**CELLULAR MORPHOMETRY OF THE BRONCHI
OF HUMAN AND DOG LUNGS**

**Annual Progress Report
For Period April 1, 1992 - March 31, 1993**

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Abstract

Quantitative data of the human bronchial epithelial cells at possible risk for malignant transformation in lung cancer is crucial for accurate radon dosimetry and risk analysis. The locations and other parameters of the nuclei which may be damaged by α particles must be determined and compared in different airway generations, among smokers, non-smokers and ex-smokers, between men and women and in people of different ages. This proposal includes extended morphometric studies on electron micrographs of human epithelium of defined airway generations and in parallel on electron micrographs of the dog bronchial lining. The second part of this proposal describes studies to quantitate the cycling bronchial epithelial population(s) using proliferation markers and immunocytochemistry on frozen and paraffin sections and similar labeling of isolated bronchial epithelial cells sorted flow cytometry.

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For the Budget Period April 1, 1993 through March 31, 1994

1. Total DOE Funds Obligated (Note A)	\$100,000
2. Estimated Total Project Costs through the Current Budget Period (Note B) chargeable to DOE	\$100,000
3. Anticipated DOE Funds Available For Carry-Over (Note C) into the Next Budget Period	NONE

NOTES

A- See \$ amount in item 16.b (3) on Grant Face Page (Form DOE F 4600.1).

B- Only costs incurred or estimated to be incurred under Section A, Budget Plan of Appendix C, to this Grant, should be used in computing this amount.

C- This amount may not necessarily agree with your cash amounts.

PROGRESS REPORT

A. Human and Dog Tissues, Electron Microscopy

All but one of the 63 human lung specimens received to date (139 samples, each with one to four different bronchi of the same generation; generations 2 to 6) and dog bronchi similarly dissected (249 samples from 39 dog lungs; generations 1 to 6 and branch points of 4 generations) have been processed through embedding in epoxy resin. Approximately 90% of our human samples have been sectioned and stained for light microscopic examination. Of these approximately 85% have been judged to be adequately preserved and usable for electron microscopic morphometric analysis. One hundred and nineteen human bronchial samples have been completely processed for electron microscopy. From the 1041 electron micrographs taken of the epithelial lining of these human bronchi 856 have been entered into the Computerized Stereological Analysis Program (COSAS, Cornacchia and Black, 1988). They have also been used for the measurement of the distances of basal and mucous cell nuclei to the apical free surface and the number of cells per unit length of basal lamina.

Table 1 summarizes the patients who were the sources of our tissues, the diagnoses of their lesions and the lung lobes from which we obtained bronchi. Table 2 summarizes our dog tissues. We are also analyzing the epithelia at areas of airway bifurcation as well as those of adjacent airways and so we have been taking branch point samples adjacent to each bronchial generation.

Using our expanding electron micrograph collection and COSAS, we continue to add to our established data bases which describe the nuclear volume density as well as other nuclear parameters such as maximum and minimum diameters and nuclear numbers per electron micrograph for five cell types (basal, mucous, intermediate/indeterminate, ciliated, and intraepithelial white blood cells) of the bronchial epithelium of humans and dogs. As sufficient human samples are

processed, we will compare these data for men and women, as well as smokers, non-smokers and ex-smokers. A paper using some of our data has been submitted (Robbins et al., 1992).

Of the 882 electron micrographs of our dog bronchial epithelium samples 730 have been entered into COSAS and also used for the measurement of the distances of basal and mucous cell nuclei to the epithelial free surface. The branch points of dog bronchi are also being examined to determine if their epithelia differ from that of the adjacent airways.

As we accumulate additional electron micrographs of bronchial gland duct epithelium we will extend our analysis to include the basal cells of these ducts. Both the ciliated and the collecting ducts of human bronchial glands clearly have basal cells (Meyrick et al. 1969). We have collected micrographs of the epithelium of 22 bronchial gland ducts but have not analyzed them as yet.

