Studies on Cystic Fibrosis Using Isoelectric Focusing. III. Correlation between Cystic Fibrosis Protein and Ciliary Dyskinesia Activity in Serum Shown by a Modified Rabbit Tracheal Bioassay

GREGORY B. WILSON,⁽³²⁾ MELVIN T. MONSHER, AND H. HUGH FUDENBERG

Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina, Charleston, South Carolina, USA

Summary

We have developed a modified rabbit tracheal bioassay for use in investigating a possible correlation between cystic fibrosis protein (CFP) and ciliary dyskinesia factor (CDF) in human serum. The bioassay requires high standards of tissue selection, and all epithelial tissue must be free of underlying connective tissue. When serum samples were collected and processed carefully and warmed to 37° before assay, CDF could be reliably detected in 31 of 31 sera from cystic fibrosis (CF) homozygotes or obligate heterozygotes in 35 min or less without prior fractionation or concentration of sera, whereas 13 of 14 normal control sera were nonreactive. CDF-positive serum reacts in three consecutive phases: (1) initial increase in ciliary beat frequency, (2) ciliary dyskinesia, and (3) tissue destruction with extrusion of single ciliated cells, mucus, and debris. Our results confirm the association of CDF with cystic fibrosis. The bioassay is not specific for CF, however, when whole sera are bioassayed, since serum from several patients with bronchial asthma also caused ciliary dyskinesia. However, this finding need not preclude using rabbit tracheal ciliated epithelial tissue as an assay for following the purification of CDF.

Isoelectric focusing showed that the presence or absence of CFP corresponded with that of dyskinesia activity in all sera tested except for the active samples from seven asthma patients, which were negative for CFP. The results indicate that CFP and CDF may be identical or closely related markers for the CF gene, and suggest that the activity detected by the rabbit tracheal bioassay in sera from patients with asthma and other diseases probably is caused by a substance different from a CF-specific CDF.

Speculation

Several substances in human serum can produce ciliary dyskinesia in rabbit tracheal epithelium, but only one appears to be a specific marker for CF. We speculate that CFP is related structurally or metabolically to a CF-specific CDF and that patients with bronchial asthma and other respiratory and autoimmune disorders harbor a substance that can produce ciliary dyskinesia but that differs from CF-specific CDF.

INTRODUCTION

Spock et al. (15,16) first described a material, termed "ciliary dyskinesia factor" (CDF), that was present in sera from cystic fibrosis (CF) patients (CF homozygotes) and their parents (obligate heterozygote carriers for CF) and disrupted the normal beating pattern of rabbit tracheal cilia. They considered ciliary dyskinesia activity (CDA) to be a specific marker for the CF gene, since sera from patients with a variety of other diseases (i.e., agammaglobulinemia, bronchial asthma, bronchiectasis, celiac syndrome, hay fever) did not cause ciliary dyskinesia (15). Their findings were questioned for several years, however, primarily because the rabbit tracheal bioassay for detection of CDF could not be reproduced by several other investigators (4,7,13).

The association of a CDF with cystic fibrosis has recently been confirmed by Conover et al. (8). However, their results indicate that CDA is not a specific characteristic of CF when whole serum is used in the bioassay, since sera from patients with other diseases produced a ciliary dyskinesia reaction indistinguishable from that caused by sera from CF homozygotes (9). More recent doubt about the existence of a CDF has been raised by the high-speed cinematographic studies performed by Cheung and Jahn (5,6). These investigators claim that sera from homozygotes and heterozygotes for CF does not cause dyskinesia of rabbit tracheal cilia. Instead, they maintain that cytolysis with cellular destruction of the ciliated epithelium is the major effect of serum, and that destruction of the membrane leads to ciliostasis as a secondary effect (6).

We have previously described a protein associated with the CF gene, designated "cystic fibrosis protein" (CFP). The CFP can be detected by isoelectric focusing in sera from CF homozygotes and obligate heterozygotes (22) and appears to be a specific marker for CF (20,22). In conjunction with another protein marker (bands B, C, D), CFP can be used to distinguish most homozygotes or heterozygotes for CF from normal unaffected individuals, and most heterozygotes for CF from CF patients (22). It is not clear whether CFP possesses any biological (e.g., enzymatic) activity, and its possible role in the pathophysiology of CF is unknown.

