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MECHANISMS *in* RESPIRATORY TOXICOLOGY

Volume I

Hanspeter Witschi
Paul Nettesheim

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Mechanisms in Respiratory Toxicology

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Editors

Hanspeter Witschi, M.D.
Senior Research Staff Member
Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

Paul Nettesheim, M.D.
Chief
Laboratory of Pulmonary Function and Toxicology
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina

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PREFACE

Over the last decade, pulmonary toxicology has become an exciting and fast moving field. A main reason for this is the realization by many investigators, which occurred about 10 years ago, that the lung is not simply a gas-exchanging organ. At the same time there was an increasing awareness that lung tissue may not become damaged by airborne agents alone, but also by chemicals carried by the bloodstream. Among these are naturally and manmade agents, some of which have considerable economic or medical value. It was also realized that the main problem in environmentally induced pulmonary disease is not so much acute, but rather long-term low-level toxicity and it became clear that very little information is available on chronic injury.

It seemed an appropriate undertaking to review some of the current knowledge on the pathobiology of toxic lung damage. This could have been done by describing the toxicology of individual agents. However, such an analysis might not provide an in-depth insight into underlying general pathogenetic principles and might also become overly repetitious, since many of the more thoroughly studied toxic agents produce similar end results. We therefore chose to focus on the cellular and biochemical mechanisms of lung tissue responses to chemical injury.

In the first volume of *Mechanisms in Respiratory Toxicology* an overview is presented on the access toxic agents have to the lung. This includes description of the anatomical features of the lung as well as a discussion of the kinetics of the delivery of chemicals either by the airways or the bloodstream. Once toxic agents reach their target, they set in motion a sequence of events such as cell death, development of edema, and changes in the activity of the mucociliary escalator.

In the second volume pulmonary defense mechanisms and endogenous factors modulating the biological response are discussed. Equally important for understanding the diversity of toxic reactions is the knowledge of biotransformation of chemicals in their target cells. Finally, two clinically important conditions resulting from chronic lung damage are discussed, namely fibrosis and emphysema.

Throughout the two volumes emphasis is placed on cellular and biochemical mechanisms. It is hoped that a discussion of general pathogenetic principles will interest all who are concerned with the action of toxic chemicals on the lung.

It is a pleasure to acknowledge the dedicated and highly competent work of all contributors to this book. Our thanks also go to the staff of CRC Press for skillfully editing the two volumes.

THE EDITORS

Hanspeter Witschi, M.D., is a Senior Research Staff Member at the Biology Division, Oak Ridge National Laboratory. Dr. Witschi received his M.D. degree from the University of Bern, Switzerland, in 1960. He worked as a research fellow in the MRC Toxicology Research Unit in Carshalton/England, the Kettering Laboratory at the University of Cincinnati, and in the Department of Pathology, University of Pittsburgh. From 1969 to 1977 he was at the Department of Pharmacology, Faculty of Medicine, University of Montreal before moving to Oak Ridge National Laboratory. His present research concerns mechanisms of acute and chronic lung damage.

Paul Nettesheim, M.D., is Chief of the Laboratory of Pulmonary Function and Toxicology at the National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services. Dr. Nettesheim received his M.D. degree from the University of Boun Medical School in 1959. His professional experience includes training in Pathology at the University of Freiburg i Br. and the University of Pennsylvania between the years 1960 to 1963. He was a member of the research staff at the Biology Division of Oak Ridge National Laboratory from 1963 to 1978. In 1978 he became a member of the National Institute of Environmental Health Sciences. His present research concerns pulmonary cell biology and respiratory tract carcinogenesis.

CONTRIBUTORS

Michael R. Boyd, M.D., Ph.D.
Chief, Molecular Toxicology Section
Clinical Pharmacology Branch
National Cancer Institute
Bethesda, Maryland

Kathryn H. Bradley
Chemist
Pulmonary Branch
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, Maryland

Arnold R. Brody, Ph.D.
Head, Pulmonary Pathology Group
Laboratory of Pulmonary Function and Toxicology
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina

Carroll E. Cross, M.D.
Professor
School of Medicine
Departments of Physiology and Medicine
University of California
Davis, California

Ronald G. Crystal, Ph.D.
Chief, Pulmonary Branch
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, Maryland

Gerald S. Davis, M.D.
Chief
Division of Pulmonary Medicine
University of Vermont
Burlington, Vermont

