Meetings

Respiratory Cilia

Cilia are found in all animal groups except Nematoda and have a wide variety of functions. The structure and function of respiratory cilia, measurement of their activity, and responses of the cilia to irritants, bacteria, and viruses were discussed at a symposium held in Durham, North Carolina, 18–19 February.

The structure and ultrastructure of the ciliated epithelial cells of the respiratory tract were summarized by Johannes Rhodin (New York Medical College). Cilia are anchored by basal corpuscles, from which rootlets extend to large numbers of interconnected mitochondria located above dense lysozymes in the upper zone of the cell. Nine peripheral and two central fibrils arise within the basal corpuscles and are fused at the tip of each cilium. Contraction and coordination depend on the interconnections of these fibrils. Ciliated cells develop from intermediate cells which develop microvilli. The life span of ciliated cells is not known, but cell death is preceded by loss of cilia.

The beating of cilia is triggered by excitation. Cilia of a cell or area of epithelium do not beat synchronously but beat one after another, metachronically. This metachronism, which ensures minimum interference between adjacent cilia, either depends upon a mechanical linkage or is neuroid, controlled by a pacemaker. Mechanical stimulation, acetylcholine, or 5-hydroxytryptamine activates cilia to a constant frequency. Although the direction of ciliary beating is changed in protozoa by stimulation, in the respiratory tract it is unidirectional.

Movement of water or mucus is produced by bending, which is propagated from one end of the cilia to the other. The mechanism of bending, its generation, and its control in flagella, as a model for cilia, were described by Charles Brokaw (California Institute of Technology). The bending waves of cilia are nearly circular arcs separated by short straight regions, and active bending occurs sequentially along the flagella. There is no direct evidence for contractility of the fibrils of cilia and, in fact, the proximal portion is probably anchored via rootlets. Because cilia and flagella stilled by glycerin are reactivated by adenosine triphosphate (ATP) and diphosphate, ATP is probably utilized for energy transport within the cell. The amount of ATP utilized is consistent with the amount of work done by flagella. ATP is probably distributed along the length of the cilia, with one molecule every 20 angstroms. This observation arouses speculation that no more than one ATP molecule is used every time a bend passes along an organelle. If those sections of a cilium which are capable of bending are sensitive to the waves passively propagated along the surface of the cilium, mechanical coordination would be possible even in the absence of independent oscillation. A special region near the base of flagella or cilia may be capable of autonomous oscillation.

Factors regulating ciliary movements were described by Robert Gosselin (Dartmouth Medical School). Although there is evidence that cilia are neurally controlled, cilia in vertebrates are not known to be innervated, except in the frog palate. It has been postulated that various substances, including acetylcholine, epinephrine, norepinephrine, serotonin, tyramine, tryptamine, and histamine may act as pacemakers for ciliary activity, with serotonin seeming most probable, particularly in Mollusca. Cations, hydrogen, potassium, and calcium are needed for ciliary activity, and sustained beating requires oxygen. More sophisticated studies are needed to understand how mammalian cilia move.

There have been difficulties in isolating ciliary movement from mucus flow ever since Leeuwenhoek described cilia in 1695. J. J. Ballenger (Northwestern University) described a rotating ciliated explant model. Human respiratory epithelium obtained by lightly scraping the trachea during tonsillectomy is examined for rotation in a hanging-drop preparation. In a Rose chamber, which permits microscopic viewing and photography during exposure of the explant to gaseous or liquid agents, rotation can be used to measure the effect of physical and chemical agents on ciliary motion. R. Rylander (National Institute of Public Health, Stockholm) pointed out that the results obtained in examining cilia are strongly influenced by the choice of preparation and method of measurement. Models which have been used include protozoa, spermatozoa, single respiratory epithelial cells or cell masses in tissue culture, mollusk gills, frog esophagus, chick nostrils, rabbit and ox trachea, and pulmonary clearance (although phagocytosis is also an important factor in this) in man. There are also many methods for measuring ciliary beating or mucus transport. The physical properties of mucus, as well as ciliary motility, determine mucus transport, and mucus-free ciliated epithelium, present in clams, behaves differently from that which is mucus-covered.

Cilia beat in a mucus blanket, the flow of which can be altered by changing hydration or electrolyte concentration and by chemicals and drugs. According to Steven Carson (Maspeth, New York), serotonin is useful in stimulating or inducing transport, while acrolein, phenol, and cigarette smoke inhibit it. Charles Kensler (Cambridge, Massachusetts) reviewed certain physical and chemical influences on particle transport by mucus-covered tracheal cilia in a preparation which permitted variation in concentration and duration of exposure. The broader implications of ciliary function in human disease and the effects of industrial dust on ciliated epithelium were described by George Wright (Cleveland). Variations in concentration of noxious agents with time are important, and the interposed mucus may determine whether dust particles have any physical or physical-chemical effects on cilia. The biophysics of mucus transport of particles is complex, depending upon the wetting, shape, mass, and perhaps smoothness of the particles. Vanadium pentoxide is an industrial dust which causes acute inflammation of bronchial mucosa. Tor Dalhamn (National Institute of Public Health, Stockholm) has measured mucus flow in the exposed trachea of rabbits by cinephotomicrography. Phenol aerosols and smoke from filtered and unfiltered cigarettes stop ciliary activity and mucus transport.