Accordingly, we have developed a slightly modified version of the rabbit tracheal bioassay (8,15,16) to explore the possible biological activity of CFP. In the present report, we describe our modified rabbit tracheal bioassay in detail; document photographically the sequence of events that follow the application of human serum to rabbit tracheal ciliated epithelium; examine the specificity of CDF as a marker for CF on the basis of our testing of sera from patients with bronchial asthma by bioassay and electrofocusing; and describe our initial attempts to correlate CFP and CDF in whole serum. In another paper (26) our analyses of crude and partially purified preparations of CFP for CDA are reported and two methods are described for distinguishing the CDA in CF and asthmatic sera.

MATERIALS AND METHODS

Collection and processing of samples. Serum was obtained from clotted blood of 16 CF patients (homozygotes), 15 obligate heterozygote carriers for CF, 7 siblings of CF patients, and 14 normal healthy controls. The blood was collected, processed, and frozen in aliquots as described previously (22). Serum was also obtained from 7 individuals with bronchial asthma ("patient controls") to compare the specificity of ciliary dyskinesia activity and CFP as markers for detecting individuals homozygous or heterozygous for CF. All of the asthmatic patients were off any form of medication for at least 7 days prior to providing a blood sample. Informed consent was obtained from each subject (or in the case of minors from their parents) before blood was drawn.

All normal healthy controls and "patient controls" had no known clinical or family history indicative of cystic fibrosis. Diagnosis of CF was confirmed in all CF patients by their clinical history and by the presence of an elevated concentration of chloride in the sweat (14). All siblings of CF patients had sweat chloride concentrations within the normal range. The age, sex, and serum immunoglobulin G (IgG) levels of the subjects studied are shown in Table 1. Serum samples were obtained from subjects deliberately selected to encompass a wide range of age and serum IgG levels, in order to eliminate possible bias in the results.

Isoelectric focusing. Serum samples were analyzed for CFP by electrofocusing in a pH 5-10 gradient as described previously (22). Additional details of and precautions concerning the technique, sample collection and processing have also been reported (19). Samples were also screened for protein bands, B, C, and D the markers that can be used to distinguish most CF homozygotes from heterozygotes (21,22). Since preliminary experiments indicated that serum positivity for bands B, C, and D was not obviously correlated with the time of onset of ciliary dyskinesia or with the presence of CDA, data for bands, B, C, and D have been omitted except where specifically relevant to the suspected genotype (i.e., siblings of CF patients). For all samples analyzed by isoelectric focusing, identical aliquots of serum were tested for ciliary dyskinesia activity, using the modified rabbit tracheal bioassay described below.

Media. Incomplete medium (M199-I) consisted of medium 199 (pH 7.2), containing Earle's salts (Grand Island Biological Co., N.Y.), sodium bicarbonate (2.2 g/liter) and 2 mM L-glutamine, supplemented with penicillin (50 units/ml) and streptomycin (50 mcg/ml). Complete culture medium (M199-C) consisted of 90% M199-I, plus 10% fetal calf serum (Grand Island Biological Co., N.Y.).

Culture of tracheal tissue. Normal female New Zealand white rabbits (5-7 pounds) were used exclusively. The rabbits were killed and the tracheas were removed under aseptic conditions, as described by Conover et al. (8). The tracheal tissue was washed twice in a 60 x 15 mm Falcon tissue culture petri dish containing 10 ml of M199-I at 37°C to remove excess blood, and the epithelium was removed with a scalpel as described previously (8), except that M199-I was used (15,16). The epithelial sheets were transferred to sterile Falcon tissue culture petri dishes containing approximately 2-3 ml of M199-I at 37°C, and cut into small pieces (roughly 5 mm square) with a scalpel. Ten to fifteen pieces were transferred to a sterile 60 x 15 mm tissue culture dish containing 3-4 ml of M199-C and placed in a humidified tissue culture incubator at 37°C and at 5% CO₂ tension until used for bioassay 24 to 48 h later. A pH of 7.2 to 7.4 was maintained throughout the culture.

Bioassay. Explants were quickly examined in the tissue culture dish by light microscopy at a magnification of 50x and then 125x using an inverted Ernst-Leitz-Wetzlar microscope (Germany) at room temperature (25-27°C) (15,16).