B. Morphometry

Since we have access to patient records and interview most of the people from whom we get bronchial samples, we are comparing the airways of ex-smokers with smokers and non-smokers. We also collect occupational and residence data. For our analyses we consider individuals who stopped smoking more than 10 years ago as ex-smokers and those who stopped more recently as smokers. Table 3 summarizes the volume density data to date for the 5 cell types of the human bronchial epithelial nuclei and intraepithelial white blood cells of smokers, non-smokers, and ex-smokers by airway generation. Overall there are trends but as yet no statistically significant differences. It seems, if one examines the VD data for basal cell nuclei, that ex-smokers may be different from non-smokers and more like smokers. Although mucous metaplasia is known to accompany smoking (Chang 1957; Hayashi et al., 1978) the volume density of mucous cell nuclei does not differ among the three groups even though the total volume occupied by the cells may. These

initial data thus suggest that smoking may cause changes in the basal (but perhaps not mucous cells) such that they do not revert to the non-smoking values even after 10 years when cancer risk has dropped (Fishman, 1988; Samet, 1992).

Areas of abnormal epithelium are seen in some bronchi and are excluded from our analyses. Fig. 1 shows disturbed epithelial polarity in the epithelium of a non-smoker. Endocrine cells are also occasionally found (Fig. 2) and clearly have hemidesmosomes as reported for other basal cells (Evans and Plopper, 1988),

The figures we generated for the distances of basal and mucous cell nuclei to the epithelial free surface have been used to update a model of the ^{222}Rn decay product dose to these nuclei in the human bronchial epithelium (Harley, et al, 1991). We continue to record the sex and age of our patients and will analyze these parameters when the data base is larger. In comparison to Baldwin et al. (1991) our data for the distances of the nuclei of the basal cells (6006 measured) and mucous cells (2103 measured) shows mean values in the same range as Baldwin et al. but slightly lower. The following gives our current unweighted mean values:

BASAL CELL NUCLEI

Gen.	Smokers		Non-smokers		Ex-Smokers	
	Dist. $\pm$ SE	# Pts.	Dist. $\pm$ SE	# Pts.	Dist. $\pm$ SE	# Pts.
3	32.0 $\pm$ 3.5	11	25.0 $\pm$ 1.1	7	-	-
4	27.5 $\pm$ 1.6	14	22.6 $\pm$ 2.1	7	27.2 $\pm$ 3.8	3
5	29.4 $\pm$ 1.8	16	29.4 $\pm$ 2.6	8	34.4 $\pm$ 8.5	3
6	26.1 $\pm$ 2.6	10	29.6 $\pm$ 3.2	6	32.5 $\pm$ 8.5	1
all	29.9 $\pm$ 1.6	30	26.4 $\pm$ 3.2	10	28.3 $\pm$ 2.6	4

MUCOUS CELL NUCLEI

Gen.	Smokers		Non-smokers		Ex-Smokers	
	Dist. $\pm$ SE	# Pts.	Dist. $\pm$ SE	# Pts.	Dist. $\pm$ SE	# Pts.
3	21.3 $\pm$ 2.9	11	16.4 $\pm$ 1.1	7	-	-
4	19.3 $\pm$ 1.6	13	15.2 $\pm$ 2.1	5	12.1 $\pm$ 2.8	6
5	19.1 $\pm$ 1.9	12	19.2 $\pm$ 2.9	6	-	-
6	22.5 $\pm$ 6.1	6	17.4 $\pm$ 2.6	6	-	-
all	20.2 $\pm$ 1.3	40	17.1 $\pm$ 1.1	24	-	-

A direct comparison with the results of Mercer et al.(1991) is not possible because of the way their data are presented. In bronchi from 3 human lobes whose generations are defined by diameter they report that basal and mucous cell nuclei are found at a range of 24 to 48 μ m from the epithelial surface.

