Studies of Cigarette Smoke Transfer Using Radioisotopically Labelled Tobacco Constituents

Part II: The Transference of Radioisotopically Labelled Nicotine to Cigarette Smoke*

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INTRODUCTION

Studies of the fate and distribution of tobacco smoke constituents within biological systems are often greatly facilitated by inclusion of radiolabelled compounds in the tobacco, because of the increased sensitivity and specificity associated with radioisotopic analyses. Use of such techniques is valid, however, only if the distribution of the added material and its combustion products in the smoke is fully known. Before such studies involving nicotine could begin it was necessary to demonstrate that the smoke transfer characteristics of endogenous and exogenous nicotine from uniformly labelled cigarettes were similar. In addition to a knowledge of the distribution of nicotine and its combustion products in the particulate and vapour phases, it was important to determine whether the specific activity of nicotine in mainstream smoke remained constant throughout the smoking. It was therefore essential to study puff-by-puff transference of alkaloids (as nicotine).

Several investigators have described apparatus for determination of the mass-balance after smoking of 14 C-activity added to cigarettes (1-4), but none of these earlier designs was considered entirely suitable for the present work. This paper describes the construction and evaluation of equipment for smoking a cigarette and collecting all the products of combustion. The apparatus was designed to permit the quantitative puff-by-puff collection of mainstream total particulate matter (TPM). In the present studies cigarettes were spiked with nicotine-z'-14C-di(p-toluoyl tartrate) using a device described by Houseman and Heneage (5).

MATERIALS, METHODS AND APPARATUS

Radiolabelled Cigarettes

The impregnation of sorted and conditioned tipped cigarettes (70 mm) with an ethanolic solution of nicotine-2'-¹⁴C-di(p-toluoyl tartrate), specific activity

20 mCi/m mole, has been described (5). The mean total ¹⁴C-activity recovered from extracted whole cigarettes was found to be 0.500 \pm 0.012 μ Ci (95%) confidence limits).

Liquid Scintillation Counting

Sample radioactivity was measured using a Packard Tri-Carb Model 3375 Liquid Scintillation Spectrometer with optimized gain and window settings. Aliquots of radioactive solutions were counted in a scintillant consisting of $8^{0}/_{0}$ w.v. naphthalene, $0.5^{0}/_{0}$ w.v. PPO [2,5-diphenyloxazole] and $0.005^{0}/_{0}$ w.v. dimethyl-POPOP[1,4-di-(2-(4-methyl-5-phenyloxazolyl))benzene] in 1:1:1 xylene:1,4-dioxan:ethanol (15 ml). The Automatic External Standard channels-ratio method of quench correction was adopted and all samples were counted to at least $1^{0}/_{0}$ accuracy.

Gas Chromatography

The mass of nicotine in methanol extracts of smoke TPM retained by mainstream and sidestream Cambridge filter discs and by the butt was determined using a Pye-Unicam Model 104 Gas Chromatograph fitted with a flame ionization detector coupled to a Honeywell Electronik Model 194 1mV strip chart recorder. A $9' \times 1/4''$ O.D. glass column containing 10% w.w. Carbowax 20M and 5% w.w. potassium hydroxide on 80–100 mesh Chromosorb W was operated isothermally at a temperature of 180° C with an argon carrier gas flow rate of 45 ml/min. (6).

Ultra-Violet Spectroscopy

The mass of total alkaloids (as nicotine) in the methanol extracts was determined by a standard spectroscopic procedure (7). A Hilger and Watts Uvispek Model H 700-9 UV/visible spectrophotometer was used to measure the optical density of an acidified steam distillate of each extract at three separate wavelengths.

Construction and Operation of the Smoking Apparatus

A block diagram of the whole assembly is shown in Figure 1. The cylindrical glass water-cooled smoking

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Figure 1. Block diagram of smoking apparatus.



