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# The effect of smoking on nicotine metabolism *in vivo* in man

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Nicotine and a basic metabolite, cotinine, were determined in the urine by gas–liquid chromatography after intravenous administration of (−)-nicotine hydrogen (+)-tartrate to groups of male and female smokers and non-smokers in whom the urine was maintained at an acid pH. The urinary recoveries of nicotine and cotinine from male smokers fell in two groups. One showed a lower recovery of both alkaloids than was seen with male non-smokers. The other showed a similar recovery of nicotine but more cotinine than the male non-smokers. Female smokers excreted less nicotine but more cotinine than female non-smokers. More nicotine but less cotinine was excreted by female non-smokers than by male non-smokers. The results show sex dependent metabolism of nicotine occurs in non-smoking humans and that smoking causes alterations in nicotine metabolism.

The nature of tolerance to nicotine and tobacco smoking has received much attention (Dixon & Lee, 1912; Edmunds & Smith, 1915; Werle & Uschold, 1948; Yamamoto, Nagai & others, 1966; Wenzel & Broadie, 1966; Welch, Harrison & others, 1968, 1969; Stalhandske & Slanina, 1970; Ruddon & Cohen, 1970); evidence for the induction of hepatic microsomal enzymes as an explanation is equivocal since reports of inhibition as well as induction have occurred.

While it is known that there are sex differences in the drug metabolizing activity of rats and mice (Quinn, Axelrod & Brodie, 1968; Booth, 1966, 1967), evidence in men is lacking.

The observations of Beckett & Triggs (1967) have now been extended by an analysis of urine for nicotine and cotinine and an examination of the different nicotine metabolism between the sexes.

## METHODS

Nicotine and cotinine were extracted and analysed by the method of Beckett & Triggs (1966) using a Perkin Elmer F11 gas chromatograph with a flame ionization detector. Phendimetrazine (Ayerst, McKenna & Harrison Ltd.) replaced chlorphentermine as the internal standard for nicotine (Rt nicotine 2·0, phendimetrazine 3·8 min). Lignocaine was used as the internal standard for the cotinine determination (Rt cotinine 6·0, lignocaine 4·2 min).

Sterile ampoules containing 3·07 mg (−)-nicotine hydrogen (+)-tartrate (BDH Ltd.) in 5 ml water (equivalent to 1 mg of nicotine base), were prepared. Randomly selected ampoules were assayed for nicotine content by gas–liquid chromatography.

Male and female volunteer smokers and non-smokers were injected intravenously with 5 ml of the above nicotine hydrogen tartrate solution: the urine of all subjects

was controlled at pH 4·8 ± 0·2 (Beckett & Tucker, 1966). Smokers ceased smoking at least 36 h before the injection and the analysis of a "blank" sample of urine before injection was used to demonstrate the absence of nicotine and cotinine. The injections were given over 5–10 min and the subjects reaction noted. Urine samples were collected at 30 min intervals for 4 h at 60 min intervals for a further 8 h, then at will until a final sample was collected at 24 h. The pH and volume of each sample was measured immediately using a pH meter and all samples were stored at 4° until analyses were complete. Duplicate experiments were carried out in two male smokers and one male non-smoker at intervals from 3 days to 15 months. Experiments where the pH of the urine was not controlled were carried out with two male non-smokers (Subjects 1 and 3). Nicotine was determined in each urine sample, cotinine was determined only in bulked 24 h samples.

## RESULTS AND DISCUSSION

When the urine is adjusted to an acid pH the excretion of cotinine but not of nicotine is affected by changes in urinary output (Triggs, 1967). Although water-loading of the subject abolishes this effect, the resultant low concentrations of nicotine preclude its accurate measurement. In the present work, therefore, pH control without water-loading has been used and as a result cotinine recoveries exhibit greater inter- and intra-subject variation than recoveries for nicotine. Amounts of cotinine recovered in 24 h (*ca* 1600 ml urine) of less than 50 µg being too small to be measured accurately have been recorded as >5% cotinine in Table 1.