We have found intraepithelial leucocytes and occasional mast cells and are quantifying these as one of our cell populations whose nuclei are within the range of α particles (Tables 3, 4, 5). Such cells are often present in bronchial epithelium although not always noted (for example Fig. 1 of McDowell et al.,1978). The intraepithelial T-lymphocytes subsets in human bronchi have been characterized (Fournier et al. 1989). A recent paper by Henshaw and colleagues (Bridges et al, 1991) suggests a possible association between mutations of the hypoxanthine-guanine phosphoribosyl transferase gene in human blood T-lymphocytes and the radon concentrations in the homes of the individuals whose cells were examined. These authors clearly indicate that their findings do not prove causality but they speculate that Rn could affect the lymphocytes while they are "resident in various organs including bone marrow and the lung" (Bridges et al.,1991, p.1187). We (Harley and Robbins, 1991) have examined the possibilities of significant lymphocyte exposure to Rn decay products while they are in various lung loci

(intraepithelial, BALT [bronchus associated lymphoid tissue], within the connective tissue space or capillaries of the interalveolar septa, within lymphatic vessels accompanying major airways or in the pleura). It is apparent that the populations of lymphocytes which may be subjected to the highest alpha particle dose while in the lung are within the epithelium or just below it within BALT (Harley and Robbins, 1991). A provocative suggestion such as the one made by Bridges and colleagues must however, explain the apparent lack of increased risk of primary lung lymphoma in miners exposed to high Rn levels. It is clear that in vitro human lymphocytes can be damaged by radon (Wolff et al., 1991). At present we are examining our electron micrograph set to determine the percentages of the different intraepithelial white blood cell types. Some preliminary figures from 408 micrographs by airway generation are summarized below.

		<u>Human Intraepithelial Wandering Cells by Type</u>					
Gen		#	PMN	Small	Med.	Large	
Mast							
#	µgphs		Lympho	Lympho	Lympho	Macroph	Cells
3	102	0	24	30	28	11	3
4	128	4	30	36	11	8	7
5	116	0	28	35	9	17	7
6	62	0	18	24	17	11	16

Table 4 summarizes the volume density data for the five types of epithelial cell nuclei of dog bronchi from generations 2 to 6 and includes as separate categories the epithelia of bronchi at four branch points (generations 2 to 3; 3 to 4; 4 to 5; 5 to 6). In view of the possible non-uniformity of cell populations at these regions of greater stresses and particle deposition, we are extending our analysis to examine bifurcation areas in comparison to the airways just proximal and just distal. Micrographs of additional branch points will be added as we complete the processing of our tissues. In comparison with the VD data for human bronchial lining cells (Table 3), dog basal cell nuclei and mucous cell nuclei occupy (Table 4) a somewhat larger percentage of the epithelial volume.

Measurements of the distances on the electron micrographs of basal and mucous cell nuclei to the epithelial free surface analogous to those of human bronchial epithelium are also being made of dog bronchial epithelium. Some of our measurements of these distances have been utilized to update the ^{222}Rn dosimetry for the dog lung (Harley et al., 1991b). Fig. 3 of dog bronchial epithelium shows a rare image of a basal cell in mitosis.

The number density of the nuclei of the 5 cell types per generation and the number of nuclei per micrograph per generation can also be derived from COSAS. Table 5 gives these data for our human bronchi and branch points and compares smokers, non-smokers and ex-smokers.

C The conversion of COSAS for the Macintosh

This process is 70% complete (the programmer has just had a baby and the conversion has taken somewhat longer than initially anticipated). Concluding the conversion will simplify and speed our research. It will also require that we have additional hard disk computer memory and RAM .

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TABLE 1. DIAGNOSES AND LUNG SITES OF HUMANS

DIAGNOSIS	FEMALES			MALES		
	SM	NS	EX-SM	SM	NS	EX-SM
SQUAMOUS CELL CARCINOMA	4			4		3
LARGE CELL CARCINOMA	2			3		
EPIDERMOID CARCINOMA	2		1			
ADENOCARCINOMA	5	6	4	5	2	2
CARCINOID CARCINOMA	1	2				
UNKNOWN AT THIS TIME	3		2	2		2
OTHER	3	1		2	1	

