Monograph on the Pharmacology and Toxicology of Nicotine

A. J. Cohen & F. J. C. Roe

TOBACCO ADVISORY COUNCIL
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Monograph on the Pharmacology and Toxicology of Nicotine

by

A. J. Cohen, B. Sc., Ph. D., C. Chem., F.R.S.C.

and

F. J. C. Roe, D. M. (Oxon), D. Sc. (Lond), F.R.C. Path.

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KEY TO ABBREVIATIONS

$[a]_D^{20}$	specific optical rotation at 20° C for D (sodium) line
bp_{745}	boiling point at 745 mm mercury pressure
°C	degrees centigrade
^{14}C	labelled with carbon-14
cis	stereochemical configuration opposite of trans
CNS	central nervous system
d_4^{20}	density (specific gravity at 20° C referred to water at 4° C)
EEG	electroencephalogram
g	gram
ibid	ibidem (at the same place)
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
kg	kilogram (1000 g)
l	laevo (rotatory)
l	litre
LD_{50}	lethal dose 50% kill
LDL_0	lowest published lethal dose
m	metre
mg	milligram (10^{-3} gram)
µg	microgram (10^{-6} gram)
ml	millilitre (cubic centimetre) (10^{-3} l)
mol. wt.	molecular weight
n_D^{20}	index of refraction at 20° C, sodium light
ng	nanogram (10^{-9} gram)
NMR	nuclear magnetic resonance
per se	by itself
pg	picogram (10^{-2} gram)
pH	log of reciprocal of hydrogen ion concentration
ppb	parts per billion (b = 10^9)
ppm	parts per million
(*s*)	sinister (left)
s.c.	subcutaneous
trans	stereochemical configuration opposite of cis
T.R.C.	Tobacco Research Counil
UV	ultraviolet light
>	greater than

1. INTRODUCTION

The purpose of this review is to summarize pharmacological and toxicological data concerning nicotine. In doing so we have concentrated on data from studies on nicotine or its salts ***per se*** and eschewed data from studies on tobacco smoke or tobacco-smoke condensate where it is difficult to distinguish the effects of nicotine from those of other constituents.

Faced with what they regard as unacceptable incidences of smoking-associated diseases, medical authorities worldwide have variously been drawn towards three different courses of action: firstly, the total discouragement or even abolition of the smoking habit; secondly, a progressive reduction of the deliveries of smoke components, including both tar and nicotine, by cigarettes; thirdly, the development of cigarettes which deliver adequate doses of nicotine without the necessity of inhaling large doses of toxic vehicle. A specific purpose of this review is to help those endeavouring to choose between the second and third of these courses of action.

In a nutshell our approach has been to regard nicotine as a "drug" to which man is exposed in various "vehicles" and by various routes. In this context, we have tried to delineate the effects of the "drug".

A monograph in German was published in 1968 on the pharmacology and toxicology of nicotine[1] and other important works of reference include four comprehensive volumes covering experimental and clinical studies on tobacco by Larson and his colleagues[16-19] and the US Surgeon General's series on "Smoking and Health".[2,5,7,8,11,13,135]

2. CHEMISTRY

2.1 Discovery and occurrence

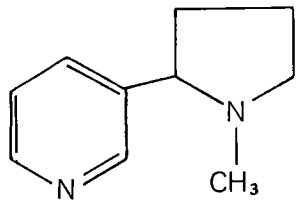
The term for the alkaloid, nicotine, is derived from "nicotiana", a genus established in 1773 by the Swedish botanist Linnaeus. He named it after Jean Nicot, the French Ambassador to Portugal who first arranged for seeds to be bought as a gift for the French Queen mother, Catherine de Medici. This was shortly after Francisco Hernandes, court physician to Philip of Spain, introduced him to tobacco plants in 1559 for medicinal purposes.[132]

Nicotine occurs in leaves, and other parts, dried, cured or fresh, in concentrations of 0% (effectively) to 10% of *Nicotiana tabacum* and *N. rustica* (the latter is used for smoking in India/Pakistan and for chewing tobacco in these areas and also West Africa). Nicotine content is sensitive to variety and cultural practices and occurs usually in smaller concentrations in other species of *Nicotiana*, some other solanaceous plants and in some unrelated species such as *Zinnia.*[42]

Nicotine was first isolated in 1829 and subsequently other alkaloids have been detected in tobacco as follows: anabasine, anatabine, isonicoteine, *1*-N-methylanabasine,

1-N-methylanatabine, nicotelline, nicotimine, nicoteine, nicotyrine and nornicotine. The proportion of nicotine to nicoteine to nicotimine to nicotelline in tobacco is 1000:20:5:1, respectively.[42] Apart from the normal alkaloid constituents of tobacco, the following alkaloids have been identified in tobacco smoke: myosmine, obeline, *α*-, *β*- and *γ*-socratine, anodmine, lathreine and lohitam.[42]

2.2 Structure



Nicotine: (*s*)-3-(1-methyl-2-pyrrolidinyl)pyridine;
1-methyl-2-(3-pyridyl)pyrrolidine;
β-pyridyl-*α*-N-methylpyrrolidine;
$C_{10}H_{14}N_2$; Mol. wt. 162.23

2.3 Physicochemical properties

Nicotine is a volatile, strongly alkaline, oily liquid, which when pure is colourless but turns yellow on oxidation. It is very hygroscopic and turns brown on exposure to air or light. It has an acrid burning taste and develops an odour of pyridine. bp_{745} 247° (partial decomposition); volatile with steam; n_D^{20} 1.5282; d_4^{20} 1.0097; $[\alpha]_D^{20}$ −169°. It forms salts with almost any acid and double salts with many metals and acids. Nicotine is miscible with water below 60°. It is very soluble in alcohol, chloroform, ether, petroleum, kerosene and oils.[43]

Nicotine is isolated from tobacco commercially by treatment with alkali and steam distillation or by extraction with solvents.[42,50]

Nicotine undergoes auto-oxidation under the influence of light, oxidation by UV light, platinized asbestos, acids, hydrogen peroxide, potassium permanganate, potassium ferricyanide and silver oxide. It is reduced by sodium/alcohol and platinum oxide — platinum black and hydrogen.[42]

On passage over red-hot porcelain, nicotine partly decomposes (20%) to a gaseous mixture of hydrogen with paraffins and olefins and a liquid product containing pyridine, picoline, collidine and myosmine. Heating at 250–280° C in a sealed tube yields methylamine and nicotinic acid.[42]

2.4 Analytical methods — analysis in biological samples

A wide range of analytical techniques has been used to quantify nicotine and its metabolites in biological fluids, the principal ones being:

(i) gas chromatography
(ii) gas chromatography — mass spectrometry
(iii) high pressure liquid chromatography
(iv) radiochemical methods
(v) radioimmunoassay.

Techniques (i)—(iv) are essentially similar and will be discussed separately from the radioimmunoassay method.

The purely physical methods can be subdivided generally into two distinct stages; an extraction and concentration stage which is common to most techniques and a separation, identification and quantification stage which is specific to the analytical technique to be used.

Nicotine and its metabolites in biological samples are usually extracted from the aqueous phase into organic solvents. The efficiency of extraction is dependent on pH and the partition coefficients of nicotine and its metabolites between the phases. In general, nicotine is extracted alone using ether,[3] while nicotine and cotinine are extracted simultaneously using chloroform or dichloromethane.[20,23]

Available sample volume, nicotine and metabolite concentrations within the sample, and sample origin are important factors for consideration when selecting phase extraction conditions. Solvents used and extraction conditions can influence considerably the co-extraction of contaminant materials from the biological samples. Nicotine and its metabolites have been isolated from blood and urine using ion-exchange chromatography followed by solvent (chloroform:methanol) elution.[53] This technique can be used for bulk samples, but does not necessarily eliminate the problem of co-extraction of contaminating materials. Nicotine and its metabolites are concentrated after extraction into an organic solvent, usually by evaporation to dryness under a stream of nitrogen. Co-extracted compounds are also concentrated at this stage, but careful choice of secondary solvents can reduce the contamination problem. The choice of the appropriate solvent for re-dissolving materials will depend on the separative technique to be used for analysis.

Nicotine and cotinine are usually separated from co-extracted contaminating compounds by using a variety of gas chromatographic conditions. If only nicotine is to be analysed, Apiezon L is reported to be a satisfactory column material.[54] Nicotine and cotinine may be determined using Carbowax, Ucon and SP2250 as gas chromatography column materials.[20,64,140]

The sensitivity of the gas chromatographic method is largely a function of the detector system selected. In general, the following absolute sensitivities for nicotine can be expected: Flame ionization detector, 5—10 ng; nitrogen phosphorus detector, 0.05—0.1 ng; electron capture detector, about 1 pg. Lower sensitivity might reasonably be expected for cotinine.

Various workers have reported problems with gas chromatographic analyses: instability of nicotine and metabolites;[141] failure to resolve co-eluting metabolites.[21] Hence, in developing methodology, it is important to establish absolute recovery levels and, if possible, to incorporate coupled gas chromatography-mass spectrometry analysis to validate the suitability of the selected chromatographic conditions.

Gas chromatographic analysis is probably the most suitable method for routine measurement of nicotine and cotinine. If the method of catalytically converting nicotine-N-1-oxide to cotinine is also included,[33] gas chromatography can be used for the analysis of the major metabolites of nicotine.

For the identification of a greater range of nicotine metabolites, high pressure liquid chromatography (and to a lesser extent thin-layer chromatography) is likely to be of considerable value, particularly if large sample volumes or high concentrations of material are available.

Radiolabelled (^{14}C) nicotine can be used for work on nicotine metabolism and for distribution studies using autoradiography. The initial specific activity of radiolabelled nicotine limits the sensitivity of the method, and in view of the levels of activity involved, this technique is likely to be useful only for animal studies.

Mass spectrometry can be used in conjunction with a range of separative techniques to characterize compounds and to assay sample homogenicity. Recent advances in mass spectrometry technology, particularly the generation of molecular ion species, have significantly increased the sensitivity of this technique.

There are several reports in the literature on the use of radioimmunoassay to estimate nicotine[143, 144] and cotinine[145] in biological samples. Although radioimmunoassay is probably the most direct technique to use, it is an extremely difficult task to develop the technique. Initially, it is necessary to synthesize (and characterize) nicotine (and/or cotinine) — bovine serum albumin conjugates, and then to raise antibodies to these antigens, which have demonstrable low cross reactivity with other metabolites. Cross reactivity of the antisera with additional nicotine metabolites can be investigated, although many nicotine metabolites are not commercially available and require chemical synthesis (mass spectrometric analysis would be required to determine product purity). The cross reactivity of naturally-occurring metabolites is an additional problem and a potential weakness of this assay. A sensitivity of 50 pg/ml for nicotine has been reported.[144] However, this was determined using non-physiological standard solutions and indeed, when plasma solutions were used, containing known amounts of nicotine, there was a distinct change in base line sensitivity. This change in base line sensitivity was also observed in the presence of other nicotine metabolites and other potentially cross-reactive material. These observations, if common to all radioimmunoassays, would cast doubt on the validity of this technique under certain circumstances.

2.5 Nicotine content of tobacco smoke

The burning cone of a cigarette attains a temperature of 800–900°C during puffing and drops to about 600°C between puffs. At the higher temperature zone, pyrolysis occurs and volatiles such as nicotine are vaporized. The temperature of the mainstream smoke as it enters the mouth is dependent on the number of puffs which have been taken, being 20–40°C for earlier puffs and increasing to 40–80°C for later ones, measured immediately the smoke leaves the cigarette.[12]

The particulate phase which accounts for 8% by weight of total mainstream smoke contains mainly organics including nicotine, the remainder of the smoke is made up of gases (nitrogen, oxygen, carbon dioxide, carbon monoxide, etc.) together with volatile organic compounds and water vapour.[12]

In the burning cigarette, typically 15% of the nicotine content is transferred unchanged into the particulate phase of the mainstream smoke, although this proportion varies with a cigarette's parameters and an individual's own smoking characteristics. About 40% is transferred into the sidestream smoke particulates and about 20% is deposited in the cigarette butt, although this latter figure particularly is dependent on the smoker's own characteristics and on particular cigarette parameters such as the efficiency of the filter. The remaining 30% is degraded into various products of pyrolysis,[12] mainly myosmine, bipyridyl and pyridines.[135]

Table 1 shows the reduction in both the tar and nicotine deliveries in cigarettes in various countries over the last 25 years. In the USA, the most popular cigarettes (filter) were reported in 1975 to deliver 15–25 mg tar and 1–1.5 mg nicotine.[12,135]

Cigarettes and cigars deliver smokes which have similar concentrations of nicotine (although cigar smoke is more alkaline) while that of pipe smoke is 2–3 times higher. The nicotine concentration in the smoke of dry cigars or cigarettes is twice that found in the smoke of moist cigars or cigarettes.[12]

Table 1: *Sales-weighted average tar and nicotine deliveries (mg) in cigarettes in various countries*[*12,139]

Year	UK		USA		Austria		Sweden		Canada		West Germany†		Switzerland†	
	T	N	T	N	T	N	T	N	T	N	T	N	T	N
1955			39.7	2.7										
1960			28.6	1.6	35.6	2.2								
1965	31.4	2.1	24.2	1.4	27.0	1.3	30.9	1.75						
1966											20.7	1.18		
1970			21.3	1.4	22.5	0.97	24.0	1.62	21.0	1.5	17.4	1.0	24.3	1.38
1973	18.7	1.4			18.95	0.83								
1974							21.8	1.57	17.5	1.1				
1975			19.2	1.18							14.1	0.66	15.6	0.95
1979			15.75†	1.16†										

Tar = Tar N = Nicotine

* Comparisons between countries are not necessarily valid because of differences in smoking parameters used by them. Some values have been read off graphs and therefore may only be accurate to one decimal place.
† Data by Philip Morris Inc., January 1979.