Paul Kotin (National Cancer Institute) described bronchogenic carcinoma induced in rats and mice by aerosolized hydrocarbons. It is not known whether hydrocarbon gains access to the cell by pinocytosis after penetrating the mucus blanket or from the backside via lymphatics after it has been cleared from airspaces. A combination of influenza virus Lee or myxovirus Sendai plus ozonized hydrocarbons produces carcinoma in certain species of mice. The cytological changes are similar to those seen in the bronchial epithelium of cigarette smokers.

The last session concerned the effects of bacteria and viruses on the respiratory epithelium. Gustave Laurenzi (New Jersey College of Medicine) described an aerosol system for exposing mice and rabbits to airborne bacteria and a method for quantitating lung clearance. Smoke inhalation and alcohol ingestion alter bacterial clearance and depress mucociliary function. Although alcohol did not interfere with mobilization of phagocytes in rabbits, it did so in mice.

The effect of viruses on nasal mucociliary function in the chick was discussed by Frederick Bang (Johns Hopkins University). Viruses, particularly Newcastle virus, produce chaotic mucus flow and death of ciliated cells. He suggested the coelomic urn cell from *Sipunculus*, which is a freely moving mucus-producing cell, as a possible model for study of the respiratory epithelial cell. C. H. Stuart-Harris (University of Sheffield, England) pointed out that rhinoviruses, respiratory enteroviruses, and influenza viruses are the active agents in causing colds and febrile illness in adults. Acute bronchitis in previously well persons should be distinguished from exacerbation of symptoms in patients with chronic chest disease. Influenza viruses and rhinoviruses produce irritation and fluid exudation and increase mucus production, but they are not associated with the persistent sputum production of patients with chronic bronchitis.

D. A. J. Tyrrell (Harvard Hospital, Salisbury, England) described an elegant organ-culture technique for observing the cytopathic effect of certain viruses in human respiratory epithelium. Common cold virus has been cultivated and serial passage has succeeded. A working culture ensures continuity of cells and permits observation of the effects of respiratory viruses on cell growth and function. Cilia of these respiratory epithelial cells may beat while the cells are hosts for large numbers of viruses. Thus, viral growth in cells may not disturb their function. Extrapolation of these findings is limited by the dissimilarity between the cells infected in tissue culture and those infected in the intact organism, and also because present techniques are inadequate for growing some of the viruses which cause respiratory disease. The cytopathologic effects of viruses, particularly those which cause influenza, on ciliated epithelium were described by J. F. Ph. Hers (University Hospital, Leiden, Netherlands). Immunofluorescent techniques make it possible to identify the site of attachment of viral subunits and to distinguish stages of synthesis. The S, or soluble, complement-fixing ribonucleic acid antigen of influenza virus appears first in the nucleus and multiplies on the nuclear border, resulting in destruction and death of the cell. The hemagglutinin or V antigen attaches and multiplies on the cell border just within the cytoplasm, and the activity of the cell continues. These techniques offer the hope that at least group-specific etiological diagnoses of diseases in humans can be made by immunofluorescence of exfoliated respiratory epithelial cells.

In summarizing the symposium, David Bates (McGill University) pointed out that studies of the long-term effects of noxious materials on biological systems are necessary for an understanding of chronic diseases in man; this type of study is difficult under present research granting policies. He cautioned against extrapolation from the effects produced on animals in minutes or hours, after large experimental doses of a substance, to diseases which appear in humans after years of exposure to much smaller quantities of the same or similar substances. Public emphasis on and expenditure for research on lung cancer should be refocused upon the more frequent and important chronic destructive lung diseases, chronic bronchitis and pulmonary emphysema.

There is a paucity of information available about the fluid in which cilia beat and the effect upon cilia of changing the properties of mucus. Little is known about the difference between mucus in small distal airways and in large bronchi and between mucus in the upper lobes and in the lower lobes of the lung. Although deficiencies of thyroid and vitamin A appear to reduce mucus transport and lung clearance, mechanisms by which they do so are unexplained. The role of macrophages in clearance of particles from the lungs and the interaction of phagocytic and mucociliary clearance require investigation. The uniformity of effects of noxious agents on widely varying preparations of ciliated cells confirms the observation that nature is economical with methods but wasteful of materials.

Anderson Hilding (Duluth), commenting from a lifetime of research on cilia, observed that there are only minor disagreements as to the harmful effects of cigarette smoke on the lung, and that these effects require no further demonstrations to provide a basis for action. Thus, what to do about cigarettes, neither a food nor a drug and the sole product of a large undiversified industry, poses a challenge to imaginative economics. Hilding's interest in cilia and mucus transport arose from the central position which the common cold and viral respiratory diseases play in the pathogenesis of serious diseases of nose, ears, throat, and lung as seen by an otolaryngologist. He recommended great care in extrapolating from ciliary behavior in protozoa, clams, and frogs to that in man.

The proceedings of the symposium will be published as a supplement to the *American Review of Respiratory Diseases*. The symposium was supported by the P. Lorillard Company.

KAYE H. KILBURN
JOHN V. SALZANO
Departments of Medicine and Physiology, Duke University Medical Center, Durham, North Carolina

Forthcoming Events

June

26–3. International **Dental** Federation, 53rd, Vienna, Austria. (G. H. Leatherman, 35 Devonshire Pl., London, W.1, England)

27–29. **Smoking and Health**, 1st intern. congr., New York, N.Y. (W. A. Scharffenberg, 6830 Laurel St., NW, Washington, D.C. 20012)

27–30. **Botanical** Soc. of America, Northeastern Section, summer field meeting, Univ. of Maine, Orono. (R. K. Zuck, Dept. of Botany, Drew Univ., Madison, N.J.)