TISSUE SOURCE	FEMALES			MALES		
	SM	NS	EX-SM	SM	NS	EX-SM
RIGHT UPPER LOBE	3	1	2	7	2	3
RIGHT MIDDLE LOBE	3	1	1	1		1
RIGHT LOWER LOBE	2	1	1	1		
LEFT UPPER LOBE	5	5	3	7		1
LEFT LOWER LOBE	5			1		1
RIGHT LUNG	2	1				1

TOTAL FEMALES = 36
TOTAL MALES = 26

Table 1. Lung samples are classified by location and pathology. This table includes only the lung samples from which useful bronchi (acceptably preserved) were derived. Compared to the expected incidence of lung cancer in the U.S. our group shows more adenocarcinoma in woman and less in men as might have been expected (Churg,1988; Yesner,1988). It has been reported that the upper lobes are a predominant site of carcinoma (Churg,1988) and our cohort shows that both the left and right upper lobes as very frequent sites of tumor occurrence.

TABLE 2

DOG BRONCHI

GENERATION	NUMBER OF SAMPLES
GENERATION 1	20
GENERATION 2	30
GENERATION 2 BRANCH	6
GENERATION 3	31
GENERATION 3 BRANCH	13
GENERATION 4	33
GENERATION 4 BRANCH	14
GENERATION 5	34
GENERATION 5 BRANCH	13
GENERATION 6	24
GENERATION 6 BRANCH	11
GENERATION 7	1
TOTAL	229

Table 2. Thirty-nine male dogs of various ages and sizes have been the source of these bronchi which were taken from all lobes. Initial fixation procedures were developed using dog tissues and of those, forty samples were found to be inadequately preserved and were discarded. Of the 229 samples 72 have been analysed to date. The total number of micrographs of dog bronchial epithelium in COSAS at present is 882, of these 743 micrographs have been analyzed.

TABLE 3

Number and Volume Density of Human Bronchial Epithelial Cells

A. Smokers

Gen	# ugrphs	Mucous Cells		Basal Cells		Indef/intermed Cells		Ciliated Cells		White Blood Cells	
		VD	#	VD	#	VD	#	VD	#	VD	#
2	4	0.02 ± 0.02	4.50 ± 0.58	0.03 ± 0.01	9.50 ± 1.91	0.04 ± 0.01	9.50 ± 3.42	0.08 ± 0.01	11.25 ± 2.50	0.01 ± 0.009	6.25 ± 3.95
3	93	0.02 ± 0.02	3.83 ± 2.81	0.04 ± 0.01	7.44 ± 2.91	0.06 ± 0.03	9.77 ± 5.49	0.07 ± 0.02	8.83 ± 3.58	0.004 ± 0.005	1.48 ± 2.17
4	104	0.02 ± 0.02	3.29 ± 2.63	0.04 ± 0.01	7.32 ± 2.63	0.05 ± 0.04	9.66 ± 6.85	0.09 ± 0.04	10.43 ± 3.69	0.006 ± 0.006	1.88 ± 1.89
4BR	9	0.01 ± 0.01	2.00 ± 1.73	0.03 ± 0.01	9.00 ± 2.12	0.05 ± 0.02	12.11 ± 6.11	0.08 ± 0.02	10.56 ± 4.13	0.002 ± 0.003	1.00 ± 1.00
5	116	0.02 ± 0.01	2.54 ± 2.04	0.03 ± 0.02	6.44 ± 2.58	0.04 ± 0.02	7.66 ± 5.28	0.10 ± 0.04	10.39 ± 3.86	0.006 ± 0.007	1.64 ± 2.34
5BR	9	0.02 ± 0.02	5.22 ± 2.91	0.03 ± 0.01	7.67 ± 3.00	0.04 ± 0.02	10.11 ± 4.19	0.08 ± 0.02	10.78 ± 3.42	0.01 ± 0.004	4.89 ± 2.03
6	84	0.02 ± 0.02	4.01 ± 3.23	0.03 ± 0.01	7.52 ± 3.06	0.05 ± 0.04	11.21 ± 10.16	0.08 ± 0.03	11.76 ± 4.75	0.007 ± 0.008	2.17 ± 2.72