cartridge shown diagrammatically in Figure 2 was approximately 25 cm in length and 8 cm in internal diameter. A detachable ash trap (A) with integral airinlet pipe was mounted at the bottom of the cartridge. Incoming filtered and dried air was dispersed by a sintered-glass disc (B) fused into the flared end of the pipe. A curved ash deflector (C) was mounted on short legs above the sinter. With an air flow rate of 2 l/min. through the cartridge, a normal burning rate for the cigarette was achieved. The flow rate was controlled by a needle valve and measured with a "Rotameter" flowmeter. The detachable glass cigarette holder (D) was fitted with a smaller ground-glass joint immediately behind the cigarette, enabling a series of numbered Cambridge filter holders (E) to be interchanged rapidly for puff-by-puff collection of mainstream TPM without disturbing the burning cigarette or its holder in any way. The filter holders were constructed of "Nylatron-GS" and were fitted with an extended nose piece to minimize the path length between the back of the cigarette and the filter holder. This reduced the area on which particulate material could condense and be effectively "lost" during puffby-puff transfer studies. The change-over of filter holders involved sliding back the smoking pump and attached holder a sufficient distance from the cartridge to enable the holder to be disconnected from the inlet port of the pump. In order to provide the required degree of flexibility, the outlet port of the pump was connected to the mainstream vapour phase trapping system by means of a spherical ball joint. A stoppered port (F) in the wall of the cylinder diametrically opposite the cigarette holder was used for lighting and extinguishing the cigarette. Sidestream TPM was trapped on two 115 mm diameter Cambridge filter discs housed in the "Nylatron-GS" sidestream filter holder (G) cemented to the top of the cartridge. The two discs were separated by a 4 mm spacing ring (H).

The mainstream and sidestream vapour phase com-





ponents of cigarette smoke were catalytically oxidized to carbon dioxide in a furnace tube packed with Hopcalite catalyst electrically heated to 200° C and the CO₂ was then trapped in a suitable absorption medium. Mounted in series with the furnace were two vapour phase absorption bottles, each containing a freshly prepared solution of 1:8 ethanolamine:2-methoxyethanol (50 ml). Volatile materials resistant to catalytic oxidation, e.g. certain hydrocarbons, were then passed through a liquid nitrogen-cooled impaction trap. The catalytic oxidation of mainstream vapour phase smoke was assisted by a stream of air (1 l/min.), introduced at a point immediately after the *outlet* port of the smoking pump. The air stream was switched off during the puffing period.

The piston-operated smoking pump shown in Figure 3 was based on a 100 ml glass syringe. The puff volume could be varied between 0 and 50 ml by adjusting the length of the connecting rod at A.

In the present studies the device was adjusted to produce a 35 ml puff of 2 seconds duration once every minute. A rotary valve assembly was constructed from "Nylatron-GS" and comprised two parts, a fixed outer half (B) containing the intake and outlet ports, and a movable half (C) with an offset port which was cemented to the end of the syringe barrel. The rotating half was actuated by a connecting rod (D), driven by a second motor (E). A gas-tight seal was achieved by lightly compressing the accurately machined faces of the two halves using an adjustable coil spring. Immediately before the puff, the connecting rod (D) rotated the syringe barrel and offset port into line with the intake port in B. The puff was taken and then the barrel was rotated so that its offset port was in line with the fixed outlet port. The vapour phase was then discharged, in 2 seconds, and the syringe returned to an intermediate resting position between the two fixed ports. The puff profile produced by this smoking pump is shown in Figure 4. The timing marks are each equivalent to 0.5 seconds.

Determination of ¹⁴C-Activity Balance, Including Puff-By-Puff Transfer of Particulate Material to Mainstream Smoke

In a typical smoking experiment ten cigarettes each containing a mean 0.50 μ Ci of nicotine-2'-¹⁴C-di(p-toluoyl tartrate) were smoked consecutively in the above apparatus. Ten 35 ml puffs of 2 seconds duration were taken at one minute intervals, leaving a 21 mm butt (tip overwrap + 2 mm). Mainstream TPM was collected puff-by-puff consecutively on ten 56 mm Cambridge filter discs. The numbered filter disc holders were removed and stoppered 45 seconds after the appropriate puff. Each filter was then used again to collect particulate material from the corresponding puff of the next cigarette. Material condensing on the walls of the smoking cartridge was included in the sidestream TPM assay.

On completion of smoking, the mainstream and sidestream Cambridge filter discs, the butts and the ash were individually extracted with cold methanol (20 ml; sidestream discs 50 ml). The smoking cartridge was rinsed out with methanol and the total eluate diluted to 100 ml. Vapour phase absorption bottles and cold traps were also rinsed out with methanol and the washings diluted to 100 ml and 50 ml respectively. Aliquots of each solution were assayed for total ¹⁴Cactivity, total nicotine (endogenous + exogenous), and total alkaloids (as nicotine) by liquid scintillation counting, gas-liquid chromatography, and ultra-violet spectroscopy respectively. ¹⁴C-alkaloids in the acidified steam distillate resulting from determination of total alkaloids (as nicotine) were measured by liquid scintillation counting.

Figure 4. Puff profile.



RESULTS AND DISCUSSION

The degree of uniformity of deposition of nicotine- $2'^{-14}$ C-di(p-toluoyl tartrate) within impregnated tipped cigarettes was demonstrated by *Houseman* and *Heneage* (5).