2.6 Variability of nicotine intake due to differences in smoking patterns

Humans smoke in many different ways. Consequently there are large inter-smoker differences in the proportions of tobacco smoke constituents that end up in mainstream smoke. Similarly there is wide variation between smokers in the proportions of inhaled constituents that are absorbed from the mainstream smoke. In both cases the variation depends on differences in smoking parameters such as duration, size and frequency of puffs, depth of inhalation, retention time, etc. Even one and the same smoker doesn't necessarily smoke in a consistent way: his puffing and inhaling habits may change as he smokes his way down a cigarette or they may be measurably different at different times of the day.

This variability has to be taken into account in the interpretation of data on the pharmacology and toxicology of nicotine where exposure is via the inhalation of tobacco smoke.

2.7 Products of pyrolysis including carcinogens

Nicotine comprises a significant proportion of the tobacco leaf and the products to which it gives rise at high temperatures influence the chemical and possibly the biological properties of cigarette smoke. Table 2 lists the products obtained by pyrolysis of nicotine at 860°C under N_2. The list includes unchanged nicotine (16.4% of pyrolysate) and polycyclic aromatic hydrocarbons but not the carcinogenic dibenzacridines[5] and dibenzcarbazole reported to be present in tobacco smoke and nicotine pyrolysate.[12,135] No benzo(a)pyrene was found present in the pyrolysate.[12]

Table 2: *Products from nicotine pyrolysis (860° under N_2)*[12]

Compound	% of pyrolysate
Basic fraction	
Pyridine	5.30
2-Methylpyridine	0.51
3- and/or 4-Methylpyridine	8.10
3-Vinylpyridine	1.00
2-Cyanopyridine	25.30
2- and/or 4-Cyanopyridine	6.20
Quinoline	7.20
Isoquinoline	1.30
Nicotine	16.40
Benzacridines	
Benzquinolines	
Neutral fraction	
Benzene	0.40
Pyrrole	1.20
Toluene	0.30
Benzonitrile	0.69
2- and/or 4-Cyanopyridine	0.89
Indole	1.20
Skatole	0.25
Styrene	
Indene	
Naphthalene	
Acenaphthene	
Anthracene/phenanthrene	

2.8 Other products in tobacco smoke

2.8.1 N'-Nitrosonornicotine

During curing and fermentation of tobacco, specific nitrosamines can be formed by nitrosation of alkaloids, as evidenced by the identification of N'-nitrosonornicotine,

4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosoanatabine in processed tobacco leaves. The yield of these compounds depends upon the concentrations of nitrate and the alkaloid in the leaf.[135]

N'-Nitrosonornicotine was the first example of a nitrosamine to be found in unburnt tobacco although benzo(a)pyrene[9] was the first organic carcinogen to be identified. The nitrosamine is derived from nornicotine or nicotine and has been detected in US tobacco at levels of 1.9—88.6 ppm, highest levels being found in fine-cut chewing tobacco, snuff and little cigar tobacco.[12, 63] It is present in mainstream cigarette smoke (US Blend non-filter) at 137 ng/cigarette,[12, 62] although the concentration of N'-nitrosonornicotine is only one-tenth to one-hundredth of that in unburnt tobacco.[12]

The 1979 US Surgeon General's report gives the following levels for N'-nitrosonornicotine: 0.3—7 ppm in cigarette tobacco; 3—45 ppm in cigar tobacco, 2—90 ppm in chewing tobacco and snuff; 0.14—3.7 μg/cigarette in mainstream smoke; 3.2—5.5 μg/cigar in mainstream smoke; 1.7—6.1 μg/cigarette in sidestream smoke; 0.9—17 μg/cigar in sidestream smoke.[135]

N'-Nitrosonornicotine may also be formed during tobacco chewing since incubation of tobacco with a high nitrite concentration with human saliva resulted in a 40% increase in N'-nitrosonornicotine concentration (127 ppm) which is 1000 times the level found in mainstream smoke of an average US non-filter cigarette.[12]

N'-Nitrosonornicotine is formed in tobacco, possibly during curing and is more likely to be derived from nicotine than nornicotine.[12] Possibly about 50% of N'-nitrosonornicotine in the smoke originates directly from tobacco while the other 50% is derived by pyrosynthesis of nicotine during smoking.

An 8-fold increase in nornicotine in tobacco resulted in a 50% increase in N'-nitrosonornicotine in smoke whereas doubling the nicotine content in tobacco led to a 3-fold increase in the amount of N'-nitrosonornicotine in the smoke.[62, 85] Thus although N'-nitrosonornicotine can be formed from nicotine and nornicotine, the nicotine/nornicotine ratio in most tobaccos is of the order 15—20:1, suggesting that nicotine is the major precursor of N'-nitrosonornicotine.[62] The transfer rate from cigarette to smoke for nornicotine is 4.1% compared with 12.8% for nicotine.[63] It has been suggested that reduction of nitrate to nitrite by bacteriological or enzymatic action during the curing of tobacco leads to the formation of N'-nitrosonornicotine from nicotine or nornicotine.[63]

N'-Nitrosonornicotine is formed chemically in significant amounts from solutions of nicotine and nitrite at pH greater than 4.4.[62]

2.8.2 Other N-nitroso compounds

Two other nitrosamines, 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and 4-(N-methyl-N-nitrosamino)-4-(3-pyridyl)-butanal (NNA) are produced in comparable amounts during smoke generation by opening of the pyrrolidine ring of nicotine. NNA is not actually found in the smoke because it is a very short-lived species, breaking down to a non-nitrosamine material.[12, 133]

NNK is present at 0.1—0.4 ppm in cigarette tobacco, 2—36 ppm in cigar tobacco, 0.11—0.42 μg/cigarette in mainstream smoke, 1.9—4.2 μg/cigar in mainstream smoke,

0.41–0.6 μg/cigarette in sidestream smoke and 0.8–16 μg/cigar in sidestream smoke.[135]

It is noteworthy that two other nitrosated alkaloids are present in smoke, nitrosanabasine and nitrosoanatabine, but these are not derived from nicotine.[133]

2.8.3 Nicotine N-oxides

Three N-oxidation products can be derived from nicotine and of these nicotine 1'-N-oxide (pyrrolidine oxide) and nicotine 1,1-di-N-oxide but not nicotine 1-N-oxide (pyridine oxide) can exist as diastereoisomers. These compounds and their isomers can easily be distinguished by paper chromatography and NMR spectroscopy. N-oxides are highly polar and of low lipid solubility.[80]

Tobacco leaves contain 0.002–0.141% nicotine-N'-oxide. During smoking it is partly converted to nicotine. Nicotine-N'-oxide has actually been reported not to be present in tobacco smoke.[9]

2.8.4 Cotinine

Cotinine is present in tobacco smoke to the extent of about 57 μg/cigarette in mainstream smoke.[16]

3. METABOLISM

3.1 Absorption

In considering metabolism and particularly absorption, account should be taken of the variability of nicotine intake due to differences in smoking patterns, as discussed earlier (section 2.6, p. 5).

3.1.1 Oral mucosa – importance of smoke pH

Absorption of nicotine in the mouth can vary considerably (4–45%), being dependent on the pH and, of course, the concentration; it is much slower than absorption from inhaled smoke in the lungs.[12]

Nicotine in cigar smoke (pH 8.5) would appear to be more readily absorbed through the mucous membrances of the mouth than is nicotine in cigarette smoke (pH 5.3), probably because of the higher concentration of unionized nicotine in the relatively alkaline cigar smoke. These findings were obtained by following the rise in blood pressure (index of absorption) in anaesthetized cats caused by introduction into the mouth of tobacco smoke from a smoking simulator and of buffered solutions of nicotine.[69]

3.1.2 Inhalation

In so far as it is true that smokers adjust their smoking parameters (i.e. puff size, duration and frequency, depth of inhalation, etc.) so as to get particular pulse doses of nicotine or a particular blood level of nicotine with which they associate satisfaction (see section 10, p. 32), the effects of pH on the rate of absorption of nicotine is obviously crucial. Too deep inhalation of alkaline smoke might lead to acute nicotine

overdosage whilst inadequately deep inhalation of acid smoke might lead to "nicotine-starvation". As a corollary of these considerations, we see that the pH of smoke is likely to determine the amount of smoke vehicle inhaled and the extent of its penetration into the respiratory tract.

Rarely more than 25% and usually only about 15% of the total nicotine content of a cigarette is likely to appear in the mainstream smoke and smokers who inhale may absorb up to 90% of this nicotine. This efficient extraction occurs in the lungs whereby the nicotine enters the pulmonary capillary blood and then, via the arterial system, reaches the brain.[21]

3.1.3 Oral

Intragastric instillation of 1 mg/kg of ^{14}C-nicotine in the cat and rabbit leads to much lower blood nicotine levels than following parenteral administration, as was shown by work carried out at the T.R.C. laboratories in Harrogate in 1974.[4]

3.1.4 Skin

The urinary excretion (up to 89 μg/100 mg creatinine) of cotinine by workers harvesting green tobacco was regarded as evidence that significant nicotine absorption takes place through the skin.[57]

3.2 Blood levels of nicotine

3.2.1 Smoking

The maximum nicotine concentration in the arterial blood ranged from 31 to 41 μg/l in four regular smokers (inhalers) who, following overnight deprivation, smoked a single cigarette labelled with ^{14}C-nicotine over 9–11 minutes; blood samples were taken during and for 50 minutes after smoking.[21, 104] In the same study, one regular smoker (non-inhaler) showed a maximum blood concentration of nicotine of 8 μg/l, compared with 2 and 4 μg/l in two subjects (non-smokers) after smoking one cigarette. Peak concentrations of blood nicotine were usually attained before smoking ceased and blood nicotine fell rapidly after smoking ceased. In one smoker (inhaler), the blood level of cotinine, the major nicotine metabolite, rose soon after smoking and exceeded that of nicotine between 15 and 60 minutes after smoking began.[21, 104]

Plasma levels of nicotine were assayed 3 minutes after completion of a cigarette in a group of 10 regular smokers (inhalers) before and after 5 hours of smoking high nicotine (mean cigarettes smoked 6.7; mean nicotine yield 3.2 mg), low nicotine (smoked 12.5; yield 0.14 mg) or their usual brands (smoked 10.7; yield 1.34 mg). Mean plasma nicotine levels after 5 hours of smoking were 29.2, 8 and 30.1 μg/l in the high nicotine, low nicotine and usual brand groups, respectively, as compared with 24.4 μg/l mid-morning level, i.e. 5 hours earlier. This study concluded that plasma nicotine just after a cigarette is smoked depends more on the way it is smoked than on its nicotine yield or the number of cigarette smoked in the preceding few hours.[101] Serum levels of nicotine in 240 smokers attained a maximum of 73 μg/l.[12]

More recent studies have compared blood nicotine levels with nicotine yields. These have shown that in general smoking a lower nicotine cigarette results in lower blood nicotine levels than those obtained after smoking a higher nicotine cigarette. However, the degree of reduction in blood levels is proportionately considerably lower than the degree of reduction in nicotine yields.[120–122]

3.2.2 Intravenous administration

Repeated injections of 4 μg/kg of ^{14}C-nicotine every 60 seconds for 1 hour results in peak blood nicotine levels of about 100 μg/l in the rat, cat and squirrel monkey but 78 μg/l in the rabbit. (Unpublished work, T.R.C. Laboratories, Harrogate, 1974.[4])

3.2.3 Subcutaneous administration

Following a 0.4 mg/kg dose of ^{14}C-nicotine, blood nicotine levels peaked at 77–100 μg/l at 10–30 minutes after injection into squirrel monkeys, cats, rats and rabbits but the rate of decline was slower than after intravenous injection.[22]

3.2.4 Oral administration

Blood nicotine levels peaked at about 25 μg/l in cats and rabbits and 154 μg/l in rats at 20–60 minutes following oral intubation of 1 mg/kg of ^{14}C-nicotine. Blood cotinine levels were considerably higher in rabbits (373 μg/l) and rats (168 μg/l) than in cats (43 μg/l) and peaked from 40 to >120 minutes after nicotine dosage. (Unpublished work done at T.R.C. Laboratories, Harrogate, 1974.)