B. Non-Smokers

Gen	# ugrphs	Mucous Cells		Basal Cells		Indef/Intermed Cells		Ciliated Cells		White Blood Cells	
		VD	#	VD	#	VD	#	VD	#	VD	#
3	50	0.02 ± 0.02	2.92 ± 2.42	0.04 ± 0.01	7.56 ± 2.43	0.06 ± 0.03	9.56 ± 5.11	0.08 ± 0.03	9.50 ± 2.91	0.004 ± 0.004	1.12 ± 1.19
4	85	0.02 ± 0.02	3.26 ± 3.07	0.09 ± 0.01	7.49 ± 2.53	0.05 ± 0.03	7.95 ± 5.30	0.09 ± 0.03	10.28 ± 3.65	0.003 ± 0.006	0.97 ± 1.33
5	55	0.02 ± 0.01	3.72 ± 2.59	0.04 ± 0.01	8.13 ± 3.36	0.05 ± 0.02	11.69 ± 6.68	0.09 ± 0.03	13.15 ± 4.79	0.005 ± 0.005	1.22 ± 1.07
6	63	0.02 ± 0.01	3.25 ± 2.85	0.04 ± 0.01	8.24 ± 2.90	0.05 ± 0.03	10.87 ± 5.79	0.09 ± 0.03	12.54 ± 3.78	0.005 ± 0.005	1.84 ± 1.57

C. Ex-Smokers

Gen	# ugrphs	Mucous Cells		Basal Cells		Indef/Intermed Cells		Ciliated Cells		White Blood Cells	
		VD	#	VD	#	VD	#	VD	#	VD	#
3	2	0.03 ± 0.01	7.00 ± 2.82	0.01 ± 0.0002	2.50 ± 0.70	0.02 ± 0.01	5.50 ± 3.54	0.09 ± 0.01	12.50 ± 2.12	0.000 ± 0.000	0.00 ± 0.00
4	46	0.02 ± 0.02	4.59 ± 3.38	0.03 ± 0.01	6.78 ± 2.58	0.05 ± 0.02	8.82 ± 4.04	0.07 ± 0.03	8.22 ± 3.66	0.007 ± 0.008	2.13 ± 2.47
5	57	0.02 ± 0.02	3.63 ± 2.88	0.03 ± 0.02	7.61 ± 3.69	0.04 ± 0.02	8.47 ± 5.51	0.09 ± 0.03	11.86 ± 4.37	0.006 ± 0.007	1.86 ± 2.09
6	29	0.02 ± 0.04	2.62 ± 2.54	0.03 ± 0.01	6.79 ± 2.29	0.06 ± 0.04	11.28 ± 7.40	0.08 ± 0.04	10.10 ± 4.03	0.004 ± 0.008	1.14 ± 1.59

Table 3. Summarizes the data from COSAS on the volume density (VD) of the nuclei of airway cell types (mucous, basal, intermediate/indeterminate, ciliated, and intraepithelial white blood cells) and the average numbers of nuclei of each type per micrograph. The data are presented for four bronchial generations and smokers, non-smokers, and ex-smokers are compared.

TABLE 4

Number and Volume Density of Dog Bronchial Epithelial Cell Nuclei by Airway Generation