Michael J. Evans, Ph.D.
Associate Director
Medical Sciences Department
SRI International
Menlo Park, California

Victor J. Ferrans, M.D.
Chief, Ultrastructure Section
Pathology Branch
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, Maryland

Joan Gil, M.D.
Associate Professor of Medicine and Anatomy
Cardiovascular-Pulmonary Division
Department of Medicine
University of Pennsylvania
Philadelphia, Pennsylvania

Arnold B. Gorin
Assistant Professor in Medicine
School of Medicine
University of California
Davis, California

Kaye H. Kilburn, M.D.
Ralph Edgington Professor of Medicine
Director
Environmental Sciences Laboratory
University of Southern California School of Medicine
Los Angeles, California

Charles Kuhn, M.D.
Professor of Pathology
Washington University School of Medicine
Associate Pathologist
Barnes Hospital
St. Louis, Missouri

Jerold A. Last, Ph.D.
Associate Professor of Internal Medicine and Biological Chemistry
University of California
Davis, California

Lee V. Leak, Ph.D.
Professor of Anatomy
Director
Ernest E. Just Laboratory of Cellular Biology
College of Medicine
Howard University
Washington, D.C.

Marvin Lesser, M.D.
Chief of Pulmonary Medicine
Bronx Veterans Administration Medical Center
Assistant Professor of Medicine
Mount Sinai Hospital
Bronx, New York

Gibbe H. Parsons, M.D.
Director
Medical and Respiratory Intensive Care Unit
University of California Davis Medical Center
Sacramento, California

John A. Pierce, M.D.
Director, Pulmonary Disease Division
Professor of Medicine
Washington University
St. Louis, Missouri

Otto G. Raabe, Ph.D.
Associate Adjunct Professor
Department of Radiological Sciences
School of Veterinary Medicine
University of California
Davis, California

Stephen I. Rennard, M.D.
Senior Staff Fellow
Pulmonary Branch
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, Maryland

Sami I. Said, M.D.
Chief, Pulmonary Disease Section
Veterans Administration Medical Center
Professor of Internal Medicine and Pharmacology
University of Texas Health Science Center
Dallas, Texas

Robert M. Senior, M.D.
Associate Professor of Medicine
Washington University School of Medicine
Co-Director, Pulmonary Disease and Respiratory Division
The Jewish Hospital
St. Louis, Missouri

Alan G. E. Wilson, Ph.D.
Senior Research Toxicologist
Metabolism Section
Environmental Health Laboratory
Monsanto Company
St. Louis, Missouri

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Delivery of Toxic Agents to the Lung

Chapter 1

COMPARATIVE MORPHOLOGY AND ULTRASTRUCTURE OF THE AIRWAYS

Joan Gil

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I. INTRODUCTION

The purpose of this chapter is to describe the anatomy of the mammalian lung with emphasis on those structures and features that are apt to be involved in early toxicologic reactions. In this context, boundaries seem particularly important, e.g., those between air and tissue or between inspired and alveolar air. The air spaces of the lung are comprised of conducting airways (trachea, bronchi, and bronchioles) and terminal airways (ducts and alveoli) where the gas exchange takes place. Both of them are lined, at least in part, by extracellular fluids of relatively unclear composition and uncommon physicochemical properties: the seromucous secretions in the conducting airways and the extracellular lining layer ("surfactant") in the alveoli. The boundary between blood and endothelium is also to some extent unique because the capillary bed seems to be subjected to frequent changes in shape, size, and level of perfusion, depending on a variety of factors. This presentation is limited to the morphological aspects of lung function. In keeping with current research trends, it includes data on morphometry of different parts of the lung.

II. CONDUCTING AIRWAYS

Conducting airways are a system of branching tubes of regular, cylindrical, or somewhat irregular cross section that extend from the trachea down to the last respiratory bronchioles. From the point of view of particle penetration and deposition and airborne environmental injury, dimensions and geometry of the bronchial tree are of outermost significance, but other important features include the cellular composition and the nature of the fluid lining of the lumen of the airways. These aspects have been the object of very intensive research in recent times.

A. Airway Morphometry

The significance of the airway geometry in connection with different problems of pulmonary physiology or environmental aggression was recognized early. First attempts at casting airways with diverse materials were already reported in the 19th century, and advanced morphometric studies on airway casts are still being published. Although not primarily anatomical in nature, the work of Rohrer[1] in 1915 deserves mention because it pioneered a mathematical approach to the basis of airflow resistance in the respiratory system. A solution to the problems raised by Rohrer's and subsequent studies on lung mechanics required a realistic knowledge of the airways both in qualitative and quantitative terms (for review see Pedley et al.[2]).

