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CELLULAR MORPHOMETRY AND CYCLING CELL POPULATIONS OF HUMAN AND DOG BRONCHI

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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 nuclei of these cells may be targets for damage by α particles. Then it is important to determine the locations and other parameters of these nuclei 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 bronchial epithelium of defined airway generations. The second part of this proposal describes the continuation of studies to quantitate the cycling tracheo-bronchial epithelial population(s) using proliferation markers and immunocytochemistry on paraffin sections. The proliferative potential of the airway mucosa of smokers, non-smokers, and ex-smokers, men and women, as well as individuals of different ages are being compared. Normal human bronchial linings are also being compared with normal adult dog bronchi and metaplastic and repairing human airways. Since cycling cells can be more sensitive to damage from carcinogens and radioactivity, the quantitative data from this project will allow the development of more accurate radon risk analysis.

I. Introduction

In order to assess the lung tissue at risk for genetic damage from radon daughter decay products, the quantitative and cycling cell parameters of the epithelial lining of the bronchi, the most common sites of cancer, must be determined in people of both sexes, individuals of different ages, and smokers, non-smokers and ex-smokers. Our research has included studies in two broad areas: (1) morphometry and (2) determination of the cycling and possible stem cell populations of all the bronchial epithelial structures (i.e. lining, glands and their ducts). These data are necessary for more accurate dosimetry and radon risk analysis and to begin to characterize the cell populations(s) to which genetic damage may result in eventual neoplasia.

First, our efforts have been directed towards expanding our current stereological data base to include larger numbers of samples from both sexes, younger individuals, ex-smokers and non-smokers, and lungs of people of all ages from transplant donors rather than from surgical specimens, i.e. not tissues adjacent to areas of lung pathology. Airways from both types of samples have been used to determine the distances of the nuclei of epithelial cells to the free surface and the volume density (V_D) as well as other parameters of the nuclei in all these human groups using electron micrographs. These data are necessary to develop more accurate weighting factors for dosimetry and radon risk analysis for the different segments of the population which may have significantly different lung functions (Hislop and Haworth, 1989; Jacobs et al., 1992; Pistelli et al., 1992), cancer risks (Bourke et al., 1992) and cellular parameters as well as Rn exposures (Svensson et al., 1989). We have been receiving lungs from transplant donors from the National Disease Research Interchange (NDRI) and our data base now includes a significant number of samples of bronchi of the very young, adolescents, and young adults as well as adult never smokers of both sexes.

Our work is a comprehensive attempt, at the electron microscopic level, to determine the volume density and several other important quantitations of human epithelial cell nuclei. In addition, we are determining the distances of putative target nuclei to the epithelial free surface in human airways defined by dissection, not size. The number of cells of different types per unit length of basal lamina of bronchial epithelium is also being derived from the data base of electron micrographs. Such numbers are permitting more accurate estimations of the dimensions of the nuclear targets in radon-induced carcinogenesis and also allow more realistic dosimetry and risk analysis. Since radon dosimetry and risk may differ in smokers, non-smokers and

ex-smokers, men and women as well as people of different ages, data about the bronchi of these groups is being obtained and compared.

Our second area of focus is the cycling cells and possible stem cell populations(s) of the bronchial mucosa and thus the cells most likely to be the epithelial targets for alpha particle induced carcinogenesis. Although there is no definitive answer yet as to the number of mutations required for lung carcinogenesis (Renan, 1993), dividing cells are at increased risk for damage to genetic material and the process of mitosis itself may be important in tumorigenesis (Mothersill et al., 1989; Preston-Martin et al., 1990; Birrer et al., 1992). Thus, it is vital to determine the capacity of the human bronchial lining epithelial cell populations of different age groups, men, women, non-smokers, smokers and ex-smokers to cycle and to compare different airway generations and bifurcation regions. Our efforts so far have been presented in two abstracts (Robbins and Meyers, 1993; Robbins and Meyers, 1994a), and a full paper has been submitted with our initial data (Robbins and Meyers, 1994b). This paper includes a new model for the stem cell populations of the human tracheobronchial epithelium. Normally, in adults, the tracheobronchial epithelium has been considered to have a very low turnover rate (Boren and Paradise, 1978; Ayers and Jeffery, 1988) but in humans little is known about the normal mitotic rates at different ages, in different airway generations or at areas of bifurcation, or possible differences between the sexes. As indicated above, the organ donor lungs which we have been receiving through our participation in the NDRI program have provided us the unparalleled opportunity to examine the cycling bronchial epithelial populations in lungs of individuals of different ages and sexes, smokers, ex-smokers and non-smokers and in airways adjacent to those used for morphometric measurements.

Quantitative data as to the extent to which the bronchial epithelial cells can be stimulated to divide and the relative contributions of basal vs. suprabasal cells to the process of repairing the bronchial mucosa is essential to improved radon dosimetry and risk analysis. We have found that because organ donors often spend several days on a respirator before the lungs are sent to us, we have the unique opportunity to compare baseline (or close to baseline) cell turnover in intact regions of the bronchial mucosa with the higher rates of cell turnover adjacent to areas of damage that are in the process of repair (Robbins and Meyers, 1993; 1994a; 1994b). It is likely that the process of human tracheobronchial epithelial repair more closely resembles that which occurs in the sheep trachea (Barrow et al., 1992) or in rats who breathed a high level of ozone for 3 days (Rajini et al., 1993) than that which has been

described in hamsters (Keenan et al., 1982; Man and Hulbert, 1988) because of the differences in cell types and the number of basal cells (Plopper et al., 1983; 1989).

Since dogs have been used in studies of lung cancer from radon and other carcinogens (Cross et al., 1982; Benfield and Hammond, 1992; Reif et al., 1992), we have analyzed the epithelia of dog bronchial generations in parallel to the human samples (Harley et al., 1992). An important similarity between human and dog lungs is that both have rather abundant submucosal glands in the walls of the upper airways (Jeffery, 1983; Hyde et al., 1990). We have continued to use dog bronchi to perfect immunostaining techniques, and to compare with our human data and thus help assess the reliability of using dog models for lung carcinogenesis. We had hoped also to use them for initial flow cytometry cell population analyses but the rather drastic cuts in funding prevented us from beginning these studies.

We will continue to use dog lungs obtained from our colleagues in cardiac surgery and thus will be able to examine the effects of short term intubation (2-4 hours) on the cycling cell populations of the bronchial epithelium of various airway generations and at regions of bifurcation. Having completed the processing of tissues and entering of electron micrographs into COSAS for morphometry studies of dog bronchial epithelium, we are preparing to run one final analysis and publish our results and then focus more on the human tissue studies.

II. Results

A. Tissues- Human and Dog

As in our past research, human bronchi for this study have been obtained from normal non-involved lung tissues (generally part of one lobe) adjacent to pathological areas removed by NYU/Tisch Hospital Pathology Residents after surgical resection. If not used for this study these tissues would normally be discarded. The margins of our surgical specimens are large and the bronchi we dissect and use are considerably more than 40 mm from the abnormal areas. Thus, the report of metaplasias near to lung cancers (Kayser et al., 1991) is not of concern. In almost all cases patients are interviewed post-op (as well as obtaining necessary information from hospital records). In addition to employment and residence (city, state), we record sex, age, pathology diagnosis and smoking status (non-smoker, smoker, ex-smoker-defined as an individual who stopped smoking > 10 years ago).

In view of the difficulties in obtaining non-surgical, normal lung tissues, we are receiving organ donor lungs from the National Disease Research Interchange (NDRI) which have been made available to us when recipients waiting for transplants cannot

be matched or lungs are unsuitable for transplantation for other reasons. Although this is an expensive means to obtain tissue, it is the only means legally available to us to obtain bronchi from younger individuals and non-smokers who are free of lung pathology. The lungs are maintained in Euro-Collins or UW (University of Washington) solutions on ice for shipment. These have been used to preserve lungs for transplantation (Cooper and Vreim, 1992, Mayer et al., 1992). Table 1 lists our NDRI supplied lungs and the age, sex and smoking status of the donor, as well as the length of time the individual spent on a respirator until aortic cross-clamp. These are invaluable samples which we have utilized for both stereology and immunocytochemistry. Thus, we obtain morphometric and cycling cell population data on adjacent regions of the same airways.

As can be seen in Table 1, 20 of the 26 human lungs supplied by the NDRI have been usable for our purposes. The non-usable ones were infected or poorly preserved. Only one lung came from an exsmoker but 13 were from non-smokers (7 children, 6 adults age 20 or older).