The criteria for selecting tissue used in bioassay generally followed those set forth by Conover et al. (8): (a) Only those pieces with vigorously active cilia, minimal outgrowth, and unobscured borders are used. (b) The ciliated borders must be synchronously beating, uniform, and free of mucous contamination. (c) The pH of the culture must be between 7.0 and 7.4.

In addition, it is important that the tissue specimen used be totally free of any underlying connective tissue. Connective tissue under an otherwise acceptable piece of ciliated epithelium can be easily seen using light microscopy at a magnification of 50-125x. Figures 1A and 1B show photographs of two separate pieces of tracheal epithelium which meet these conditions, except that the sample shown in Figure 1B contains some underlying connective tissue. Both pieces of tissue have an active, synchronously beating ciliated border free of mucus (Figs. 1C and 1D). In preliminary experiments we found that epithelial tissue with underlying connective tissue showed a ciliary dyskinesia reaction in response to normal control serum similar to that caused by CF serum samples. Identical aliquots of the same normal control sera failed to cause a reaction in tissue specimens devoid of underlying connective tissue. Also, addition of connective tissue to previously nonreactive normal sera causes conversion of the sera to a positive status and will produce a dyskinesia reaction in tissue devoid of connective tissue (18). The connective tissue apparently activates a component in all human sera which causes a non-specific ciliary dyskinesia reaction.

Samples that met the above criteria were removed as required for bioassay with a wooden applicator stick, transferred to a glass slide, washed with M199-I at 37°C to remove old M199-C, and covered with a solution containing 2 drops of fresh M199-I at 37°C and 2 drops of serum to be tested. The serum was thawed and warmed to 37°C just prior to the assay. Prewarming the sample to 37°C just prior to bioassay serves two purposes (18). (A) When one compares the results obtained by Spock et al. (15,16) and Conover et al. (8) for the time of onset of ciliary dyskinesia it is obvious that the kinetics of the reaction like many biological reactions is temperature dependent. To achieve a time interval for the assay that is conducive for using the system to follow the purification of various CDA's, we found that starting the reaction at 37°C (both the tissue, medium, and sera are at 37°C) allowed for completion of the test by 35 min (35 of 42 samples react by 30 min, see Results) and did not necessitate using a 37°C warm room or a temperature regulated microscope stage. It was felt that many investigators would not have access to a heated microscope stage and/or would prefer not to work in a 37°C warm room, therefore, the present alternative procedure was worked out. (B) In addition, preheating prevents traumatization of the tissue which is already at 37°C for reasons mentioned above.

A cover slip was placed over the sample, and the assay was monitored at room temperature using a magnification of 400x and phase-contrast illumination (15,16). An appropriate border of cilia was localized within 60 sec after the tissue was exposed to the serum. The ciliary activity was observed continuously for the first 30 min and then at 5-min intervals for an additional 30 min. Each serum specimen was assayed at least twice by an investigator who was unaware of the source of the samples. Duplicate readings usually did not vary by more than 10%. When two assays for a single sample did not agree, the sample was retested in the same manner until consistent results were obtained. Ciliary dyskinesia, not cessation of the ciliary beat was used as the sole criterion for a positive reaction (see Results).

With each bioassay, normal control serum (shown by electrofocusing to be CFP negative) was tested initially to evaluate the vitality of the tissue. In addition, a control sample of normal, nonreactive serum was assayed simultaneously with several of the unknown serum samples tested. Two microscopes were used, so that any changes in ciliary beating caused by application of the unknown sample could immediately be compared with the control.

Photomicrography. Photomicrography was performed with a 35-mm Minolta camera (Model SR-T201, Minolta Camera Co., Ltd. Ramsey, New Jersey) mounted onto a Nikon Model LV9-Ke microscope. Exposure was automatically controlled with a Nikon Microflex (Model AFM), and Kodak Tri-X ASA 400 film was used.

RESULTS

Ciliary dyskinesia activity and cystic fibrosis protein in whole human serum. The results of screening whole human sera for ciliary dyskinesia activity (CDA) are shown in Figure 2. All sera from 16 CF homozygotes and 15 obligate heterozygotes produced ciliary dyskinesia in 35 min or less. Sera from CF homozygotes tended to cause ciliary dyskinesia to occur in less time than those sera from obligate heterozygotes (14 ± 8 and 25 ± 8 min respectively (mean $\pm$ S.D.)). There was extensive overlap, however, between values for the two groups, making it impossible to distinguish between homozygotes and heterozygotes on an individual basis.

