Russian], Moscow (1961).
10. B. M. Carlson, The Regeneration of Minced Muscles, Basel (1972).

GLYCEROL MODELS OF CILIATED EPITHELIUM OF THE BRONCHIAL MUCOSA AND THEIR USE FOR THE DIAGNOSIS OF CHRONIC NONSPECIFIC LUNG DISEASES

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By treating cells with glycerol to render their membrane permeable and by carefully extracting the cell contents it is possible to obtain what are called cell models, namely cells from which the soluble components have been removed, but whose contractile apparatus remains intact. If the method of extraction is suitable, the contractile apparatus of the extracted cells retains the power of techanical movement of the same kind as *in vivo*, in the presence of exogenous ATP and appropriate inonic conditions. The first model of this kind was obtained from muscle fibers by extraction with a solution of low ionic strength, containing glycerol [9, 10]. Later, models were obtained by glycerol extraction from a whole range of different cell objects [2] and a method of obtaining glycerol models of the ciliated epithelium of some vertebrates was devised [1].

The aim of this investigation was to determine the characteristics of models of ciliated epithelial cells of the human bronchial mucosa and to discover whether these models may be used to evaluate the functional state of the ciliated epithelium in various forms of bronchopulmonary pathology.

EXPERIMENTAL METHOD

Brush biopsy specimens of the bronchial mucosa, obtained during diagnostic bronchoscopy on adults and children with chronic bronchopulmonary diseases were used for investigation. Cell models of the human ciliated bronchial epithelium were obtained by the method in [1] with some modifications. The sample of epithelium of the bronchial mucosa was kept in 45% glycerol containing 20 mM sodium-phosphate buffer, pH 7.0, and 120 mM KCl and incubated for 24 h at 4°C to render the epithelial cell membranes permeable. The permeable preparation was transferred to a slide and throughly washed at room temperature with buffer containing 20 mM sodium phosphate buffer, pH 7.0, 120 mM KCL, and 5 mM $MgCl_2$ to remove the glycerol and soluble cell components. Testicular hyaluronidase also was added to the buffer in a concentration of 2 μg/ml to produce hydrolysis of viscous mucopolysaccharides, which could prevent beating of the cilia, The models were reactivated by addition of ATP solution to a final concentration of 5 mM. The observations were made by phase-contrast and luminescence microscopy. Preparations for luminescence microscopy were stained with a 0.1 mM solution of acridine orange in 0.9% NaCl.

EXPERIMENTAL RESULTS

The results of phase-contrast microscopy of models of ciliated epithelial cells from the human bronchial mucosa, washed free from glycerol and viscous mucopolysaccharides, are illustrated in Fig. 1a, b. The specimen of the models consisted of a suspension of cell groups, single ciliated cells, and cell fragments. The cilia were nonmotile. After staining with acridine orange, the cytoplasm of the models fluoresced brightly, a characteristic feature of nonviable cells.

On addition of 5 mM ATP the cilia of most cells began to beat. The frequency of beating

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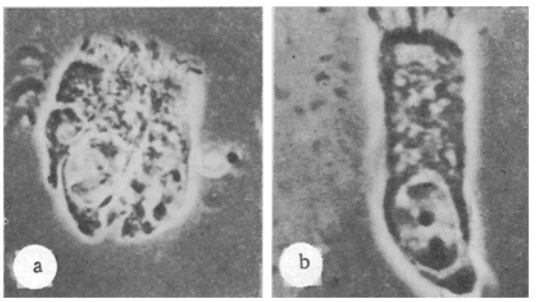

Fig. 1. Phase-contrast microscopy of glycerol models of ciliated epithelium of the human bronchus. Magnification: a) 600, b) 900×.

largely depended on temperature. At room temperature the maximal frequency did not exceed 3 Hz. With a rise of reactivation temperature to 37°C a considerable increase in the frequency of beating was observed: It could not be determined with the naked eye (the cilia quivered).

Addition of 20 M KVO_3 to the reactivated models caused instant cessation of ciliary movement. As was shown p reviously [7, 8] vanadate, in a concentration of 1-10 μM, inhibits dynein ATPase activity *in vitro*, but in a concentration of 10-100 μM it inhibits ATP-dependent movement of models of flagella and cilia [5, 7]. Catechol and noradrenalin abolished the inhibitory action of vanadate [7]. KOV_3 also restores ciliary movement to cell models of the ciliated epithelium of the bronchial mucosa.

It is thus possible to obtain cell models of the ciliated epithelium of the human bronchus, specifically reactivated by ATP, by the method described above.

In the modern view, functional activity of ciliated epithelial cells determined the mucociliary clearance and plays a difinite role in the pathogenesis of various forms of chronic bronchopulmonary pathology. One cause of the disturbance of mucociliary clearance is the presence of defects in motility of the cilia of ciliated epithelial cells. Motility defects can be explained either by organic disturbances of the contractile system of the cilia (tubulin–dynein complex) or by functional (reversible) changes due, for example, to inflammation of the bronchial mucosa and an increase in viscosity of the secretion, and so on.