Weibel offered the first comprehensive quantitative treatment of airway branching in his book.[3] Division of an airway takes place by irregular dichotomy, i.e., a mother branch divides into two daughter airways of different diameter which, in turn, become parent branches. As we shall see below, irregularity of the branching pattern is one of the major problems encountered in morphometry of the conducting airways. Information needed for the study of important items, such as dead space, type of airflow (turbulent or laminar), airflow resistance, and pattern of particle deposition, includes the following anatomic features: number of dichotomic divisions, length-to-diameter ratios of the simple branches, number of units in each generation, particularly number of units in the last generation, and changes in airway diameter after each division. For special purposes, knowledge of branch angles and inclination to gravity may also be necessary. Weibel studied detailed casts of the more proximal generations of airways beginning with the trachea, and additionally he performed measurements on the total amount of peripheral airways visible in histologic specimens. From this he established that after assigning to the trachea the generation number 0, the average number of

generations down to the last airways (alveolar sac) was 23, with the terminal bronchioles, the last purely conducting airways, being on the average generation 16.

Most airflow studies are based on the above-described Model A of Weibel which, without ignoring the branching irregularity, imposes a workable pattern by simply taking the mean values of length and diameter for each generation. The effective length of individual paths could be anywhere between 15 and 30 generations. Tables listing data for individual airway generation (number, diameter, length, and total cross section and volume)[3] and airflow data (Reynolds number at different airflow rates) have been published.[2] Plotting the progressive reduction of diameter d of conducting airways against the generation number, Weibel derived the relationship

$$d_Z = d_0 \cdot 2^{-\frac{Z}{3}} \qquad (1)$$

(where d_0 is the diameter of the trachea (1.8 cm), and d_z is the average diameter of the branches in generation Z) which is favorable from the point of view of hydrodynamics. This relationship holds only until Z = 16 (terminal bronchioles, d_z = 0.06 cm). Subsequent branchings, from Z = 17 to 23 show much larger diameters. In this model, the total number of branches (n) of a generation is evidently $n = 2^z$ which explains why in this system the trachea must be assigned the generation number 0. Useful as this model was, certain relevant parameters were missing from this model. These have been listed by Phalen et al.[4] as follows:

1. The model did not provide branching angles.
2. It did not consider the effects of asymmetry in daughter segments.
3. It did not describe local differences in anatomy among lobes.

Irregular dichotomy was to become the major concern of many workers. According to Horsfield and Cumming,[5] an asymmetrical dichotomously branching system is one with variation in the diameters or lengths of the branches in a given generation or a variation in the number of divisions down the end branches. These authors stressed that in an irregular system, it is an unjustified restraint to count generations from the trachea (generation 0) down to end branches because any intermediate generation number embodies branches different in size and possibly in function. They proposed to number the branches upward starting with branches of a diameter equal to 0.07 cm ("lobular branches") to which the order number 1 was arbitrarily assigned. Thinner branches were designated 0. Horsfield and Cumming[5] performed a painstaking counting of the amount and individual measurements of all the airways between the above-indicated 0.07-cm bronchioles and the trachea (a total of 8298). The shortest path length was reached after 8 branchings, and the longest was found after 25 divisions, with the mean being 14.6. Additionally these authors measured samples of the last distal airways between the 0.07-cm branches and the end. They found the number of distal divisions to range from two to seven.

This careful study in fact showed that Weibel's model was reasonably accurate in spite of the assumptions made. Additionally it has proven very difficult to use asymmetrical systems in modeling for flow dynamics.[2] In a comprehensive, careful study of compared airway morphometry in humans and three other mammals, Phalen et al.[5] point out that asymmetry and its effects are more pronounced in larger than in smaller airways. The most common branching angle appears to be 37.5°.[4,6]

B. Airway Organization

1. Histology of Airway Epithelium

The conducting airways are lined by a pseudostratified ciliated epithelium on a thick basement membrane. Its extraordinary complexity has only recently been recognized: it is an epithelial lining, a mechanical and an immunological defense organ, and an exocrine and an endocrine gland. At the onset, one must point out at the existence of numerous species differences and the variability of the quantitative relationship between cell types.

a. Cells

Several detailed reviews on the structure of epitheliel cells have been recently published by Kuhn,[7] Breeze and Wheeldon,[8] and Jeffery and Reid.[9] The enumeration of the cell types is surprisingly long.

