicotine, (−)-methylanabasine and (−)-anabasine excreted in the urine after oral administration to man was dependent on urinary pH while that of (−)-cotinine depended on urinary pH and on urine flow rate. No unchanged β-nicotyrine, β-nornicotyrine or myosmine was detected in urine. More nornicotine and anabasine than their corresponding tertiary amines nicotine and methylanabasine were excreted unchanged in the urine. Correlations of pKa_1 and lipid solubility and the urinary excretion of these compounds were made.

The pharmacology and toxicology of nicotine and some structurally related minor alkaloids in animals has been reported by Clark, Rand & Vanov (1965), but their fate in animals and man is unknown. We have examined the urinary excretion and buccal absorption of some of these alkaloids along with their pKa_1 values and lipid solubility.

MATERIALS AND METHODS

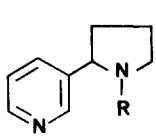
The amounts of alkaloids in urine were measured by g.l.c. using a Perkin-Elmer F11 gas chromatograph with a flame ionization detector and a Leeds and Northrup Speedomax G recorder, Model S. pH Values were measured using a Pye-Dynacap pH meter. (−)-Nicotine (Ib) was supplied as the hydrogen (+)-tartrate salt by B.D.H. Ltd., (−)-anabasine (IIa) and β-nicotyrine (IIIb) by Fluka, and (−)-nornicotine (Ia), β-nornicotyrine (IIIa), (−)-methylanabasine (IIb) and myosmine (IV) (Fig. 1) were gifts from Imperial Tobacco Co. Cotinine (V) was prepared by the method of Bowman & McKennis (1959). Phendimetrazine bitartrate and lignocaine hydrochloride, used as internal standards, were supplied by Ayerst, McKenna & Harrison Ltd., and the Pharmaceutical Manufacturing Co., respectively.

Gas-chromatographic assays

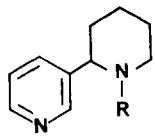
Assays were carried out on a 1 m $\frac{1}{8}$ in o.d. stainless steel column, packed with acid washed D.M.C.S. treated Chromosorb G coated with 5% potassium hydroxide and 2% Carbowax 20M. H_2 18 p.s.i.; air 25 p.s.i.; N_2 50 ml/min at 25°; stream split ratio 3 : 1; injection block setting 4·5.

Alkaloids were extracted with ether, or, for cotinine, with dichloromethane, under alkaline conditions according to Beckett, Gorrod & Jenner (1971); conditions and retention times are given in Table 1.

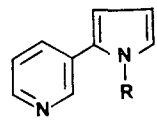
N-Oxide metabolites (see Beckett & others, 1971) and *N*-demethylated products were also measured.



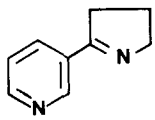
Ia R = H Nomicotine
Ib R = Me Nicotine



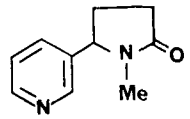
IIa R = H Anabasine
IIb R = Me Methylanabasine



IIIa R = H β-nomicotyrine
IIIb R = Me β-nicotyrine



IV Myosmine



V Cotinine

FIG. 1. Structural formulae of tobacco alkaloids used.

Table 1. *Analysis by g.l.c. of some tobacco alkaloids in urine.*

Alkaloid	No. of solvent extractions used	Oven temp. (°C)	Retention time of alkaloids (min)	Internal marker	Retention time of marker (min)	Standard error of assay procedure
Methylanabasine	4	125	6·2	Nicotine	3·8	$< \pm 6\%$
Nicotine	3	135	2·6	Phendi-metrazine	5·2	$< \pm 6\%$
Nornicotine*	6	145	5·6	"	3·4	$> \pm 20\%$
Anabasine	4	145	6·0	"	3·4	$< \pm 6\%$
Myosmine	5	145	6·2	"	3·4	$< \pm 6\%$
β-Nicotyrine	4	145	10·4	"	3·4	$< \pm 6\%$
Cotinine	3	204	6·0	Lignocaine	4·2	$< \pm 6\%$
β-Nornicotyrine	4	204	8·0	"	4·2	$< \pm 6\%$

* Urine saturated with sodium chloride (3 g).

Determination of buccal absorption characteristics

Buccal absorption determinations were made as described by Beckett & Moffat (1968).

Trials

Alkaloids (≡ 2 mg base, except cotinine) were administered in aqueous solution as the hydrogen tartrate salts orally to subjects; 10 mg cotinine base was given to subjects with normal urine flow but 20 mg base was given to those with enhanced urine flow (see below). Urine samples were collected at 30 min intervals for 4 h, at 60 min intervals for a further 8 h, then at suitable times until amounts of unchanged drug fell below detectable concentrations; the pH and volumes were recorded immediately after collection and the samples stored at 4° until analyses were complete.

