Studies of Cigarette Smoke Transfer Using Radioisotopically Labelled Tobacco Constituents

Part I: The Preparation of Radioisotopically Labelled Cigarettes*

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INTRODUCTION

The transference of tobacco constituents to cigarette smoke is often most easily investigated by incorporating known radioisotopically labelled compounds with the tobacco. Two such studies carried out at these laboratories (1, 2) have used nicotine-2'- ^{14}C -di(p-toluoyl tartrate) and the paraffinic hydrocarbon dotriacontane-16, 17- ^{14}C . Ideally, the added material should be distributed homogeneously throughout the tobacco and the smoke transfer characteristics of endogenous and exogenous material should be similar.

Thornton and Valentine (3) determined the balance after smoking of ^{14}C -activity added to cigarettes. Solutions of radioactive chemicals were added along the entire length of intact cigarettes by means of a hypodermic syringe, the syringe being withdrawn from the axis of the cigarette at a uniform rate as injection proceeded. In other smoke transfer studies reported by Jenkins et al. (4, 5) and Maddox et al. (6) cigarettes were prepared by hand from tobacco sprayed with a solution of radiolabelled compound. The high rejection rate arising from a greater variation of weight and draw resistance in hand made cigarettes was unacceptable and in the present studies intact cigarettes were impregnated evenly along their length with a solution of radioactive material. An apparatus, which offers certain advantages over earlier designs, was constructed for this purpose and is described in this paper.

using the method of Decker (7). The chemical and radiochemical purities were checked by two-dimensional thin-layer chromatography. A single Koenig-positive spot was found to contain 99.1% of the total radioactivity recovered from the developed TLC plate.

Liquid Scintillation Counting

Sample radioactivity was measured using a Packard Tri-Carb Model 3003 Liquid Scintillation Spectrometer with optimized gain and window settings. Aliquots of radioactive solutions were counted in a 0.6% w.v. solution of 2-(4-tert-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole (Butyl-PBD) in 4:1 toluene: ethanol (15 ml). Powdered silica from TLC plate scrapings was added directly to vials containing scintillator and 4% w.v. Cab-O-Sil (Packard) [Snyder and Stephens (8), Snyder (9)]. The channels-ratio method of quench correction was adopted and all samples were counted to at least 1% accuracy.

Gas Chromatography

The chemical purity of dotriacontane-16, 17- ^{14}C was established using a Pye Unicam Model 104 Gas Chromatograph fitted with a flame ionization detector coupled to a Honeywell 1 mV chart recorder. A 3 ft. $\times$ 1/4" O.D. glass column containing 3% w.w. poly-m-phenoxyline on 80-100 mesh Chromosorb W was operated isothermally at a temperature of 280°C with a nitrogen carrier gas flow rate of 45 ml/min.

Liquid Chromatography

A solution of dotriacontane-16, 17- ^{14}C in toluene (5 ml) containing an accurately known weight of added unlabelled dotriacontane (ca. 25 mg) and a trace of anthracene was passed down a column of Woelm neutral alumina (10 g, activity grade 1). The sample was eluted with toluene (6 $\times$ 5 ml) and six fractions (5 ml each) were collected. Aliquots of each fraction and the unchromatographed solution were assayed by gas chromatography and liquid scintillation counting.

MATERIALS, METHODS AND APPARATUS

Radiochemicals

Dotriacontane-16, 17- ^{14}C , specific activity 58.6 mCi/m mole, was purchased from C.E.A., France. The chemical and radiochemical purities were shown to be greater than 98% by gas chromatography and liquid chromatography respectively.

Nicotine-2'- ^{14}C -di(p-toluoyl tartrate), specific activity 20 mCi/m mole, was synthesised in these laboratories

* Received for publication: 9th May, 1973.

Figure 1. The cigarette-spiking apparatus.

Thin-Layer Chromatography

Radiolabelled nicotine was examined using standard glass plates (20 cm $\times$ 20 cm) layered with Kieselgel G (E. Merck A.G.) 0.25 mm thick. The plates were heated at 110°C for 30 min. (10) and then ascending chromatography was performed for 30 min. with the solvent system benzene-ethanol-acetone-aq. NH_3 (sp.gr. 0.88) (50:5:40:5 by vol.). The plate was then dried and re-run for 2 hrs. at right angles to the first direction using *n*-butanol-ethanol-2.5 M- NH_3 (4:1:1 by vol.). Developed thin-layer chromatograms were dried and fixed with ethanolic 1% w. v. *p*-aminobenzoic acid and spots were located by exposure to cyanogen bromide vapour. Unsprayed plates were divided into 1 cm squares and the silica scraped off into counting vials for measurement of radioactivity.