In the present transfer studies nicotine was found only in the particulate phase of smoke. The efficiency with which Cambridge filter discs retained smoke TPM was clearly demonstrated by the fact that after smoking ten cigarettes consecutively, 90-95 % of total nicotine recovered from the two sidestream filter discs was retained by the first and none was carried through the second into the vapour phase. With smaller smoking cartridges the proximity of the burning cigarette to the sidestream filter and the smaller area of the filter disc caused nicotine to vaporise and pass through the disc.

The carbon dioxide trapping efficiency of the vapour phase absorption bottles was demonstrated by the fact that for both sidestream and mainstream smoke $98-99^{0/0}$ of the recovered vapour phase ¹⁴C-activity was retained by the first trap, even with an air flow as high as 2 l/min.

The smoke distribution of total alkaloids (as nicotine), total nicotine, total ¹⁴C-activity, and exogenous ¹⁴Calkaloids is shown in Table 1. The 49 mm of tobacco rod consumed during smoking to a 21 mm butt contained a mean 98.6% of the total ¹⁴C-nicotine added to each cigarette (5). All percentage transfer figures quoted in Table 1 are therefore based on levels of exogenous and endogenous alkaloids originally present in this radiolabelled portion of tobacco rod. The mean mass of endogenous total alkaloids (as nicotine) originally present in the 49 mm portion was shown by assay of unsmoked tobacco to be 10.43 mg (as free base). The additional contribution of exogenous ¹⁴Cnicotine was 3.99 µg (as free base). The 16 mm filter rod and 5 mm of unburnt tobacco which made up the butt were assayed together. Two sets of data relating to the mean amount of material retained by the butts are shown in Table 1. In the first set the butt assay includes alkaloids filtered out of the mainstream smoke as well as the alkaloids initially present in the 5 mm of tobacco prior to smoking. It is not meaningful, therefore, to express these figures as a percentage of the alkaloids originally contained in the burnt portion of tobacco. In the second set of butt figures the 1.06 mg

Table 1. Smoke distribution of total alkaloids (as nicotine), total nicotine, total ¹⁴C-activity and ¹⁴C-alkaloids (mean of ten 0.50 μ Cl cigarettes smoked consecutively).

| | Total alkaloids (as nicotine) | | Total nicotine | | Total ¹⁴ C-activity | | 14C-alkaloids | |
|--|----------------------------------|---|--------------------|---|--------------------------------|--|---|--|
| Phase of smoke | (mg/ cigarette) | as a % of original con- tained in tobacco burnt | (mg/ cigarette) | as a % of original con- tained in tobacco burnt | (nCi/ cigarette) | as a % of original con- tained in tobacco burnt* | (nCi/ cigarette) | as a % of original con- tained in tobacco burnt* |
| Mainstream TPM | 1.49 | 14.3 | 1.47 | 14.1 | 76.1 | 15.4 | 73.3 (96.3 %) of total '4C- activity) | 14.9 |
| Mainstream vapour | 0 | 0 | 0 | 0 | 20.0 | 4.1 | 0 | 0 |
| Sidestream TPM | 4.16 | 39.9 | 4.09 | 39.2 | 202.6 | 41.1 | ⇒ 182.1 | 37.0 |
| Sidestream vapour | 0 | 0 | 0 | 0 | 80.5 | 16.3 | 0 | 0 |
| Butt + Butt, assay adjusted for contribution made by material present in butt | 3.47 | - | 3.43 | - | 106.9 | · _ | 98.1 | - |
| tobacco before smoking. | 2.41 | 23.1 | 2.37 | 22.7 | 99.7 | 20.2 | 90.9 | 18.5 |
| Ash | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 8.06 | 77.3 | 7.93 | 76.0 | 478.9 | 97.1 | 346.3 | 70.4 |

* Mean total 14C-activity originally contained in tobacco burnt == 492.8 nCi per cigarette.

+ includes material retained by 16 mm filter rod and 5 mm of unburnt tobacco.

of total alkaloids (as nicotine) and 7.25 nCi of 14 Cnicotine initially present in the butt tobacco have been subtracted. The remaining activity in the butt 'was 20.2% of the mean total 14 C-activity originally contained in the burnt tobacco rod.

Excellent agreement was obtained between the gaschromatographic (6) and spectroscopic (7) methods used for determining nicotine in mainstream and sidestream TPM and the butt.