Table 1. NDRI Supplied Lungs

SAMPLE #	DATE	SEX	AGE yrs.	SMOKING STATUS	HRS. INTUBATED	USABLE Y/N
1	8/17/92	F	44	Nonsmoker	48	Y
2	11/24/92	F	11	Nonsmoker	48	N
3	12/3/92	M	30	Smoker	24	N
4	1/22/93	F	52	Nonsmoker	48	Y
5	1/30/93	M	19	Smoker	48	Y
6	3/4/93	F	5 mo	Nonsmoker	24	N
7	3/22/93	M	30	Smoker	48	Y
8	3/27/93	M	2 dy	Nonsmoker	48	Y
9	4/9/93	M	10	Nonsmoker	24	Y
10	5/20/93	M	23	Smoker	48	Y
11	7/9/93	M	33	Nonsmoker	48	Y
12	8/14/93	M	20	Nonsmoker	168	Y
13	8/20/93	M	5	Nonsmoker	24	Y
14	9/27/93	F	13	Nonsmoker	24	Y
15	11/17/93	M	16	Nonsmoker	48	Y
16	1/4/94	M	37	Smoker	96	N
17	3/23/94	F	36	Smoker	72	Y
18	3/30/94	F	60	Nonsmoker	72	Y

19	4/19/94	M	14	Nonsmoker	96	N
20	5/5/94	M	32	Smoker	18	Y
21	5/17/94	M	10	Nonsmoker	96	Y
22	8/5/94	F	47	Smoker	24	N
23	9/4/94	M	21	Nonsmoker	24	Y
24	10/17/94	M	19	Nonsmoker	120	Y
25	10/27/94	F	66	Ex-Smoker	96	Y
26	11/25/94	F	48	Smoker	24	Y

All but one of the 88 human lung specimens to date (396 samples, each with one to four different bronchi of the same generation; generations 1 to 6 and branching regions) and dog bronchi similarly dissected (288 samples from 45 dog lungs; generations 0 to 6 and branch points of 5 generations) have been processed through embedding in epoxy resin. Using light microscopic examination approximately 85% of the samples have been judged to be adequately preserved and usable for electron microscopic morphometric analysis.

The dogs we have used are legally supplied research animals and all the requisite animal use forms have been supplied and permission from the proper institutional committee (IACUC) has been obtained. Because the cost per dog is high (close to \$500. per animal at present), we have shared dogs and costs with other researchers whenever possible.

Table 2 summarizes our dog tissues. We have also analyzed the epithelia at areas of airway bifurcation as well as those of adjacent airways thus branch point samples adjacent to each bronchial generation.

Table 2 Dog Bronchi

GENERATION	NUMBER OF SAMPLES
GENERATION 1	15
GENERATION 1 BRANCH	4
GENERATION 2	38
GENERATION 2 BRANCH	13
GENERATION 3	37
GENERATION 3 BRANCH	13
GENERATION 4	40
GENERATION 4 BRANCH	20
GENERATION 5	41
GENERATION 5 BRANCH	20
GENERATION 6	32
GENERATION 6 BRANCH	14
GENERATION 7	1
TOTAL	288

Table 2. Forty-five adult 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, fifty-five samples were found to be inadequately preserved and were discarded. Of the 288 samples 276 have been analysed to date. The total number of micrographs of dog bronchial epithelium in COSAS at present is 1388, of these 1352 micrographs have been analyzed.

B. COSAS - Stereological Analysis

1. Humans

From the 1869 electron micrographs taken of the epithelial lining of human bronchi 1766 have been entered into the Computerized Stereological Analysis Program (COSAS, Cornacchia and Black, 1988). This same set of electron micrographs also has been used for the measurement of the distances of basal and mucous cell nuclei to the apical free surface and the number of epithelial cells per unit length of basal lamina which have been used to update a model (Harley et al., submitted).

Using our electron micrograph collection and COSAS, we have continued 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 human bronchial epithelium.

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 generations and branch points. Overall there are trends, but as yet no statistically significant differences. It seems, if one examines the V_D data for basal cell nuclei, that ex-smokers may be different from non-smokers and more like smokers. Mucous metaplasia is known to accompany smoking (Chang 1957; Hayashi et al., 1978) and the volume density of mucous cell nuclei of smokers in our set is also higher; the V_D of ex-smokers resembles non-smokers. These data thus suggest that smoking may cause changes such that mucous cells revert to the non-smoking values after 10 years when cancer risk has dropped (Fishman, 1988; Samet, 1992). In addition, there are already some provocative findings. In the bronchial generations studied it is clear that the V_D of basal cell nuclei is relatively constant even though the prevalence of basal cells is thought to diminish peripherally (Breeze and Wheldon, 1977; Jeffrey and Corrin, 1984). Because of the small size of our stratified samples, the differences do not yet have a consistent pattern, nevertheless the airways of ex-smokers appear different than those of non-smokers or smokers.

The number density and surface density of the nuclei of the 5 cell types per generation can also be derived from COSAS. Tables 4 and 5 gives these data for our human bronchi and branch points and compares smokers, non-smokers and ex-smokers. The number density of mucous cell nuclei is higher in almost all generations of smokers and ex-smokers seem to more closely resemble smokers than non-smokers. At branching regions of smokers and ex smokers there seem to be no differences in the number density of basal cells when compared with adjacent airways. In non-smokers especially in generations 4B and 5B branching regions have much lower numerical density of basal cell nuclei.

Table 6 presents our current COSAS derived values for volume density, number density and surface density of human bronchial basal, mucous and intermediate / indeterminate cell nuclei and compares males and females of all ages and is stratified by smoking status but not by age group. In male smokers the volume density of basal cell nuclei is clearly less than that of basal nuclei in non-smokers and ex-smokers whose values are very similar. In females smokers, however, no such difference is discernable. This may reflect the wider sample of age differences in our male lung samples. Again in males, the volume density of mucous cell nuclei in smokers is less than that of non- and ex-smokers and the non- and ex-smoker values are identical.

Table 3.

Volume Density of the Nuclei of Human Bronchial Epithelial Cells

A. Smokers

Gen	# Micrographs	Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
		Volume Density		Volume Density		Volume Density		Volume Density		Volume Density	
2	41	0.018 ± 0.002		0.037 ± 0.002		0.054 ± 0.003		0.068 ± 0.005		0.007 ± 0.001	
2B	12	0.024 ± 0.003		0.027 ± 0.003		0.038 ± 0.006		0.050 ± 0.007		0.005 ± 0.001	
3	132	0.022 ± 0.002		0.034 ± 0.001		0.049 ± 0.003		0.074 ± 0.003		0.006 ± 0.001	
3B	2	0.041 ± 0.016		0.038 ± 0.001		0.046 ± 0.002		0.059 ± 0.031		0.000 ± 0.000	
4	128	0.019 ± 0.001		0.034 ± 0.001		0.053 ± 0.003		0.083 ± 0.003		0.006 ± 0.001	
4B	19	0.013 ± 0.002		0.028 ± 0.002		0.039 ± 0.004		0.059 ± 0.009		0.008 ± 0.002	
5	115	0.015 ± 0.001		0.035 ± 0.002		0.043 ± 0.002		0.081 ± 0.004		0.007 ± 0.005	
5B	24	0.021 ± 0.002		0.029 ± 0.002		0.048 ± 0.005		0.088 ± 0.007		0.009 ± 0.001	
6	103	0.021 ± 0.002		0.030 ± 0.001		0.049 ± 0.003		0.088 ± 0.004		0.007 ± 0.001	
6B	25	0.018 ± 0.003		0.026 ± 0.003		0.041 ± 0.004		0.051 ± 0.004		0.004 ± 0.001	

B. Non-Smokers

Gen	# Micrographs	Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
		Volume Density		Volume Density		Volume Density		Volume Density		Volume Density	
2	22	0.019 ± 0.005		0.027 ± 0.003		0.056 ± 0.011		0.061 ± 0.013		0.003 ± 0.003	
2B	14	0.021 ± 0.009		0.036 ± 0.011		0.071 ± 0.013		0.039 ± 0.019		0.008 ± 0.006	
3	35	0.017 ± 0.001		0.031 ± 0.008		0.056 ± 0.015		0.079 ± 0.016		0.005 ± 0.002	
3B	35	0.016 ± 0.001		0.030 ± 0.003		0.048 ± 0.019		0.071 ± 0.013		0.036 ± 0.041	
4	114	0.017 ± 0.006		0.038 ± 0.004		0.039 ± 0.018		0.088 ± 0.023		0.003 ± 0.002	
4B	22	0.018 ± 0.006		0.036 ± 0.004		0.041 ± 0.008		0.080 ± 0.007		0.005 ± 0.004	
5	127	0.017 ± 0.004		0.034 ± 0.004		0.052 ± 0.013		0.085 ± 0.018		0.005 ± 0.002	
5B	39	0.015 ± 0.004		0.027 ± 0.005		0.062 ± 0.008		0.070 ± 0.009		0.008 ± 0.003	
6	119	0.018 ± 0.006		0.033 ± 0.006		0.052 ± 0.007		0.083 ± 0.019		0.004 ± 0.002	
6B	64	0.026 ± 0.013		0.032 ± 0.006		0.052 ± 0.007		0.057 ± 0.013		0.009 ± 0.002	

TABLE 3.