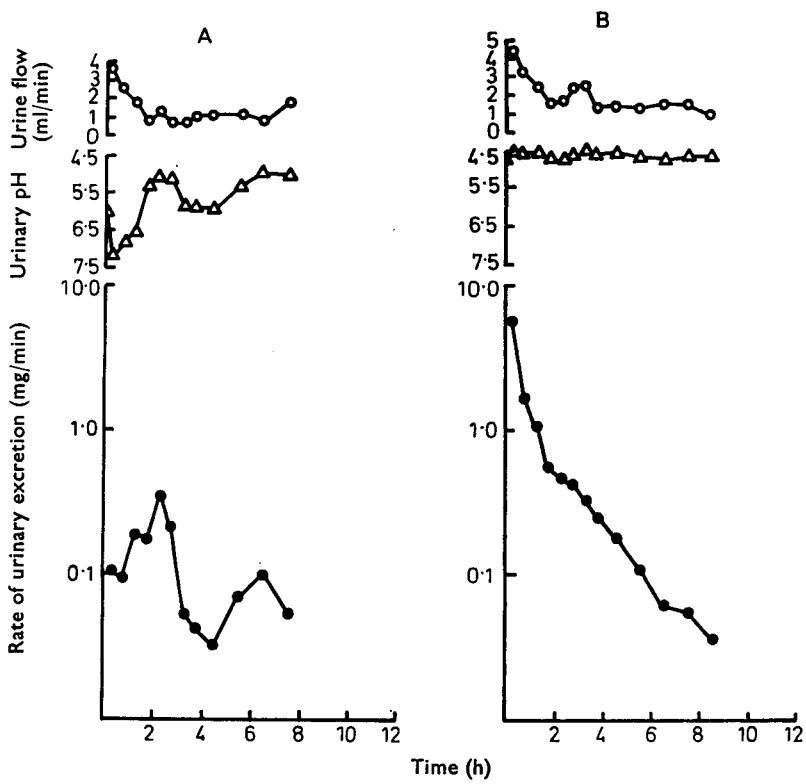


FIG. 1. Urinary excretion of nicotine after the intravenous administration of 3·07 mg (—)-nicotine hydrogen (+)-tartrate to a subject with, (A) fluctuating and (B) acidic controlled urinary pH.

Table 1. *Urinary recoveries of nicotine and cotinine from male and female smokers and non-smokers, with fluctuating or acidic urinary pH, after intravenous administration of* 3·07 *mg* (−)*-nicotine hydrogen* (+)*-tartrate.*

| Subject | Age | Approximate number of cigarettes smoked daily | % Nicotine excretion in 12 h | | % Cotinine excretion in 24 h | | Urine volume 24 h |
|---|---|---|---|---|---|---|---|
| *Male non-smokers* | | | | | | | |
| 1 | 23 | — | 31·0 | | ND | | 2822 |
| 1* | 23 | — | 31·8 | | 11·2 | | 1497 |
| 2 | 34 | — | 31·7 | | 7·3 | | 3945 |
| 3 | 23 | — | 31·0 | | 8·1 | | 1701 |
| 4 | 23 | — | 35·5 | Av. | ND | Av. | 1683 |
| 5 | 51 | — | 36·8 | 33·5 | ND | 9·2 | 1754 |
| 6 | 22 | — | 32·1 | | ND | | 1394 |
| 7 | 23 | — | 35·5 | | ND | | 1386 |
| 8 | 22 | — | 34·5 | | 9·2 | | 1666 |
| 9 | 24 | — | 35·4 | | 10·1 | | 1180 |
| 1‡ | 23 | — | 4·8 | | <5 | | 2132 |
| 3‡ | 23 | — | 4·7 | | <5 | | 1846 |
| *Male smokers* (low recovery) | | | | | | | |
| 1 | 24 | 40 | 21·6 | | <5 | | 1625 |
| 2 | 23 | 10 | 21·2 | | <5 | | 1752 |
| 3 | 26 | Pipe smoker | 21·2 | Av. | ND | Av. | 1464 |
| 4 | 48 | 20 | 24·0 | 22·5 | <5 | <5 | 1426 |
| 5 | 24 | 30 | 23·6 | | ND | | 2170 |
| 6 | 24 | 30 | 23·1 | | <5 | | 2470 |
| *Male smokers* (high recovery) | | | | | | | |
| 1 | 35 | 15 | 30·1 | | 21·3 | | 1384 |
| 1* | 35 | 15 | 32·0 | | 27·7 | | 1353 |
| 2 | 40 | 40 | 34·8 | | 21·6 | | 1550 |
| 2* | 40 | 40 | 35·5 | Av. | 20·8 | Av. | 1275 |
| 3 | 25 | 25 | 32·8 | 34·4 | 24·5 | 25·3 | 1537 |
| 4 | 21 | 25 | 34·8 | | 24·5 | | 1514 |
| 5 | 21 | 20 | 38·1 | | 31·3 | | 2935 |
| 6 | 23 | 20 | 37·4 | | 30·4 | | 1740 |
| *Female non-smokers* | | | | | | | |
| 1 | 22 | — | 44·0 | | <5 | | 1890 |
| 2 | 23 | — | 49·3 | Av. | <5 | Av. | 1969 |
| 3 | 22 | — | 44·7 | 45·4 | <5 | <5 | 1455 |
| 4 | 28 | — | 43·5 | | <5 | | 2863 |
| *Female smokers* | | | | | | | |
| 1 | 23 | 20 | 34·0 | | 22·5 | | 1726 |
| 2 | 21 | 15 | 40·5 | Av. | 35·2 | Av. | 3408 |
| 3 | 23 | 25 | 37·3 | 35·9 | 23·4 | 26·4 | 982 |
| 4 | 21 | 15 | 38·4 | | 24·5 | | 1943 |
| 5 | 23 | 15 | 29·3 | | ND | | 1655 |

