Influence of Puff Frequency and Puff Volume on the Alkaloid Content of Smoke

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Alkaloid delivery to the mainstream smoke of the University of Kentucky Reference Cigarette 1R1 and a commercial cigarette increased with an increase in puff volume or puff frequency. On a per cigarette basis, puff frequency had a greater effect than puff volume on the delivery of individual alkaloids. Percentage delivery of the individual alkaloids from the cigarette to the mainstream smoke was highest for anabasine and was followed in descending order by nicotine, nornicotine, and anatabine. Mainstream smoke of a University of Kentucky Reference Cigarette smoked with standard smoking conditions contained 2.63 mg, 33.5 μg, 10.0 μg, and 11.3 μg/cigarette of nicotine, nornicotine, anabasine, and anatabine, respectively.

The total alkaloid content, often expressed as nicotine content, of tobacco and tobacco smoke has been reported frequently (Harlan and Moseley, 1955; Kuhn, 1965; Stedman, 1968). The alkaloid content of smoke is readily altered by alkaloid content of the tobacco, the technique of smoking, and the presence of filters and their efficiency. Bogen (1929) reported increased nicotine delivery in the mainstream smoke with a more rapid rate of smoking. Jenson (1935) demonstrated increased nicotine delivery with increased puff volume, in effect an increased smoking rate. Also, he observed increased nicotine delivery in the mainstream smoke with shorter length of butt, lower moisture content of tobacco, and higher alkaloid content of tobacco. Newsome and Keith (1957) found linear relationships between number of puffs or puff volume and smoke weight. Wynder and Hoffman (1960) reported higher yields of condensate at higher puff frequencies. In a detailed study, Kaburaki *et al.* (1965) observed increased tar yield per cigarette with increased puff volume, puff frequency, and puff duration. In general, any factor that increases the delivery of particulate matter will increase delivery of nicotine.

Commercial cigarette tobacco contains primarily nicotine and only very small quantities of nornicotine, anabasine, and anatabine. Approximately two-thirds of the nicotine present in the tobacco is found in the smoke, while one-third is destroyed. The mainstream smoke is of greatest interest because this largely determines exposure of smokers to alkaloids. Preiss (1936), Pyriki (1948), and Harlan and Moseley (1955) have defined the distribution of nicotine during the smoking process. Depending upon smoking conditions, 20 to 30% of the nicotine in the tobacco was found in the mainstream smoke of nonfilter cigarettes. Nicotine in the sidestream smoke, in the butt, and the amount pyrolyzed was also well documented; however, little is known about the minor alkaloids. Mostly qualitative data relating to their presence in the smoke have been reported (Stedman, 1968). However, Quin (1959) reported 11 μg of anabasine and 14 μg of anatabine per cigarette in the mainstream smoke and Larson and Haag (1944) reported 4% of the nornicotine passed from tobacco to mainstream smoke. The objective of the present investigation was to study the influence of smoking parameters on the quantitative transfer of nicotine, nornicotine, anabasine, and anatabine from cigarette to mainstream smoke.

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METHODS

The University of Kentucky Reference Cigarette Lot 1R1 and a commercial, nonfilter, 85-mm cigarette were used in these investigations. The University of Kentucky Reference Cigarette is a nonfilter, 85-mm cigarette with standard low-porosity phosphate paper and a 7.6-cm (H_2O) pressure drop. Additives are 5.5% (w/w) corn syrup and 2.9% (w/w) glycerine. After equilibration to 11.3% mositure, average weight of the University of Kentucky Reference Cigarette and the commercial cigarette was 1.12 and 1.07 g, respectively. Cigarettes were analyzed or smoked immediately after removal from equilibration chambers. Four replications of five cigarettes each were smoked for each set of determinations according to the procedure of Ogg (1964). These were smoked to an approximate 23-mm butt length on a R. W. Mason Mark III smoking machine. Puff volume was 20, 35, or 50 ml; puff frequency was 30, 60, or 90 sec and all puffs were of 2-sec duration. Puff volume, puff frequency, and puff duration were all within the range observed or calculated for smokers by several investigators (Wynder and Hoffmann, 1967). Smoke from five cigarettes was collected on a Cambridge filter. Total particulate matter (TPM), including moisture, of the cigarettes from each smoking condition was determined by weighing. The alkaloid fraction was removed from the Cambridge filter by placing the filter in 30 ml of 1 *N* HCl and shaking for 30 min. Fifteen milliliters of the acid extract was transferred to a separatory funnel and extracted four times with 5-ml portions of chloroform. These chloroform extracts were discarded. Three milliliters of 10 *N* NaOH was added to the acid extract and again extracted four times with chloroform. Chloroform fractions were combined, and the chloroform was removed *in vacuo* at 40°C. The extract was made up to known volume with methyl acetate. Alkaloids of the tobacco leaf were extracted with benzene–chloroform (9 to 1 v/v) by the procedure of Keller *et al.* (1969). The benzene–chloroform was removed and the alkaloid extract made up to known volume with methyl acetate. Individual alkaloids of the smoke and tobacco leaf were separated and quantified by gas–liquid chromatography. The gas chromatograph was a Victoreen Model 4000 equipped with a flame ionization detector. The column was 2.15-mm i.d. × 3.0-m stainless steel packed with acid-washed, dimethyldichlorosilane treated 60–80 mesh Chromosorb W coated with 10% DC 550. The oven temperature, injection port temperature, and detector bath temperature were maintained at 170, 225, and 270°C, respectively. The carrier gas was helium, metered at 40 ml/min at 50 psi. Quantitation of the individual alkaloids was made from stan-

dard curves of nicotine, nornicotine, and anabasine. Anatabine was quantified from anabasine standards.