Sera from 13 of 14 normal control individuals and 3 of 7 siblings of CF patients did not cause ciliary dyskinesia by the end of the assay period (60 min) and were therefore considered negative for the ciliary dyskinesia factor (CDF) (Fig. 2). Sera that failed to react by 60 min were negative for longer periods, but for practical purposes observations were generally discontinued after 60 min.

No correlation was found between the age or sex of the subjects tested and the reactivity of their sera. No attempt was made to correlate severity of the disease in the CF patients tested with the time required for ciliary dyskinesia to occur, since all obligate heterozygote carriers for CF, and 4 of 7 siblings of CF patients that were positive on bioassay (Fig. 2) were totally asymptomatic.

When the serum samples were assayed for CFP by isoelectric focusing (Fig. 2), all those positive for CDA were also found to be positive for CFP. Three of four siblings of CF patients were positive for CFP and CDA, but lacked either band B, C, or D, indicating that they were heterozygote carriers for CF. One normal control and one sibling positive for CFP and CDA did not lack band B, C, or D. (In previous studies, approximately 25% of the obligate heterozygote carriers analyzed were positive for all three bands.) Sera from patient controls (bronchial asthma) were found to elicit a ciliary dyskinesia reaction identical to that produced by sera from individuals homozygous or heterozygous for CF except that several sera from asthmatic patients also produced subsequent ciliostasis. However, none of the patient controls were positive for CFP and all were positive for bands B, C, and D, excluding the possibility that they might be carriers of the CF gene (Fig. 2).

Documentation of ciliary dyskinesia and membrane destruction. When sera positive for CFP or CDF were applied to rabbit tracheal ciliated epithelium,

the following sequence of events was observed: (a) The first noticeable effect was an increase in the ciliary beat frequency. The cilia continued to beat synchronously, but the beat was faster than that of the normal controls run simultaneously or of the same preparation at the beginning of the assay. (b) The ciliary beat became disorganized. Ciliary asynchrony or dyskinesia is shown in Fig. 3B and 3C. The cilia appeared quite different from the normal control photographed at the same time (Fig. 4B and 4C) and also from the same preparation at the time of application of the serum sample (Fig. 3A). This change in the appearance of the ciliary beat (e.g., ciliary dyskinesia) was the only criterion used to characterize a sample as being CDA-positive. (c) Approximately 10-40 min after the onset of ciliary dyskinesia, destruction of the tissue usually occurred. The border became obscure, single cells, mucus and debris were extruded from the tissue (Fig. 3D), and many but not all of the cilia ceased to beat. In contrast, the normal control preparation continued to show synchronously beating cilia (Fig. 4D). This sequence of events (a to c) was observed easily, without special optical equipment (see Materials and Methods).

Not all CDA-positive sera (Fig. 2) caused destruction of the epithelial tissue by the end of the assay period (60 min). Although ciliary dyskinesia was the only criterion used to characterize a positive reaction, 3 of 16 CF homozygote sera and 2 of 15 obligate heterozygote sera were also ciliotoxic (i.e., immobilized the cilia prior to tissue destruction). The 3 CF patients and 2 obligate heterozygote carriers were not related. Whenever a ciliotoxic reaction occurred, it was usually preceded by ciliary dyskinesia.

DISCUSSION

This investigation was designed to establish a reliable and reproducible rabbit tracheal bioassay for use in exploring the possible biological activity and role of CFP (21,22) in the pathophysiology of cystic fibrosis. We chose the rabbit tracheal bioassay instead of the oyster gill bioassay primarily because the latter is subject to seasonal and regional variations in the accessibility of *Crassostrea virginica*, the oyster used by Bowman and co-workers (11), whereas rabbits are readily accessible.