Absorption of nicotine after oral administration in dogs is much greater for a nicotine solution of pH 10.2 than for one of pH 7.4.[41]

3.2.5 Multiple intravenous injections to simulate smoking

Two cigarette smokers (inhalers) received 1 mg ^{14}C-nicotine in 10 divided doses at 1 minute intervals. The peak arterial nicotine concentration was 16 μg/l for 1 mg nicotine, compared with 41 μg/l for 1.9 mg nicotine in smoke attained by inhalation. The rate of disappearance of nicotine from arterial blood was slower than when nicotine was given by smoking.[21,104]

Blood levels of nicotine achieved by inhaling cigarette smoke may be mimicked closely by a series of intravenous injections both in man and the cat. Hence in model experiments not involving smoke inhalation the preferred route for administration is by intravenous injection with the subcutaneous route as a reasonable alternative.[22, 58] The intravenous dose of nicotine simulating a puff of tobacco smoke is likely to be between 1 and 2 μg/kg.[58]

3.3 Tissue distribution

Whole-body autoradiography to plot the distribution of radioactivity in mouse, rat and cat tissues after injection of ^{14}C-nicotine indicated a rapid accumulation of ^{14}C in the brain[10] and other tissues, especially the salivary glands, and a gradual increase in radioactivity in gastric juice and stomach contents.[39]

Whole-body autoradiography in several mouse strains treated with ^{14}C-nicotine by parenteral administration or by inhalation revealed accumulation of ^{14}C in the epithelium of the bronchi and nasal mucosa; the metabolites, cotinine and nicotine-1'-N-oxide, did not localize in the bronchial epithelium.[59]

In cats given an intravenous dose of ^{14}C-nicotine, all tissues examined showed a maximum ^{14}C-nicotine content 5 minutes after injection, and thereafter radioactivity declined. Highest levels of ^{14}C-nicotine were seen in the kidney (111 μg/kg), followed

by brain (96.9 μg/kg), liver and stomach (76 μg/kg), adrenals (66 μg/kg) and lung (47.5 μg/kg). Cotinine levels rose slowly over a 2-hour period in most tissues except in the liver where the highest level of 29 μg/kg was recorded after only 15 minutes. Of the total radioactivity present after 5 minutes in either the adrenals, brain, skeletal muscle or stomach, 83–95% was accounted for by ^{14}C-nicotine, compared with only 25% (lungs), 43% (liver) and 57% (kidney). After 2 hours the brain nicotine level is 2–8 times greater than in any of the other tissues.[39] There is evidence of regional concentration of nicotine in the thalamus and hypothalamus.[41]

In experimental animals, nicotine has been shown to cross the placenta into the foetus.[135]

3.4 Biotransformation of nicotine

The pathways of metabolism of nicotine are illustrated in Figure 1.

Metabolism of nicotine occurs principally in the liver. Its main metabolite, cotinine, appears in the blood within a few minutes and significant amounts of other metabolites appear in the tissues after only 5 minutes. Kidney and lung may also contribute slightly to nicotine's metabolism.[10, 34, 39] Selective oxygen availability in tissues as well as plasma nicotine levels may influence nicotine catabolism in experimental animals.[135]

Nicotine 1'-N-oxide has been detected in the urine of smokers[48] and in the urine of cats[25] or rabbits given nicotine. This metabolite is formed *in vitro* by hepatic and lung preparations[32, 34] from several mammalian species. The N-oxide can be reduced back to nicotine *in vivo* (human, oral;[38] rat, intraperitoneal) and *in vitro.* This reduction following oral administration to man is attributed to the action of the gut flora or intestinal enzymes.[38] The urine of cigarette smokers contains both the R,S-cis and especially the S,S-trans diastereoisomers of nicotine 1'-N-oxide; both compounds are present in rabbit tissues *in vivo* and in cat liver and urine.[80]

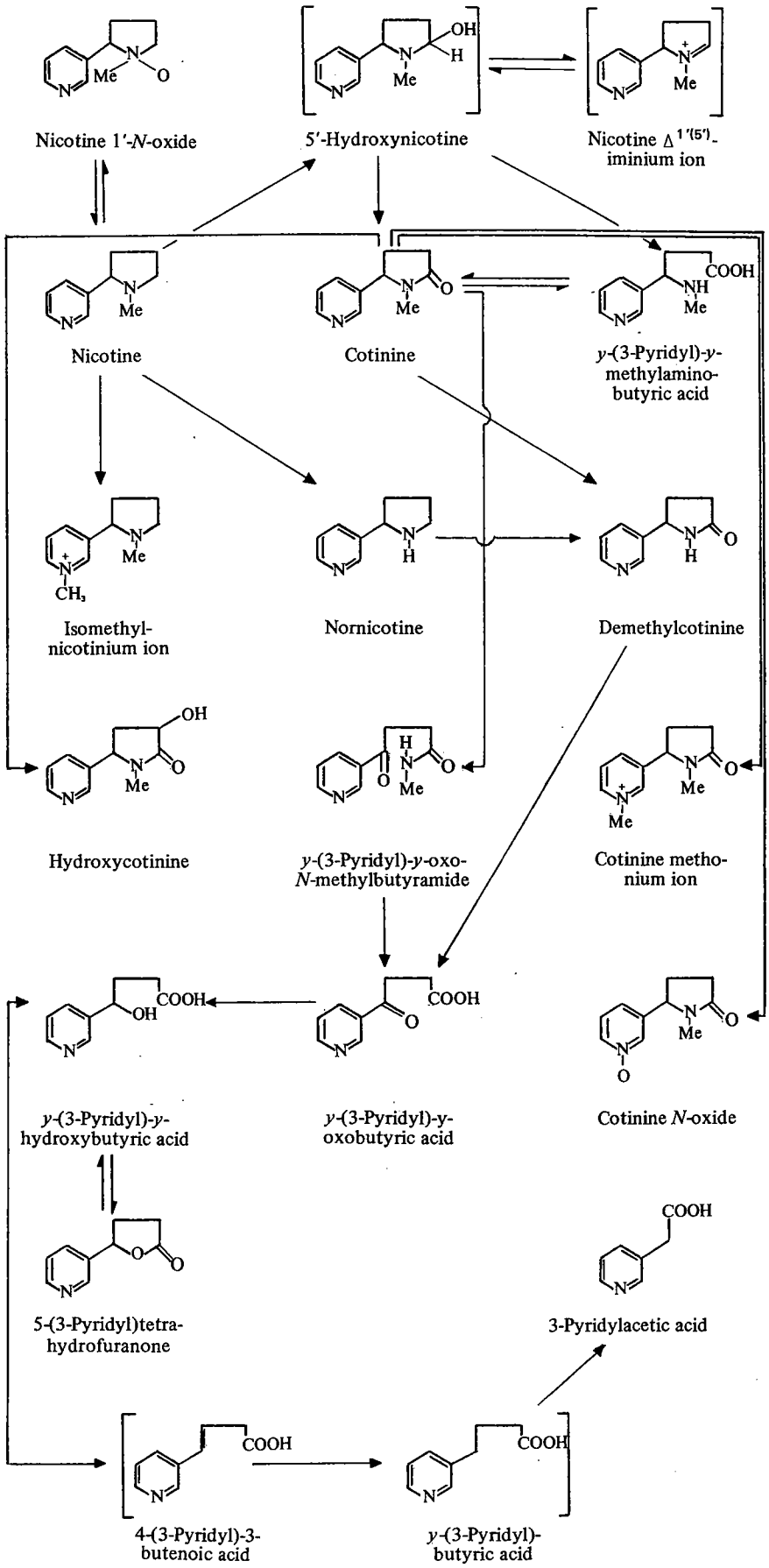
Nornicotine arises by N-demethylation of nicotine, observed both *in vivo* and *in vitro.*[25, 80]

Demethylcotinine is excreted in the urine of cats or man (non-smoker) given nicotine and in the urine of smokers. This metabolite arises via demethylation of cotinine, as has been reported to occur after cotinine administration to dogs, mice and rats but not after cotinine administration to man.[80] Demethylcotinine is also formed *in vitro* from nicotine in isolated perfused dog lung.[34]

Cotinine is the major *in vivo* metabolite of nicotine in most species. It is found in the urine of dogs, rabbits, rats and mice given nicotine[80] and also in the urine of a non-smoker given nicotine orally as well as in smokers' urine.[31, 48] Cotinine is formed *in vitro* from nicotine in liver, lung[34] and kidney tissues of several species including man.[80] The biotransformation of nicotine to cotinine proceeds via two possible intermediates: 5'-hydroxy nicotine and nicotine $\Delta^{1'(5')}$-iminium ion.[30]

Cotinine is rapidly formed from nicotine with significant amounts of the metabolite being formed within 1 minute after intraperitoneal injection of nicotine in mice.[35]

Figure 1: **Pathways of nicotine metabolism**[80]



4-Hydroxycotinine arises by pyrrolidone ring hydroxylation of cotinine and is found in smokers' urine[31] and in the urine of dogs, rats, mice, monkeys and human subjects receiving cotinine. It is also produced from nicotine by mouse liver *in vitro.*[80]

Nicotine isomethonium ion is found in the urine of dogs given nicotine and its formation provides evidence for the *in vivo* direct N-methylation of the pyridine ring of nicotine.[80]

Cotinine methonium ion is found in the urine of dogs and humans given cotinine orally.[80]

γ(3-Pyridyl)-γ-methylaminobutyric acid is believed to arise by cleavage of the pyrrolidone ring of cotinine (or demethylcotinine) through hydrolysis of the amide linkage. It is found in the urine of dogs given nicotine or cotinine, in the urine of rats given cotinine and in the urine of smokers. It re-arranges chemically to give cotinine.[80]

γ-(3-Pyridyl)-γ-oxo-N-methylbutyramide is thought to arise by oxidative 1,2 cleavage of the pyrrolidone ring of cotinine (or demethylcotinine). It is found in the liver and kidneys of mice given ^{14}C-nicotine and in the urine of dogs, rats, mice and humans given either nicotine or cotinine. It is also formed from nicotine *in vitro* in mouse tissue slices. It undergoes further metabolism in the dog and rat to γ-(3-pyridyl)-γ-oxobutyric acid, possibly via oxidative deamination.[80]

3-Pyridylacetic acid is apparently the end product of nicotine metabolism and is found in the urine of cats,[25] dogs, mice and humans given nicotine or cotinine. It arises possibly from γ-(3-pyridyl)-γ-hydroxybutyric acid or from dihydrometanicotine.[80]

Dihydrometanicotine is a urinary metabolite of nicotine in the rat and following its own administration to rats and dogs it gives rise to 4-(3-pyridyl)butyric acid and 3-pyridylacetic acid in the urine.[80]

3.5 Excretion of nicotine and metabolites

Following an intravenous dose of ^{14}C-nicotine to cats, 55% of the dose was excreted in the urine within 24 hours and 70% within 72 hours. Only 0.5% of the dose was excreted in the faeces within 24 hours and negligible amounts thereafter. Only small amounts of unchanged nicotine (1% of dose) and cotinine (0.4% of dose) appeared in the 24-hour urine. Over the pH range of the urine 6.7—7.2 about 10—20% of the nicotine would be present as free base thus facilitating re-absorption from the renal tubules. Over a 4-hour period, only 0.5% of the injected dose was excreted in the bile.[39]

In another study in cats given 20 intravenous injections of ^{14}C-nicotine at 1-minute intervals, 77% of the total dose administered was excreted in the urine at 24 hours and 90% at 72 hours; in the 24-hour urine, unchanged nicotine and cotinine accounted for 2.5% and 0.5% of the dose, respectively. The 24-hour faeces contained only 0.7% of the total ^{14}C administered.[25]

Little or no $^{14}CO_2$ appears in the expired air of rats and dogs given ^{14}C-nicotine.[39]

In human subjects, the 24-hour urinary excretion of nicotine, cotinine, and nicotinine 1'-N-oxide after oral (2 mg) or intravenous (1 mg) administration of nicotine is shown in Table 3. By the oral route, the urinary excretion of unchanged nicotine is pH dependent, that of cotinine slightly pH dependent and that of nicotine-1'-N-oxide independent of pH. The urinary recoveries and excretion profiles of nicotine, cotinine and nicotine-1'-N-oxide after intravenous nicotine and cigarette smoke are similar.[33, 37]

Nicotine 1-N'-oxide is quantitatively excreted unchanged following an intravenous dose.[24]

Table 3: ***Urinary excretion of nicotine, cotinine and nicotine 1'-N-oxide in man after oral and intravenous administration***[33]