Construction and Operation of the Cigarette Spiker

The cigarette is held in the channelled holder A (Fig. 1) by means of a contoured plastic spacer, the whole being secured by a removable split cylindrical cover A'. This assembly rotates, by virtue of the gear train B, when the shaft C is rotated. The cigarette holder and the gear train are moved on a carriage which engages with a worm on the shaft. Rotation of the shaft causes the cigarette holder to travel towards the syringe D. As this occurs the cigarette is revolving continuously and the needle penetrates the longitudinal axis of the cigarette for a predetermined distance. During this part of the movement the micrometer which operates the syringe is not driven.

When the rotation of shaft C is reversed a solution of the compound of interest is injected into the cigarette as the latter is withdrawn from the needle. The volume delivered is dependent on the ratio of the gear train E, the pitch of the micrometer screw, and the bore of the

syringe. The needle is a 17- or 18-gauge hypodermic needle modified to have a symmetrical closed point and is drilled through the sides approximately 2 mm behind the tip. The following data relate to a machine constructed for use with untipped cigarettes 70 mm long:

Ratio of gear train B, 3:1.

Ratio of gear train E, 1:55.

Pitch of worm, 4 turns per inch (1.57 per cm).

Two complete revolutions of the gear E advanced the micrometer 1 mm which caused the syringe used to deliver 112 μl of solution. By replacing the worm with one of a different pitch and choosing a needle of appropriate length one may adapt the machine to impregnate cigarettes of non-standard length, or filter-tipped varieties.

Radiolabelled Cigarette Preparation

(a) *Untipped Cigarettes*: Standard untipped flue-cured cigarettes (70 mm) were conditioned at 70°F and 60% R.H. and selected on the basis of average weight (± 20 mg) and average pressure drop ($\pm 4\%$ at a flow rate of 1050 ml/min.). Sorted cigarettes were impregnated with 112 μl of a cyclohexane solution of dotriacontane-16, 17- ^{14}C and conditioned for a further week. Batches of cigarettes containing approximately 1 μCi of radioactivity per cigarette were prepared; the first and last cigarettes of the batch being rejected. Whole cigarettes, selected at random, were extracted with toluene (25 ml) and aliquots of extract (5 ml) were chromatographed on alumina. Duplicate aliquots of column eluate and parent extract were assayed for radioactivity by liquid scintillation counting. The axial distribution of radioactivity within the tobacco rod was checked by cutting a randomly selected cigarette into equal sections. Each section corresponded to the length of tobacco rod consumed during a puff cycle, leaving a 20 mm butt.

Individual sections and the butt were weighed and extracted with toluene (10 ml) and the extracts assayed by liquid scintillation counting. The radial distribution of radioactivity was checked by autoradiography. Cigarettes were mounted in a brass holder and carefully bisected along their longitudinal axis using a skin grafting knife. The upper half of the cigarette was discarded, and the lower half, retained in the holder, was overlaid with Kodirex X-ray film (Kodak Ltd.) which was exposed at room temperature for seven days.

(b) *Tipped Cigarettes*: Conditioned and sorted tipped flue-cured cigarettes (70 mm) were each impregnated with 112 μ l of an ethanolic solution of nicotine-2'- ^{14} C-di(p-toluoyl tartrate). Cigarettes containing a nominal 0.5 μ Ci or 50 μ Ci of radioactivity were prepared. The distribution of added radioactivity along the cigarette rod was checked as before, except that methanol was used as solvent and the cigarettes were sectioned to a 21 mm butt (tip overwrap + 2 mm). Longitudinally sectioned 50 μ Ci cigarettes were overlaid with X-ray film which was exposed at room temperature for three hours.

RESULTS AND DISCUSSION

The reproducibility of operation of the cigarette-spiking apparatus was checked initially by weighing the amount of water injected into previously weighed cigarettes.

Table 1. Distribution of dotriacontane-16, 17- ^{14} C within impregnated untipped cigarettes (1.0 μ Ci)*.

Section no.	Specific activity of section (μ Ci/g)			
	Cigarette A	Cigarette B	Cigarette C	Cigarette D
1	1.436	1.393	1.659	1.257
2	1.169	0.882	1.503	1.122
3	1.085	1.139	1.011	1.105
4	0.961	1.159	0.965	1.363
5	0.992	1.402	1.014	1.292
6	0.786	1.121	0.805	0.944
7	1.134	1.098	0.822	0.963
8	1.236	1.012	1.064	1.018
9	1.042	1.057	0.841	0.892
10	0.820	0.877	0.855	0.848
Butt	0.579	0.576	0.627	0.666
Mean (excluding butt section)	1.066	1.114	1.054	1.080
95 % confidence limits	± 0.438	± 0.404	± 0.665	± 0.401
Total ^{14} C-activity recovered, including butt (μ Ci)	0.996	0.988	0.987	1.012

* Total ^{14} C-activity recovered from extracted whole cigarettes = $0.996 \pm 0.036 \mu$ Ci (mean of 4; 95 % confidence limits).

