Respiratory Tract Retention of Blue Tetrazolium Reducing Substances from Tobacco Smoke (33761)

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A prerequisite to proper biological evaluation of the effects of tobacco smoke after short-time exposure is the ability to study the retention characteristics of smoke through the respiratory tract. Recently, a technique was developed based on blue tetrazolium reduction by substances in tobacco smoke which should permit estimation of the deposition of the particulate and gas phases (1). The application of this procedure would overcome the limited capabilities of exisiting methods which require complex analyses for monitoring gas phase or have yielded data mainly on particle size and mass for whole smoke (2–5).

The blue tetrazolium technique in effect detects certain reducing substances in tobacco smoke representing a cross section of constituents which affords a method for comparing the composition and quantity of smoke from various tobaccos. The present investigation describes a biological application in which the levels of reducing material of cigarette tobacco smoke exposed to the buccal cavity and the lung were determined.

Methods and Material. Eighteen human volunteer subjects comprised of 14 females between the ages of 18–52 and 4 males between the ages of 16–33 years were asked to smoke their own brand of cigarettes in as normal a fashion as possible. Four of the subjects have been smoking cigarettes for 15–32 years, while the remaining ones have been smoking for 3–11 years. For the meaurements of buccal absorption they were required to hold the smoke in their mouths for the approximate length of time they would ordinarily use for lung inhalation. The expelled mouth or lung air volume after each puff was directed through a Cambridge CM-113 filter in its holder. The subsequent expiration was also discharged through the same filter pad. Control air samples before a smoking trial began, in addition to the third expiration after smoking, were separately collected in the manner just described.

The trapped material on the filter pads was extracted four times with approximately 7 ml of absolute ethanol each time and the final volume was adjusted to 30 ml. To aliquots of 0.1–0.5 ml of the ethanolic extract were added 0.25 ml of 0.15% blue tetrazolium (3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-bis[2,5-diphenyl-2*H*-tetrazolium chloride]) in absolute ethanol followed by the addition of 0.25 ml of 0.3% tetramethylammonium hydroxide in absolute ethanol (v/v). The reaction was carried out in a 10 × 75 mm cuvette at 45° for 15 min and the color product was measured at 530 mμ.

Similar smoking trials were performed with vapor phase by having the subject smoke through a Cambridge filter. The expired air was directed through a Midget Impinger bubbler trap (Gelman Instrument Co.) containing 15 ml of absolute ethanol at room temperature. The color reaction was carried out as above.

Results and Discussion. The data presented in the Tables are direct measures of the blue tetrazolium reducing capacity in terms of absorbance units per fractional portion of the recovered smoke in ethanolic solution. The data are not transformed to relate or normalize these values to some "typical" standard reducing substance found in tobacco smoke because of the wide range of reactivity with blue tetrazolium that would be expected from some of the hundreds of identified compounds (6).

The data on the recovery of reducing substances from cigarette smoke after respiratory tract exposure are presented in Tables I-IV. In all subjects the retention of particulate and gaseous reducing components from smoke is much higher after pulmonary ex-

TABLE I. Recovery of Blue Tetrazolium Reducing Substances from the Particulate Matter of Expired Air from Human Subjects after Inspiration of Whole Cigarette Smoke.

		Absorbance of 1/60 aliquot/6 puffs					
Trial:		1		2		3	
Subject	Cigarette brand[a]	Mouth	Lung	Mouth	Lung	Mouth	Lung
CG	M	2.05	0.40	2.15	0.65	1.90	0.62
LHN	M	1.55	0.04	1.70	0.08	1.90	0.17
LMF	M	2.80	0.56	1.67	0.65	—	—
WR	M	1.55	0.19	1.70	0.30	—	—
SG	M	5.30	0.59	3.60	0.47	—	—
LF	S	2.70	0.07	1.95	0.12	2.15	0.10
SL	N (m)	1.50	0.10	2.10	0.56	1.90	0.38
DM	O	1.95	0.33	1.75	0.41	2.90	0.48
BO	P	1.45	0.07	1.60	0.16	—	—
HR	T	2.30	0.42	3.00	0.49	—	—
JG	H	1.77	0.38	2.70	0.55	2.90	0.53
CCC	C	4.25	0.30	2.05	0.54	3.60	0.33
MW	D	2.70	0.11	2.85	0.23	—	—
PB	D	1.50	0.25	—	—	—	—
CF	R (m)	4.50	0.49	5.25	0.45	—	—
DK	Q (m)	2.30	0.31	1.60	0.78	3.90	0.52

[a] All cigarettes were of the filter type and their lengths were 85 mm except for brands S and T which were 100 mm; (m) denotes menthol.

posure than it is for buccal cavity exposure. This is consistent with the data of others based largely on particle size and total mass determinations (2–5).

The higher absorbance values listed in Table I for particulate matter as compared to those for gas phase shown in Table III reflect the greater quantity of reducing material in the former. This was expected since the particulate phase of mainstream smoke contains approximately 10 times more reducing material than gas phase (1).

The values in Table II for inspired particulate phase were estimated from parallel trials in which the total particulates generated by each smoker were determined after trapping with a Cambridge filter. This was assumed to represent the initial level before exposure to respiratory tissue although puff volumes may have varied in the two trials. When gas phase was inhaled, the trapped particulate phase from each trial indicated that the puff volume varied widely among different smokers. The relative standard deviation of 4–6 repetitive tests on the same subjects ranged from ±14% to ±37%.