Route	Dose	Subject	Urinary pH	Urinary recovery in 24 hours (% of dose)		
				Nicotine	Cotinine	Nicotine 1'-N-oxide
Oral	2 mg	1	Uncontrolled	3.8	6.3	4.1
			Acidic	11.4	9.4	3.6
			Alkaline	0.0	7.9	4.1
		2	Uncontrolled	0.9	5.1	3.0
			Acidic	11.5	6.3	3.0
			Alkaline	–	–	–
Intravenous	1 mg	1	Acidic	34.8	21.6	4.2
		2	Acidic	35.5	20.8	3.8
		3	Acidic	35.4	11.2	3.8

The presence of nicotine in human breast milk (smokers on 20–25 cigarettes daily) was reported as long ago as 1927 and the elimination of nicotine by lactating breasts was confirmed by biological tests (leech bioassay or frog bioassay – skeletal muscle and vagus cell paralysis) in 1931 and 1933. In one subject, about 0.06–0.24 mg nicotine was estimated to be eliminated in her milk in 24 hours.[26]

In 1942, again using biological test (*Daphna magna* paralysis bioassay), the average concentration of nicotine in breast milk of occasional (1–4 cigarettes), moderate (5–10 cigarettes) and heavy (11–20 cigarettes) smokers was reported as 0.116–0.16, 0.225–0.278 and 0.445–0.5 mg/l and none was detected in the breast milk of non-smokers. The main excretion of nicotine appears in the milk and urine 4–5 hours after smoking. About 11–17 times as much nicotine was excreted in the urine as in breast milk.[27]

More recently, gas-chromatographic analysis revealed an average of 0.091 mg/l (range <0.020–0.512 mg/l) in 28 samples of breast milk from 9 smokers (mostly moderate, 0.5–1.5 packs per day) but none was found in 6 samples from 6 non-smokers. These results were remarkably similar to those obtained by the earlier bioassay methods. Large fluctuation in the nicotine content in the milk of a single donor makes it difficult to estimate an infant's oral intake of nicotine.[28]

The nicotine concentration in breast milk is proportional to the number of cigarettes smoked.[135] However, it is important to note (i) that no evidence is available to suggest that nicotine is present in human breast milk at concentrations sufficient to impair the development of the nursing infant and (ii) that ***in utero*** exposure to nicotine resulting from maternal smoking is not associated with an increased risk of developing congenital malformations[135] (see also section 8.2, p. 27).

3.6 Enzyme induction/inhibition

The effect of nicotine on drug-metabolizing enzyme activity is complex and the conflicting results obtained reflect differences in species, dosage, duration of dosage and stress-induced effects.[80,135]

For example, nicotine pretreatment brought about a rise in drug-metabolizing activity in rabbits but not in a similarly-conducted study in dogs. In rats, nicotine pretreatment decreased activity in one experiment and was without effect in another experiment. Lower dosage over a prolonged period slightly increased hepatic metabolizing activity but increased by 100% activity in the lungs, kidneys and brain.[80]

Some experiments suggest that whilst acute high administration produces inhibition of metabolism, lower daily doses of chronic administration will elicit enzyme induction. Thus a single dose of nicotine (5—40 mg/kg) caused induction of acetylaminofluorene and benzopyrene hydroxylases[111] but pretreatment with 40 mg/kg for 2 or 3 days caused a reduction in enzyme activity. Similarly whilst 3 daily injections of 5 mg/kg of nicotine into mice caused a 50% decrease in nicotine metabolism, nicotine administration in the drinking water (24—27 mg/kg for 10—17 days) hardly affected nicotine metabolism.[135]

On the other hand, high daily doses of nicotine (16 mg/kg for 3 days) enhanced hepatic ethylmorphine and norcodeine metabolism whilst this effect was not maintained following administration in the drinking water if the nicotine level fell below 4.4 mg/kg/day for 7 days, suggesting that the increased activity is a stress-related event.[80]

Differences have been observed in nicotine metabolism between smokers and non-smokers. Thus following an intravenous injection of nicotine, one group of 6 male smokers exhibited a lower urinary output of both nicotine over 12 hours and of cotinine over 24 hours, as compared with 10 male non-smokers whilst another group of 8 male smokers showed a similar nicotine output but a higher cotinine output than the 10 male non-smokers. In contrast, 5 female smokers excreted less nicotine but more cotinine than 4 female non-smokers but the combined output of nicotine and cotinine was higher in female smokers than female non-smokers. The fact that male non-smokers excreted less nicotine but more cotinine than female non-smokers may represent an important sex difference in nicotine metabolism in man. A similar comparison of nicotine metabolism in male and female smokers is complicated by the apparent metabolic changes induced by smoking.[36,80]

Evidence of induction of nicotine-metabolizing enzymes in man by tobacco smoking has been presented, based on unchanged nicotine output in the urine following intravenous injection of nicotine or inhalation of nicotine vapour and smoking.[29]

Tobacco smoke is known to inhibit various enzyme systems including dehydrogenases and an oxygenase and a component of tobacco smoke other than nicotine may well interfere with the metabolism of nicotine or its metabolites.[80] The smoking process is thought to inhibit nicotine metabolism in the lungs.[59]

Various drugs including enzyme inducers are capable of altering nicotine metabolism. Thus phenobarbital pretreatment in mice enhanced ^{14}C-nicotine metabolism ***in vivo*** (conversion to cotinine) and ***in vitro*** (conversion to cotinine and increased $^{14}CO_2$) and also stimulated the breakdown of cotinine. In addition, phenobarbital pretreatment reduced the uptake of nicotine in the brain and liver following intraperitoneal administration of nicotine.[35]

Pretreatment of rats with 2-acetamidofluorene, 3-methylcholanthrene or benzo(a)-pyrene enhanced nicotine metabolism (elevated nicotine oxidase activity in rat-liver microsomes).[102]

4. PHARMACOLOGY

4.1 General

Apart from toxicological considerations, the pharmacology of nicotine in doses similar to those absorbed from tobacco smoke inhalation is also relevant to the evaluation of any health hazard. Nicotine is regarded as the most pharmacologically-active compound in tobacco smoke.[12, 108, 135]

The pharmacological effects of nicotine at doses absorbed by inhalers (i.e. 1 mg approx. per cigarette) are comparatively small. The response at any one time represents a summation of stimulant and depressant actions from direct, reflex and chemical mediator influences on several organs. The main actions are central stimulation and/or depression (which vary with the individual), transient hyperpnoea, peripheral vasoconstriction (usually associated with a rise in systolic pressure), suppression of appetite, stimulation of peristalsis and, at larger nicotine intakes, nausea of central origin, associated with vomiting.[4–6]

4.2 Nervous system

Nicotine acts directly on the sympathetic and parasympathetic ganglion cells, manifest as transient excitation (facilitating transmission of impulses) followed by depression (blockade of transmission) or even paralysis at effective doses.[5] The effects of nicotine on the neuromuscular junction are similar to those on the ganglion cells.[106]

In the central nervous system, as in ganglia, primary stimulation is succeeded by depression. Also nicotine, like acetylcholine, discharges adrenaline from the adrenal glands and other chromaffin tissue. Nicotine releases noradrenaline from the hypothalamus.[68, 75] It also releases antidiuretic hormone from the pituitary[105] by stimulating the supraoptico-hypophyseal system. By excitation of chemoreceptors in the carotid body, nicotine also augments various reflexes.[5]

Its action on central nervous system functions in both animals and man includes increased respiratory, vasomotor and emetic activity at moderate doses and tremors and convulsions at high doses. Amounts absorbed from heavy smoking may produce transient hyperpnoea through carotid and aortic arch reflexes. Increased blood pressure is partly central in origin. EEG arousal patterns have been observed in various mammalian species given nicotine at 10–20 μg/kg, a dose level commonly attained in man by smoking.[5] Regional blood flow in the cerebral cortex is enhanced by nicotine and tobacco smoke in cats.[66]

The Annals of the New York Academy of Sciences devoted a whole volume in 1967 to the effects of nicotine on the central nervous system.[10]

4.3 Cardiovascular system

Nicotine possesses a very pronounced cardiovascular action which in general is due to stimulation of sympathetic ganglia and of the adrenal medulla, coupled with the

discharge of catecholamines from sympathetic nerve endings and chromaffin tissues of various organs. Contributing to the sympathomimetic response is the activation of chemoreceptors of the aortic and carotid bodies.[106]

Because nicotine has both stimulant and depressant phases of action,[115] the ultimate response of any one system represents the summation of several different and opposing effects of nicotine. Thus nicotine can increase heart rate by excitation of sympathetic or paralysis of parasympathetic cardiac ganglia or can decrease heart rate by paralysis of sympathetic or stimulation of parasympathetic cardiac ganglia.[106] A dose of 1 mg nicotine is deemed insufficient to stimulate heart rate and to elevate blood pressure.[112]

Under experimental conditions, low doses of nicotine stimulate the ganglia whereas high doses can paralyse them; however, during smoking by humans stimulation occurs and it is unlikely that levels of nicotine achieved during smoking would result in paralysis. The release of catecholamines and other biogenic amines during smoking[49] and the dose-related increase in pulse rate and blood pressure from cigarette smoking[56] provide evidence that nicotine is absorbed in pharmacologically-active amounts in the human smoker. Non-inhalers do not absorb nicotine fast enough to achieve pharmacologically-active levels.[49]

Normal subjects respond to nicotine in the form of increases in heart rate, cardiac output, blood pressure and coronary blood flow, the increase in the latter being proportional to the nicotine-induced increase in myocardial oxygen demand. However in animals[100] with damaged myocardium and in humans with coronary heart disease, nicotine may not elicit an increase in myocardial oxygen consumption or in coronary blood flow. Thus patients with coronary heart disease may not respond to nicotine in tobacco smoke in the same way as normal individuals and cardiac output may fall.[12]

Reviews of the effects of nicotine on the cardiovascular system appeared in 1960[14] and in 1979.[142]

4.4 Gastro-intestinal tract

The effects of nicotine on the gastro-intestinal tract are largely due to parasympathetic stimulation.[106] The reduction of appetite by smoking has been attributed both to direct effects on gastric secretion and motility and to reflexes arising from local effects on the taste buds and mucous membranes in the mouth. Effects on the hypothalamus, similar to amphetamine-induced depression of appetite, and psychological mechanisms may also be involved. Nausea and vomiting are the most common symptoms related to the gastro-intestinal tract and probably originate centrally in the medullary emetic chemoreceptor trigger zone. Nicotine stimulates peristalsis, probably involving local, central and reflex actions. Nicotine tends to augment motility in the colon in smokers, possibly due to the action on the parasympathetic ganglia in the bowel. Excessive smoking may be associated with diarrhoea or constipation.[5] Study of the effects of nicotine on gastro-intestinal function has gained importance in view of the association between smoking and peptic ulceration (see section 9.4, p. 31).

4.5 Immunology of nicotine

Nicotine is not the responsible antigenic component of tobacco leaf although the possibility that nicotine can act as a hapten cannot be excluded.[7,135]

4.6 Pharmacology of nicotine metabolites and analogues

Cotinine shows no remarkable pharmacological activity.[16]

Nicotine N-oxide is 50–100 times less active than nicotine in the blockade of stimulation of the vagus and visceral nerves and 70 times less active than nicotine in its action on the isolated guinea-pig intestine.[16]

Nicotine was invariably far more potent than nornicotine, metanicotine, nicotyrine, dihydrometanicotine, cotinine and nornicotyrine in comparative pharmacological studies on the following systems: contraction of guinea-pig ileum; pressor action in pithed rat; release of catecholamines from cat adrenals; contraction of frog rectus; blockade of contraction of guinea-pig diaphragm; inhibition of cat knee-jerk and flexor reflexes; inhibition of chick flexor reflex; inhibition of chick crossed extensor reflex.[18,135]

Cotinine and demethylcotinine exert a nicotine-like effect on isolated smooth muscle.[18]

Nicotine-like actions of cis- and trans-metanicotine have been observed using isolated rabbit aortic strips and ileal segments.[109]

4.7 Tolerance to nicotine

By definition, tolerance is manifested by a decreasing response to repeated administration of the same dose of a drug or by the requirement for increasing doses in order to produce the same response. Tolerance to nicotine can develop. Thus initial exposure may result in sweating, nausea, vomiting, etc., but the effects disappear after repeated exposure and this period of adjustment allows for a higher nicotine intake. Tolerance to the depressant activity of nicotine in rats has been demonstrated. The metabolic type of tolerance occurs and smokers metabolize nicotine faster than non-smokers.[40] Specific organ tolerance to nicotine is relatively low grade and comparatively short-lived and the tolerance which permits administration of nicotine in doses several fold greater than those producing toxic signs and symptoms initially varies with age, sex and duration of exposure.[5,135]