Using this instrument all cigarettes received within $\pm 1\%$ of the nominal 112 μ l.

The distribution of radioactivity along the tobacco rod of cigarettes impregnated with dotriacontane-16, 17- ^{14} C and nicotine-2'- ^{14} C-di(p-toluoyl tartrate) is shown in Tables 1 and 2 respectively. These data relate to the measured specific activity of numbered sections of cigarette, each equivalent to the length of tobacco rod (including paper) consumed during a puff and smoulder cycle under the particular smoking conditions employed, leaving 20 and 21 mm butts respectively for the two cigarette types. The section nearest the butt was designated 10; the needle entered and was withdrawn through the other end. The radial distribution was examined autoradiographically and was shown to be uniform. Since both the tipped and untipped cigarettes used in these studies were 70 mm long, the length of tobacco rod consumed during smoking to the standard butt was 49 mm for tipped cigarettes and 50 mm for untipped cigarettes. For each cigarette-type examined, the deposition of radioactive material in this portion of the tobacco rod was uniform, apart from a slightly higher level at the front of each rod. The increased deposition may have been due to capillary action when the loaded hypodermic needle entered and was withdrawn from the tobacco. The position of the cross-drilled holes in the hypodermic needle

Table 2. Distribution of nicotine di(p-toluoyl tartrate)-2'- ^{14} C within impregnated tipped cigarettes (0.5 μ Ci)*.

Section no.	Specific activity of section (μ Ci/g)			
	Cigarette A	Cigarette B	Cigarette C	Cigarette D
1	0.731	0.749	0.831	0.903
2	0.758	0.606	0.676	0.865
3	0.698	0.704	0.603	0.669
4	0.638	0.639	0.718	0.627
5	0.780	0.688	0.663	0.697
6	0.767	0.604	0.646	0.692
7	0.734	0.595	0.711	0.765
8	0.699	0.693	0.650	0.656
9	0.730	0.651	0.666	0.741
10	0.600	0.629	0.397	0.652
Butt	0.094	0.099	0.089	0.096
Mean (excluding tobacco in butt section)	0.714	0.656	0.656	0.727
95 % confidence limits	± 0.129	± 0.115	± 0.248	± 0.210
Total ^{14} C-activity recovered, including butt (μ Ci)	0.518	0.487	0.482	0.513

* Total ^{14} C-activity recovered from extracted whole cigarettes = $0.500 \pm 0.012 \mu$ Ci (mean of 4; 95 % confidence limits).

relative to the closed pointed tip resulted in reduced deposition of radioactivity in the butt portion of both tipped and untipped cigarettes. Since this portion is never smoked, however, the instrument is suitable for use in smoke transfer studies of the type referred to earlier (1, 2).

SUMMARY

An apparatus for the preparation of radiolabelled cigarettes is described. The apparatus dispensed a fixed volume of liquid from a hypodermic syringe, the volume being controlled by a gear-driven micrometer assembly. The liquid was deposited along the axis of an intact cigarette as the latter was withdrawn from the syringe needle at a uniform rate. The distribution of added radioactive material within the tobacco rod was checked and shown to be uniform.

ZUSAMMENFASSUNG

Es wird eine Apparatur zur Herstellung radioaktiv markierter Cigaretten beschrieben. Das Gerät liefert eine gegebene Flüssigkeitsmenge aus einer Injektions-spritze, wobei das Volumen durch eine Mikrometerschraube dosiert wird. Die Flüssigkeit wird in Richtung der Längsachse in eine intakte Cigarette eingespritzt, wobei diese von der Injektionsnadel gleichmäßig zurückgezogen wird. Die Verteilung von zugesetztem radioaktiven Material innerhalb des Tabakstranges wurde untersucht und erwies sich als einheitlich.

RESUME

On décrit un appareil pour la préparation de cigarettes marquées aux isotopes radio-actifs. Par une seringue hypodermique, l'appareil déverse une quantité déterminée de liquide, dont le volume est contrôlé par un assemblage micrométrique entraîné par un engrenage. Le liquide est déposé le long de l'axe de la

cigarette intacte, au fur et à mesure que celle-ci est retirée à intervalle constant de l'aiguille de la seringue. La distribution de substances radio-actives dans le boudin de tabac a été mesurée, et l'on a pu démontrer qu'elle était uniforme.

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Acknowledgement

The technical assistance of Mr. J. B. Hopper is gratefully acknowledged.

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