Volume Density of the Nuclei of Human Bronchial Epithelial Cells**C. Ex-Smokers**

Gen	# Micrographs	Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
		Volume Density		Volume Density		Volume Density		Volume Density		Volume Density	
2	12	0.012 ± 0.003		0.034 ± 0.004		0.049 ± 0.007		0.075 ± 0.004		0.008 ± 0.001	
3	43	0.019 ± 0.002		0.031 ± 0.002		0.049 ± 0.004		0.069 ± 0.004		0.006 ± 0.001	
4	72	0.018 ± 0.002		0.035 ± 0.002		0.046 ± 0.003		0.082 ± 0.004		0.007 ± 0.001	
4B	9	0.016 ± 0.003		0.030 ± 0.017		0.051 ± 0.009		0.093 ± 0.013		0.005 ± 0.002	
5	106	0.020 ± 0.001		0.029 ± 0.002		0.041 ± 0.002		0.093 ± 0.003		0.006 ± 0.001	
5B	34	0.022 ± 0.002		0.028 ± 0.002		0.041 ± 0.004		0.078 ± 0.004		0.007 ± 0.002	
6	70	0.017 ± 0.002		0.043 ± 0.003		0.043 ± 0.003		0.084 ± 0.005		0.005 ± 0.001	
6B	40	0.016 ± 0.002		0.029 ± 0.002		0.040 ± 0.004		0.011 ± 0.005		0.011 ± 0.001	

Table 3. Summarizes the data from COSAS on the volume Density (V_D) of the nuclei of airway cell types (mucous, basal, intermediate/indeterminate, ciliated, and intraepithelial white blood cells per micrograph). The data are presented for 5 bronchial generations and four bifurcation areas. Smokers, non-smokers, and ex-smokers are compared.

Table 4.

Number Density of the Nuclei of Human Bronchial Epithelial Cells

A. Smokers

Gen	# Micrographs	Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
		Number Density		Number Density		Number Density		Number Density		Number Density	
2	41	0.016 ± 0.002		0.048 ± 0.004		0.060 ± 0.004		0.046 ± 0.004		0.017 ± 0.002	
2B	12	0.026 ± 0.004		0.012 ± 0.006		0.052 ± 0.026		0.031 ± 0.002		0.009 ± 0.003	
3	132	0.022 ± 0.001		0.053 ± 0.003		0.059 ± 0.003		0.050 ± 0.002		0.016 ± 0.002	
3B	2	0.019 ± 0.014		0.034 ± 0.007		0.026 ± 0.002		0.017 ± 0.009		0.000 ± 0.000	
4	128	0.022 ± 0.002		0.051 ± 0.002		0.063 ± 0.003		0.057 ± 0.002		0.017 ± 0.002	
4B	19	0.025 ± 0.004		0.069 ± 0.007		0.076 ± 0.008		0.050 ± 0.005		0.021 ± 0.005	
5	115	0.015 ± 0.001		0.052 ± 0.003		0.056 ± 0.004		0.062 ± 0.003		0.042 ± 0.003	
5B	24	0.022 ± 0.003		0.045 ± 0.004		0.069 ± 0.007		0.055 ± 0.005		0.023 ± 0.005	
6	103	0.028 ± 0.003		0.062 ± 0.004		0.091 ± 0.011		0.084 ± 0.006		0.022 ± 0.003	
6B	25	0.019 ± 0.003		0.031 ± 0.003		0.041 ± 0.005		0.027 ± 0.003		0.011 ± 0.003	

B. Non-Smokers

Gen	# Micrographs	Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
		Number Density		Number Density		Number Density		Number Density		Number Density	
2	22	0.015 ± 0.001		0.028 ± 0.013		0.073 ± 0.017		0.038 ± 0.009		0.009 ± 0.006	
2B	14	0.011 ± 0.002		0.036 ± 0.011		0.062 ± 0.001		0.033 ± 0.013		0.008 ± 0.006	
3	35	0.017 ± 0.003		0.046 ± 0.015		0.067 ± 0.012		0.050 ± 0.011		0.013 ± 0.005	
3B	35	0.016 ± 0.005		0.039 ± 0.010		0.062 ± 0.025		0.043 ± 0.009		0.021 ± 0.007	
4	114	0.019 ± 0.007		0.061 ± 0.019		0.052 ± 0.019		0.065 ± 0.023		0.009 ± 0.004	
4B	22	0.019 ± 0.002		0.019 ± 0.013		0.060 ± 0.012		0.058 ± 0.007		0.017 ± 0.011	
5	127	0.021 ± 0.005		0.050 ± 0.012		0.073 ± 0.011		0.066 ± 0.018		0.013 ± 0.004	
5B	39	0.014 ± 0.004		0.015 ± 0.008		0.061 ± 0.039		0.057 ± 0.007		0.015 ± 0.008	
6	119	0.018 ± 0.004		0.053 ± 0.006		0.072 ± 0.011		0.061 ± 0.012		0.009 ± 0.005	
6B	64	0.025 ± 0.009		0.048 ± 0.005		0.079 ± 0.009		0.048 ± 0.009		0.024 ± 0.005	

TABLE 4.

Number Density of the Nuclei of Human Bronchial Epithelial Cells

C. Ex-Smokers

Gen	# Micrographs	Mucous Cells		Basal Cells		Indet./Intermed Cells		Ciliated Cells		White Blood Cells	
		Number Density		Number Density		Number Density		Number Density		Number Density	
2	12	0.016 ± 0.003		0.065 ± 0.012		0.083 ± 0.011		0.070 ± 0.005		0.016 ± 0.003	
3	43	0.018 ± 0.002		0.041 ± 0.002		0.068 ± 0.007		0.045 ± 0.004		0.019 ± 0.005	
4	72	0.025 ± 0.002		0.065 ± 0.003		0.074 ± 0.005		0.068 ± 0.004		0.024 ± 0.003	
4B	9	0.025 ± 0.006		0.056 ± 0.008		0.066 ± 0.012		0.067 ± 0.007		0.016 ± 0.009	
5	106	0.022 ± 0.002		0.049 ± 0.002		0.060 ± 0.004		0.074 ± 0.004		0.017 ± 0.002	
5B	34	0.033 ± 0.003		0.051 ± 0.005		0.061 ± 0.006		0.056 ± 0.005		0.016 ± 0.003	
6	70	0.027 ± 0.004		0.047 ± 0.004		0.082 ± 0.007		0.095 ± 0.005		0.015 ± 0.002	
6B	40	0.019 ± 0.002		0.056 ± 0.004		0.076 ± 0.009		0.088 ± 0.008		0.021 ± 0.003	

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

Table 4.

Number Density of the Nuclei of Human Bronchial Epithelial Cells

A. Smokers

Gen	# Micrographs	Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
		Number Density		Number Density		Number Density		Number Density		Number Density	
2	41	0.016 ± 0.002		0.048 ± 0.004		0.060 ± 0.004		0.046 ± 0.004		0.017 ± 0.002	
2B	12	0.026 ± 0.004		0.012 ± 0.006		0.052 ± 0.026		0.031 ± 0.002		0.009 ± 0.003	
3	132	0.022 ± 0.001		0.053 ± 0.003		0.059 ± 0.003		0.050 ± 0.002		0.016 ± 0.002	
3B	2	0.019 ± 0.014		0.034 ± 0.007		0.026 ± 0.002		0.017 ± 0.009		0.000 ± 0.000	
4	128	0.022 ± 0.002		0.051 ± 0.002		0.063 ± 0.003		0.057 ± 0.002		0.017 ± 0.002	
4B	19	0.025 ± 0.004		0.069 ± 0.007		0.076 ± 0.008		0.050 ± 0.005		0.021 ± 0.005	
5	115	0.015 ± 0.001		0.052 ± 0.003		0.056 ± 0.004		0.062 ± 0.003		0.042 ± 0.003	
5B	24	0.022 ± 0.003		0.045 ± 0.004		0.069 ± 0.007		0.055 ± 0.005		0.023 ± 0.005	
6	103	0.028 ± 0.003		0.062 ± 0.004		0.091 ± 0.011		0.084 ± 0.006		0.022 ± 0.003	
6B	25	0.019 ± 0.003		0.031 ± 0.003		0.041 ± 0.005		0.027 ± 0.003		0.011 ± 0.003	

B. Non-Smokers

Gen	# Micrographs	Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
		Number Density		Number Density		Number Density		Number Density		Number Density	
2	22	0.015 ± 0.001		0.028 ± 0.013		0.073 ± 0.017		0.038 ± 0.009		0.009 ± 0.006	
2B	14	0.011 ± 0.002		0.036 ± 0.011		0.062 ± 0.001		0.033 ± 0.013		0.008 ± 0.006	
3	35	0.017 ± 0.003		0.046 ± 0.015		0.067 ± 0.012		0.050 ± 0.011		0.013 ± 0.005	
3B	35	0.016 ± 0.005		0.039 ± 0.010		0.062 ± 0.025		0.043 ± 0.009		0.021 ± 0.007	
4	114	0.019 ± 0.007		0.061 ± 0.019		0.052 ± 0.019		0.065 ± 0.023		0.009 ± 0.004	
4B	22	0.019 ± 0.002		0.019 ± 0.013		0.060 ± 0.012		0.058 ± 0.007		0.017 ± 0.011	
5	127	0.021 ± 0.005		0.050 ± 0.012		0.073 ± 0.011		0.066 ± 0.018		0.013 ± 0.004	
5B	39	0.014 ± 0.004		0.015 ± 0.008		0.061 ± 0.039		0.057 ± 0.007		0.015 ± 0.008	
6	119	0.018 ± 0.004		0.053 ± 0.006		0.072 ± 0.011		0.061 ± 0.012		0.009 ± 0.005	
6B	64	0.025 ± 0.009		0.048 ± 0.005		0.079 ± 0.009		0.048 ± 0.009		0.024 ± 0.005	

TABLE 4.