The ciliated cells account for the bulk of cells (see Figures 1 to 4) and are responsible for moving the mucous secretions. They are columnar, electron lucent with abundant mitochondria, secondary lysosomes, rough endoplasmic reticulum, multivesicular bodies, smooth vesicles, and well-developed Golgi. Their lumenal surface is covered with long, slender microvilli and approximately 250 cilia per cell, each 6-μm long and 0.3-μm wide (see Figure 2). The cilia contain an axoneme, a system of nine peripheral double tubules with dynein arms arranged forming a ring around two single central microtubules. Each cilium is anchored to basal bodies, identical with the centrioles active in cell mitosis, and these are fixed by a dense system of "root" microtubules, believed to be a cytoskeletal feature. The tip of the ciliar shaft is provided with claw-like projections which may adhere to the underface of the mucous blanket. The molecular organization and physiology of ciliar beat have been extensively discussed.[7,10]

The goblet cells (see Figure 1) are tall columnar, with slender basis and broad apex. Their cytoplasm is dark; it contains extensive rough endoplasmic reticulum and a supranuclear large Golgi. The apical portion contains variable amounts of secretion granules, sometimes fusing with each other. These granules which often show a dense core by electron microscopy contain some form of mucus, and their histochemical characteristics are well known.[9] Accounts of the normal amount of goblet cells vary greatly; an often-repeated figure for humans is 6800 cells per square millimeter. At the root of the problem, we face the fact that they undergo hyperplasia as unspecific response to irritation (together with increase of the epithelial mitotic index and hypertrophy of the seromucous glands).[9] It has been claimed by several workers that goblet cells are rare in specific pathogen-free adult animals. These cells evidently secrete mucus, but it is dubious at what extent they contribute to the movable blanket of bronchial mucus. In the intestinal epithelium, similar cells perform a general protective function.

The small, pyramidal *basal cells* are generally regarded as the progenitors of the other cell types.

Brush cells are columnar, with a broad basis. They are characterized by the presence of a brush border of very thick microvilli (200 μm in diameter and 800 μm in length) with conspicuous tonofilaments.

Intermediate cells seem to represent stages between young basal cells and fully differentiated cells.

Special type cells occur only in some species. They usually do not reach the lumenal surface; they interdigitate heavily with neighboring cells and contain specific, membrane-bound granules of moderate electron density placed peripherally.

Epithelial serous cells were recently described in the rat trachea. They are thought to be similar to the serous secretory cells of the seromucous glands and contain homogeneous, ovoidal, large inclusions.

Endocrine cells include Kultschitsky, amine uptake and decarboxylation (APUD) or

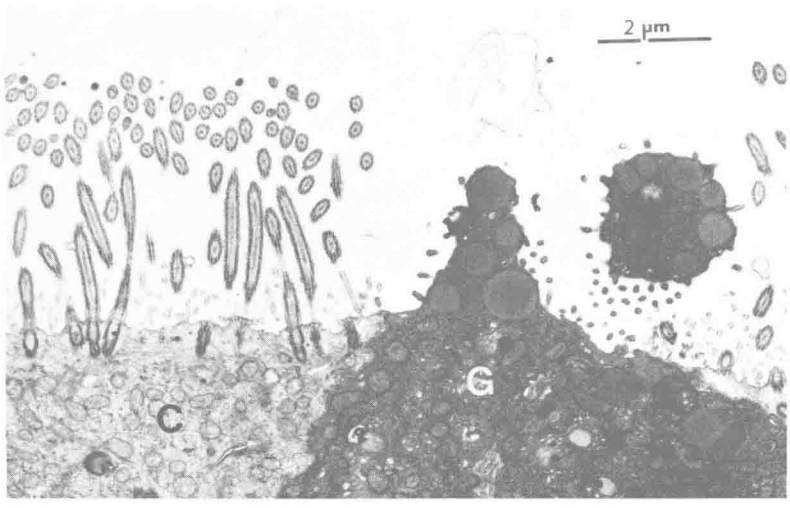

FIGURE 1. Trachea of rat lung fixed by filling the airspaces with glutaraldehyde; C, ciliated cell; G, goblet cell. Note that tips of cilia and of goblet cell reach similar height.

