

## PAPERS AND ORIGINALS

## Absorption and metabolism of nicotine from cigarettes

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*British Medical Journal*, 1975, 4, 313-316

## Summary

Eight men volunteers each smoked a single cigarette containing  $^{14}\text{C}$ -nicotine and gave arterial blood samples during and for 50 minutes after smoking. The maximum concentration of nicotine in the arterial blood ranged from 31 to 41  $\mu\text{g/l}$  in four regular cigarette smokers who inhaled. Two non-smokers achieved maximum levels of 2 and 4  $\mu\text{g/l}$ . On a separate occasion two of the inhalers received 1 mg  $^{14}\text{C}$ -nicotine in 10 divided doses injected intravenously. In both cases the peak arterial nicotine concentrations bore a similar relationship to the intravenous dose, as did the peak nicotine concentrations to the retained doses during smoking.

## Introduction

Though nicotine consumption is thought to be the main reason for the smoking of tobacco, there is little information on the fate of nicotine in cigarettes. The present study had two main aims—firstly, to draw up a balance sheet of the nicotine in a cigarette by measuring all the fractions of smoke, both inhaled and sidestream, the amount exhaled, and the amount remaining in the butt; and, secondly, to measure the concentration of nicotine in

blood during and after smoking. Inhaled nicotine is delivered into the pulmonary capillary blood and thence, via the arterial system, into the brain. To interpret the significance of short-term changes in concentration it was desirable to measure the nicotine concentration in the arterial compartment.

Previous work has been handicapped by the difficulty of measuring nicotine and its metabolites in blood. Gas chromatographic methods are available for nicotine,<sup>1,2</sup> but there is evidence of interference from a nicotine-like substance present in the blood of both smokers<sup>1</sup> and non-smokers and in animals<sup>3</sup> not exposed to tobacco smoke. To obviate this problem we used a radiochemical assay for nicotine, which, in addition, measures the principal metabolite cotinine.

## Materials and methods

Eight men aged 29 to 51 years volunteered to participate in the study. Five were habitual smokers and three did not smoke.

**Cigarettes**—Filter-tipped cigarettes (70 mm) similar to a commercially available medium tar and nicotine brand were spiked with 1-(2'- $^{14}\text{C}$ )-nicotine bis-(di-*p*-toluoyl-D-tartrate).<sup>4</sup> Either 60.7 or 67.6  $\mu\text{Ci}$   $^{14}\text{C}$ -nicotine of specific activity 20 Ci/mol was added to each cigarette in an ethanolic solution. The puff-by-puff transfer characteristics of endogenous and exogenous  $^{14}\text{C}$ -nicotine in such cigarettes have been determined, and it has been established that the mean specific activity of nicotine in smoke remains constant from one puff to the next.<sup>5</sup>

**Smoke collection**—All smoke from the cigarette was collected. The cigarette was mounted in a smoking cartridge, which consisted of a vertically mounted glass cylinder with a port in one side.<sup>6</sup> The burning tip of the cigarette projected into the cylinder and the butt protruded out of the port. Air was drawn through the cartridge at a constant rate of 2 l/min. This supported combustion, maintained static burning characteristics, and swept the sidestream smoke from the tip of the cigarette on to a glass-fibre filter (Cambridge Filter Corporation, Syracuse, NY), which trapped the particulate phase of smoke and hence almost all of the  $^{14}\text{C}$ -nicotine. The vapour phase of smoke containing  $^{14}\text{CO}$  passed through an oxidising furnace, which converted it to  $^{14}\text{CO}_2$ . The vapour was then drawn through two Drechsel absorption bottles, each containing 75 ml of a mixture of ethanolamine and 2-methoxyethanol (1:8 v/v). The exhaled smoke was collected into an anaesthetic facemask applied intermittently. Two exhalations were made into the mask after each puff and the smoke passed via a flap valve into a polyethylene bag, which was emptied continuously by a pump, the smoke being drawn on to a filter disc and vapour phase absorption system identical with that used for the collection of sidestream smoke.