Gen	#	Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
		V/D	#	V/D	#	V/D	#	V/D	#	V/D	#
2	108	0.019 ± 0.017	2.72 ± 1.86	0.035 ± 0.013	8.59 ± 2.44	0.039 ± 0.023	8.45 ± 5.28	0.097 ± 0.029	11.74 ± 3.66	0.006 ± 0.007	1.97 ± 2.31
2,3	7	0.031 ± 0.007	3.57 ± 12.13	0.036 ± 0.011	7.14 ± 2.34	0.045 ± 0.018	6.57 ± 2.44	0.098 ± 0.027	9.86 ± 3.53	0.0002 ± 0.0004	0.14 ± 0.38
3	132	0.022 ± 0.017	3.28 ± 2.19	0.039 ± 0.014	8.62 ± 2.72	0.046 ± 0.027	7.96 ± 5.32	0.105 ± 0.038	11.92 ± 3.51	0.002 ± 0.004	0.78 ± 1.42
3,4	18	0.015 ± 0.012	3.12 ± 2.39	0.033 ± 0.018	8.22 ± 2.13	0.039 ± 0.019	8.00 ± 4.12	0.089 ± 0.031	12.06 ± 2.15	0.003 ± 0.006	0.83 ± 0.99
4	163	0.032 ± 0.017	4.34 ± 2.35	0.036 ± 0.014	8.00 ± 3.02	0.042 ± 0.025	7.72 ± 5.17	0.114 ± 0.041	13.13 ± 4.17	0.025 ± 0.028	0.88 ± 1.29
4,5	45	0.029 ± 0.013	4.56 ± 2.37	0.030 ± 0.013	7.1 ± 2.5	0.033 ± 0.019	6.60 ± 4.66	0.113 ± 0.030	14.64 ± 4.34	0.003 ± 0.005	0.98 ± 1.51
5	145	0.039 ± 0.025	4.90 ± 2.62	0.033 ± 0.011	7.95 ± 2.91	0.044 ± 0.030	8.31 ± 7.10	0.109 ± 0.035	13.43 ± 4.74	0.002 ± 0.003	0.70 ± 1.55
5,6	9	0.026 ± 0.016	5.00 ± 2.92	0.037 ± 0.014	7.44 ± 2.65	0.036 ± 0.019	6.89 ± 3.76	0.151 ± 0.027	17.78 ± 2.39	0.003 ± 0.003	0.44 ± 0.53
6	108	0.028 ± 0.016	4.33 ± 2.66	0.033 ± 0.013	6.84 ± 2.62	0.035 ± 0.023	6.19 ± 4.13	0.113 ± 0.036	13.22 ± 4.37	0.002 ± 0.003	0.68 ± 0.96

Table 4. Summary of the data from COSAS on the volume density (V/D) of the nuclei of airway cell types (mucoi's, basal, intermediate/indeterminate, ciliated, and intraepithelial white blood cells) and the average numbers of nuclei of each type per micrograph. The data are presented for 5 generations as well as 3 branch points (generations 2 to 3; 3 to 4; 4 to 5; and 5 to 6). A total of 730 dog micrographs entered and analyzed.

TABLE 5

Number and Number Density of Human Bronchial Epithelial Cells

A. Smokers

Gen	# ugrphs	Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
		ND	#	ND	#	ND	#	ND	#	ND	#
2	4	0.01 ± 0.02	4.50 ± 0.58	0.04 ± 0.01	9.50 ± 1.91	0.03 ± 0.01	9.50 ± 3.42	0.03 ± 0.004	11.25 ± 2.50	0.03 ± 0.01	6.25 ± 3.95
3	93	0.02 ± 0.02	3.83 ± 2.81	0.06 ± 0.04	7.44 ± 2.91	0.07 ± 0.04	9.77 ± 5.49	0.05 ± 0.03	8.83 ± 3.58	0.01 ± 0.02	1.48 ± 2.17
4	104	0.02 ± 0.02	3.29 ± 2.63	0.06 ± 0.02	7.32 ± 2.63	0.06 ± 0.04	9.66 ± 6.85	0.06 ± 0.03	10.43 ± 3.69	0.02 ± 0.02	1.88 ± 1.89
4BR	9	0.02 ± 0.01	2.00 ± 1.73	0.07 ± 0.02	9.00 ± 2.12	0.09 ± 0.04	12.11 ± 6.11	0.06 ± 0.02	10.56 ± 4.13	0.01 ± 0.01	1.00 ± 1.00
5	116	0.02 ± 0.02	2.54 ± 2.04	0.05 ± 0.03	6.44 ± 2.58	0.05 ± 0.04	7.66 ± 5.28	0.06 ± 0.03	10.39 ± 3.86	0.02 ± 0.02	1.64 ± 2.34
5BR	9	0.04 ± 0.02	5.22 ± 2.91	0.05 ± 0.02	7.67 ± 3.00	0.08 ± 0.03	10.11 ± 4.19	0.06 ± 0.03	10.78 ± 3.42	0.04 ± 0.02	4.89 ± 2.03
6	84	0.03 ± 0.03	4.01 ± 3.23	0.07 ± 0.04	7.52 ± 3.06	0.09 ± 0.12	11.21 ± 10.16	0.08 ± 0.05	11.76 ± 4.75	0.02 ± 0.02	2.17 ± 2.72