Table 4: *Acute toxicity of nicotine in various species*

Species	Route	LD_{50} dose (mg/kg)	LDL_0 dose (mg/kg)	Reference
Mouse	Oral	24		50
		24–35		16
		3.3		45
	Subcutaneous	16		50
		16–45		16
			16	45
	Intravenous	7.1		50
		0.8		45; 16
		0.55		16
		0.7		18
	Intraperitoneal	63.5		50
		5.9		45
		9.99–11.77		16
Rat	Oral	50–60		50
		188		16
	Skin	140		45
	Intravenous	2.8		16
			1	45; 16
	Intraperitoneal		20–23.5	16
		34–39		17
	Parenteral		34	45
	Subcutaneous	36.5–48	1.5	16
Rabbit	Intravenous	9.4		50
		5.9–9	12	16
	Dermal	50		50
	Subcutaneous		1.8	16
		30		16
	Intraperitoneal	14		16
Dog	Intravenous	5		50; 16
	Oral	9.2		45
	Intramuscular		7.7	45
	Parenteral		5.7	45
Cat	Intravenous	2		50
Guinea-pig	Subcutaneous		15	45
		26		16
	Intraperitoneal		32–33	16
	Intravenous		4.5	16
Pigeon	Oral	75		45
Duck	Oral	75		45
Monkey	Intramuscular		6	16

5. TOXICITY

5.1 Acute toxicity in animals

The acute LD_{50} and LDL_0 values of nicotine in various species by different routes of administration are shown in Table 4. Nicotine has a very high acute toxicity and acts with the speed of cyanide, producing death in minutes. Fractionating the dose with respect to time decreases acute toxicity in cats and mice.[16]

The major effects are transient stimulation followed by depression and paralysis of the central and peripheral nervous systems and skeletal muscle nerve endings with rapid death often due to paralysis of the respiratory muscles.[50]

Nicotine is locally irritating. It is absorbed rapidly through the skin.[50]

Cilia toxicity is possibly important since the loss of cilia is a feature of the metaplastic change in the epithelium (ciliated columnar to squamous) which is thought by some to be a step in the direction of malignancy. Under *in vitro* conditions,[9] a solution of nicotine at a concentration found in cigarette smoke was found to be without cilia toxicity although a 2% solution caused cessation of ciliary movement in excised respiratory epithelium from rats and rabbits within 5–10 minutes. In the light of subsequent developments in methodology it is not certain whether the *in vitro* studies referred to above are reliably indicative of the *in vivo* effects of nicotine.

Neither nicotine nor its pyrolysis products contribute significantly to the oedema-producing properties of cigarette smoke although nicotine may increase the sensations of irritation, as evidenced by rabbit eye experiments.[97]

5.2 Acute toxicity in man

The toxic signs and symptoms observed in man are quite varied in view of the complex and phasic pharmacological actions of nicotine. The commonest effects found in moderate intoxication include nausea, vomiting, abdominal pain, diarrhoea, headache, sweating, palpitation and fatigue. More severe signs and symptoms include faintness, dizziness, weakness and confusion, progressing to prostration with increasing tachycardia, dyspnoea, coma, convulsive seizures and respiratory arrest.[17, 50]

Most deaths occur within a few minutes of ingestion and recovery usually occurs if survival exceeds 1–4 hours. Approximately 60 mg nicotine orally is estimated to be lethal to most adults.[16, 50]

Autopsy findings include oedema and hyperaemia in the lungs, oedema of the brain, swelling and erosion of the inner surface of lips and mucous membranes of the throat, oesophagus and stomach.[16–18] In 34 cases of acute nicotine poisoning in Hungary, post-mortem examination revealed the following: mobilization of liver cells, loosening of alveolar epithelium due to pulmonary oedema, renal necrosis and large changes caused by concentration of the myofibrillae of the cardiac muscle.[19] There appear to be no characteristic gross or microscopic changes in the tissues of persons dying from acute nicotine poisoning.[16, 18]

Many cases of fatal nicotine intoxication have occurred from accidental or suicidal ingestion of nicotine insecticides. In the USA, 288 such deaths were reported between 1930–1934.[16, 50] Cases of poisoning have also arisen from swallowing chewing-tobacco or tobacco juice.[16]

Although about 60 mg as a single dose is deemed fatal to man, a dose of 30–50 mg nicotine taken in the drinking water over 24 hours produces only symptoms of intoxication and it has been estimated that "up to 280 mg nicotine/day can be taken in the form of tobacco without harm".[16]

Following an oral dose, toxic effects were elicited by 1–2 mg in non-smokers but 5–10 mg produced virtually no symptoms in heavy smokers. Subcutaneously, a dose of 1.5 mg is tolerated by non-smokers compared with 6 mg by smokers. Intravenously, a dose of 0.6 mg resulted in great individual variability in the symptoms elicited. Dermally, 3 g for a single exposure or 2.4 g/day for multiple exposures were estimated to be dangerous.[16]

Toxic signs and symptoms followed intravenous injection of 1 mg/minute of nicotine for 3 minutes.[17]

5.3 Chronic toxicity in animals

Although numerous studies have been conducted on the effects of chronic exposure to nicotine in experimental animals, no formal lifespan dose-response study with full histology of a wide range of organs appears to have been published.

In 1964, the Report of the Advisory Committee to the Surgeon General of the Public Health Service in the USA concluded that most of the chronic studies on nicotine were poorly designed and controlled and were of little value for extrapolation to man. For example, even in the most relevant studies, the daily dose of nicotine was close to the maximum tolerated dose (just subconvulsive), i.e. far in excess of that encountered in human smoking exposure. Whilst weight loss and degenerative vascular changes were seen in rats and dogs[72] under these extreme conditions, weight loss but no histological change was found by other workers. In lifespan studies in rats exposed to tobacco smoke at levels simulating human exposure, very little systemic toxicity was noted. Despite the inadequacy in the experimental data, it was felt that chronic systemic toxicity is quite low in small to moderate dosage.[5]

An authoritative review in 1961 also drew attention to the limitations of chronic toxicity data in animals – either the doses used were too low to detect untoward effects or were so high as to produce manifestations of acute toxicity and eventually death through non-specific cachexia.[16]

Excellent summaries of chronic toxicity experiments in mammals (mainly in mice, rats, rabbits, guinea-pigs and hamsters) have appeared over 1961–1975.[16–19] Whilst negative gross and histological findings have been obtained in various organs under different experimental conditions (usually involving oral administration or injections) it is instructive to list effects that have been reported as follows: growth retardation or weight loss; adrenal enlargement; changes in the conjunctiva, retina and optic nerve; degenerative changes in the pituitary, thyroid, and various components of the nervous system.

Pulmonary exposure to inhaled cigarette smoke or to injected nicotine in dogs can result in pulmonary vasoconstriction, causing increased pulmonary arterial pressure; this effect is possibly due to histamine release from lung tissue.[2]

Presumably in relation to the cardiovascular system, it has been claimed that there are no recognized chronic or cumulative specific physiological or histological effects of nicotine in experimental animals.[18]

5.4 Chronic poisoning in man

The chronic toxicity of nicotine has been mainly studied in connection with the use of tobacco. Effects linked to chronic nicotine absorption in smokers include gastro-intestinal symptoms, disturbance in heart rhythm, vasoconstriction and occasional visual impairment.[50]

The chronic toxicity of nicotine in amounts normally derived from tobacco is considered to be very low.[18,19]

A threshold limit value of 0.5 mg/m^3 for occupational inhalation exposure to nicotine has been set in the USA; even though the air concentration may be below the limit value, significant additional exposure to the skin may be dangerous.[45]

5.5 Acute and chronic toxicity of pyrolysed nicotine

From acute and chronic studies in mice and rats, the pyroformed bases, resulting from the pyrolysis of nicotine, are distinctly less toxic than nicotine.[16]

5.6 Toxicity of metabolites of nicotine and related compounds

When tested by subcutaneous injections in mice, nicotine is 50 times as toxic as cotinine and 20 times as toxic as nicotine-1'-oxide.[32] Nicotine is also considerably more acutely toxic than nicotine-N-oxides by the intravenous, subcutaneous and oral routes in mice (Table 5). Nornicotine has the same high order of toxicity as nicotine (Table 5).

N'-Nitrosonornicotine, which has a subcutaneous LD_{50} in rats of more than 1g/kg, produces haemorrhages in the lungs and abdominal organs and epithelial-cell necrosis in the posterior nasal cavities and liver.[79]

5.7 Effect of drugs and other agents on nicotine toxicity

Various studies in animals have explored the influence of drugs (cerebral or psychic stimulants, central nervous system depressants, local anaesthetics, autonomic drugs), vitamins, hormones and miscellaneous agents on the acute toxicity of nicotine.

Thus the acute toxicity of nicotine can be antagonized by spasmolytic and ganglion-blocking agents, local anaesthetics and central nervous system depressants (e.g. chlorobutanol, phenobarbital) and it appears that the anti-nicotine effects of these

Table 5: *Acute toxicity of nicotine metabolites and related compounds in various species*

Species	Route	LD_{50} dose (mg/kg)	LDL_0 dose (mg/kg)	Reference
		Nornicotine		
Mouse	Intraperitoneal	14.66–21.7		16
	Intravenous	3.4		16
Dog	Intravenous	4.0		16
Rabbit	Intravenous	3.0		16
	Intraperitoneal	>13.7		16
		Nicotine N-oxide		
Mouse	Subcutaneous	940		16
		Nicotine 1-N-oxide		
Mouse	Intravenous	24.1		17
	Subcutaneous	295		17
	Oral	295		17
		Nicotine 1'-N-oxide		
Mouse	Intravenous	60		17
	Subcutaneous	730		17
	Oral	195		17
		Nicotine 1,1'-di-N-oxide		
Mouse	Intravenous	1450		17
	Subcutaneous	1650		17
	Oral	225		17

compounds is related to various mechanisms involved at the different sites of action of nicotine.[16] Certain vitamins (e.g. B_1) and hormones (e.g. luteohormone) also antagonize nicotine toxicity.[16,17]

Some drugs (e.g. reserpine) can influence nicotine toxicity in either direction in that 0.1 mg/kg reserpine raised the LD_{50} of nicotine, 1 mg/kg had little effect but 3 mg/kg decreased the LD_{50}.[17]

Various anticonvulsant, tranquillizing, antihistaminic, parasympatholytic, sympatholytic, ganglion blocking and nicotinolytic drugs were investigated for protection against nicotine-induced tremor, clonic and tonic convulsions and death in mice. The most effective drugs affording protection against death were tranquillizers such as reserpine, perphenazine, chlorpromazine and diethazine.[18]

Nicotine-induced toxicity was antagonized by α-2-amino-1-methylethyl(benzhydrol HCl), a potent CNS-active agent, but not by nialamide. Other drugs and agents such as 6-azauracil, 6-azauridine, γ-butyrolactone, certain diphenylalkylamine derivatives and hepzidine maleate were also protective.[18]

Drugs acting on various sites of the autonomic nervous system can also afford protection against the risk of death from nicotine. These include: pempidine, phencarbamide, phentolamine, pronethalol, idrobutamine, propranolol, compounds RP 904 and RP 2222, tetraethylammonium, trihexyphenidyl and Mydeton. Conflicting results have been obtained with hexamethonium. Atropine and scopalamine had little protective effect.[18]

Phenobarbital pretreatment in mice raised the intraperitoneal LD_{50} from 14 to 34 mg/kg and also increased tolerance to repeated sublethal doses. Phenobarbital pretreatment had no effect on the intravenous LD_{50} of nicotine in mice.[35] Lethal effects of nicotine can be blocked by a nicotine metabolite, possibly 5'-hydroxynicotine or nicotine $\Delta^{1'(5')}$iminium ion.[30,129]

5.8 Effect of nicotine on toxicity of other drugs and agents

Nicotine pretreatment increased adrenaline-induced mortality in mice when given orally at 6 mg/kg[16] but afforded protection when given intravenously at 1–4 mg in rabbits.[17] Nicotine pretreatment however failed to protect against the lethal effects of various drugs in mice.[19] Nicotine antagonized the toxicity of an intravenous infusion of potassium chloride in dogs.[19] Nicotine augments the toxicity of procaine in dogs but afforded protection against parathion.[16]

6. CARCINOGENICITY

6.1 Experimental studies

Early indications of a possible relationship between smoking and cancer prompted a search for carcinogens in tobacco, tobacco smoke and smoke condensates. Tests of

nicotine and its metabolites in animals have been undertaken and the findings are summarized below.

6.1.1 Nicotine

6.1.1.1 Oral route: CBA mice treated orally with commercial nicotine showed no increase in lung tumour incidence although many animals survived over 2 years of treatment.[16]

An oral study on nicotine hydrochloride in Swiss mice is in progress.[119]

In 1936 long-term feeding of nicotine to rats was reported to produce adrenal adenomas[77,92,94] but in another study no tumours were observed.[94]

Nicotine was given in the drinking water providing a dose of 10 mg/kg body weight/day to 45 Wistar rats for 19 months; one animal died at 15 months and showed malignant tumours in the liver and intestines.[76] Since the authors do not mention the fate of the remaining 44 animals, it is to be presumed that none of these animals developed tumours.