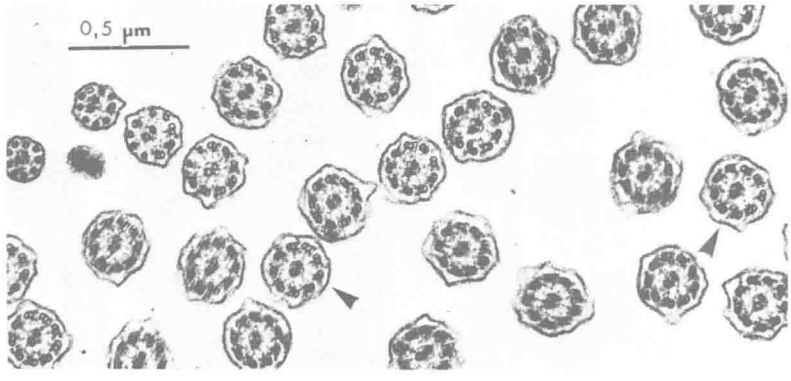

FIGURE 2. Same material as Figure 1, cross sectional views of cilia. Arrows point at cilia where the subcellular arrangement of tubules is well visible. (See complete description in the reviews by Kuhn[7] and Sleigh.[10])

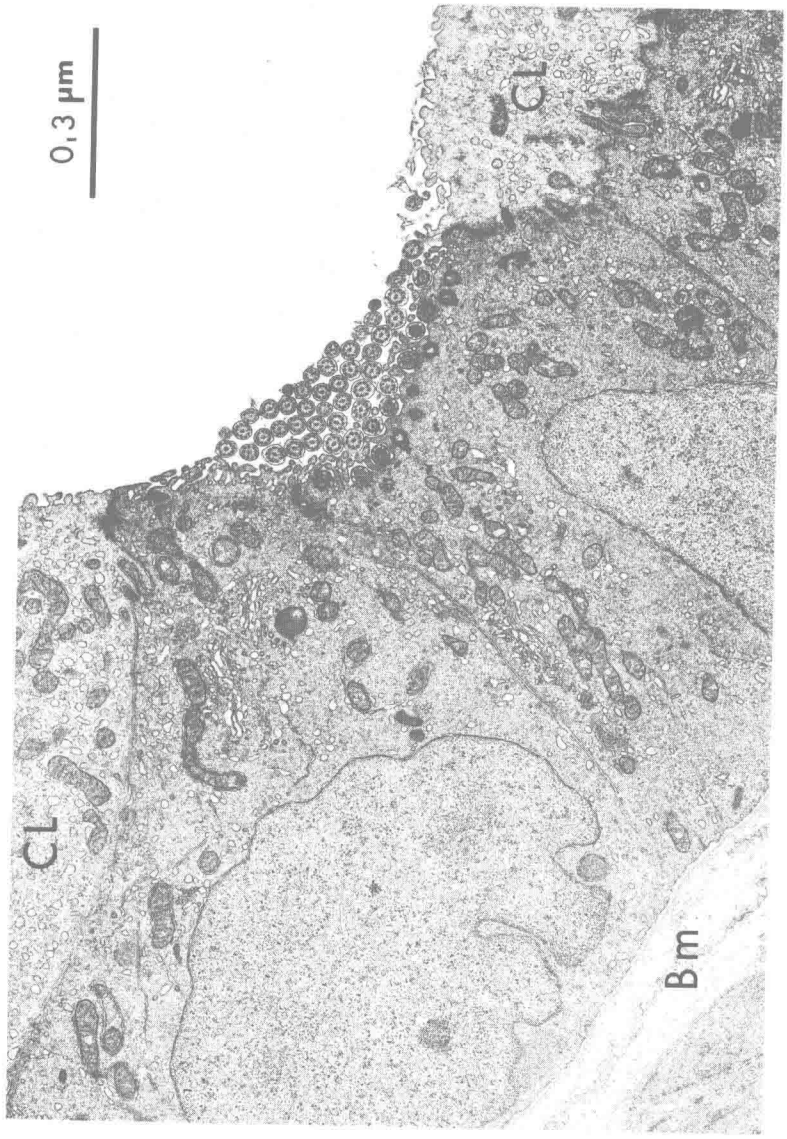

FIGURE 3. Bronchiole of rat lung fixed by perfusion of the pulmonary artery. Two cuboidal, moderately dark ciliated cells rest on a basement membrane (Bm) and are flanked by two Clara cells (CL) with smooth vesicles. On left, lower corner, is a smooth muscle cell.