Number Density of the Nuclei of Human Bronchial Epithelial Cells

C. Ex-Smokers

Gen	# Micrographs	Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
		Number Density		Number Density		Number Density		Number Density		Number Density	
2	12	0.016 ± 0.003		0.065 ± 0.012		0.083 ± 0.011		0.070 ± 0.005		0.016 ± 0.003	
3	43	0.018 ± 0.002		0.041 ± 0.002		0.068 ± 0.007		0.045 ± 0.004		0.019 ± 0.005	
4	72	0.025 ± 0.002		0.065 ± 0.003		0.074 ± 0.005		0.068 ± 0.004		0.024 ± 0.003	
4B	9	0.025 ± 0.006		0.056 ± 0.008		0.066 ± 0.012		0.067 ± 0.007		0.016 ± 0.009	
5	106	0.022 ± 0.002		0.049 ± 0.002		0.060 ± 0.004		0.074 ± 0.004		0.017 ± 0.002	
5B	34	0.033 ± 0.003		0.051 ± 0.005		0.061 ± 0.006		0.056 ± 0.005		0.016 ± 0.003	
6	70	0.027 ± 0.004		0.047 ± 0.004		0.082 ± 0.007		0.095 ± 0.005		0.015 ± 0.002	
6B	40	0.019 ± 0.002		0.056 ± 0.004		0.076 ± 0.009		0.088 ± 0.008		0.021 ± 0.003	

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) per micrograph. The data are presented for four bronchial generations and four regions of bifurcations, Smokers, non-smokers, and ex-smokers are compared.

Table 5.

Surface Density of the Nuclei of Human Bronchial Epithelial Cells**A. Smokers**

Gen	# Micrographs	Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
		Surface Density		Surface Density		Surface Density		Surface Density		Surface Density	
2	41	0.094 ± 0.011		0.207 ± 0.012		0.288 ± 0.016		0.316 ± 0.026		0.046 ± 0.007	
2B	12	0.129 ± 0.013		0.161 ± 0.016		0.208 ± 0.031		0.223 ± 0.026		0.029 ± 0.008	
3	132	0.122 ± 0.008		0.206 ± 0.008		0.278 ± 0.014		0.353 ± 0.013		0.039 ± 0.004	
3B	2	0.172 ± 0.077		0.196 ± 0.011		0.207 ± 0.013		0.236 ± 0.014		0.00 ± 0.000	
4	128	0.121 ± 0.007		0.217 ± 0.008		0.343 ± 0.040		0.434 ± 0.016		0.044 ± 0.004	
4B	19	0.083 ± 0.013		0.204 ± 0.014		0.263 ± 0.033		0.315 ± 0.046		0.052 ± 0.016	
5	115	0.090 ± 0.008		0.227 ± 0.011		0.252 ± 0.013		0.479 ± 0.017		0.307 ± 0.024	
5B	24	0.120 ± 0.016		0.184 ± 0.013		0.279 ± 0.022		0.412 ± 0.030		0.064 ± 0.011	
6	103	0.139 ± 0.011		0.217 ± 0.008		0.359 ± 0.044		0.499 ± 0.022		0.054 ± 0.007	
6B	25	0.095 ± 0.013		0.141 ± 0.016		0.204 ± 0.018		0.214 ± 0.017		0.027 ± 0.007	

B. Non-Smokers

Gen	# Micrographs	Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
		Surface Density		Surface Density		Surface Density		Surface Density		Surface Density	
2	22	0.091 ± 0.013		0.137 ± 0.033		0.308 ± 0.062		0.282 ± 0.064		0.024 ± 0.019	
2B	14	0.088 ± 0.025		0.186 ± 0.067		0.349 ± 0.024		0.262 ± 0.010		0.057 ± 0.038	
3	35	0.094 ± 0.015		0.185 ± 0.060		0.318 ± 0.078		0.379 ± 0.086		0.032 ± 0.011	
3B	35	0.087 ± 0.015		0.166 ± 0.020		0.275 ± 0.112		0.335 ± 0.040		0.054 ± 0.013	
4	114	0.099 ± 0.032		0.235 ± 0.049		0.233 ± 0.102		0.454 ± 0.015		0.019 ± 0.010	
4B	22	0.098 ± 0.019		0.204 ± 0.038		0.248 ± 0.048		0.414 ± 0.055		0.041 ± 0.031	
5	127	0.101 ± 0.028		0.203 ± 0.037		0.309 ± 0.058		0.203 ± 0.037		0.032 ± 0.011	
5B	39	0.079 ± 0.022		0.119 ± 0.056		0.365 ± 0.058		0.354 ± 0.041		0.051 ± 0.023	
6	119	0.095 ± 0.032		0.291 ± 0.012		0.296 ± 0.051		0.412 ± 0.010		0.030 ± 0.012	
6B	64	0.141 ± 0.063		0.185 ± 0.029		0.306 ± 0.031		0.292 ± 0.072		0.060 ± 0.012	

TABLE 5.

Surface Density of the Nuclei of Human Bronchial Epithelial Cells**C. Ex-Smokers**

Gen	# Micrographs	Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
		Surface Density		Surface Density		Surface Density		Surface Density		Surface Density	
2B	12	0.070 ± 0.016		0.215 ± 0.028		0.300 ± 0.043		0.391 ± 0.20		0.047 ± 0.007	
3	43	0.101 ± 0.009		0.177 ± 0.011		0.286 ± 0.025		0.330 ± 0.021		0.045 ± 0.010	
4	72	0.115 ± 0.009		0.237 ± 0.010		0.286 ± 0.016		0.417 ± 0.018		0.053 ± 0.007	
4B	9	0.111 ± 0.022		0.201 ± 0.034		0.293 ± 0.055		0.452 ± 0.056		0.038 ± 0.019	
5	106	0.124 ± 0.009		0.189 ± 0.008		0.247 ± 0.015		0.481 ± 0.018		0.044 ± 0.005	
5B	34	0.147 ± 0.011		0.188 ± 0.015		0.245 ± 0.023		0.411 ± 0.025		0.047 ± 0.011	
6	70	0.117 ± 0.013		0.214 ± 0.011		0.287 ± 0.019		0.507 ± 0.023		0.038 ± 0.006	
6B	40	0.096 ± 0.011		0.196 ± 0.011		0.272 ± 0.027		0.562 ± 0.032		0.069 ± 0.008	

Table 5. Summarizes the data from COSAS on the Surface Density (SD) of the nuclei of airway cell types (mucous, basal, intermediate/indeterminate, ciliated, and intraepithelial white blood cells) per micrograph. The data are presented for four bronchial generations and four bifurcation regions. Smokers, non-smokers, and ex-smokers are compared.

Table 6.

Stereological Parameters of the Human Bronchial Epithelium:
A comparison of male and female smokers, non-smokers and ex-smokers

FEMALES			MALES		
	Volume Density	Number Density	Surface Density	Volume Density	Number Density
Smokers	Basal Cells	0.035 ± 0.001	0.227 ± 0.006	0.021 ± 0.001	0.026 ± 0.001
	Mucous Cells	0.019 ± 0.001	0.117 ± 0.005	0.028 ± 0.001	0.048 ± 0.002
	Indet/Inter Cells	0.049 ± 0.002	0.314 ± 0.022	0.047 ± 0.002	0.064 ± 0.003
Non - Smokers	Basal Cells	0.036 ± 0.001	0.223 ± 0.005	0.032 ± 0.001	0.047 ± 0.001
	Mucous Cells	0.019 ± 0.001	0.108 ± 0.005	0.019 ± 0.001	0.017 ± 0.001
	Indet/Inter Cells	0.043 ± 0.002	0.266 ± 0.009	0.061 ± 0.001	0.079 ± 0.002
Ex- Smokers	Basal Cells	0.032 ± 0.001	0.194 ± 0.006	0.031 ± 0.001	0.059 ± 0.002
	Mucous Cells	0.019 ± 0.001	0.109 ± 0.006	0.019 ± 0.001	0.026 ± 0.001
	Indet/Inter Cells	0.043 ± 0.002	0.254 ± 0.010	0.045 ± 0.002	0.078 ± 0.004
					0.289 ± 0.011

Both the large ducts of human bronchial glands clearly have basal cells (Meyrick et al. 1969) and we have taken 30 micrographs of the epithelium of 6 human bronchial gland ducts. The V_D of the basal cell nuclei in these ducts is similar to that of basal cell nuclei in generation 2 bronchial epithelium. The duct epithelium also contains a minor population of serous cells which are rarely encountered in the human large airways. Our own results with PCNA labeling indicate (see section F) that the basal cells of these ducts are capable of extensive proliferation in adult bronchi as well as newborn and young and thus they may indeed serve as a stem cell pool capable of possible damage from trauma, ionizing radiation or other carcinogens.