B. Non-Smokers

Gen	# ugrphs	Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
		ND	#	ND	#	ND	#	ND	#	ND	#
3	50	0.02 ± 0.02	2.92 ± 2.42	0.06 ± 0.02	7.56 ± 2.43	0.07 ± 0.04	9.56 ± 5.11	0.06 ± 0.02	9.50 ± 2.91	0.01 ± 0.01	1.12 ± 1.19
4	85	0.02 ± 0.02	3.26 ± 3.07	0.07 ± 0.03	7.49 ± 2.53	0.07 ± 0.05	7.95 ± 5.30	0.08 ± 0.03	10.28 ± 3.65	0.01 ± 0.02	0.97 ± 1.33
5	55	0.03 ± 0.02	3.72 ± 2.59	0.06 ± 0.03	8.13 ± 3.36	0.08 ± 0.05	11.69 ± 6.68	0.08 ± 0.04	13.15 ± 4.79	0.01 ± 0.02	1.22 ± 1.07
6	63	0.02 ± 0.02	3.25 ± 2.85	0.06 ± 0.03	8.24 ± 2.90	0.07 ± 0.05	10.87 ± 5.79	0.07 ± 0.03	12.54 ± 3.78	0.02 ± 0.01	1.84 ± 1.57

C. Ex-Smokers

Gen	# ugrphs	Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
		ND	#	ND	#	ND	#	ND	#	ND	#
3	2	0.03 ± 0.02	7.00 ± 2.82	0.02 ± 0.003	2.50 ± 0.70	0.02 ± 0.01	5.50 ± 3.54	0.05 ± 0.02	12.50 ± 2.12	0.00 ± 0.00	0.00 ± 0.00
4	46	0.03 ± 0.02	4.59 ± 3.38	0.05 ± 0.02	6.78 ± 2.58	0.06 ± 0.04	8.82 ± 4.04	0.05 ± 0.03	8.22 ± 3.66	0.02 ± 0.03	2.13 ± 2.47
5	57	0.02 ± 0.02	3.63 ± 2.88	0.06 ± 0.02	7.61 ± 3.69	0.06 ± 0.04	8.47 ± 5.51	0.07 ± 0.04	11.86 ± 4.37	0.02 ± 0.03	1.86 ± 2.09
6	29	0.02 ± 0.02	2.62 ± 2.54	0.05 ± 0.02	6.79 ± 2.29	0.08 ± 0.06	11.28 ± 7.40	0.06 ± 0.05	10.10 ± 4.03	0.01 ± 0.02	1.14 ± 1.59

Table 4. Summarizes the data from COSAS on the Number density (ND) of the nuclei of airway cell types (mucous, basal, intermediate/indeterminate, ciliated, and intraepithelial white blood cells). The data are presented for four bronchial generations and smokers, non-smokers, and ex-smokers are compared.

Legend for FIGURE 1

a. Bronchial epithelium from a 53 year old non-smoker demonstrating some disturbed polarity in the form of tight junction sealed, ciliated intrapithelial channels. (I). In b. one such space appears to communicate with the free surface. Note the intrapithelial lymphocyte (arrow)

Magnification 2,500x.

Fig. 1

FIGURE 2

Among the basal cells of this micrograph from a 74 year old male smoker is the cytoplasm of an endocrine cell which also rests on the basal lamina. It clearly has hemidesmosomes (arrows).

Magnification 15,000 x

FIGURE 3

Slightly obliquely sectioned dog bronchial epithelium demonstrating a basal cell in mitosis (arrow). Note the intraepithelial lymphocytes and thin basement membrane which is typical of the dog airway lining. This is a rare finding in our electron micrograph set.

Magnification 2,500x.

END

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