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**Procedure**—The volunteers were asked not to smoke after midnight on the evening before the experiment. On the morning of the experiment a polyethylene catheter was passed into the brachial artery under local anaesthesia using the Seldinger technique with the subject seated. Arterial pressure was recorded direct from the catheter using a Bell and Howell transducer and a Devices recorder. An ECG was also recorded. After 10 minutes of observation the subjects smoked the test cigarette, taking one puff every minute until it was finished (9-11 puffs). Each smoker was asked to try to puff in his usual way, and non-smokers were asked to smoke as deeply as possible. The procedure was rehearsed, without catheterisation, before the day of the experiment. Arterial blood was drawn at intervals during smoking and at intervals during the next 50 minutes. The samples were taken at various times during the smoking period but always at the same time relative to the puff. On average each sample (10 ml) took 20 seconds to withdraw, and a median sampling time of 30 seconds after the start of the appropriate puff was chosen. The subjects emptied their bladder before smoking the cigarette, being given 500 ml water to ensure a good flow. Urine samples were collected 20 and 60 minutes after smoking began. The arterial catheter was removed at 60 minutes. All urine and faeces passed in the next 72 hours were collected.

**Intravenous nicotine**—Two separate experiments were performed in which cigarette smokers were injected with 1 mg  $^{14}\text{C}$ -nicotine, specific activity 2.43 Ci/mol. The protocol was identical with that for the smoking experiments except that the nicotine was given in 10 separate injections of 100  $\mu\text{g}$  at one-minute intervals. The injection was made through a 16-gauge butterfly cannula inserted into an antecubital vein, the nicotine being flushed into the vein using a free flow of saline from an intravenous infusion set. Arterial blood samples were taken as before.

**Analyses**—Blood was analysed for nicotine and cotinine by a radiochemical method.<sup>7</sup> Aliquots of urine were similarly extracted and analysed. Faeces were homogenised in 5 volumes of saline and aliquots combusted to  $^{14}\text{CO}_2$  with a Packard model 305 sample oxidiser. The liberated gas was absorbed in a methanolic ethanolamine mixture and counted. Radioactivity in all samples was measured by liquid scintillation spectrometry (Packard Instrument Co) using a modified Brays scintillator.<sup>8</sup> Samples were counted to an accuracy of 1%. The glass-fibre filters were extracted with methanol; total  $^{14}\text{C}$  activity and  $^{14}\text{C}$ -nicotine in the extracts were determined by the method of Houseman.<sup>5</sup> The mean specific activity of nicotine in smoke was calculated from the mass and radioactivity of nicotine in a steam distillate of the methanol extract, as measured by ultraviolet spectroscopy and liquid scintillation spectrometry respectively.  $^{14}\text{CO}_2$  in the absorption bottles was determined by direct counting of aliquots of the ethanolamine solutions. Arterial carboxyhaemoglobin levels (COHb) before and after smoking were determined by the method of Commins and Lawther.<sup>9</sup>

## Results

### FATE OF NICOTINE IN CIGARETTE

Table I shows the amounts of radioactivity in each of the smoke fractions for each experiment. More than half of the radioactivity in the cigarette was in the sidestream smoke, the next largest fraction being in the unsmoked butt (filter tip and unsmoked tobacco). The amount of radioactivity exhaled in the mainstream smoke ranged from 1.3 to 8.8  $\mu\text{Ci}$ . The difference between the sum of these fractions in terms of radioactivity gives the dose apparently absorbed by the smoker, from which the apparent dose of  $^{14}\text{C}$ -nicotine may be calcu-