2. Dogs

We have also been using COSAS to study the morphometry of dog bronchial epithelium on electron micrographs. Of the 1388 electron micrographs of our 170 dog bronchial epithelium samples 1352 have been entered into COSAS. As in the human airways the branch points of dog bronchi are also being analyzed to determine if their epithelia differ from those of the adjacent airways. We are waiting for the conversion of the VAX mainframe computer to an Alpha and then we will run one final analysis of our dog data. We will analyze the volume density data for the five types of epithelial cell nuclei of dog bronchi from generations 2 to 6 and include as separate categories the epithelia of bronchi at five branch points (generations 1 to 2; 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. If our expanded analysis is similar to the one run last year, dog basal cell nuclei and mucous cell nuclei will occupy a somewhat larger percentage of the epithelial volume in comparison with the V_D data for human bronchial lining cells. A paper using our dog data has been published (Harley et al., 1992)

C. Measurements of nuclear distances from the epithelial free surface and number of cells per unit length of basal lamina

1. Humans.

Radon dosimetry (Harley, 1988; 1989) requires information about the distances of the nuclei at risk for damage from radon daughter decay of the luminal α -particle source. To date we have measured the distances of 9132 basal and 5986 mucous cell nuclei on approximately 85% of the electron micrographs also used for COSAS. Measurements are made from the most superficial aspect of a nuclear profile to the nearest luminal plasmalemma (at the base of the cilia and microvilli). Although the human trachea has a continuous mucous "blanket", this layer is reduced to

"plaques" in the bronchi (Sleigh et al., 1988). In hamsters, at least, it is clear that the bronchial periciliary fluid is accessible to particulates (Schurch et al., 1990) and so it is our supposition that the apical plasmalemma to the superficial aspect of a nucleus represents the minimum distance that must be traversed by α particles. Fig. 1A. shows the frequency distribution of the depths of all the human basal cells and Fig. 1B the distribution of mucous cell nuclear depths from the same micrographs. It is clear that there is a wide range of depths and that almost all of the basal cell nuclei, which are the most deeply located within the epithelium, are within the path length of decay of radon daughter emitted α particles. Table 7 A gives the mean values for the depth of human basal and mucous cell nuclei.

We are analyzing the differences between airway generations and between men and women, smokers, non-smokers and ex-smokers. At present, as with the volume density data, stratification produces small sample sizes and figures with large standard errors. Thus, larger data sets are required to yield significant results. As can be seen in Table 8 there are only trends. To date 35 smokers, 24 non-smokers, 13 ex-smokers and 11 unclassified individuals constitute our data base. If data from branch points and all generations is included, then there are some interesting possible difference between smokers and non-smokers. In male smokers basal cell nuclei of generations 5 and 6 are approximately 2.5 μm closer to the free surface than those of non-smokers. This could result in α dose differences of up to 30 % (NCRP, 1984). In both mucous and basal cell nuclei of generations 3 and 4 however, the nuclei of non-smokers are closer to the lumen than those of smokers. Ultimately, it will be of interest to compare the incidence of tumors in airways of different generations in male and female smokers and non-smokers to see if they can correlate with the differences in nuclear depth we find. We need to enlarge and diversify our sample population by including bronchi from individuals who are younger and lack lung pathology and to expand the bronchial generations obtained from older individuals, especially ex- and non-smokers.

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, 1992). 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 and mucous cells shows mean values in the same range as Baldwin et al. but slightly lower.

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 lung lobes whose generations are defined by diameter they report that basal and mucous cell nuclei are found at a range of 24 to 48 μm from the epithelial surface. Our data, which do not include the cilia or surface mucus layers, find these nuclei at both more superficial and deeper distances as can be seen in our frequency distributions (Fig. 1).

2. Dogs

Measurements of the depths of mucous and basal cell nuclei of dog bronchi of different generations and in regions of bifurcation have also been made. Some early values have been used to determine ^{222}Rn dosimetry in the dog lung (Harley et al., 1992). Table 7 B gives the average depths from the free surface of 7813 basal cell nuclei and 3836 mucous cell nuclei as determined on electron micrographs of dog bronchial epithelium of generations 1 to 6. Additional measurements have been made but are not yet included in this figure. Additional measurements of the distances of dog bronchial basal and mucous cell nuclei to the free surface by generation at branching areas have also been made but not yet analyzed. In our data so far, basal cell distances to the free surface are similar overall when airways and branch points are compared but mucous cell nuclei at branch points are deeper.

E. Examination of the ducts of bronchial glands

The major excretory ducts of human bronchial glands have basal cells (Meyrich et al., 1969; Jeffery et al., 1992). Since the bronchial luminal surface is not totally covered by a layer of mucus (Sleigh et al., 1988), the duct opening is a possible pathway for the exposure of the ductal epithelium to airborne particulates bearing carcinogens such as radioactive radon daughters. The concept that these basal cells can be reached by airborne substances is strengthened by the observation that they undergo hyperplasia in smokers (Auerbach, 1967) and it has been suggested (Auerbach, 1967; Ochs, 1988) that they are a possible source of carcinomas. Adenocarcinomas and basaloid carcinomas have been reported (Kodama et al., 1982; Hirata et al., 1989; Brambilla et al., 1992). This hypothesis is also strengthened by the observation that the ducts of tracheal glands can repopulate a denuded epithelial lining in neonates (Stahlman et al., 1989). In view of the general phenomenon from other glandular systems in the human body such as salivary and mammary glands, where duct cells are the stem cells (Alberts et al., 1989), duct basal cells should be considered in radon / carcinogenesis risk modeling. As our micrograph set enlarges we will measure the distances of duct basal cell nuclei to the free surface and compare them with the adjacent airway epithelium.

Our results with PCNA labeling indicate that the basal cells of these ducts are capable of extensive proliferation in adult bronchi as well as newborn and young and thus they may indeed serve as a stem cell pool that may possibly be damaged by ionizing radiation and other carcinogens (Robbins and Meyers, 1993; 1994a, 1994b).

F. Immunocytochemistry

Although there have been many studies on the proliferation and differentiation of the mammalian respiratory epithelium and the effects of age, sex, and noxious agents in animals (Ayers and Jeffery, 1988; Jeffrey et al., 1992) there is little direct information and there are many questions about the proliferative capacity of mature human airway epithelial population(s) *in vivo*. Based on animal studies, the literature supports the thesis that both mucous and basal cells are potentially mitotic (Ayers and Jeffery, 1988; Ford and Terzaghi-Howe, 1992a; Johnson et al., 1990; Johnson and Hubbs, 1990; McDowell et al., 1990; Nettlesheim et al., 1990; Jetten, 1991) but there is controversy as to the identity of the ultimate, long-lived stem cells which presumably are the targets in the genesis of human bronchial carcinomas which take many years to develop (Yesner, 1988). Although recent cell separation and culture studies on animal tracheobronchial epithelium favor the interpretation that the basal cells are the stem cell of the airway epithelium (Inayama et al., 1988; Nettlesheim et al., 1990, Ford and Terzaghi-Howe, 1992a; 1992b) a highly plausible scheme for cell replacement in mature respiratory epithelium should contain two proliferative compartments. Our proposed model for the renewal of the human tracheobronchial epithelium (Robbins and Meyers, 1994b) draws heavily on the models of Potten (1983) and Potten and Morris (1988) for the epidermis and the small intestine. It proposes that there are two stem cell compartments within respiratory epithelium - a reserve stem cell population and a transient amplifying stem cell population. Both compartments are mitotic but the long lived, reserve stem cells cycle less frequently, replace themselves and contribute as necessary to the transient amplifying stem cell population. The amplifying compartment cells divide more frequently and this is the compartment that can greatly expand in response to trauma or during growth.

In injured hamster airways and the rat trachea, mucous granule containing cells have been considered to constitute the stem cell population (Keenan et al., 1982; McDowell et al., 1983; Evans and Shami, 1989; Johnson et al., 1990) but other investigators have suggested, consistent with the view held by many, that the basal cells are the stem cells in adult hamster bronchi (Breuer et al., 1990) and rabbit trachea (Inyama et al., 1988, Nettlesheim et al., 1990). Our own recent results on epithelial renewal in human bronchial mucosa *in vivo* supports the model with two

proliferative compartments described above and provides evidence for the idea that the transient amplifying stem cell compartment (located suprabasally and perhaps containing some basally located cells as well) is responsible for the immediate replacement of epithelial cells in the short term (Robbins and Meyers, 1993; Robbins and Meyers, 1994a; Robbins and Meyers, 1994b).

To detect PCNA (Hall et al., 1990; Carey et al., 1992; Theunissen et al., 1992) airway segments were fixed in 10% formalin or were preserved in methcarn fixative (Puchtler et al. 1970) at 4°C for 16 - 24 hours. Included with each was an autologous mediastinal lymph node to serve as a positive control. After dehydration in graded methanols, the tissues were cleared with xylene and embedded in Paraplast (Polysciences, Inc.). Five μ m sections were cut and a hematoxylin and eosin stained slide of each was examined to determine adequate preservation and for identification of metaplastic, damaged and / or repairing areas.