More than 97% of the ¹⁴C-activity originally added to the cigarettes was recovered. ¹⁴C-alkaloids accounted for 96.8% of the measured total ¹⁴C-activity of mainstream TPM. The overall figure of 49.2 nCi/mg ob-

Table 2. Puff-by-puff transference of total alkaloids (as nicotine) and ¹⁴C-alkoloids to mainstream smoke (mean of ten 0.50 μ Ci cigarettes smoked consecutively).

| Total alkaloids (as nicotine) (μg/cigarette) | ¹⁴ C-alkaloids (nCi/ cigarette) | Overall specific activity of alkaloids in mainstream smoke (nCi/mg) |
|--|---|--|
|) 63.8 | 3.64 | 57.1 |
| 105.0 | 5.67 | 54.0 |
| 139.3 | 6.88 | 49.4 |
| 139.9 | 6.87 | 49.1 |
| 143.4 | 7.51 | 52.4 |
| 188.0 | 9.49 | 50.5 |
| 159.8 | 7.94 | 49.7 |
| 161.3 | 7.81 | 48.4 |
| 193.7 | 9.30 | 48.0 |
| 194.5 | 8.21 | 42.2 |
| | Total alkaloids (as nicotine) (μg/cigarette)) 63.8 105.0 139.3 139.9 143.4 188.0 159.8 161.3 193.7 194.5 | Total alkaloids (as nicotine) (μg/cigarette) '4C-alkaloids (nCi/ cigarette)) 63.8 3.64 105.0 5.67 139.3 6.88 139.9 6.87 143.4 7.51 188.0 9.49 159.8 7.94 161.3 7.81 193.7 9.30 194.5 8.21 |

tained experimentally for the specific radioactivity of alkaloids transferred to mainstream smoke [1.49 mg of total alkaloids (as nicotine) and 73.3 nCi of ¹⁴Calkaloids] compared favourably with a figure af 47.2 nCi/mg calculated from the known levels of endogenous and exogenous alkaloids (10.43 mg and 492.8 nCi respectively) originally present in the burnt portion of tobacco. Data are presented in Table 2 which illustrate the puff-by-puff transference of total alkaloids (as nicotine) and ¹⁴C-alkaloids to mainstream smoke. The specific activity of alkaloids in mainstream smoke was constant from puff to puff, apart from the first and last puffs. The higher value obtained for the first puff may have resulted from the increased deposition of radioactive material at the front of the unsmoked tobacco rod (5) and the last puff presumably contained a small amount of endogenous nicotine distilled from the unspiked butt tobacco. In conclusion, the smoke transfer characteristics of the two types of material are similar.

SUMMARY

The construction and evaluation of equipment for smoking a cigarette and collecting all the products of combustion is described. The apparatus was designed to permit the quantitative puff-by-puff collection of mainstream TPM. It has been used to study the transference of nicotine-2'.¹⁴C-di(p-toluoyl tartrate) to tobacco smoke. The smoke transfer characteristics of endogenous and exogenous alkaloids were similar. Alkaloids (as nicotine) were found only in the particulate phase of smoke, ¹⁴C-alkaloid accounting for approximately 95% of the total ¹⁴C-activity recovered from mainstream and sidestream TPM.

ZUSAMMENFASSUNG

Es werden Aufbau und Auswertung einer Apparatur zum Verrauchen einer Cigarette und zum Erfassen aller Verbrennungsprodukte beschrieben. Die Vorrichtung wurde entwickelt, um zugweise die Partikelphase (TPM) des Hauptstromrauches quantitativ auffangen zu können. Sie wurde zur Untersuchung des Überganges von Nikotin-z'-¹⁴C-di(p-toluoyl-tartrat) in den Tabakrauch benutzt. Die Rauchübergangseigenschaften endogener und exogener Alkaloide waren vergleichbar. Alkaloide (als Nikotin) wurden nur in der Partikelphase des Rauches gefunden, wobei ¹⁴C-Alkaloid etwa 95 % der ¹⁴C-Aktivität ausmachte, die insgesamt in der Gesamtpartikelphase von Haupt- und Nebenstromrauch wiedergefunden wurde.

RESUME

On décrit la construction et l'évaluation d'un appareil à fumer capable de collecter la totalité des produits de combustion. Le dispositif a été conçu pour permettre le recueillement quantitatif et bouffée par bouffée de la matière particulaire totale (TPM) du flux principal de fumée. Il a été utilisé pour étudier le transfert du di-p-toluoyl-tartrate de nicotine-2-¹⁴C dans la fumée de cigarette. Les caractéristiques de transfert des alcaloïdes endogènes et exogènes étaient similaires. Les alcaloïdes (comme la nicotine) ont été retrouvés uniquement dans la phase particulaire de la fumée, les alcaloïdes marqués au ¹⁴C représentant à peu près 95% de l'activité totale du ¹⁴C recueilli dans la phase particulaire des flux principal et secondaire.

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