* Repeat after 15 month interval.
‡ Fluctuating urinary pH.

pH dependent fluctuations in the urinary excretion of nicotine under normal conditions (Fig. 1a) disappear when the urine is maintained at an acid pH (Fig. 1b) and a higher recovery of unchanged nicotine is then obtained (Beckett & Triggs, 1967) (Table 1). The elimination of nicotine does not appear to be a single first order process.

Typical urinary excretion patterns of male and female smokers and non-smokers (Fig. 2) show no significant differences in the time taken to eliminate half of the nicotine dose. The differences in nicotine recoveries arise from differences in the

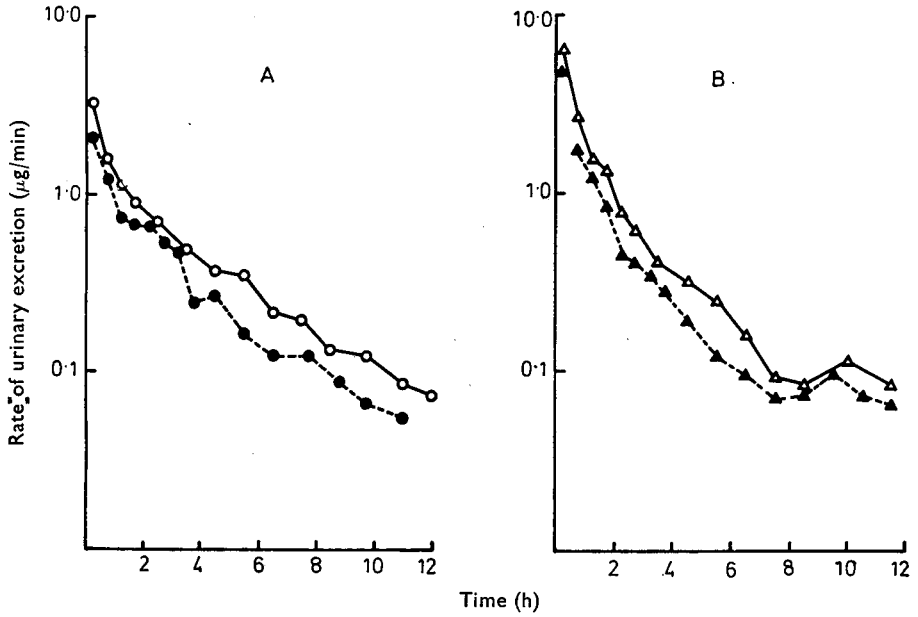


FIG. 2. Typical urinary excretion patterns of nicotine from (A) a male and (B) a female smoker and non-smoker after intravenous administration of 3·07 mg (—)-nicotine hydrogen (+)-tartrate under conditions of controlled acidic urinary pH. Open points—non-smokers. Closed points—smokers.

rate of excretion (μg/min) at any given time. The peak rate of nicotine excretion occurs within the first 30 min after injection; excretion is complete within 12 h in all groups.

