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On cystic fibrosis factor (CFF) and its proposed influence on mucociliary function

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SUMMARY

Cystic fibrosis is a systemic disease where symptoms from the respiratory tract are important. The frequent chronic infections in paranasal sinuses as well as in the lower respiratory tract are related to the occurrence of thick, viscous secretions. Spock et al. (1967) described an abnormal serum factor in patients with cystic fibrosis. This cystic fibrosis factor has been associated with dyskinetic ciliary motion induced by serum and cell culture media from patients with cystic fibrosis.

In this study a sensitive method for photoelectric recording of the mucociliary function was used to examine the effect of sera, cell culture media and bronchial lavage fluids from patients with cystic fibrosis. No sign of decreased mucociliary activity was found. Electron microscopy showed morphologically normal cilia.

INTRODUCTION

In cystic fibrosis or mucoviscoidosis the exocrine glands are affected which leads to complications especially from the respiratory tract where the main symptom is accumulation of thick viscous secretion (Di Sant-Agnese and Davis, 1976; Wood et al., 1976). Alexander Spock (1967) observed that serum from a patient with cystic fibrosis caused dyskinetic ciliary motion in rabbit tracheal explants. This effect was caused by a substance which Spock (1967) called cystic fibrosis factor. However, the chemical nature of this factor is still unknown (Barnett et al., 1973 and 1973; Bowman et al., 1975; Danes, 1976) and furthermore, no reliable and sensitive biological assay has been found for the demonstration of the effect of this factor on the mucociliary function (Baur et al., 1976; Gabridge et al., 1979; Cheung and Jahn, 1976 and 1976).

In the present study we used a photoelectric method (Håkansson and Toremalm, 1965; Reimer et al., 1977) for recording of the mucociliary activity and also electron microscopy in an effort to demonstrate the cystic fibrosis factor in vitro.

MATERIAL AND METHODS

Serum samples. Blood samples from 5 children with cystic fibrosis and from 5 age-matched healthy children were used for testing.

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Cell cultures. Skin fibroblasts were obtained from 3 children with cystic fibrosis and from 3 age-matched healthy controls. Serum samples and cell culture media were obtained from Dr. Hans Kollberg, Department of Pediatrics, University of Umeå, Umeå, Sweden.

Bronchial lavage fluids. Bronchial lavage specimens were obtained from 12 patients with cystic fibrosis. Because of extensive lung disease these patients were submitted to bronchial lavage under general anesthesia. The lavage specimens were obtained from Dr. Alexander Spock, Department of Pediatrics, Duke University Medical Center, Durham, North Carolina, USA.

Recording of mucociliary activity. Trachea from healthy rabbits were placed in an experimental chamber (Håkansson and Toremalm, 1965). For the recording of the mucociliary activity a sensitive photodiod on a microscope was used (Håkansson and Toremalm, 1965; Reimer et al., 1977). The temperature was kept at 37 °C and the relative humidity above 90%.

Electron microscopy. After the mucociliary function tests were finished the tracheal specimens were fixed and prepared for electron microscopic examination in transmitted light and for scanning electron microscopy (v. Mecklenburg et al., 1974).

RESULTS

Sera from 5 children with cystic fibrosis were tested. These children had typical clinical signs from the lungs and/or the gastrointestinal tract and elevated sweat electrolytes as measured on two occasions. A group of 5 age-matched healthy children was used as a control. According to previous reports (Texas Reports on Biology and Medicine, Special Issue: Cystic Fibrosis, 1976; Barnett et al., 1973 and 1973; Bowman et al, 1975; Danes, 1976) the test procedures were modified in certain aspects. The serum samples, diluted as well as undiluted, were incubated with pieces of rabbit trachea and also added as drops every 5th minute. The tracheal preparations were used both fresh and after a prior incubation with culture medium containing fetal calf serum. The mucociliary activity was recorded at intervals during 3–4 hours without any sign of dyskinetic or reduced ciliary activity. The ciliated epithelium incubated with cystic fibrosis serum was analyzed by electron microscopy. The cilia ultrastructure on a cross-section through the basal bodies was found to be normal. The basal feet had approximately the same orientation (Figure 1) (Tegner et al., 1981).