An estimated absorbance of inspired reducing material between 5.0–6.6 is given in Table II. Since the measured reducing material in the trapped expired particulates was between 3.3–5.3, a mouth retention between 10-48% was calculated. A corresponding calculation for lung yielded an estimated inspired level between 5.5–8.2 and on the basis of the directly determined expired particulates of 0.2–1.2, a lung retention of 79-97% was found.

Although comparable data for the deposition of the gas phase of smoke have not been found in the literature to permit direct comparison, it appears that the retention values listed in Table IV are similar to those reported for smoke particulate matter in general. Using rather sophisticated techniques, Mitchell (2) observed 20–50% smoke particle retention in the buccal cavity and 82–97% retention in the lungs. Others (2, 4, 5, 7) reported retention values, observed by similar procedures, ranging from 19 to 77% for mouth and 82 to 98% for lungs. Since the test subjects used in these experiments were not trained in any way before the

TABLE II. Retention of Blue Tetrazolium Reducing Material by Mouth and Lung Tissue after Inspiration of 6 Puffs of Cigarette Whole Smoke.

	Absorbance of 1/30 aliquot							
	Est. particulate phase, inspired[a]		Particulate phase expired		Difference		Retained (%)	
Subject	Mouth	Lung	Mouth	Lung	Mouth	Lung	Mouth	Lung
CG	5.8	5.8	4.1	1.1	1.7	4.7	29	81
LMF	5.0	5.6	4.5	1.2	0.5	4.4	10	79
WR	6.3	5.5	3.3	0.5	3.0	5.0	48	91
SL	6.6	5.7	3.7	0.7	2.9	5.9	44	88
LF	6.3	8.2	4.5	0.2	1.8	8.0	29	97
HR	5.9	7.1	5.3	0.9	0.6	6.2	10	87

[a] See text for explanation of how estimate achieved.

experiments, it is possible that much of the variation in the buccal cavity trials may be caused by swallowing or inhaling a portion of the smoke. All smokers were normally inhalers; thus, holding the smoke in the mouth was not natural for them.

The simplicity and sensitivity of this blue tetrazolium procedure should be useful in other applications such as routine monitoring of tobacco smoke reducing materials as they come from a cigarette, cigar or pipe, as they traverse the respiratory tract and as they enter the tissues of laboratory animals. An estimate of the amount of reducing material absorbed on lung tissue as compared with that quantity that enters the lung air space may be possible. Lung washings or homogenates may contain tissue tetrazolium reductase which must first be inhibited to prevent interference with the nonenzyme reduction of blue tetrazolium. In such a system a comparison could then be made of absorbed (lung washings) and penetrating (lung homogenates) reducing material with and without smoke exposure.

Summary. A new biological application of blue tetrazolium as a measure of reducing components in particulate and vapor phase portions of cigarette smoke was studied. Quantitative comparisons of reducing material trapped with other particulate on a Cambridge filter and those present in the gas phase permitted estimation of the percentage of reducing material retained by the human

TABLE III. Recovery of Blue Tetrazolium Reducing Substances in Expired Air after Inspiration of Gas Phase Only.

		Absorbance of 1/30 aliquot/6 puffs					
	Trial:	1		2		3	
Subject	Cigarette brand	Mouth	Lung	Mouth	Lung	Mouth	Lung
CG	M	0.49	0.02	0.31	0.03	0.26	0.01
LMF	M	0.19	0.04	0.18	0.03	0.09	0.01
WR	M	0.39	0.04	0.10	0.01	—	—
LHN	M	0.22	0.02	0.10	0.01	0.31	0.02
MC	M	0.32	0.06	0.29	0.02	—	—
GN	M	0.10	0.02	0.12	0.01	0.15	0.01
DM	O	0.23	0.03	0.60	0.06	0.67	0.02
SL	N	0.49	0.02	0.43	0.02	—	—
DO	Q	0.34	0.03	0.57	0.02	—	—
LF	S	0.45	0.02	0.26	0.01	0.29	0.02
HF	T	0.32	0.14	0.30	0.03	—	—

TABLE IV. Retention of Blue Tetrazolium Reducing Material by Mouth and Lung Tissue after Inspiration of 6 Puffs of Gas Phase of Cigarette Smoke.

	Absorbance of 1/30 aliquot							
	Est. gas phase, inspired[a]		Gas phase expired		Difference		Retained (%)	
Subject	Mouth	Lung	Mouth	Lung	Mouth	Lung	Mouth	Lung
CG	0.72	0.62	0.49	0.02	0.23	0.60	32	97
LMF	0.53	0.46	0.19	0.04	0.34	0.42	64	91
WR	0.79	0.65	0.39	0.04	0.40	0.61	51	94
LHN	0.27	0.34	0.22	0.02	0.05	0.32	19	94
MC	0.54	1.04	0.32	0.06	0.22	0.98	41	94
GN	0.33	0.65	0.10	0.02	0.23	0.63	70	97
DM	0.98	0.77	0.23	0.03	0.75	0.74	77	96
SL	1.02	0.66	0.49	0.02	0.53	0.64	52	97
D O'D	0.98	0.69	0.34	0.03	0.64	0.66	65	96
LF	0.85	0.90	0.45	0.02	0.40	0.88	47	98
HR	0.56	0.77	0.32	0.14	0.24	0.63	43	82

[a] Since it has been established that there is approximately 10 times more reducing material in particulate phase as compared to vapor phase (1), an estimate of reducing material in the inspired vapor phase was obtained by direct measure of the reducing material in the particulate matter that had been trapped on the Cambridge filter during the gas phase smoking of each individual.

respiratory tract during a smoking trial. The technique of measuring blue tetrazolium reducing materials can be utilized to assess the deposition of gas and particulate constituents of tobacco smoke in various regions of the respiratory tract.

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