lated.<sup>10</sup> Subjects 1-4 exhaled less radioactivity (mean 2.3  $\mu\text{Ci}$ ) than subjects 5-8 and therefore had a higher calculated dose (mean 15.2  $\mu\text{Ci}$ ). Subjects 5-8 exhaled a larger amount of mainstream smoke (mean 7.4  $\mu\text{Ci}$ ) and therefore had a smaller calculated retained dose (mean 6.4  $\mu\text{Ci}$ ). Subjects 1-4 retained between 82% and 92% of the nicotine in the smoke that they took into their mouths and were probably habitual inhalers. By contrast, the non-smokers exhaled 34%, 47%, and 70% of the smoke that they took into their mouths. Subject 5 was a habitual cigarette smoker who considered that he inhaled. The data, however, indicate that he did not because his absorbed dose of nicotine and arterial blood concentration of nicotine were low and he exhaled 71% of the radioactivity he took into his mouth.

### ARTERIAL BLOOD CONCENTRATIONS OF NICOTINE AND COTININE

Fig 1 shows the arterial blood concentration profiles of nicotine in the seven subjects for whom measurements were made. The maximum concentrations of nicotine achieved are shown in table II. In subjects 1-4, who retained most of the mainstream smoke they inhaled, the maximum arterial concentration ranged from 31.3 to 41.0  $\mu\text{g/l}$  (mean 34.5  $\mu\text{g/l}$ ). The remaining three subjects (numbers 5-7) had much lower concentrations, ranging from 2.5 to 8.0  $\mu\text{g/l}$ .

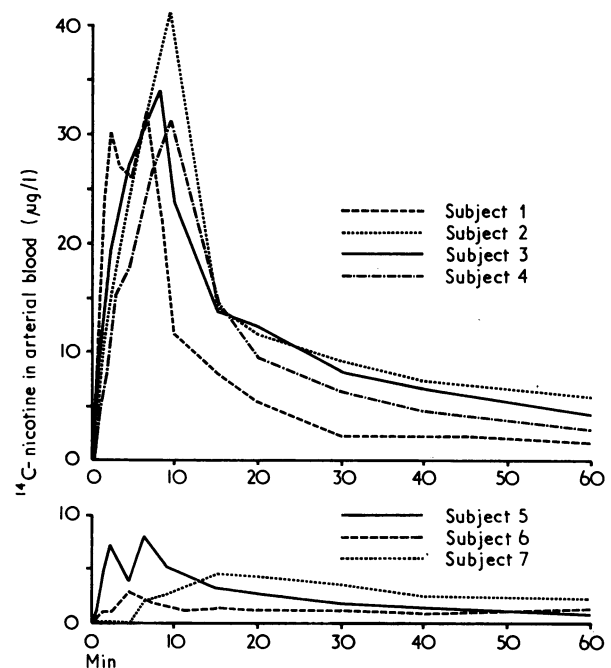


FIG 1—Arterial blood levels of  $^{14}\text{C}$ -nicotine in habitual smokers who inhaled (subjects 1-4), in a habitual smoker who did not inhale (subject 5), and in non-smokers (subjects 6 and 7) after smoking one cigarette labelled with  $^{14}\text{C}$ -nicotine.

The half lives of nicotine in arterial blood were calculated assuming a one-compartment model and using the points between 20 and 60 minutes. The nicotine distribution may not have been complete at

TABLE I—Fate of  $^{14}\text{C}$ -nicotine in single cigarettes smoked by the eight men

Subject	Presumed smoking habit	$^{14}\text{C}$ -activity in cigarette ( $\mu\text{Ci}$ )	$^{14}\text{C}$ -activity recovered after smoking ( $\mu\text{Ci}$ )			Mean specific activity of nicotine in smoke (mCi/mol)	Apparent dose to smoker	
			Sidestream smoke	Exhaled mainstream smoke	Unsmoked cigarette		$\mu\text{Ci}$	% total
1	Inhaler	67.6	37.4	3.0	13.2	1.05	14.0	20.7
2	Inhaler	67.6	34.3	2.9	14.6	1.02	15.8	23.4
3	Inhaler	60.7	28.4	1.9	13.6	0.84	16.8	27.7
4	Inhaler	60.7	33.4	1.3	11.9	0.87	14.1	23.2
5	Non-inhaler	67.6	39.7	7.7	17.0	1.13	3.2	4.7
6	Non-smoker	60.7	37.5	4.4	10.1	0.99	8.7	14.3
7	Non-smoker	60.7	33.0	8.8	9.0	0.81	9.9	16.3
8*	Non-smoker	60.7	37.8	8.6	10.7	0.80	3.6	5.9

\*Subject exhaled every breath after each puff through facemask.