Skin fibroblasts were obtained from 3 children with cystic fibrosis. Three age-matched healthy children served as a control group. Some of the cell-lines were cultured in medium to which protein A from stafylococcus aureus or human immunoglobulin G had been added. According to previous reports (Hösli et al., 1979) protein A and human immunoglobulin was found to induce the production of the cystic fibrosis factor. As human immunoglobulin have been described to

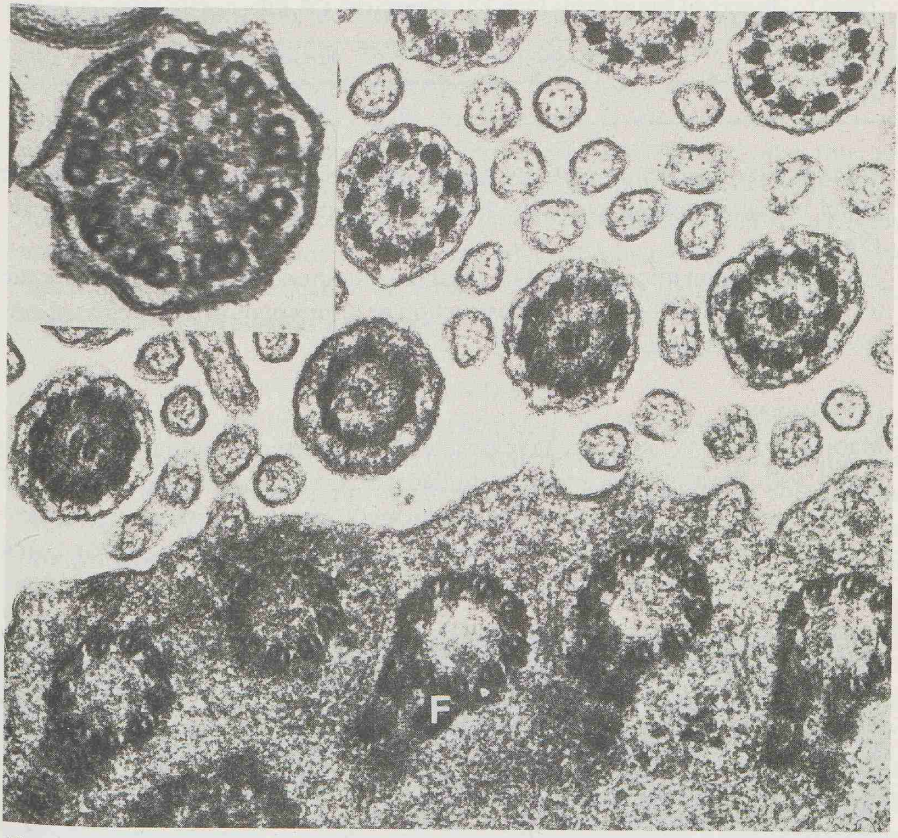

Figure 1. Ciliated tracheal epithelium from a rabbit incubated with cystic fibrosis serum for 1 hour. Cross-section through the basal bodies, some of which are cut at the level of the basal foot (F). All basal feet have approximately the same orientation. Magnification 74.000 ×. Insert: Higher magnification of a cross-section through a cilium. Dynein arms can be seen. Magnification 152.000 ×. (Tegner et al., 1981).

be important for the function of cystic fibrosis factor (Danes et al., 1973) we also tested culture media to which human immunoglobulin had been added. None of these cell culture media had any inhibitory effect on the mucociliary activity (Tegner et al., 1981).

Besides serum and fibroblast cell culture the cystic fibrosis factor has also been described in bronchial secretions (Spock et al., 1967; Doggett et al., 1973). At the Cystic Fibrosis Center, Duke University, bronchial lavage is used as a treatment for patients with extensive pulmonary affection. Samples of bronchial lavage fluids from 12 patients were analyzed. Most of the patients were treated on at least two occasions. Tracheal preparations were incubated with bronchial lavage

Table 1. Bronchial lavage fluids from 12 patients with CF. Effect on mucociliary activity.

No of assay	Observ. time (hrs)	No arrest	Arrest
41	5	34	7

fluids and the mucociliary activity was recorded at intervals during 5 hours. We found no significant effect on the mucociliary activity (Table 1) (Spock et al., 1981).

Scanning electron microscopy of tracheal mucous membrane incubated with the test solutions did not reveal any destruction or disorientation of the ciliated surface (Figure 2) (Spock et al., 1981).