Nicotine, administered in increasing doses (0.17–0.45 mg/mouse) in the drinking water of mice starting 1 day before intraperitoneal injections of urethane, showed no co-carcinogenic activity with urethane after 18 weeks, the incidence of pulmonary adenomas being comparable with the controls given urethane alone.[16]

A study in mice exposed to UV light for 21 weeks and given increasing doses of nicotine in the drinking water (0.07 to 0.45 mg/mouse) showed no additive or co-carcinogenic effect of nicotine with UV light.[16]

6.1.1.2 Skin route: CBA mice treated with commercial nicotine by skin application showed no increase in lung tumour incidence, although many animals survived over 2 years of treatment.[16]

No difference was found in mouse skin carcinogenicity between smoke-condensate with normal tar to nicotine ratio and condensate from filter tip cigarettes with reduced tar to nicotine ratio.[19]

Nicotine, when added at 1.5–6 mg/ml to solutions containing benzo(a)pyrene (10 μg/ml) and 12-O-tetradecanoylphorbol-13-acetate (0.6 μg/ml), increased mouse skin tumour incidence from 37% (nicotine absent) to 68–80% (nicotine present) at 37 weeks; female ICR Swiss mice were painted 10 times weekly with 0.2 ml of the solutions. The results were taken to provide evidence of nicotine's co-carcinogenic potential.[93] Similar results were obtained using benzo(a)pyrene (5 μg/ml) instead of the mixture and according to Bock a mixture of 10 μg/ml benzo(a)pyrene and 0.6 μg/ml 12-O-tetradecanoylphorbol-13-acetate is equivalent in terms of tumour response to 30% cigarette smoke condensate solution (i.e. 0.2 ml applied 10 times weekly).[81]

In an unpublished mouse-skin painting experiment, carried out at T.R.C. Laboratories, animals received thrice-weekly applications of 19 μg benzo(a)pyrene with/without 2.5–10.0 mg nicotine (as tartrate). Up to 68 weeks of treatment, the skin tumour incidence was significantly lower in the nicotine/benzo(a)pyrene group than in the

benzo(a)pyrene group but after 68 weeks nicotine had no effect on tumour incidence. Statistical analysis of the reduction in tumour incidence up to 68 weeks showed that the reduction was statistically significant up to 44 weeks but not statistically significant between 44 and 68 weeks. The tumour-inhibitory effect was dose-related. Statistical analysis of a similar experiment showed that nicotine (as tartrate) had no significant effect on skin tumorigenesis by the neutral or hydrocarbon fraction of smoke condensate.

Removal of nicotine from smoke condensate did not diminish the mouse skin tumour response to the condensate.[5] However this early experiment is regarded as inadequate to have demonstrated an effect in either direction.[82]

More convincing evidence of a lack of mouse skin tumorigenic potential stems from more recent fractionation experiments. These show that virtually all the tumorigenic activity of whole smoke condensate remains in the cyclohexane fraction G, all nicotine alkaloids having been removed in the water-soluble fraction B and the aqueous methanol fraction F.[123]

Despite suggestions in the USA of a strong correlation between the mouse skin carcinogenicity of certain fractions of smoke condensates and their nicotine contents,[12] analysis of mouse skin painting experiments carried out at the T.R.C. Laboratories in Harrogate in 1975, concluded that the carcinogenicity of smoke condensate would appear to depend more on smoking parameters (i.e. puff frequency, volume and duration) than on the yields of nicotine or condensate from particular cigarettes. The smoking parameters may be such that they lead not only to higher yields of nicotine but also to more carcinogenic pyrolytic products.

6.1.1.3 Inhalation route: There is need to treat with caution the claim that mice exposed to the smoke of cigarettes containing 2.09% nicotine showed a higher incidence of lung tumours compared with mice exposed to cigarette smoke containing 0.78% nicotine.[16] Specific methods of cigarette smoke exposure are not detailed and were not controlled from the point of view of dosimetry. Moreover, the smoke of cigarettes which differed in nicotine yield almost certainly differed in tar yield.

6.1.1.4 Intraperitoneal route: Nicotine was given at 2 mg/kg/week intraperitoneally to 67 Sprague-Dawley rats but no evidence of carcinogenicity was obtained; animals survived 85 weeks compared with 95 weeks in controls.[61]

6.1.1.5 Subcutaneous route: Subcutaneous administration of nicotine (1 mg/kg, 3 times daily) did not affect the onset or growth of benzo(a)pyrene-induced tumours either in control or in "immunosympathectomized" mice.[19]

Pretreatment of newborn mice with 3 daily subcutaneous injections of 0.5 mg/kg nicotine delayed the onset and reduced the incidence of benzo(a)pyrene-induced tumours. The rate of growth of tumour, once developed, was however increased by nicotine treatment.[19]

6.1.1.6 Ames test for mutagenicity: The Ames test is used as a short predictive test for carcinogenicity. Nicotine proved negative in the Ames Salmonella/liver microsome test.[86]

6.1.2 Oxidized nicotine

Application of a crude preparation of oxidized nicotine to the skin of 14 mice of a mixed stock resulted in lung adenomas in 7 animals. These findings have been taken to be of doubtful significance and further study in a different mouse strain is recommended.[16]

Twice-weekly skin painting of a 0.8% solution of oxidized nicotine in acetone to 61 mice for 10 months resulted in no adenomas; 3 mice died over this period.[16]

6.1.3 Nicotine pyrolysate

Thirty Wistar rats received for 2 years drinking water containing a rapidly-pyrolysed preparation of nicotine (combustion temperature 700°C), equivalent to a dose of 10 mg/kg body weight/day; 3 animals developed intestinal sarcomas.[76] A basic fraction of the pyrolysate, when administered in the drinking water, also produced malignant intestinal tumours (embryonic carcinoma) in 3/45 rats.[76] No tumours were seen in controls in either study.

Subcutaneous injections of nicotine pyrolysate (5 mg/kg) given weekly to rats failed to produce lesions.[17]

6.1.4 Cotinine

In 1964, Truhaut and his colleagues[73] reported that administration of cotinine at 0.05% in the drinking water (daily dose 30 mg/kg; total dose 7–10 g/kg) for 8–11 months to Wistar rats resulted in malignant tumour development. 12/15 rats which died between 8 and 18 months from the start of treatment showed malignant tumours at death mainly lymphoreticular sarcomas in the large intestinal wall – no tumours were seen in controls.

The French claim was disputed by Schmähl and his colleauges in 1968.[83] Wistar rats (BR46 strain) received cotinine at 0.05% in the drinking water for up to 2 years (average daily dose 30 mg/kg; total dose 19 g/kg over average survival period of 630 days). Tumour incidence was in the range of controls and cotinine was deemed non-carcinogenic to rats despite the fact that the total dosage was twice that used by French workers who claimed cotinine to be carcinogenic in rats.

Reference has been made to tumour-induction following bladder implantation of cotinine-containing pellets into mice and following treatment of mice with cotinine during early neonatal life but no reference was given to the original studies.[41] (The validity of the bladder implantation test for assessing carcinogenicity has been questioned in view of the number of false positive results obtained by this method.)

6.1.5 Nicotine-N-oxide

It has been suggested that the R- and S-stereoisomers of nicotine 1'-N-oxide, nicotine metabolites produced in the lungs and liver and excreted in the urine, should be tested for carcinogenicity because they are similar to the 7-N-oxides of xanthine and guanine claimed to be carcinogenic on injection into Wistar rats.[41,84]

Nicotine N-oxide and its pyrolysis product (2-methyl-6(3-pyridyl)tetrahydro-1,1-oxazine) were tested for co-carcinogenicity – the compounds were given in the drinking water of mice in increasing doses starting 1 day before intraperitoneal injections of urethane but no co-carinogenic action was apparent after 18 weeks, the

incidence of pulmonary adenomas being comparable with the control groups given urethane alone.[16]

Mice exposed to UV light for 21 weeks and receiving increasing doses (0.3–1.4 mg/mouse) of nicotine N-oxide in the drinking water showed no evidence of additive or co-carcinogenic action in 30 weeks on test.[16]

6.1.6 N'-Nitrosonornicotine

Weekly intraperitoneal injections of 0.1 ml of 2% N'-nitrosonornicotine in arachis oil for 41 weeks to Chester-Beatty mice resulted in multiple pulmonary adenomas in 7/8 mice surviving over 8 months.[17] These results were confirmed in another study.[79]

Male Fischer rats given N'-nitrosonornicotine at 0.02% in the drinking water for 30 weeks (total average dose 630 mg/animal) exhibited after 11 months tumours in 14/20 animals, mostly oesophageal papillomas and carcinomas but also tumours of the nasal cavity and pharynx. N'-Nitrosonornicotine is regarded as a moderately-active carcinogen compared with the strong oesophageal carcinogen, dinitrosopiperazine.[12]

In another study in rats given 7 mg/rat/day in the drinking water for 44 weeks, all rats had olfactory adenocarcinomas and in addition 1 oesophageal papilloma, 1 fore-stomach papilloma and 1 liver tumour occurred in 3 rats surviving 43 weeks. No rats survived more than 46 weeks.[79]

Swiss mice given dermally 0.1 ml of a 0.3% solution thrice weekly for 50 weeks showed no skin tumours.[79]

Hamsters injected subcutaneously or intramuscularly thrice weekly with 5 mg/animal for 25 weeks showed papillary tumours of the trachea (12/19 survivors at 83 weeks). One nasal adenocarcinoma was seen at 45 weeks.[45,79]

6.2 Clinical/epidemiological studies

Evidence from epidemiological and experimental studies reviewed in 1975[12] and in 1979[135] showed that lowering the tar and nicotine content of smoke will lower the death rate of lung cancer.

In an appraisal of epidemiological studies comparing the mortality of smokers of different types of cigarettes, Lee[124] reported that compared with smokers of higher nicotine plain cigarettes, smokers of lower nicotine filter cigarettes exhibit a substantially reduced mortality from lung cancer as well as other types of cancer (buccal cavity and pharynx, oesophagus, larynx and bladder but not pancreas). It should be realized however that this evidence does not demonstrate whether nicotine *per se* influences the risk of development of cancer.[124]

The association between tobacco chewing and cancer of the oral cavity and oesophagus has prompted examination of unburned tobacco for specific components which are known carcinogens. Amongst these is the nitrosamine, N'-nitrosonornicotine, which is known to induce tumours in the oesophagus, nasal cavity and pharynx of rats given the carcinogen in the drinking water.[12] Whilst N'-nitrosonornicotine may be one of the causal agents responsible for cancer of the oesophagus in tobacco chewers it is not

known whether the carcinogen can exert its action locally at the site of chewing since nitroso compounds normally require prior metabolic activation to the proximate carcinogenic molecule.[18]

The ratio of cotinine to nicotine 1'-N-oxide is higher in smokers with bladder cancer than in smokers without bladder cancer but the significance, if any, of this finding in relation to the aetiology of bladder cancer is unknown.[48]

6.3 Assessment of carcinogenic potential

Despite the various experiments on nicotine, no adequate dose-response carcinogenicity study has been undertaken in experimental animals upon which a sound evaluation can be based.

Nevertheless it is clear from the results so far obtained that nicotine is not a potent carcinogen; nor is there any convincing evidence that nicotine possesses weak carcinogenic activity. The conflicting outcome of the co-carcinogenicity studies makes it difficult to draw any definitive conclusions but there is no consistent evidence that nicotine can act as an incomplete carcinogen.

Of greater relevance is the ability of N'-nitrosonornicotine, which is also present in unburned tobacco and tobacco smoke, to produce tumours in rats, mice and hamsters. Whether N'-nitrosonornicotine is present in sufficient concentrations to contribute to the carcinogenicity of tobacco smoke is however unknown.

Following assessments of the experimental data in 1962,[87] 1964,[5] and 1966,[89] it was accepted that nicotine was not carcinogenic. Moreover in 1971, nicotine was not included in the US Surgeon's List of Identified or Suspected Tumorigenic Agents in Cigarette Smoke.[11,19]

There is no supportive evidence for the hypothesis[18] that nicotine enhances the lung cancer risk in cigarette smokers by disrupting defences dependent upon the autonomic nervous system. Similarly, there is no experimental evidence to support the view[17] that nicotine enhances the lung cancer risk by impairing pulmonary clearance (from tobacco smoke and polluted air) of carcinogens as a consequence of ciliastasis.

More specifically, it has been claimed (despite evidence to the contrary) that nicotine may account for part of the tumorigenic activity observed when smoke condensates are applied to mouse skin[12] and even if some involvement by this route was established the relevance of such a finding to carcinogenesis in smokers would be open to question.