Duplicate experiments in three male subjects—two smokers and one non-smoker—at intervals from three days to 15 months showed no variation in the urinary excretion pattern or in the percentage recoveries of nicotine and cotinine. This suggests that the enzyme activity in the individual is little changed over a long period.

None of the smokers reported any nausea from the nicotine injections; this was reported in varying degrees by all non-smokers, thus indicating some degree of tolerance to nicotine among smokers.

The urinary recoveries of nicotine and cotinine obtained after intravenous administration of nicotine to subjects with acidic urinary pH (Table 1; Fig. 3) show that subjects may be divided into distinct groups.

The urinary recoveries of nicotine and cotinine from male smokers fall in two groups. One showed a lower recovery of both alkaloids than was seen with male non-smokers (low recovery group). The other showed a similar recovery of nicotine but more cotinine (high recovery group) than male non-smokers. Female smokers excreted less nicotine but more cotinine than female non-smokers and gave a higher combined recovery of these compounds.

Possibly tobacco smoke may have caused the induction of nicotine and continine metabolism in "low recovery" male smokers. On the other hand, the results for "high recovery" male smokers indicates that smoking has either inhibited the metabolism of cotinine or emphasized the cotinine route of metabolism at the expense of an alternative route of nicotine metabolism.

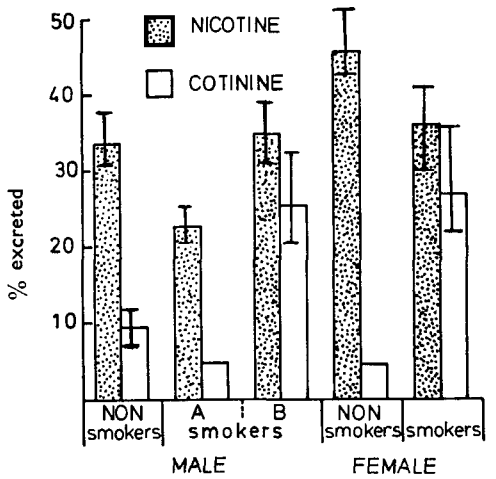


FIG. 3. Comparison of mean urinary recoveries of nicotine and cotinine in male and female smokers and non-smokers after the intravenous administration of 3·07 mg (—)-nicotine hydrogen (+)-tartrate. A, low recovery B, high recovery groups.

Smoking in females apparently caused increased total metabolism of nicotine and either increased cotinine formation or inhibition of the further metabolism of cotinine. In neither male nor female smokers is there a correlation between the urinary recoveries of nicotine and cotinine and the approximate number of cigarettes smoked daily before the trials were carried out.

The complex picture of human nicotine metabolism is in accord with many previously reported data where pretreatment with nicotine, tobacco smoke or other xenobiotics has led to either induction or inhibition of nicotine metabolism (or both) under similar experimental conditions (see Stalhandske & Slanina, 1970). However, tobacco smoke is a known inhibitor of many enzyme systems (Benedict & Stedman, 1968; Sato, Suzuki & Fayuyama, 1962; Schievelbein, 1967; Schievelbein, Werle & others, 1969) including dehydrogenases and an oxygenase, so that inhibition of the further metabolism of cotinine or the formation of nicotine-1′-*N*-oxide, recently observed as a metabolite of nicotine in man (Booth & Boyland, 1970), is possible.

Male non-smokers excrete less nicotine but more cotinine than female non-smokers (Fig. 3) showing that sex-dependent metabolism of nicotine occurs in humans. A comparison of nicotine metabolism in male and female smokers is complicated by the metabolic changes induced by smoking and the validity of such a comparison is dubious.

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