Trials were conducted under conditions of either fluctuating or acidic controlled urinary pH (4·8 ± 0·2) maintained according to Beckett & Tucker (1966).

Additional trials with cotinine were made with water loaded subjects under conditions of fluctuating and acidic urinary pH. High urine flow rates were maintained by subjects drinking 600 ml of water hourly before and during the course of the trial (Beckett & Wilkinson, 1965).

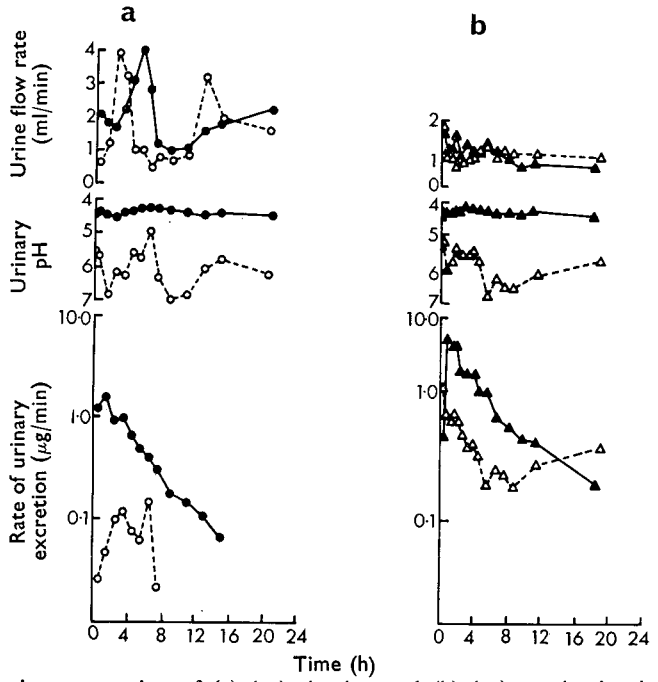


FIG. 2. The urinary excretion of (a) (−)-nicotine and (b) (−)-nornicotine in a subject with fluctuating (open symbols) or controlled acidic urinary pH (closed symbols).

RESULTS AND DISCUSSION

Excretion

The amounts of nicotine, nornicotine, methylanabasine and anabasine excreted in the urine fluctuated with changes in urinary pH; these fluctuations were minimized

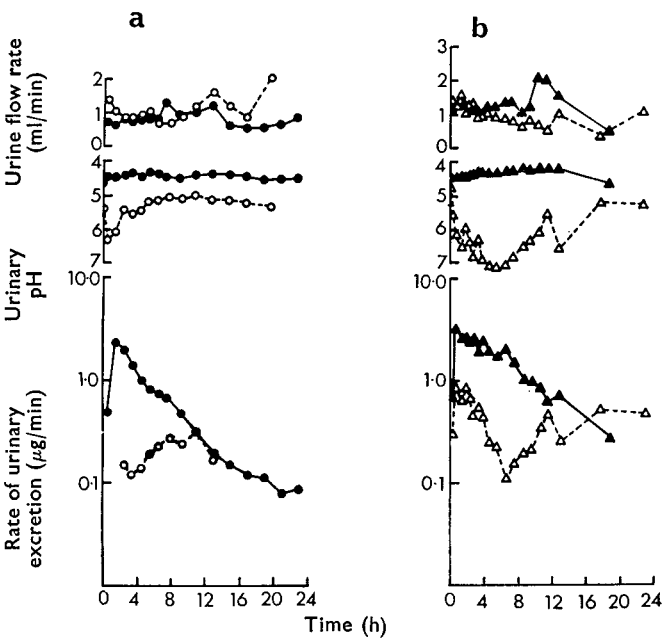


FIG. 3. The urinary excretion of (a) (−)-methylanabasine and (b) (−)-anabasine in a subject with fluctuating (open symbols) or controlled acidic urinary pH (closed symbols).