DISCUSSION

Many previous investigators have stressed the occurrence and function of a so called cystic fibrosis factor and have studied it from a pathogenetic and diagnostic point of view (Texas Reports on biology and medicine, special issue: Cystic fibrosis, 1976; Barnett et al., 1973 and 1973; Bowman et al., 1975; Danes, 1976). Using a sensitive photoelectric recording of the mucociliary activity we have not been

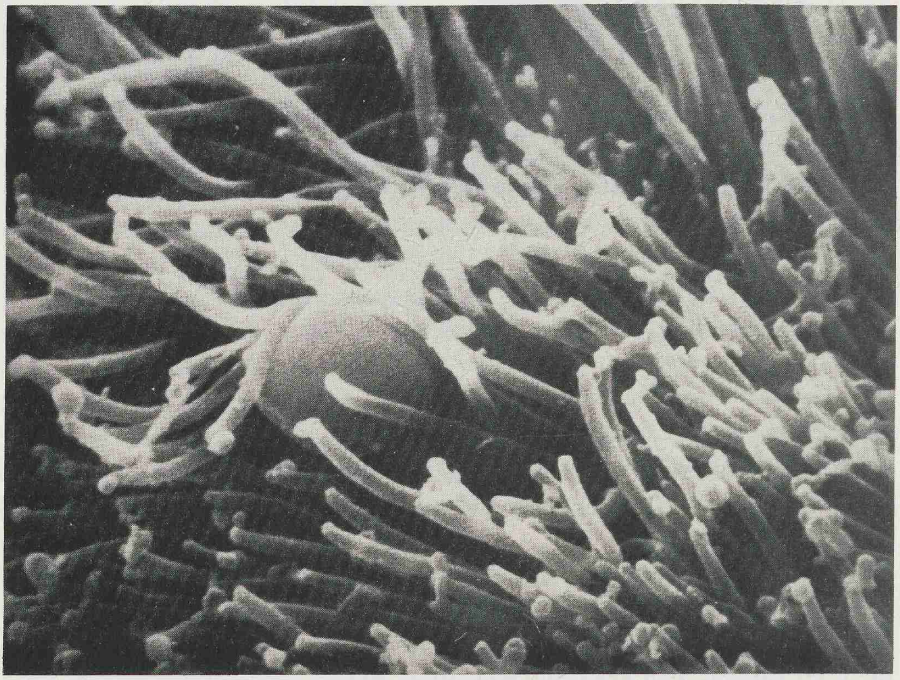

Figure 2. Scanning electron microscopy of ciliated tracheal epithelium from a rabbit incubated for 3 hours with bronchial lavage fluid from a patient with cystic fibrosis. Magnification 12.000 ×.

able to demonstrate any decreasing ciliary activity by adding cystic fibrosis serum, fibroplast cell culture media or bronchial lavage fluids. This is accordance with the findings recently described by Cheung and Jahn (1976 and 1976) and also by Gabridge et al., (1979). Furthermore, by electron microscopy we found a morphological normal ciliated surface and also a normal ultrastructure and orientation of cilia after exposure to the test solutions. (Tegner et al., 1981; Spock et al., 1981). The mucociliary function is the result of two factors, that is the propulsive cilia activity and the retarding effect of mucus. Our results indicate that there is no direct effect on the cilia in cystic fibrosis. Further research concerning mucus rheology is necessary.

ZUSAMMENFASSUNG

Die zystische Fibrose ist eine Systemerkrankung, bei der Symptome der Atemwege vorherrschen. Die häufigen chronischen Nebenhöhlenentzündungen so wie auch Infekte der unteren Luftwege hängen mit dem Vorkommen von dickem, zähflüssigem Sekret zusammen. Spock et al. (1967) haben einen abnormen Serumfaktor bei Patienten mit zystischer Fibrose beschrieben. Dieser zystische Fibrose-Faktor ist mit dyskinetischen Bewegungsstörungen der Zilien in Zusammenhang gebracht worden, die angeblich durch Blutsera oder Zellkulturmedien von Patienten mit zystischer Fibrose hervorgerufen werden könnten.

In dieser Arbeit ist eine sehr empfindliche photoelektrische Methode zur registrierung der mucoziliaren Funktion verwendet worden, um die Wirkung von Sera, Zellkulturen und Bronchenspülwasser von Patienten mit zystischer Fibrose zu registrieren. Hierbei wurden keinerlei Anzeichen einer verminderten mucoziliaren Funktion festgestellt. Die elektronen-mikroskopische Untersuchung der Zilienmorphologie ergab einen normalen Befund.