Immunocytochemical staining for PCNA utilized clone PC10 monoclonal antibody and a detection kit from Signet Laboratories, Inc. (Dedham, MA; Casasco et al. 1993). The protocol used is modified from that supplied with the kit (Robbins and Meyers, 1994b). We have used methcarn as a fixative because it has been reported to give better PCNA results (Hall et al., 1990; Carey et al., 1992). In our hands it does not increase the number of PCNA positive nuclei detected in a tissue (we ran QC on adjacent samples of the same airways fixed in formalin and methcarn) but it results in a more intense PCNA reaction product. It is clear that the cycling cell populations of the bronchial epithelium of adults, as well as children, may be greatly expanded in areas of airway repair. Thus, the existence of areas of epithelial replacement in the airways of lungs that have been subjected to respirator damage presents a unique experiment of nature that allows the quantification of this proliferative capacity in human lungs *in situ*, in a manner not otherwise possible.

We have quantitated the epithelial cycling cell populations (at least 1,000 cells per sample) of 2 human tracheas and 65 bronchi as well as 2 dog tracheas and 26 bronchi. We have compared the following human subgroups: smokers vs. non-smokers, airways vs. their adjacent branching regions, the samples (with normal morphology) with the highest levels of cycling cells (PCNA +) with those with the lowest, and vs. areas of squamous metaplasia. In addition, we have compared the lowest value human samples with normal dog tracheobronchial linings. The results are presented in Tables 9 to 11.

As assessed by PCNA positive nuclei in all of our human bronchial samples with normal morphology, on average 4.29% of the tracheobronchial epithelial cells

were cycling. Of these 2.72% were suprabasal cells and 1.57% were basal cells with a ratio of the two of 1.73 (Table 9). The values for percent PCNA (+) basal and suprabasal nuclei in the epithelium of smokers and non-smokers are also shown in Table 9. The differences between the values for these groups, although suggestive, are not statistically significant at present.

When the cell turnover of the airway linings at branch points was compared to the values in adjacent regions the percentages (3.11% vs. 5.29%, respectively, Table 9) are also indicative of possible real differences because these values are statistically significant. Since branching regions are the sites of greater deposition of airborne particulates that may carry radioactivity or carcinogens, it seems plausible that having fewer cells that divide in these areas would serve as a protection.

A comparison of the cycling cell populations of the epithelia of airways with the highest percent of PCNA (+) nuclei with those with the lowest percentages demonstrates an impressive expansion of the capacity for cell division in normal appearing areas adjacent to repairing human tracheobronchial regions (Table 10). The ten highest samples of normal morphology averaged a total of 16.20% cycling cells (10.02% suprabasal and 6.17% basal in a ratio of 1.62), while the lowest ones averaged 0.31% PCNA positive cells (0.19% suprabasal and 0.12% basal in a ratio of 1.61). The differences between the total percent cycling cells is highly statistically significant ($p < .001$).

The lowest values for human bronchial cycling cells can be compared with the values for adult normal male mongrel dogs who have not had their airways damaged by being on a respirator. As can be seen in Table 11, the percent of cycling cells in dog tracheobronchial epithelia is also less than 1% and it is not significantly different from the lowest human values. This lends support to the concept that the lowest human values that we found are close to the baseline ones in the human general population. The lower value for dog airways (.37%), which is very close to the lowest human value (.31%), was obtained by eliminating 6 apparently abnormally high dog samples from the analysis. Each of these eliminated samples had greater than 1% cycling cells in the epithelium.

The cycling cell populations of areas of squamous metaplasia in human airways were also quantitated. The total percent cycling cells in these abnormal regions was 12.64% with 8.13% suprabasal nuclei and 4.50% basal ones. This is not statistically different from the highest value for the proliferating population in repairing regions with normal morphology (Table 10). The ratio of suprabasal to basal PCNA (+) nuclei in these two groups is also very similar (1.61 vs 1.81, Table 10). It seems

possible, therefore, that these values are in the range of the maximal increase in proliferation that the human tracheobronchial mucosa is capable of reaching. If the highest and lowest human airway samples are examined it is apparent that in both the basal and suprabasal cell groups the increase in proliferative capacity is approximately 50 fold.

PCNA (+) cycling cell nuclei were also seen in submucosal glands and their ducts, as well as in endothelia, fibroblasts and mononuclear cells of the lamina propria and in cells of the bronchus associated lymphoid tissue (BALT). Gland and duct epithelia had the most cycling cells when they were just adjacent or entering into, repairing bronchial epithelium. A quantitation of some of these large ducts gave total cycling cell percentages similar to those for the adjacent repairing epithelia but with a suprabasal cell to basal positive nuclear ratio closer to 1. A plate showing PCNA (+) nuclei in both repairing airway lining and a major gland duct has been sent to accompany an article in one of the next issues of Radon Research Notes.

Table 9. Percent PCNA (+) Suprabasal and Basal Cells in Human Airway Epithellum

Human Groups	Total # Cells Counted	Total % Cycling Cells $\pm$ SE	% Suprabasal Cells (+) $\pm$ SE	Basal Cells (+) $\pm$ SE	Ratio of Suprabasal/Basal (+) Cells
All Samples*	92,510	4.29 $\pm$ 0.64	2.72 $\pm$ 0.37	1.57 $\pm$ 0.32	1.73
Smokers	23,537	3.68 $\pm$ 1.15	2.37 $\pm$ 0.67	1.31 $\pm$ 0.56	1.81
Nonsmokers	57,771	5.43 $\pm$ 0.86	3.42 $\pm$ 0.48	2.00 $\pm$ 0.44	1.71
Branching	31,185	3.11 $\pm$ 0.49	1.91 $\pm$ 0.31	1.21 $\pm$ 0.22	1.58
Non-Branching	54,765	5.29 $\pm$ 0.99	3.39 $\pm$ 0.56	1.90 $\pm$ 0.51	1.78
Males	54,251	4.56 $\pm$ 0.69	3.05 $\pm$ 0.48	1.51 $\pm$ 0.27	2.02
Females	38,259	3.91 $\pm$ 1.22	2.25 $\pm$ 0.56	1.66 $\pm$ 0.68	1.36

* Includes 3 samples from bronchi of individuals with unknown smoking status. % = of all epithelial cells.

Table 10. PCNA Analysis of Human Airways With the Highest % Cycling Cells vs. the Lowest * and Areas of Squamous Metaplasia

Human Sample	Total # Cells Counted	Total % Cycling Cells $\pm$ SE	% Suprabasal Cells (+) $\pm$ SE	Basal Cells (+) $\pm$ SE	Ratio of Suprabasal/Basal (+) Cells
Highest	10,435	16.20 $\pm$ 2.33	10.02 $\pm$ 1.13	6.17 $\pm$ 1.76	1.62
Lowest	11,007	0.31 $\pm$ 0.08	0.19 $\pm$ 0.07	0.12 $\pm$ 0.27	1.61
Metaplastic**	7,637	12.64 $\pm$ 5.21	8.13 $\pm$ 3.55	4.50 $\pm$ 1.82	1.81

* 10 samples in each group, except ** which had 6 samples.

Table 11. Cycling Cells of Dog Bronchi as Assessed by PCNA Labeled Nuclei

Airways	Total # Cells Counted	Total % of Cycling Cells $\pm$ SE	% Suprabasal Cells (+) $\pm$ SE	Basal Cells (+) $\pm$ SE	Ratio of Suprabasal/Basal (+) Cells
All Dogs	32,332	0.60 $\pm$ 0.09	0.31 $\pm$ 0.06	0.29 $\pm$ 0.05	1.09
Lowest human*	11,007	0.31 $\pm$ 0.08	0.19 $\pm$ 0.07	0.12 $\pm$ 0.27	1.61
Dog**	24,091	0.37 $\pm$ 0.05	0.18 $\pm$ 0.02	0.20 $\pm$ 0.04	0.92