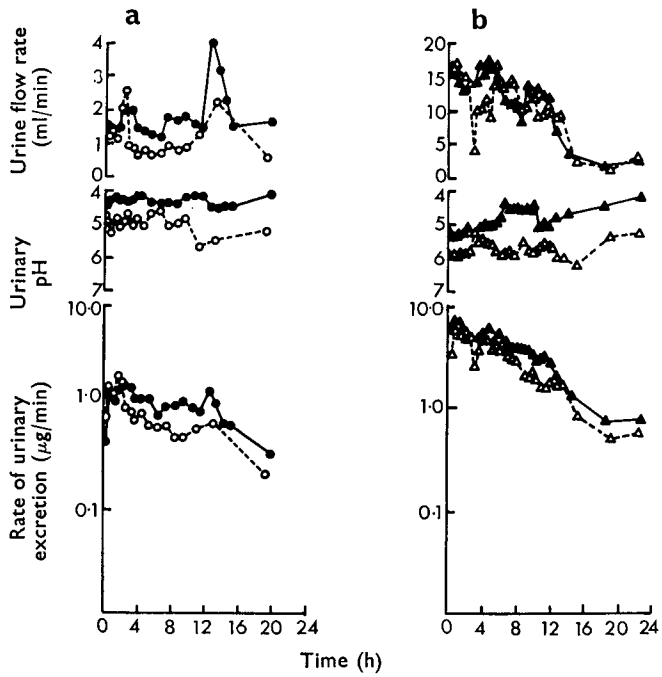


FIG. 4. The urinary excretion of (−)-cotinine under conditions of (a) normal urine flow and (b) enhanced urine flow in a subject with fluctuating (open symbols) or controlled acidic urinary pH (closed symbols).

under conditions of controlled acidic pH (Figs 2 and 3). The amount of cotinine excreted was not only dependent on pH but also on urine flow rate (Fig. 4), and a high urine flow rate did not abolish volume dependent fluctuations.

Myosmine, β-nicotyrine or β-nornicotyrine could not be detected in urine up to 24 h after administration.

Maintainance of an acidic urinary pH led to a higher recovery of those alkaloids for which the pH is a factor in excretion (Table 2). Cotinine excretion, although enhanced under conditions of acidic urinary pH, was further increased by a high urine flow rate, even though this procedure caused the pH to rise above 5·0. Lower recoveries of nicotine and methylanabasine compared to those of nornicotine and anabasine were obtained and the tertiary amines recoveries were more susceptible to changes in urinary pH.

The approximate elimination half-life ($t_{\frac{1}{2}}$) of nornicotine and anabasine was 4 h compared with 2 h for nicotine and methylanabasine; recoveries of cotinine were low and excretion rates slow ($t_{\frac{1}{2}}$ for cotinine 7 h).

Subject 2 gave recoveries of nicotine and nornicotine differing from those of other subjects under acidic controlled conditions.

No *N*-demethylated products of nicotine, methylanabasine, β-nicotyrine and cotinine or *N*-oxides of any alkaloid except nicotine were found; cotinine was also present in urine after nicotine administration (see also Beckett & others, 1971).

Buccal absorption

No more nicotine, anabasine and methylanabasine were absorbed after a buccal contact time of 5 min (Fig. 5a); pH dependence of the absorption of these alkaloids

Table 2. *Urinary recoveries of some tobacco alkaloids after oral administration under conditions of fluctuating and acidic urinary pH.*

Alkaloid	Subject	% Urinary excretion: Acidic urinary pH (4·8 ± 0·2)	% Urinary excretion: Fluctuating urinary pH	pH range
Nicotine	1	11·4	3·8	5·20–6·30
	2	27·9	—	—
	3	11·5	0·9	6·04–7·60
	4	13·8	0·8	5·76–7·40
	5	9·2	—	—
	6	15·1	—	—
Nornicotine	1	70·3	37·8	5·80–7·30
	2	41·8	—	—
Methylanabasine ..	1	16·5	3·6	5·10–6·90
	2	17·5	—	—
	3	—	2·9	5·90–6·90
Anabasine	1	70·3	27·9	5·20–7·60
	2	69·0	—	—
Cotinine	1	13·6 (4,501)*	6·9 (1,352)*	5·13–6·20
		19·9 (11,500)*†	15·9 (11,260)*	5·70–6·60
	2	13·1 (1,835)*	—	—
		20·1 (11,230)*†	—	—

* Total urine volume (ml) shown in parentheses.

† Enhanced urine flow caused urinary pH to rise above 5·0.

was exhibited (Fig. 5b). No meaningful results were obtained for the buccal absorption of nornicotine because of high assay error (>20%).

Anabasine was Class 1 according to Beckett & Triggs (1967) and nicotine and methylanabasine Class 4. Myosmine, cotinine, β-nicotyrine and β-nornicotyrine, which are much weaker bases than those previously studied require separate classification, now designated as Class 5; these drugs exhibit only slight pH dependence of buccal absorption (Fig. 5b). Compounds described by Bickel & Weder (1969) constitute members of this class.