To elucidate the character of disturbances of the working of the ciliary epithelium, a very promising method is to use cell models. As a control for reactivated preparations of cell models, motility of cilia in biopsy material immediately after sampling, without measures for rendering it permeable or reactivating it, i.e., motility on account of endogenous ATP, can be used. The absence of ciliary movement in the control preparation and its presence in the reactivated preparation are evidence that in a concrete chronic lung disease the motor apparatus of the patient's cilia is unaffected. The absence of movement both on reactivation and in the control points to disturbances of the tubulin–dynein complex, which plays a key role in beating of the cilia, and is evidence of primary ciliary dyskinesia, the basis for the formation of a chronic bronchopulmonary lesion.

The writers have studied a group of children with Kartagener's syndrome, consisting of a triad of features: total transposition of the viscera, bronchiectasis, and disease of the accessory nasal sinuses (sinusitis) (for children). This genetically determined syndrome is linked with the absence of dynein "arms" in the structure of the axonemes. Loss of the dynein "arms," which possess ATPase activity and convert the chemical energy of ATP into mechanical energy of ciliary movement, leads to nonmotility of the cilia [3, 4]. In these patients no cells with motile cilia could be found either in preparations rendered permeable after reactivation of ATP or in a control preparation of the bronchial mucosa, due to the organic (irreversible) character of the changes in their motor apparatus.

The other group of subjects consisted of adult patients with bronchial asthma, in whom endobronchitis was diagnosed by bronchoscopy (25 patients). In biopsy material of the bronchial mucosa taken from five such patients, ciliated epithelial cells were found immediately after the material was obtained, with only nonmotile cilia. Meanwhile, in preparations rendered, permeable, motility of the cilia was restored after reactivation with ATP, indicating

the absence of any organic damage to their motor apparatus. Retesting of the patients (after the end of a course of treatment) revealed cells with motile cilia actually in biopsy material from the bronchial mucosa. Inhibition of ciliary movement can perhaps be explained by the presence of a factor, disturbing ciliary activity [6], in the bronchial secretion of patients with bronchial asthma.

Motile glycerol models of ciliated epithelial cells can thus be obtained from the human bronchial mucosa and used to determine the presence or absence of organic disturbances of structure and function of the ciliary apparatus in various forms of bronchopulmonory pathology.

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LITERATURE CITED

1. V. Ya. Aleksandrov and N. I. Arronet, Dokl. Akad. Nauk SSSR, 110, 457 (1956).
2. N. I. Arronet, Motile Muscle and Cell Models, New York (1973).
3. B. A. Afzelius, Lancet, 2, 950 (1978).
4. B. A. Afzelius, P. Cammer, and B. Mossberg, Eur. J. Resp. Dis., 64, Suppl. 127, 5 (1983).
5. W. Z. Cande and S. M. Wolniak, J. Cell. Biol., 79, 573 (1978).
6. M. J. Dulfano and C. K. Luk, Thorax, 37, 646 (1982).
7. I. R. Gibbons, M. P. Cosson, J. A. Evans, et al., Proc. Natl. Acad. Sci. USA, 75, 2220 (1978).
8. T. Kobayashi, T. Matensen, J. Nath, and M. Flavin, Biochem. Biophys. Res. Commun., 81, 1313 (1978).
9. A. Szent-Gyorgyi, Biol. Bull., 96, 140 (1949).
10. L. Varga, Acta Physiol. Acad. Sci. Hung., 1, 1 (1946).

REACTION OF FIBROBLASTS OF LOOSE CONNECTIVE TISSUE TO POLY-4-VINYLPYRIDINE

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Loose connective tissue is involved in many processes taking place in the body, and its cells actively respond to the action of both endogenous and exogenous factors. For example, after injection of toxins or of a reacting dose of an antigen into a sensitized recipient, intensive vacuolation of the cytoplasm and enlargement of the processes of fibroblasts and macrophages are observed [3, 8]. However, it is virtually impossible to detect all the cells and to estimate the size of their processes in histological tissue sections. For this purpose total (film) preparations of loose connective tissue are required [1]. By means of histological stains it is difficult to identify electively the boundaries of the cytoplasm of fibroblasts, and to do this it is possible to use animal sera containing antibodies against cytoplasmic components of fibroblasts; if these are used, the immunofluorscence method can reveal the outlines of the cells, including processes and fragments of cytoplasm isolated as a result of clasmacytosis. Human or animal sera containing natural antibodies or antibodies appearing as the result of development of a pathological process can be used. Such antibodies as a rule possess high tissue specificity and are often used in immunomorphological research as a tool [12, 13, 15]. Antibodies to fibroblast antigens have been found in human and animal sera [4, 7, 15]. Since the character of action of polymers, including poly-4-vinyl-pyridine (PVP), which has been used experimentally to stimulate the immune response [6, 9, 11], on different cells has been inadequately studied, it is interesting to investigate, in particular,

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