** 20 dogs; 6 samples, apparently abnormal were omitted from this analysis, all had greater than 1% cycling cells

* 10 Samples

BIBLIOGRAPHY

- Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., & Watson, J. (1989). Molecular Biology of the Cell (second ed.) p. 972. New York: Garland Publishing.
- Auerbach, O., Hammond, E. C., Kirman, E. C., Garfinkel, L., & Stout, A. P. (1967). Histologic changes in bronchial tubes of cigarette-smoking dogs. Cancer, 20, 2055-2066.
- Ayers, M. M., & Jeffery, P. K. (1988). Proliferation and differentiation in mammalian airway epithelium. Eur. Resp. J., 1, 58-80.
- Baldwin, F., Hovey, A., McEwen, T., O'Connor, R., Unruh, H., & Bowden, D. (1991). Surface to nuclear distances in human bronchial epithelium: Relationship to penetration by Rn daughters. Health Phys., 60, 155-162.
- Barrow, R., Wang, C.-Z., Cox, R., & Evans, M. (1992). Cellular sequence of tracheal repair in sheep after smoke inhalation injury. Lung, 170, 331-338.
- Benfield, J., & Hammond, W. (1992). Bronchial and pulmonary carcinogenesis at focal sites in dogs and hamsters. Can. Res., 52, 2687s-2693s.
- Bickenbach, J. R., McCutcheon, J., & Mackenzie, I. C. (1986). Rate of loss of tritiated thymidine label in basal cells in mouse epithelial tissues. Cell Tiss. Kinet., 19, 325-333.
- Birrer, M., Alani, R., Cuttitta, F., Preis, L., Sabich, A., Sanders, D., Siegfried, J., Szabo, E., & Brown, P. (1992). Early events in the neoplastic transformation of respiratory epithelium. J. Natl. Canc. Inst. Monogr., 13, 31-37.
- Boren, H. G., & Paradise, L. J. (1978) Cytokinetics in the lung. In Harris (Eds.), Pathogenesis and Therapy of Lung Cancer (pp. 369-418). New York: Dekker.
- Bourke, W., Milstein, D., Giura, R., Donghi, M., Luisetti, M., Rubin, A. H. E., & Smith, L. J. (1992). Lung cancer in young adults. Chest, 102(6), 1723-1729.
- Brambilla, E., Moro, D., Veale, D., Brichon, P., Stoeber, P., Paramelle, B., & Brambilla, C. (1992). Basal cell (Basaloid) carcinoma of the lung: A new morphologic and phenotypic entity with separate prognostic significance. Hum. Pathol., 23, 993-1003.
- Breeze, R. G., & Wheeldon, E. B. (1977). The cells of the pulmonary airways. Am. Rev. Resp. Dis., 116, 705-777.
- Breuer, R., Zajicek, G., Christensen, T. G., Lucey, E. C., & Snider, G. L. (1990). Cell kinetics of normal adult hamster bronchial epithelium in the steady state. Am. J. Resp. Cell Mol. Biol., 2, 51-58.
- Carey, F., Fabbroni, G., & Lamb, D. (1992). Expression of proliferating cell nuclear antigen in lung cancer: A systematic study and correlation with DNA ploidy. Histopathol., 20, 499-503.
- Casasco, A., et al. PC10 monoclonal antibody to proliferating cell nuclear antigen as a probe for cycling cell detection in developing tissues. Histochem. 99: 191-199; 1993.
- Chang, S.-C. (1957). Microscopic properties of whole mounts and sections of human bronchial epithelium of smokers and nonsmokers. Cancer, 10, 1246-1259.

Chin, N., Zhang, R., Nostro, D., & Lanks, K. (1992). A novel monoclonal antibody to cytokeratin-18 with reactivity toward lung squamous cell carcinomas and adenocarcinomas of various sites. Am. J. Pathol., **140**, 1061-1069.

Cooper, J., & Vreim, C. (1992). Biology of lung preservation for transplantation. Am. Rev. Resp. Dis., **146**, 803-807.

Cornacchia, L., & Black, V. H. (1988). Development of stereological analysis software for the calculation of volume, number and surface density. Comput. Biol. Med., **18**, 75-88.

Cross, F., Palmer, R., Filipy, R., Dagle, G., & Sturat, B. (1982). Carcinogenic effects of radon daughters, uranium ore dust and cigarette smoke in beagle dogs. Health Phys., **42**, 33-52.

Evans, M. J., & Shami, S. G. (1989). Lung cell kinetics. In D. Massaro (Eds.), Lung Cell Biology (pp. 1-36). New York: Dekker.

Fishman, A. P. (1988). Epidemiology of cancer of the lung. In Pulmonary Diseases and Disorders (pp. 1905-1912). New York: McGraw-Hill Book Company.

Ford, J., & Terzaghi-Howe, M. (1992 a). Characteristics of magnetically separated rat tracheal epithelial cell populations. Am. J. Physiol., **263**, L568-L574.

Ford, J., & Terzaghi-Howe, M. (1992 b). Basal cells are the progenitors of primary tracheal epithelial cell cultures. Environ. Cell Reser., **198**, 69-77.

Hall, P., Levison, D., Woods, A., Yu, C., Kellock, D., Watkins, J., Barnse, D., Gillett, C., Camplejohn, R., Dover, R., Waseem, N., & Lane, D. (1990). Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: An index of cell proliferation with evidence of deregulation expression in some neoplasms. J. Pathol., **162**, 285-294.

Harley, N. H. (1988). Comparing radon daughter dose: Environmental versus underground exposure. Radiat. Protect. Dosim., **7**, 371-375.

Harley, N. H. (1989). Environmental lung cancer risk from radon daughter exposure. In D. J. Paustenbach (Eds.), The Risk Assessment of Environmental and Human Health Hazards New York: John Wiley & Sons, Inc.

Harley, N. H., Meyers, O. A., & Robbins, E. S. (1992a). ^{222}Rn Dosimetry in the dog lung. Rad. Protect. Dosimet., **45**, 611-618.

Harley, N. H., & Robbins, E. S. (1992b). ^{222}Rn alpha dose to organs other than lung. Radiat. Protect. Dosimet., **45**, 619-622.

Harley, N. H., Meyers, O. A., & Robbins, E. S. (199-). Biomodeling of ^{222}Rn decay product dose. Health Phys., Submitted

Harley, N. H., & Robbins, E. S. (1992) The importance of recent results from epidemiology. Proc. Int. Conf. on Radiation Safety in Mines, Saskatoon, Saskatchewan.

Hayashi, M., Sorenberger, G., & Huber, G. (1978). Differential response in male and female tracheal epithelium following exposure to tobacco smoke. Chest, **73**, 515-518.

Hayashi, Y., Fukayama, M., Koike, M., Kaseda, S., Ikeda, T., & Yokoyama, T. (1993). Cell-cycle analysis detecting endogenous nuclear antigens: Comparison with BrdU-*in vivo* labeling and a application to lung tumors. Acta Pathol. Jap., **43**, 313-319.

- Hirata Y., Nose, A., Kobayashi, S., & Takeichi, M. (1989). Expression and role of E- and P-cadherin adhesion molecules in embryonic histogenesis I. Lung epithelial morphogenesis. Develop., 105, 263-270.
- Hislop, A., & Haworth, S. (1989). Airway size and structure in the normal fetal and infant lung and the effect of premature delivery and artificial ventilation. Am. Rev. Resp. Dis., 140, 1717-1726.
- Hyde, D., Plopper, C., St. George, J., & Harkema, J. (1990). Morphometric cell biology of air space epithelium. In D. E. Schraufnagel (Eds.), Electron Microscopy of the Lung (pp. 71-120). New York: Marcel Dekker, Inc.
- Inayama, Y., Hook, G. E. R., Brody, A. R., Cameron, G. S., Jetten, A. M., Gilmore, L. B., Gray, T., & Nettesheim, P. (1988). The differentiation potential of tracheal basal cells. Lab. Invest., 58, 706-716.
- Jacobs, D., Nelson, E., Dontas, A., Keller, J., Slattery, M., & Higgins, M. (1992). Are race and sex differences in lung function explained by frame size? Am. Rev. Resp. Dis., 146, 644-649.
- Jeffery, P. (1983). Morphologic features of airway surface epithelial cells and glands. Am. Rev. Resp. Dis., 128, S14-S20.
- Jeffery, P. K., & Corrin, B. (1984). Structural analysis of the respiratory tract. In J. Bienenstock (Ed.), Immunology of the Lung and Upper Respiratory Tract (pp.1-27) New York: McGraw-Hill.
- Jeffery, P., Gaillard, D., & Moret, S. (1992). Human airway secretory cells during development and in mature airway epithelium. Eur. Resp. J., 5, 93-104.
- Jetten, A. M. (1991). Growth and differentiation factors in tracheobronchial epithelium. Am. J. Physiol., 260, L361-L373.
- Johnson, N., & Hubbs, A. (1990). Epithelial progenitor cells in the rat trachea. Am. J. Resp. Cell Mol. Biol., 3, 579-585.
- Johnson, N., Wilson, J., Habbersett, R., Thomassen, D., Shopp, G., & Smith, D. (1990). Separation and characterization of basal and secretory cells from the rat trachea by flow cytometry. Cytometry, 11, 395-405.
- Kayser, K., Hagemeyer, O., & Runtsch, T. (1991). Morphologic lesions in non-neoplastic bronchial mucosa associated with bronchial carcinomas. Morphologische veränderungen der tumorfreien bronchialschleimhaut bei patienten mit lungenkarzinomen. Zentralbl. Pathol., 137, 425-429.
- Keenan, K., Combs, J., & McDowell, E. (1982). Regeneration of hamster tracheal epithelium after mechanical injury. II Multifocal lesions: Stathmokinetic and autoradiographic studies of cell proliferation. Virchows Arch. (Cell Pathol.), 41, 215-229.
- Kodama, T., Shimosato, Y., & Kameya, T. (1982). Histology and ultrastructure of bronchogenic and bronchial gland adenocarcinoma (including adenoid cystic and mucoepidermoid carcinomas) in relation to histogenesis. In M. R. Melamed & P. Nettesheim (Eds.), Morphogenesis of Lung Cancer (pp. 147-166). Boca Raton, Fla: CRC Press.
- Leube, R., & Rustad, T. (1991). Squamous cell metaplasia in the human lung: Molecular characteristics of epithelial stratification. Virch. Arch. B Cell Pathol., 61, 227-253.
- Mackenzie, I. E. (1990). Proliferative subpopulations of the epidermis. Cell Tiss. Kinet., 22, 76.