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REFERENCES

1. Barnett, D. R., Kurosky, A., Bowman, B. H. and Barranco, S. C., 1973: Loss of the ciliary inhibitory effect of the cystic fibrosis factor following proteolytic digestion and heat denaturation. Tex. Rep. Biol. Med. 31, 697–701.
2. Barnett, D. R., Kurosky, A., Bowman, B. H., Hutschison, T., Schmoyer, I. and Carson, S. D., 1973: Cystic fibrosis: Molecular weight estimation of the ciliary inhibitor. Tex. Rep. Biol, Med. 31, 703–708.
3. Baur, P. S., Brinkley, B. R. and Bowman, B. H., 1976: Effects of cystic fibrosis serum ciliary inhibitor on oyster gill ultrastructure. Analysis by scanning and transmission electron microscopy. Tex. Rep. Biol. Med. 34, 155–174.

4. Bowman, B. H., Lankford, B. J., Fuller, G. M., Carson, S. D., Kurosky, A. and Barnett, D. R., 1975: Cystic fibrosis. The ciliary inhibitor is a small polypeptide associated with immunoglobulin G. Biochem. Biophys. Res. Commun. 64, 1310–1315.
5. Cheung, A. T. W. and Jahn, T. L., 1976: High speed cinemicrographic studies on rabbit tracheal (ciliated) epithelia. I. Determination of the beat pattern of tracheal cilia. Pediatr. Res. 10, 140–143.
6. Cheung, A. T. W. and Jahn, T. L., 1976: High speed cinemicrographic studies on rabbit tracheal (ciliated) epithelia. Cytolytic effect of cystic fibrosis serum on tracheal epithelial cells. Pediatr. Res. 10, 144–147.
7. Danes, B. S. S., Litwin, D., Hutteroth, T. H., Cleve, H. and Bearn, A. G., 1973: Characterization of cystic fibrosis factor and its interaction with human immunoglobulin. J. Exp. Med. 137, 1538–1543.
8. Danes, B. S., 1976: Expression of the cystic fibrosis genotype in cultured somatic cells. Tex. Rep. Biol. Med. 34, 135–150.
9. Di Sant-Agnese, P. A. and Davis, P. D., 1976: Research in cystic fibrosis. N. Engl. J. Med. 295, 481–485, 534–541, 597–602.
10. Doggett, R. G. and Harrison, G. M., 1973: Cystic fibrosis: In vitro reversal of the ciliostatic character of serum and parotid secretions by heparin. Nature (Lond.) 243, 250.
11. Gabridge, M. G., Bright, M. J., Agee, C. C., Mickerson, J. M. and Henderson, N. S., 1979: Development of an improved tracheal explant bioassay for the detection of ciliary dyskinesia factor in cystic fibrosis serum. Pediatr. Res. 13, 31–35.
12. Håkansson, C. H. and Toremalm, N. G., 1965: Studies on the physiology of the trachea. I. Ciliary activity indirectly recorded by a new "light beam reflex" method. Ann. Otol. Rhinol. Laryngol. 74, 954–969.
13. Hösli, P., Kollberg, H. and Vogt, E., 1979: Operational definition of cystic fibrosis. I. Monogr. Paediat. 10, 90–95.
14. Mecklenburg, C. von, Mercke, U., Håkansson, C. H. and Toremalm, N. G., 1974: Morphological changes in ciliary cells due to heat exposure. A scanning electron microscopic study. Cell Tiss. Res. 148, 45–56.
15. Reimer, Å., Håkansson, C. H., Mercke, U., and Toremalm, N. G., 1977: The mucociliary activity of the upper respiratory tract. I. A method for use in experimental studies on human material. Acta Otolaryngol. (Stockh.) 83, 491–497.
16. Spock, A., Heick, H. M. C., Cress, H. and Logan, W. S., 1967: Abnormal serum factor in patients with cystic fibrosis of the pancreas. Pediatr. Res. 1, 173–177.
17. Spock, A., Tegner, H. and Toremalm, N. G., 1981: Bronchial lavage fluids from patients with cystic fibrosis. The effect on the mucociliary activity. In manuscript.
18. Tegner, H., Ceder, O., Roomans, G. M., Kollberg, H. and Toremalm, N. G., 1981: Effects of cystic fibrosis serum and cell culture medium on the mucociliary activity of the respiratory tract. An in vitro study in rabbits. Acta Pediatr. Scand. 70, 000–000.
19. Texas Reports on biology and medicine, 1976: Special issue: Cystic Fibrosis. Tex. Rep. Biol. Med. 34, No. 1.
20. Wood, R. E., Boat, T. F. and Doershuk, C. F., 1976: Cystic fibrosis. Am. Rev. Resp. Dis. 113, 833–878.

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