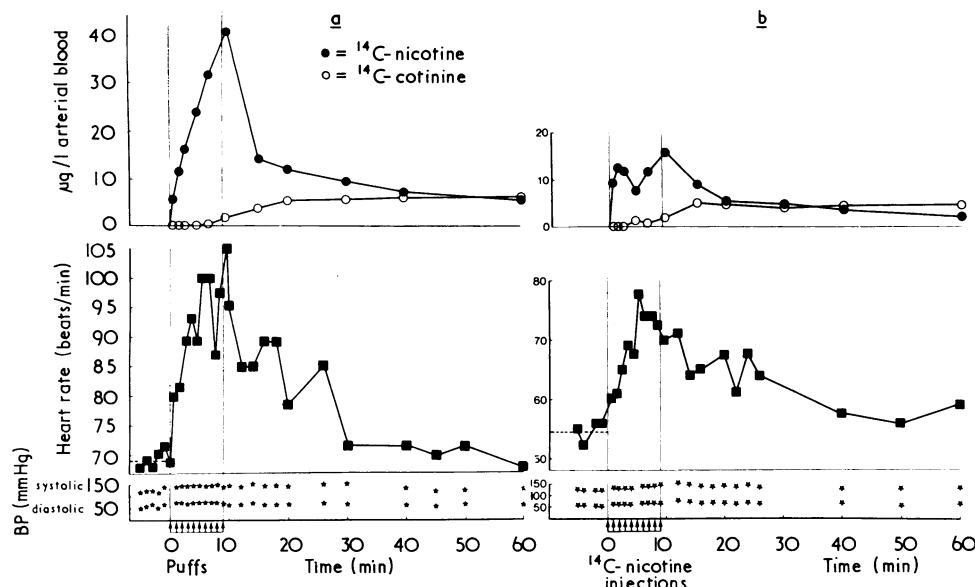


FIG 2—Subject 2. Arterial blood levels of  $^{14}\text{C}$ -nicotine (●) and  $^{14}\text{C}$ -cotinine (○), heart rate (■), and blood pressure (★) during and after smoking a cigarette labelled with  $^{14}\text{C}$ -nicotine (a), and during and after intravenous administration of 1 mg  $^{14}\text{C}$ -nicotine in 10 divided doses (b).

TABLE II—Recovery (in 72 h) of apparent dose of  $^{14}\text{C}$ -nicotine absorbed during smoking and after intravenous (IV) dosing, maximum arterial blood concentrations of nicotine achieved, and calculated half life ( $t_{1/2}$ ) of clearance of nicotine from arterial blood between 20 and 60 minutes after smoking or beginning intravenous administration

Subject	Apparent dose of nicotine (mg)	% Apparent dose recovered	% Apparent dose in faeces	Maximum arterial blood concentration of nicotine (µg/l)	$t_{1/2}$ nicotine (min)
1	1.83	72		31.9	31
2	1.91	73	1.4	41.0	41
3	2.62	66	1.6	33.8	37
4	2.07	59	0.7	31.3	24
5	0.36	66	0.4	8.0	28
6	1.08	10	0	2.5	84
7	1.38	4	0.004	4.3	37
8*	0.51	16	0.23		
2	1.00 (IV)	69	0	15.6	34
4	1.00 (IV)	79	0	16.9	18

\*Blood sample not taken.

this time and the results may therefore be an overestimate. The half lives ranged from 24 to 84 minutes with a mean of 40 minutes when only the inhalation experiments were taken into account.