RESULTS AND DISCUSSION

Quantitative data on the individual alkaloids in the University of Kentucky Reference Cigarette and the commercial cigarette used in this investigation are summarized in Table I. The total alkaloid content of the tobacco of the UK Reference Cigarette on a dry weight basis was 2.13% and is essentially equal to the 2.09% reported earlier (Benner, 1970). Previous information is not available on the individual alkaloids for either cigarette. The influence of smoking conditions on quantitative delivery of total particulate matter (TPM), nicotine, nornicotine, anabasine, and anatabine is presented in Tables II and III. As expected, the TPM delivery increased with an increase in puff volume and an increase in puff frequency (Table II). This was most noticeable at the puff frequency of 30 sec. For the UK Reference Cigarette, the treatment of standard smoking conditions, one 35-ml puff per min of 2-sec duration, was the median treatment of the nine smoking conditions but was not significantly different from the treatments of a 50-ml puff each 90 sec or 20-ml puff each 30 sec. Data are reported on a per cigarette basis and one must consider that not all the tobacco was consumed by smoking. On a dry weight basis approximately 70% or 0.7 g of the tobacco was consumed. The same TPM delivery pattern was observed with the commercial cigarette as with the UK Reference Cigarette, but TPM delivery for each smoking treatment was lower.

Data on delivery of the individual alkaloids in the smoke of the University of Kentucky Reference Cigarette are summarized in Table III. The delivery of nicotine under standard smoking conditions was the same as the average reported earlier by 11 separate laboratories (Benner, 1970). Delivery of the individual alkaloids was consistent with the TPM data, as they also increased as puff frequency and puff volume increased. The standard smoking condition was the median treatment for all alkaloids except nornicotine. Treatments of 20 ml–90 sec and 35 ml–90 sec always had the lowest alkaloid delivery, and the 50 ml–30 sec treatment always had the highest delivery of alkaloids. Similar delivery patterns for the individual alkaloids were observed with the commercial cigarette (Table III). Treatments of 20 ml–90 sec and 20 ml–60 sec were the lowest two treatments for alkaloid delivery and, as in the UK Reference Cigarette, the 50 ml–30 sec treatment had the highest delivery of alkaloids. If one considers delivery of the individual alkaloids per cigarette, puff frequency had a greater effect than puff volume.

Table I. Alkaloid Content of Tobacco from the University of Kentucky Reference 1R1 Cigarette and a Commercial Cigarette

Alkaloid	mg/cigarette: UK reference cigarette	mg/cigarette: Commercial cigarette
Nicotine	23.00	18.10
Nornicotine	0.49	0.53
Anabasine	0.07	0.07
Anatabine	0.38	0.40
Total	23.94	19.10

Table II. TPM (Wet) of University of Kentucky Reference Cigarette and a Commercial Nonfilter 85-mm Cigarette Under Several Smoking Conditions

Puff volume, ml	Puff frequency, sec	TPM[a] mg/cigarette[b]: UK reference	TPM[a] mg/cigarette[b]: Commercial
20	90	25.5a	12.8a
	60	33.3b	18.7b
	30	46.4c	35.5e
35	90	35.7b	23.1c
	60	45.7c	30.0d
	30	59.4d	49.8g
50	90	41.2c	32.6de
	60	61.1d	39.7f
	30	79.5e	57.5h

[a] TPM in these experiments refers to smoke particulates including nicotine and water. [b] In each column treatments followed by the same letter are not significant at the 0.05 probability level.

Table III. Nicotine, Nornicotine, Anabasine, and Anatabine Delivery to Smoke by the University of Kentucky Reference Cigarette and a Commercial Cigarette Under Several Smoking Conditions