The finding that nicotine can inhibit pancreatic bicarbonate secretion in the dog has led to speculation that this could lead to intracellular pH changes that may subsequently play a role in the pathogenesis of pancreatic cancer.[135]

Clearly there are difficulties in assessing nicotine's carcinogenicity in relation to the risk to smokers. First and foremost no adequate inhalation animal model has yet been developed. The Dontenwill-hamster-larynx model (larynx not lungs), rat inhalation models (end points short of cancer) and mouse skin painting model (bypasses influence of vapour phase and short-life constituents in smoke) all have serious drawbacks.

Apart from a lifespan study of daily inhalation of nicotine aerosol in rats, nicotine could also be tested directly in the drinking water, skin or intratracheal route with full histology. In addition, testing of N'-nitrosonornicotine by the intratracheal route in the mouse or rat merits consideration, especially to see if it can produce both lung and liver tumours by this route. Consideration should also be given to more thorough testing of the other nicotine metabolite, cotinine.

7. MUTAGENICITY STUDIES

Ames and his colleagues reported a negative result for nicotine when tested in their Salmonella/liver microsomes test for mutagenicity.[86] (This result has also been taken as indirect evidence of the lack of carcinogenicity of nicotine as discussed earlier in this report.)

Some cytotoxic effects but no chromosomal damage were seen in human leucocyte cultures exposed to nicotine.[88]

Gross chromosomal abnormalities were observed in mice given low and well-tolerated doses of nicotine.[88]

Nicotine impairs the mitosis of fertilized ovum of the sea urchin and induces an abnormal mitosis in the epithelium of the oral cavity of the Salamander.[74]

8. REPRODUCTION AND TERATOGENICITY STUDIES

8.1 Experimental studies in animals

Studies in pregnant mice, given high subcutaneous doses of up to 25 mg/kg/day during days 5–15 of gestation, revealed embryotoxic effects and malformations mainly of the skeletal system, the most susceptible period of administration being days 7–12.[74] It was concluded that nicotine is a teratogen despite the high doses used.[89]

Nicotine is transferred across the mouse placental barrier in amounts sufficient to evoke a cardiovascular response.[7]

Studies in pregnant rats given nicotine (e.g. 2.2 mg/kg/day subcutaneously throughout pregnancy) revealed reductions in the number of live births and birth weight.[7,18]

Pregnant rats given 50–100 ppm nicotine in the diet showed no change in foetal weight or survival. Pregnant rats given twice daily injections of 0.5–5 mg/kg of nicotine showed growth retardation and impaired food intake at high doses; delivery dates were prolonged, young were underweight but there were no abortions or premature young.[11]

In a recent study in rats, nicotine when given in subcutaneous doses of 0.1 mg/kg/day from day 14 of pregnancy exerted no adverse effect on food intake, weight gain, length of gestation, litter size or foetal development but higher doses of 1 mg/kg/day reduced litter size and increased the incidence of still-births. Continued maternal administration of nicotine at 0.1 or 1 mg/kg/day had no effect on post-partum development in the first week after birth but reductions in body weight, weights of heart and lungs and in stomach contents were observed in 2-week-old pups, the last effect being attributed to less milk being available to pups of nicotine-treated mothers (0.1 mg/kg/day). The authors claim that administration of nicotine to pregnant rats at levels compatible with human smoking does not affect foetal or early neonatal development but affects growth during the second week after birth probably by impairing milk production.[136] The authors also draw attention to the fact that in many studies of the effect of nicotine on foetal development in rats doses of 1–6 mg/kg/day were given causing severe reactions such as convulsions, periods of apnoea and sometimes death. The effect of these high doses was to prolong gestation, an effect opposite to that seen in human smoking which is generally linked to a premature delivery.[136]

Foetal weight reduction and impaired maternal food intake were observed in groups of pregnant rats inhaling smoke from either normal cigarettes, non-nicotine cigarettes (lettuce leaves) or non-nicotine cigarettes to which 15 mg nicotine were added.[11]

Neither intravenous nicotine nor smoke inhalation impaired cardiovascular functions in pregnant ewes or their foetuses.[11]

Although an outbreak of congenital arthrogrypois (skeletal deformity) was associated with the ingestion of tobacco stalks by pregnant sows, no reference was made to a possible aetiological role of nicotine and instead contamination of the tobacco stalks by insecticides, fertilizers and other chemicals was suspected.[70]

Exposure of sperm of ***Xenopus laevis*** to concentrations of 1–5 mg/ml nicotine for 30–120 minutes resulted in sterility attributed mainly to the impairment of sperm motility at high concentrations and to gross deformities in developing embryos at low concentrations.[98] Because the deformities found are also seen with radiomimetic and mutagenic alkylating agents, the antifertility effect of nicotine could be attributed to an impaired sperm motility as well as to a mutagenic action leading to a variety of embryopathies.[98]

Studies using the dubious chick embryo method also gave positive results for nicotine (disturbance of vertebral morphogenesis and developmental defects in the heart) following exposure of 0.5 to 6.5 mg per embryo.[90a–e]

Injection of nornicotine and other nicotine derivatives into hens' eggs also produced malformations in the developing embryos.[19, 91]

8.2 Human smoking, pregnancy and infant health

Foetal respiratory movements were impaired by maternal smoking of two cigarettes during 32–38 weeks of gestation. Several explanations were proposed for this effect including the placental transfer of some constituent of tobacco smoke such as

nicotine or carbon monoxide. Preliminary observations with cigarettes not containing nicotine suggest that smoking does not affect foetal breathing movements.[103]

In women smoking during pregnancy, smaller babies and higher neonatal mortality have been observed, compared with non-smokers.[11, 28] Smoking had little if any effect on lactation, despite the presence of nicotine in breast milk.[27]

Although in 1971, the US Surgeon General considered that there was insufficient evidence to assess whether maternal smoking carries a significant teratogenic risk,[11] the vast majority of numerous studies of the effects of smoking on the outcome of pregnancy have given negative results in respect of teratogenicity. From this we may deduce that nicotine at levels absorbed by smokers is not teratogenic, a view endorsed by the 1979 US Surgeon General's report which could find no evidence that maternal smoking increases the risk of congenital malformations.[135]

Although some animal studies indicate a reduction in milk production (but not in milk release) following nicotine administration, human studies have failed to provide evidence of reduced lactation following periods of heavy smoking.[135]

8.3 Conclusions

No adequate reproduction or teratogenic studies on nicotine in mammals, either at dose levels associated with smoking or at higher levels, appear to have been published. However, no evidence exists to implicate nicotine in the possible effects of maternal smoking on the foetus during pregnancy. Moreover, there is evidence to show that maternal smoking is not associated with an increased risk of congenital malformations. However, further research is needed[135] to establish whether the pharmacological activity of nicotine is such as to affect foetal development at levels encountered in smoking.

9. AETIOLOGICAL ROLE OF NICOTINE IN "SMOKING-ASSOCIATED DISEASES"

9.1 General

The Health Consequences of Smoking 1975[13] highlighted four major health consequences of smoking as follows:

(i) Cardiovascular disease (coronary heart disease in particular; myocardial infarction, angina pectoris, atherosclerosis, cerebrovascular disease)
(ii) Cancer (mainly lung; also larynx, pharynx, oral cavity, oesophagus, pancreas, urinary bladder)
(iii) Non-malignant respiratory disease (e.g. chronic bronchitis, emphysema)
(iv) Risks during pregnancy.

Reference has been made elsewhere in this report to the possible aetiological role of nicotine in cancer of the lungs, oral cavity and bladder and also to the potential hazard of nicotine during pregnancy.

Little, if any, information appears to be available to assess whether or not nicotine *per se* contributes to the development of non-malignant respiratory disease and most studies on the chronic action of smoking (excluding those on carcinogenesis) have been aimed at the aetiology of cardiovascular disease, especially arteriosclerosis.

In a recent review[124] of epidemiological data comparing mortality in smokers of different types of cigarettes, it was found that compared with smokers of higher nicotine plain cigarettes, smokers of lower nicotine filter cigarettes have highly significant reductions in chronic bronchitis, statistically-significant reductions in emphysema, coronary heart disease and stroke and slight but non-significant reductions in aortic aneurysm and gastro-intestinal ulcer. These findings cannot however be regarded as showing that nicotine *per se* influences the risk of developing these diseases.

9.2 Cardiovascular disease

Aronow (1976) claims that nicotine and carbon monoxide both aggravate angina pectoris and contribute to an increased incidence of fatal and non-fatal myocardial infarction and sudden death from coronary disease in cigarette smokers.[12]

There is some evidence from animal studies that exposure to high levels of CO enhances atherogenesis in animals fed on high cholesterol diets but not in animals fed on normal diets. By comparison there is no evidence from animal studies to suggest that nicotine enhances atherogenesis irrespective of diet.[12, 135] However specific experimental data are not available to allow a conclusion about a possible effect on experimental atherogenesis of nicotine inhaled in smoke in doses experienced chronically by smokers.[135]

Aronow (1976) further claims that aggravation of exercise-induced angina pectoris was related to the nicotine content of cigarette smoke as was the increase in arterial pressure and heart rate both of which raise myocardial oxygen demand. Therefore patients with angina pectoris due to coronary heart disease develop angina pectoris sooner after exercise following cigarette smoking for at least two reasons: (i) nicotine-induced increase in myocardial oxygen demand and (ii) carbon monoxide-induced increase in carboxyhaemoglobin causing a decrease in oxygen delivery to the myocardium.[12, 135] However, it is difficult to be sure that the effects claimed by Aronow were attributable to differences in the delivery of nicotine *per se*.

The increase in fatal and non-fatal myocardial infarction and in sudden death from coronary heart disease in cigarette smokers may be theoretically related to (i) nicotine increasing the myocardial oxygen demand during an episode of myocardial ischaemia; (ii) nicotine increasing platelet adhesiveness and thereby increasing a thrombotic tendency and (iii) nicotine lowering the ventricular fibrillation threshold during an episode of myocardial ischaemia.[12, 135] There is no clear evidence to indicate that nicotine either augments experimental atherosclerosis (e.g. combining nicotine and dietary cholesterol in rabbits) or is associated with increased atherosclerosis found in human cigarette smokers. Definitive evidence from human studies would in any case be hard to obtain due to the difficulty in separating the effects of nicotine from those of other cigarette smoke components.[12]

Studies on the effect of nicotine administration on the arterial wall in animals have revealed no injurious effect on the intima and that the occurrence of moderate fibrosis

and of calcifications[55] in the media of the aorta is mediated by catecholamines. Nicotine administration to hypercholesterolaemic or hypertensive animals imposed little if any additive effect on arterial wall injuries despite the high doses used in rabbits corresponding to a nicotine intake derived from smoking of up to 1200 cigarettes per day. Thus it was concluded that nicotine was most unlikely to be responsible for the increased incidence of atherosclerosis in smokers.[12]

Nicotine administration in common with cigarette smoking causes an increase in serum free fatty acids, mediated by catecholamine release.[47,52] This is not accompanied by a change in serum levels of trigylcerides,[99] cholesterol and phospholipids.[44] Rabbits given 1.14 mg/kg twice daily for 20 months did not exhibit lipid changes in the heart or aorta. Apart from decreased neutral fat and increased free fatty acids in the liver and increased lipoprotein lipase activity in heart and aorta, no significant influence of nicotine on lipid metabolism has been demonstrated.[12,49]

Partly based on these findings, it was argued that the increased risk to coronary heart disease cannot be explained by the pharmacological action of nicotine alone and at the outside nicotine may act as an additional aetiological factor in cardiovascular disease.[12]

In contrast, evidence has been obtained in rabbits that nicotine, either alone or in conjunction with a hypercholesterolaemic diet induced haemovascular changes frequently associated with cardiovascular pathology.[71] Induction by nicotine of elevated serum cholesterol levels in dogs, possibly secondary to a rise in free fatty acids, has been reported.[46]

The 1972, 1975 and 1979 reports of the Surgeon General on the Health Consequences of Smoking implicated both carbon monoxide and nicotine as the main candidates most likely to contribute to the health hazards of smoking.[7,13,135]

In a heavy smoker inhaling 50—100 mg nicotine daily, the principal effect of the absorbed nicotine appears to be to stimulate catecholamine production and release of noradrenaline causing sympathetic overactivity leading to increases in heart rate, blood pressure, cardiac output and as a result myocardial oxygen demand. Normal humans respond with a compensatory increase in coronary blood flow but in those with atherosclerosis this may not occur and ischaemia results.[12] In contrast to these cardiodynamic effects, such atherogenic effects as have been seen appear to be minor and reversible.[12] It has been suggested that the risk of coronary disease can be minimized in part by denicotinizing cigarettes and further suggested that the acute precipitating effects of nicotine and carbon monoxide contribute to the excess risk in the cardiovascular disease prone smoker.[12]

Nicotine-induced release of catecholamines can increase platelet stickiness and aggregation, thereby accelerating thrombus formation in damaged vessels and possibly contribute to enhancing thromboembolic episodes in persons with advanced atherosclerosis.[12]

It is not known whether the nicotine-induced increase in adrenocorticoid secretion in smokers has any relevance to atherogenesis.[116]

Unlike its effect on cardiac metabolism, there is no evidence that nicotine affects cerebral oxidative metabolism at levels equivalent to smoking. In any case, the association between smoking and increased risk of stroke is inconclusive.[135]

9.3 Parkinson's disease

A negative association between Parkinson's disease and smoking was first reported in 1966[125] and confirmed in subsequent prospective and retrospective studies.[126–129] The full range of the effects of smoke constituents, and in particular nicotine, on the central nervous system has by no means been completely evaluated. Nicotine is known to release from brain tissue biogenic amines including dopamine. Antiparkinsonian drugs act by replacing depleted brain dopamine in patients with Parkinson's disease or enhance dopaminergic activity in the brain. Whether nicotine, at levels derived from smoking, exerts a preventative role in the development of Parkinson's disease remains to be established although it is theoretically plausible that nicotine may act as a consequence of a neuropharmacological effect on the hypothalamic region of the brain.