Fig 2 gives the results of two experiments in subject 2, who smoked a cigarette and had intravenous injections of nicotine on different occasions. The ratios of peak arterial nicotine concentration to total dose of nicotine (41 µg/l for 1.9 mg nicotine in smoke, and 16 µg/l for 1 mg nicotine given intravenously) are similar and the profiles of the two concentrations are closely comparable.

Plasma levels of cotinine, the principal metabolite of nicotine,<sup>11</sup> are also shown in fig 2. Cotinine was detectable in plasma soon after smoking or the injections began and rose progressively. The levels eventually exceeded those of nicotine between 15 and 60 minutes after smoking began.

#### PHYSIOLOGICAL CHANGES

Smoking raised the heart rate and blood pressure in most cases (table III). The largest increases in heart rate occurred in the inhalers and appeared to be related to the dose of nicotine absorbed. Arterial blood carboxyhaemoglobin concentration also increased with smoking.

#### RECOVERY OF NICOTINE

The proportion of the total dose of radioactivity recovered from each subject (table II) ranged from 59% to 79% in the smokers and from 4% to 16% in the non-smokers. The low blood concentrations of nicotine in subjects 6 and 7 indicate that the real dose of nicotine absorbed by these subjects was probably lower than the calculated

TABLE III—Physiological changes after smoking single cigarette and after intravenous (IV) nicotine

Subject	Apparent dose of nicotine (mg)	Mean increase in heart rate (beats/min)	Blood pressure (mm Hg)		COHb (%)	
			before	after	before	after
1	1.83	16	140/85	150/90		
2	1.91	23	125/60	145/73	1.2	3.8
3	2.62	12	160/80	160/83	1.2	2.4
4	2.07	17	120/70	125/75	2.8	6.2
5	0.36	2	190/115	200/120	1.3	2.1
6	1.08	10	122/65	142/72		
7	1.38	0	140/70	145/62	0.2	0.5
2	1.00 (IV)	15	122/15	130/63		
4	1.00 (IV)	7	90/45	95/50		

dose. The experiment on subject 8 was carried out to see whether this discrepancy in recovery of radioactivity occurred primarily in the non-smokers. This subject gargled with a 0.15M ascorbic acid solution (2 × 50 ml) immediately after smoking and exhaled every breath between puffs into the smoke trap. The mouth washings yielded 4.3% of the apparent dose and the exhaled smoke radioactivity was higher in this subject. As a result his apparent dose was lower than in the other two non-smokers. The overall recovery of even this smaller apparent dose, however, although greater (16%) than in the other two non-smokers, was still poor.

The amount of radioactivity recovered in the faeces was low. The 20-minute urine samples contained relatively large amounts of  $^{14}\text{C}$ -nicotine (>50%) and the maximum excretion of radioactivity occurred in the first 24 hours. By such time, however, there was little or no activity due to  $^{14}\text{C}$ -nicotine in the urine, and by 72 hours there was also little or no  $^{14}\text{C}$ -cotinine. This remaining  $^{14}\text{C}$  activity, as separated by paper chromatography,<sup>12</sup> contained at least six  $^{14}\text{C}$  metabolites, of which two were  $^{14}\text{C}$ -nicotine-1'-oxide and  $^{14}\text{C}$ -demethyl cotinine.

#### Discussion

These results confirm that no more than 25% of the total nicotine content of a cigarette is likely to appear in the mainstream smoke. Most of the nicotine is lost into the surrounding air and sidestream smoke or is retained in the butt. Nevertheless, smokers who inhale may absorb up to 90% of the nicotine in the mainstream smoke drawn into their mouths. Our data suggest that this efficient extraction is due to inhalation of the smoke into the lungs. Non-smokers who do not inhale absorb much less nicotine.

This is the first study to use a radiochemical determination of nicotine in man, and these are the first data on arterial blood concentrations of nicotine and cotinine during smoking.