- Man, S., & Hulbert, W. (Ed.). (1988). Airway repair and adaptation to inhalation injury. In J. Loke (Ed.), Pathophysiology and Treatment of Inhalation Injuries. (pp. 1-47). New York: Marcel Dekker.
- Mayer, E., Puskas, J., Cardoso, F., Shi, S., Slutsky, A., & Patterson, G. (1992). Reliable eighteen-hour lung preservation at 4° and 10° C by pulmonary artery flush after high-dose prostaglandin E₁ administration. Cardiovasc. Surg., 103, 1136-1142.
- McDowell, E., & Trump, B. (1983). Histogenesis of preneoplastic and neoplastic lesions in tracheobronchial epithelium. Surv. Synth. Pathol. Res., 2, 235-279.
- McDowell, E., X.-M. Zhang and A. DeSanti (1990). Localization of healing tracheal wounds using bromodeoxyuridine immunohistochemistry. Stain Tech., 65: 25-29.
- Mercer, R., Russell, M., & Crapo, J. (1991). Radon dosimetry based on the depth distribution of nuclei in human and rat lungs. Health Phys., 61, 117-130.
- Meyrick, B., Sturgess, J. M., & Reid, L. (1969). A reconstruction of the duct system and secretory tubules of the human bronchial submucosal gland. Thorax, 24, 729-736.
- Mothersill, C., Cusack, A., & Seymour, C. (1989). Enhanced proliferation of cells from human explants following irradiation in the presence of Environmental carcinogens. Radiat. Environ. Biophys., 28, 203-212.
- Nettesheim, P., Jetten, A. M., Inayama, Y., Brody, A. R., George, M. A., Gilmore, L. B., Gray, T., & Hook, G. E. R. (1990). Pathways of differentiation of airway epithelial cells. Environ. Health Persp., 85, 317-329.
- Nimi, T., Imaizumi, M., Abe, T., Haimoto, H., & Nagaura, H. (1987). Immunohistochemical characteristics of proliferative and metaplastic lesions in bronchial mucosa. Am. J. Clin. Pathol., 88, 545-551.
- Ochs, R. (1988). Neoplasms of the lung other than bronchogenic carcinoma. In A. P. Fishman (Eds.), Pulmonary Diseases and Disorders (pp. 2011-2032). New York: McGraw-Hill Book Company.
- Ohashi, Y., Motojima, S., Fukuda, T., & Makino, S. (1992). Airway hyperresponsiveness, increased intracellular spaces of bronchial epithelium, and increased infiltration of eosinophils and lymphocytes in bronchial mucosa in asthma. Am. Rev. Resp. Dis., 145, 1469-1476.
- Pabst, R. (1992). Is BALT a major component of the human lung immune system. Immunol. Today, 13, 119-122.
- Padovan, C., Behr, J., Allmeling, A., Gerlach, J., Vogelmeier, C., & Krombach, F. (1992). Immunophenotyping of lymphocyte subsets in bronchoalveolar lavage fluid. J. Immuno. Met., 147, 27-32.
- Pesci, A., Foresi, A., Bertorelli, G., Chetta, A., & Oliveri, D. (1993). Histochemical characteristics and degranulation of mast cells in epithelium and lamina propria of bronchial biopsies from asthmatic and normal subjects. Am. Rev. Resp. Dis., 147, 684-689.
- Pfeifer, A., Jones, R., Bowden, P., Mann, D., Spillare, E., Klein-Szanto, A., Trump, B., & Harris, C. (1991). Human bronchial epithelial cells transformed by the *c-ras*-1 and *c-myc* protooncogenes induce multidifferentiated carcinomas in nude mice: A model for lung carcinogenesis. Can. Res., 51, 3793-3801.

Pistelli, R., Brancato, G., Forastiere, F., Michelozzi, P., Corbo, G., Agabiti, N., Ciappi, C., & Perucci, C. (1992). Population values of lung volumes and flows in children: Effect of sex, body mass and respiratory conditions. Eur. Resp. J., 5, 463-470.

Plopper, C., Heidsiek, J., Weir, A., St. George, J., & Hyde, D. (1989). Tracheobronchial epithelium in the adult rhesus monkey: A quantitative histochemical and ultrastructural study. Am. J. Anat., 184, 31-40.

Plopper, C., Mariassy, A. T., Wilson, D. W., Alley, J. L., Nishio, S. J., & Nettesheim, P. (1983). Comparison of nonciliated tracheal epithelial cells in six mammalian species: Ultrastructure and population densities. Exp. Lung Res., 5, 281-294.

Potten, C. (Ed.). (1983). Stem Cell Concepts. In Stem Cells. Their Identification and Characterization., (1-11) New York: Churchill Livingstone.

Potten, C., & Morris, R. (1988). Epithelial stem cells *in vivo*. J. Cell Sci. Suppl., 10, 45-62.

Preston-Martin, S., Pike, M., Ross, R., Jones, R., & Henderson, B. (1990). Increased cell division as a cause of human cancer. Can. Res., 50, 7415-7421.

Puchtler, H.; Waldrop, F.; Meloan, S.; Terry, M. and Conner, H. Methacarn (methanol-carnoy) fixation. Histochemie 21: 97-116; 1970.

Rajini, P.; Gelzleichter, T.; Last, J. and Witschi, H. Airway epithelial labeling index as an indicator of ozone induced lung injury. Toxic. 83: 159-168; 1993.

Reid, L. M. (1988). Chronic obstructive pulmonary diseases. In A. P. Fishman (Eds.), Pulmonary Diseases and Disorders (pp. 1247-1269). New York: McGraw-Hill Book Company.

Reif, J. S., Dunn, K., Ogilvie, G. K., & Harris, C. K. (1992). Passive smoking and canine lung cancer risk. Amer. J. Epide., 135, 234-239.

Renan, M. (1993). How many mutations are required for tumorigenesis? Implications from human cancer data. Molec. Carcin., 7, 139-146.

Robbins, E.S., & Meyers, O. (1993). Cell proliferation in the human bronchial mucosa. Mol. Bio. Cell. Vol.4 (Suppl.), p.332A

Robbins, E.S., & Meyers, O. (1994a). Cycling cell populations of human and dog tracheobronchial mucosa: A two compartment model for cell renewal in human respiratory epithelium. Mol. Bio. Cell. Vol. 5 (Suppl.), p. 119a.

Robbins, E.S., & Meyers, O. (1994b). Cycling cells of human and dog tracheobronchial mucosa: Normal and repairing epithelia. Submitted for publication to Enviroment International.

Robbins, E., Meyers, O., & Harley, N. (199-). Target cells in lung carcinogenesis: Human bronchial morphometry. In preparation.

Samet, J. (1992). The health benefits of smoking cessation. Med. Clin. N.A., 76, 399-418.

Schürch, S., Gehr, P., Im Hof, V., Geiser, M., & Green, F. (1990). Surfactant displaces particles toward the epithelium in airways and alveoli. Resp. Physiol., 80, 17-32.

Sleigh, M., Blake, J. R., & Liron, N. (1988). The propulsion of mucus by cilia. Am. Rev. Resp. Dis., 137, 726-741.

Söderberg, M., Hellström, S., Sandström, T., Lundgren, R., & Bergh, A. (1990). Structural characterization of bronchial mucosal biopsies from healthy volunteers: A light and electron microscopical study. Eur. Resp. J., 3, 261-266.

Spandidos, D., Zakinthinos, S., Petraki, C., Sotsiou, F., Yignisis, M., Dimopoulos, A., Roussos, C., & Field, J. (1990). Expression of *ras* p21 and *myc* p62 oncoproteins in small cell and non small cell carcinoma of the lung. Antican Res., 10, 1105-1114.

Stahlman, M., Orth, D., & Gray, M. (1989). Immunocytochemical localization of epidermal growth factor in the developing human respiratory system and in acute and chronic lung disease in the neonate. Lab. Invest., 60, 539-547.

Svensson, C., Pershagan, G., & Klominek, J. (1989). Lung cancer in women and type of dwelling in relation to radon exposure. Can. Res., 49, 1861-1865.

Theunissen, P., Leers, M., & Bollen, E. (1992). Proliferating cell nuclear antigen (PCNA) expression in formalin-fixed tissue of non-small cell lung carcinoma. Histopathol., 20, 251-255.

Tockman, M., Anthonisen, N., Wright, E., & Donithan, M. (1987). Airway obstruction and the risk for lung cancer. Ann. Int. Med., 106, 512-518.

Yesner, R. (1988). Pathology of carcinoma of the lung. In A. P. Fishman (Eds.), Pulmonary Diseases and Disorders (pp. 1913-1930). New York: McGraw-Hill Book Company.