9.4 Peptic ulceration

Various epidemiological studies have pointed to an association between smoking and peptic ulceration[5, 11, 19] although studies of the effect of either smoking or nicotine on gastrointestinal function in animals and man have failed to explain how smoking might contribute to peptic ulceration.[134, 135] Neither smoking nor nicotine appears to stimulate acid secretion or total gastric pepsin output or to alter mucus production. Several effects have however been observed that may be related to ulcer development: (i) pancreatic secretion of bicarbonate is inhibited by smoking and nicotine; (ii) reflux of duodenal contents into the stomach is increased by smoking, possibly rednering the mucosal surface more vulnerable to attack by pepsin 1; (iii) concentration of collagen-degrading pepsin 1 is raised in smokers.

Thus the available evidence cannot indicate whether nicotine (especially at levels present in smoke) may be involved in the development of peptic ulceration or in the rate of ulcer healing.[134, 135]

9.5 Relevance of involuntary inhalation of nicotine (passive smoking)

Except under conditions of grossly inadequate ventilation, atmospheric nicotine in the general environment does not exceed the maximum threshold limit value of 500 $\mu g/m^3$ for occupational exposure in the USA. However, under conditions where ventilation was grossly inadequate, smoking was found to give rise to atmospheric nicotine concentrations of up to 1040 $\mu g/m^3$.[13]

Small amounts of nicotine can be absorbed by non-smokers in involuntary smoking situations, e.g. a smoke-filled room. Under these conditions non-smokers excreted less than 1% of the amount of nicotine and cotinine excreted by smokers. At this low level of absorption, nicotine is unlikely to present a hazard to the non-smoker.[13, 135]

Under conditions of severe tobacco smoke pollution, non-smokers showed a rise in plasma nicotine to 90 $\mu g/l$ and in urinary nicotine to 80 $\mu g/l$, the latter being well below that for urinary nicotine in smokers, i.e. 1236 $\mu g/l$.[135]

10. SMOKING BEHAVIOUR: ROLE OF NICOTINE IN THE SMOKING HABIT

Smoking seems to satisfy a smoker's physiological and psychological needs and it is generally accepted that nicotine is the principal constituent responsible for a smoker's pharmacological responses. It is not part of this review to explore in detail the motives for smoking and the putative effects of nicotine in the performance or wellbeing of smokers. In this section, we consider briefly recent evidence for "compensation", i.e. evidence of smokers who change the way they smoke in order to obtain particular pulse doses of nicotine or maintain particular blood levels of nicotine.

Nicotine has been described as a psychoactive agent with tranquillizing, anti-anxiety, stimulant, depressant, antiaggression, mood stabilizing and stress-attenuating properties.[110] But inhaling smokers are clearly more likely to experience these effects than non-inhalers. It is to be presumed therefore that non-inhalers smoke mainly for reasons other than the pharmacological effects of nicotine. The range of motives for smoking which were investigated by McKennell[137] have been the subject of considerable research and debate, e.g. Dunn[15] and Thornton,[114] which is outside the scope of this review.

Inhaling smokers appear to modify their smoking behaviour to attempt to maintain the total dosage of nicotine when they smoke cigarettes of varying nicotine content.[65] The smoking rate has been shown to decrease when subjects are in addition given nicotine either orally or intravenously. Studies of nicotine antagonists indicate that smokers seek an effective brain level of nicotine when modifying their smoking behaviour. Apart from variations in puffing parameters, the nicotine intake is regulated mainly by variations in the degree of inhalation[96, 113] but partly perhaps by a small increase in consumption.[133] This compensation is only partial so that reduced nicotine yield does mean reduced intake to the smoker, although the reduction in intake will be relatively smaller than the reduction in yield. It is possible that smokers are less able to compensate in the case of very low nicotine cigarettes. Whilst trough maintenance appears to be the main motive for the heavy smoker, optimal peak effects are probably more important to light smokers.[15, 110, 114, 117] Despite much evidence, the role of nicotine in the smoking habit has still been questioned.[78] And regulation of the nicotine intake has not been consistently seen in all studies on human smokers.[114]

It is doubtful whether the cigar smoker, even though he can receive a dose of nicotine without inhaling, absorbs enough nicotine into the bloodstream rapidly enough to produce pharmacological effects.[67] In this connection a comparison[130] of life-time cigar smokers and subjects who switched from cigarettes to cigars is relevant. These workers reported that, whereas the former maintained low carboxyhaemoglobin levels indicating that they were not inhalers, the switchers who had been used to inhaling cigarette smoke continued to inhale after switching. This paper was subsequently criticized on methodological grounds.[131] Clearly there is a paucity of information on blood nicotine levels in life-time cigar smokers and therefore a lack of knowledge of the reasons why they smoke.

Behavioural studies in animals have shown that nicotine can afford protection against inborn emotional environmental stress. Thus animals trained to perform a stressful avoidance task under the influence of nicotine become dependent on nicotine for continuation of a successful performance. Dependence is related to the degree of

stress to which the animal is exposed.[60] Nicotine may alleviate a stress response in animals and also in human subjects by both peripheral and central actions, possibly involving a hypothalamic-pituitary-adrenocortical axis. Nicotine dependence in animals subjected to stress may also be mediated via its effects on the pituitary-adrenal system. An early report of impairment of intelligence in rats chronically exposed to nicotine[51] has not in general been confirmed in the course of extensive subsequent behavioural research.

Cigarette smoking may induce psychological dependence in certain individuals as a result of action of nicotine on brain reward systems, possibly mediated by noradrenaline release at central synapses.[110]

Experimental studies in man indicate nicotine-induced improvements in attentional efficiency,[114] thus supporting the subjective impressions that smoking can improve concentration and efficiency.[60] Nicotine given in small quantities orally to human volunteers has a positive effect on cortical arousal.[107] All in all it seems likely that the pleasure factor of smoking could be derived, at least in part, from the release of noradrenaline from its brain stores.[110]

It is anticipated that the increasing interest in the endocrine effects of smoking will be extended to examination of the role they play in the smoking habit.[135]

Although smokers develop some degree of dependence on the practice,[138] common sense dictates that habituation to smoking is distinguishable from addiction to drugs such as morphine and barbiturates.[5,40]

As discussed earlier (section 4.6, p. 16), acquired tolerance, even though of a low order, is important in overcoming nausea and other nicotine effects and is a factor in the continued use of tobacco.[5]

Sudden withdrawal of certain drugs may elicit withdrawal effects in people and the same holds true for nicotine. However there is no reliable indication of the likely prevalence of such effects if smoking were to be totally abolished or made prohibitively expensive.

Earlier in the report, reference was made to the low pharmacological activity of cotinine. However both behavioural and biochemical effects have been attributed to this nicotine metabolite.[67]

Because of the weak absorption of nicotine from buccal and alimentary systems, chewing nicotine gum as a possible alternative vehicle to smoke inhalation would prove much less satisfying to the regular cigarette inhaler.[95]

11. SUMMARY AND CONCLUSIONS

The present review, essentially concerned with the pharmacological and toxicological properties of nicotine, has attempted to clarify the effects of nicotine likely to

be elicited at levels of human exposure associated with tobacco smoking. Effects not attributable, either in whole or in part, to the action of nicotine, have largely been ignored in this review.

Rarely more than 25% and usually only about 15% of the total nicotine content of a burning cigarette appears unchanged in the mainstream smoke. Inhaling cigarette smokers absorb up to 90% of the nicotine content of mainstream smoke drawn into their mouths. Cigarette smokers who do not inhale absorb far less nicotine – almost certainly only in amounts that are pharmacologically inactive.

Blood levels of nicotine vary considerably according to the parameters adopted by smokers. Levels of 31–41 μg/l have been seen in inhaling cigarette smokers who, after overnight deprivation, smoked one cigarette. Under the same conditions, a level of only 8 μg/l was seen in non-inhaling smokers. Blood nicotine levels usually peak before smoking ceases. Mean plasma nicotine levels observed after 5 hours of smoking of high nicotine cigarettes (yield 3.2 mg) and low nicotine cigarettes (yield 1.34 mg) were 29.2 and 8 μg/l, respectively.

Following absorption, nicotine is readily taken up in the brain and other organs. It undergoes transformation, principally in the liver, to cotinine (major metabolite) and to various other metabolites. Significant amounts of unchanged nicotine can be excreted in the urine under certain conditions (acid pH of urine). Nicotine has been detected in breast milk of human smokers in amounts below 1 ppm.

Although nicotine is the principal pharmacologically-active component of tobacco smoke, the pharmacological effects of nicotine in the amounts absorbed by inhaling smokers from one cigarette (up to 1 mg approx.) are relatively small.

Owing to its many actions, the overall effect of nicotine obtained from smoke is complex. Nicotine exerts predominantly stimulant effects on the central nervous system, cardiovascular system and adrenal glands but a dual action can operate in that nicotine both stimulates and arouses and sedates and relaxes.

Toxicologically, nicotine is acutely very toxic and the acute effects in both animals and man have been reasonably well documented. However, assessment of the possible chronic toxic effects from prolonged exposure to nicotine has proved difficult because of the inadequacies in the animal studies that have been reported. There is no really suggestive evidence and certainly no conclusive evidence to indicate that nicotine ***per se*** acts either as a complete or an incomplete carcinogen. Whether the carcinogenic analogue, N'-nitrosonornicotine, is present in sufficient concentrations to contribute to the carcinogenicity of tobacco smoke is doubtful and needs to be assessed.

No adequate reproduction or teratogenicity studies on nicotine, either at dose levels associated with smoking or at higher levels, in mammals appear to have been published. On the other hand, there is no consistent evidence to implicate nicotine in the possible effects of maternal smoking on the foetus during pregnancy. Moreover evidence is available showing that maternal smoking is not associated with the risk of developing foetal malformations.

No experimental or epidemiological evidence is available to support the possibility that nicotine plays a contributory role in smoking-associated diseases, especially

cardiovascular disease, an area of research in which nicotine has attracted much attention. Theoretically, the pharmacological effects of nicotine on the cardiovascular system might pose an additional strain on the hearts of subjects suffering from myocardial insufficiency. However, primary toxic effects on the cardiovascular system have not to our knowledge been unequivocally demonstrated.

The importance of nicotine in the smoking habit is briefly discussed, special attention being focussed on compensation in smokers. It seems that under some circumstances some, but not all, inhaling cigarette smokers subconsciously modify their smoking patterns to regulate their nicotine intake.

The present worldwide campaign towards low-tar, low-nicotine cigarettes faces the problem that nicotine-seeking smokers might inhale more smoke to obtain their nicotine requirement and in so doing inhale more tar. As Russell[110] succinctly but poignantly puts it:

> "If people smoke for nicotine but die from tar, the logical approach to safer cigarettes would seem to lie in the development of low-tar, medium-nicotine cigarettes."

Further study and a better understanding of the pharmacological and toxicological actions of nicotine should assist authorities throughout the world to decide whether this course of action is to be preferred over the other alternatives.

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CORRIGENDA

Key to abbreviations,
T.R.C. :— for Counil read *Council*

Page 8. 3.1.1. line 5 :— for membrances read *membranes*

Page 8. 3.1.2. line 4 :— for p32 read *p37*

Page 15. 3.5. line 6 :— for p27 read *p32*

Page 18. 4.4. line 13 :— for p31 read *p36*

Page 35. 9.2. line 9 :— for trigylcerides read *triglycerides*

Page 36. 9.4. line 8 :— for rednering read *rendering*

Page 38. 10. line 22 :— for section 4.6, p16 read section *4.7, p19*

Page 41. ref.22 :— for en read *on*

Page 42. ref.56 :— for fiverses read *diverses*

Page 42. ref.58 :— for Soking read *Smoking*

Page 43. ref.69 :— for Armitage, A.J. read *Armitage, A.K.*

Page 43, ref.74 :— for anomalities read *abnormalities*

Page 43. ref.86 :— for nutagens read *mutagens*