Nicotine in venous blood from smokers has been measured using gas chromatographic methods,<sup>1, 2</sup> but the results should be viewed with caution because of the presence of a nicotine-like substance, the nature of which is disputed.<sup>1-3</sup> More recently venous nicotine levels have been measured by radioimmunoassay.<sup>13</sup> The concentrations reported here are somewhat higher than those found in venous blood, and the rate of rise and fall in the arterial blood was also greater. The rapid increase in arterial nicotine concentrations was similar in all four smokers who inhaled, and the peak levels achieved were also similar. This suggests that smokers may adjust the way in which they smoke cigarettes in order to achieve a particular arterial nicotine concentration that is associated with a desirable psychological effect.<sup>14</sup> The peak levels attained by the smokers who inhaled were of the same order as those found in animals,<sup>15</sup> and these levels produce pharmacological effects on the central nervous system.<sup>16</sup> Our observations further support the concept that many smokers smoke to dose themselves with nicotine.

All the smokers who inhaled had an increase in their heart rate, which also occurred after intravenous doses of nicotine. As the nicotine levels fell so did the heart rate. Blood pressure also rose but was not so clearly related to the apparent dose of nicotine or the concentration in the blood. The response of heart rate to smoking a cigarette appears to be a simple way of classifying inhalers and non-inhalers.

The absorbed radioactivity was rapidly excreted into the urine after both inhalation and intravenous administration. Although the amounts of nicotine and cotinine were measured in the urine fractions, no firm conclusion was drawn from these data since neither urine flow nor urinary pH was controlled during the collection.<sup>17</sup> Overall recoveries of radioactivity were poor in the non-smokers, which was almost certainly because the absorbed dose was overestimated.

Cotinine levels in blood rose continuously over the period of sampling after four to five minutes' delay, and by 60 minutes the levels usually exceeded those of nicotine. Cotinine levels have been measured by radioimmunoassay<sup>18</sup> in plasma from

smokers but the data are not strictly comparable to ours since the assays were performed on samples from people who had smoked more than one cigarette. In our subject 1, cotinine was detectable after three puffs of smoke and blood levels increased rapidly thereafter. Interestingly, he had a short plasma anti-pyrene half life, indicating an active liver microsomal drug oxidising system. Urine excretion was also faster in this subject, which was consistent with the rapid production of more polar, less lipid-soluble metabolites.

These studies were supported by the Tobacco Research Council of Great Britain. We thank Mr J B Hopper, Mr P B Minty, and Miss M Lillis for skilful technical help, and Mr B Emmett for preparing figs 1 and 2.

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# Plasma renin activity and aldosterone concentration in children

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*British Medical Journal*, 1975, **4**, 316-319

## Summary

Using semi-micro methods, plasma renin activity (PRA) and plasma aldosterone concentration (PA) were measured concurrently in 79 healthy children aged 1 month to 15 years to establish a reference range. PRA and PA varied inversely with age. Eleven children with renal hypertension had higher PRA and PA than age-matched controls. In contrast, PRA was much greater in 38 saline-depleted children. PA was not uniformly increased in this group and was within the normal range in children with adrenal diseases compared with the high values seen in

other salt-wasting states. The findings emphasise the need to relate data from patients to age-matched control values before attempting interpretation and suggest that sodium depletion is a more potent stimulator of renin-aldosterone release than renovascular disease or renal scarring in children. Plasma renin-aldosterone profiles were also valuable in discriminating between renal and adrenal causes of salt loss in childhood.

## Introduction

The renin-angiotensin-aldosterone system has been investigated extensively in adults, but relatively little information has been reported in children, partly because of the large quantities of blood hitherto needed for measuring the system's components and the sampling difficulties encountered in young children. Studies in animals have shown increased activity of the renin-angiotensin-aldosterone system in the newborn of various species.<sup>1-5</sup> Early studies in the human provided inconclusive but suggestive evidence of higher plasma renin levels in children than in adults,<sup>6-12</sup> and in 1972 Kotchen *et al*<sup>13</sup> and Hayduk

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