The pKa_1, partition coefficient, buccal absorption and urinary excretion data in one subject for these alkaloids are shown in Table 3.

Table 3. *The observed partition coefficients,* pKa_1 *and buccal absorption values and urinary recoveries under fluctuating and acidic urine conditions in the same subject of some tobacco alkaloids.*

Alkaloid	Observed partition coefficient at pH* 6·0	7·0	8·0	pKa_1	% buccal absorption (a) and % unionized (b) at pH 5·0 (a)	5·0 (b)	7·4 (a)	7·4 (b)	% urinary recovery at acidic urinary pH	% urinary recovery at fluctuating urinary pH
Nicotine	0·29	2·05	5·59	7·9†	0	0·13	15	24·03	13·8	0·8
Nornicotine ..	0·013	0·08	0·46	9·0†	—	0·01	—	2·45	70·3	37·8
Methylanabasine ..	—	—	—	—	0	—	12	—	16·5	3·6
Anabasine	0·03	0·14	0·86	8·7†	0	0·02	5	4·77	70·3	27·9
β-Nicotyrine ..	12·90	13·40	13·60	4·7†	23	66·60	27	99·80	0	0
β-Nornicotyrine ..	14·00	14·10	14·40	4·3*	21	83·37	26	99·92	0	0
Myosmine	4·70	5·40	5·60	5·5†	8	24·03	17	98·76	0	0
Cotinine	—	—	—	4·5†	0	75·97	2	99·87	19·9	13·6

* From Badgett, Eisner & Walens (1952) using a t-amyl alcohol-buffer system.

† From Yamamoto (1966).

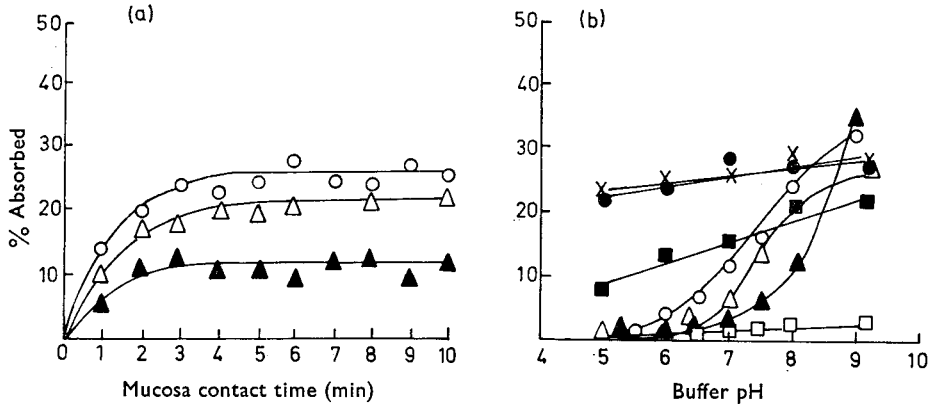


FIG. 5. The effect of (a) mucosa contact time (at pH 8·05) and (b) buffer pH on the buccal absorption of some tobacco alkaloids. ○ Nicotine. △ Methylanabasine. ▲ Anabasine. □ Cotinine. ■ Myosmine. ● β-Nornicotyrine. × β-Nicotyrine.

The higher urinary recoveries of the more water-soluble stronger bases, nornicotine and anabasine, relative to their tertiary amines nicotine and methylanabasine, is explicable in terms of the latter being metabolized more extensively and being reabsorbed in kidney tubules because of its increased lipid solubility at physiological pH. The fluctuation in the excretion of the tertiary bases with urinary pH to a greater extent than their nor-derivatives is a further reflection of the increased lipid solubility of the former compounds. The absence of myosmine, β-nicotyrine and β-nornicotyrine in the urine is explicable in terms of their weakly basic character and high lipid solubility of the unionized form.

The high urinary recovery of nornicotine and anabasine after oral administration indicates complete absorption of these alkaloids; complete absorption from the gastrointestinal tract of the other more lipid soluble alkaloids is therefore assumed. The differences in excretion among the tobacco alkaloids therefore, reflect differences in metabolism rather than in absorption.

The longer $t_{\frac{1}{2}}$ of cotinine compared to that of nicotine, the volume dependent excretion of cotinine, but not nicotine, and the similar recoveries of unchanged drug in urine indicates that cotinine is metabolized more slowly than nicotine and that possibly, unlike other basic drugs (Beckett & others, 1969), it is not transferred into the urine flowing down the kidney tubules after glomerular filtration because of its low lipid solubility.

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