Puff volume, ml	Puff frequency, sec	Delivery of: Nicotine mg/cigarette	% transfer	Nornicotine g/cigarette	% transfer	Anabasine g/cigarette	% transfer	Anatabine g/cigarette	% transfer
		University of Kentucky reference cigarette							
20	90	1.64a	9.8	27.8ab	7.8	5.2a	10.2	7.4a	2.7
	60	2.17b	12.9	33.5bc	9.3	9.0b	17.6	11.1b	4.0
	30	2.68c	16.0	40.5d	11.4	13.5c	26.4	14.6c	5.3
35	90	1.76ab	10.5	27.5a	7.7	7.2ab	14.1	9.9ab	3.6
	60	2.63c	15.7	33.5bc	9.3	10.0b	19.5	11.3b	4.1
	30	3.84de	22.9	47.8e	13.4	13.5c	26.4	17.9d	6.5
50	90	2.60c	15.5	33.8c	9.5	8.2ab	16.1	11.2b	4.0
	60	3.44d	20.5	48.0e	13.4	10.8bc	21.1	17.2cd	6.2
	30	4.06e	24.2	55.0f	15.4	19.5d	38.2	23.2e	8.4
		Commercial cigarette							
20	90	0.88a	6.7	7.2a	1.9	3.5a	6.9	5.0a	1.7
	60	1.15b	8.7	8.3a	2.1	4.6a	9.0	6.7b	2.3
	30	2.09d	15.8	23.6c	6.1	9.4c	18.3	12.4e	4.2
35	90	1.65c	12.5	24.9c	6.4	11.6d	22.7	8.4c	2.9
	60	2.33e	17.6	20.0b	5.2	6.8b	13.3	10.2d	3.5
	30	2.81f	21.2	25.2c	6.5	11.6d	22.7	19.1f	6.5
50	90	1.94d	14.6	19.4b	5.0	7.1b	13.9	11.4de	3.9
	60	2.06d	15.5	23.9c	6.2	9.2c	18.0	12.6e	4.3
	30	3.02g	22.8	37.2d	9.6	12.6d	24.6	18.3f	6.3

In each column for each cigarette treatments followed by the same letter are not significant at the 0.05 probability level. No comparisons were made between cigarettes.

The amount of the individual alkaloids present in the smoke compared with that in the leaf is reflected by the percent transfer data. With the UK Reference Cigarette, the high and low extremes for nicotine delivery were 24.2 and 9.8%, respectively. For nornicotine these respective extremes were 15.4 and 7.7%; for anabasine they were 38.2 and 10.2%, and for anatabine the high and low extremes were 8.4 and 2.7%. With the commercial cigarette, percentage of nicotine transferred ranged from 22.8% to 6.7%. The high and low extremes for nornicotine, anabasine, and anatabine were 9.6 and 1.9%, 24.6 and 6.9%, and 6.5 and 1.7%, respectively. The percentage transfer for the individual alkaloids for both cigarettes is similar, with that for the UK Reference Cigarette being slightly higher. The quantities found in the smoke may include a portion synthesized during pyrolysis as well as the portion that is transferred directly from leaf to smoke (Stedman, 1968).

The percentages of nornicotine, anabasine, and anatabine were, of the total minor alkaloid fraction, essentially equal for all smoking treatments. In the UK Reference Cigarette, nornicotine, anabasine, and anatabine accounted for 61, 17, and 20%, respectively, of the total minor alkaloids. In the commercial cigarette their values were 52, 21, and 27%, respectively.

These data indicate that deliveries of TPM, nicotine, nornicotine, anabasine, and anatabine may be altered by changing the smoking pattern. Exposure to the alkaloid fraction can be decreased by decreasing puff volume and/or puff frequency. Changes in the smoking pattern appeared to affect delivery of the individual alkaloids similarly, but the delivery of the minor alkaloids in the mainstream smoke with respect to the delivery of nicotine tended to increase with increased puff frequency. However, transfer of the individual alkaloids from tobacco to the mainstream was different. Delivery of anabasine and nicotine was greater than delivery of nornicotine and anatabine.

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Effect of Cooking on Selenium Content of Foods

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The effect of cooking on the selenium content of a variety of foodstuffs typically found in the American diet has been studied. Little or no loss of selenium occurred as a result of broiling meats, baking seafoods, frying eggs, or boiling cereals. Dry heating of cereals, however, led to 7 to 23% losses of selenium and boiling two vegetables that contain relatively high amounts of selenium, asparagus and mushrooms, led to 29 and 44% losses of selenium, respectively. It is concluded that most ordinary cooking techniques probably do not result in major losses of selenium from most foods.

Although selenium first assumed public health importance as a result of its toxic properties (Rosenfeld and Beath, 1964), more recent work has shown that the element can also have beneficial nutritional effects when present in the diet in trace amounts (Schwarz and Foltz, 1957; Thompson and Scott, 1970). Aside from preventing a number of diseases in various animals (Hartley and Grant, 1961; Nesheim and Scott, 1961; Schubert *et al.*, 1961), selenium has also been implicated in the etiology of kwashiorkor, a human protein–calorie deficiency disease (Schwarz, 1965) and the sudden death in infants syndrome (Money, 1970). There now exist several reports in the literature which deal with the selenium content of human foods (Morris and Levander, 1970; Oelschlager and Menke, 1969; Schroeder *et al.*, 1970). In two of the above papers attention was called to the fact that cooked or processed foods tended to contain less selenium than raw foods. This was attributed to the well-known instability and volatility of many selenium compounds. However, there have been no published accounts on the effect of cooking *per se* on the selenium content of foods. The purpose of the present study was to investigate the influence of various cooking and heating

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