The rabbit tracheal bioassay described in this report is a modification of the bioassays described by Spock *et al.* (15,16) and Conover *et al.* (8). Tissue was selected according to the criteria of Conover *et al.*, and the bioassay followed the protocol of Spock *et al.* with the following modifications: (a) tissue was cultured for 1-2 days instead of 4-6 days; (b) Falcon tissue culture petri dishes were used rather than Falcon culture flasks; and (c) the medium (Medium 199) was supplemented with 10% fetal calf serum rather than 20% rabbit serum. In addition to the requirements for tissue selection proposed by Conover *et al.* (8), we have found that the rabbit tracheal epithelium must be free of any connective tissue. Unless this requirement is met, the rabbit tracheal bioassay is totally unreliable. In that normal sera invariably produce ciliary dyskinesia identical to that produced by sera from CF homozygotes or heterozygotes. Additional research is required to determine whether the material in normal serum apparently activated by the presence of connective tissue is identical to the CDF found in sera from homozygotes or heterozygotes for CF. Nonetheless, our finding might explain the results of other investigators who found CDA in sera from normals as well as CF homozygotes (4,7,13), especially those of Cherry *et al.* (4), who used whole tracheal ring explants rather than just the ciliated epithelium.

Our modified bioassay can detect ciliary dyskinesia activity in serum from homozygotes or heterozygotes for CF without prior isolation or concentration of the euglobulin fraction (15,16). The bioassay requires more time than that of Conover *et al.* (8) but does not have to be performed in a 37°C warm room. We feel that warming of the serum to 37°C, use of only tissue of high quality, and careful processing and storage of the serum samples prior to bioassay collectively increase the sensitivity of the assay sufficiently to permit detection of a CDA in serum from heterozygote carriers as well as from CF homozygotes.

Both in our assay (22) and in that of Conover *et al.* (8), the blood is clotted, and the serum is separated at 4°C and then frozen in plastic at -20°C or lower (-70°C (22)). Variations in processing and storage, such as clotting at room temperature or 37°C, storage at 4°C, storage in glass, or freezing and thawing, may cause loss of CF-specific activity and/or increased nonspecific activity (18). Our findings may explain in part the problems encountered by other investigators (4,6,7,13) in detection of a CDF. That erroneous results can result from improper processing and storage of serum samples is also evident from the report of Bowman *et al.* (3).

Recently, Cheung and Jahn (6) reported that serum from homozygotes or heterozygotes for CF, or even from normal controls, did not cause ciliary dyskinesia, and that cytotoxicity was the major effect of human serum on rabbit tracheal explants, with ciliostasis as a secondary effect following total destruction of the epithelial membrane. In contrast to the work of Cheung and Jahn (6), the present studies demonstrate that ciliary dyskinesia is the first effect produced by human serum on rabbit tracheal epithelium; cytotoxicity and/or obvious destruction of the tissue follow much later. Ciliary dyskinesia can be distinguished easily from normal synchronously beating cilia without the aid of special optical equipment (i.e., an ordinary microscope can be used) and can be documented photographically without the aid of high-speed cinematography as used by Cheung and Jahn (6) (see Figs. 3 and 4).

Disparity between the results of Cheung and Jahn and others (4,6,7,13), and our results those of Conover *et al.* (8) and Spock *et al.* (15,16) might best be explained by assuming differences in tissue culture technique, bioassay or processing and storage of the serum samples. For instance, Cheung and Jahn (5,6) did not culture the rabbit tracheal epithelium prior to bioassay, the tissue was not free of debris (see Fig. 4 in Ref. 5), and some of the sera used for bioassay were not processed at 4°C and/or were not stored at temperatures below -20°C (17).

Although our results agree with those of Spock *et al.* (15,16) and Conover *et al.* (8) in that sera from individuals homozygous or heterozygous for CF caused ciliary dyskinesia whereas sera from normal healthy controls did not (the one normal healthy control positive for CDA might be expected from a heterozygote carrier frequency of 1 in 20 in the Caucasian population (12)), we do not agree with the finding of Spock *et al.* (15,16) concerning the specificity of the CDA in whole serum as a marker for the CF gene. As our results show (Fig. 2), 7 of 7 samples from patients with bronchial asthma produced ciliary dyskinesia nearly identical to that caused by sera from CF homozygotes. This finding confirms the results of Conover *et al.* (9), which indicated that sera from patients with a variety of respiratory and autoimmune disorders produced a ciliary dyskinesia reaction indistinguishable from that

produced by CF sera. We strongly feel, however, that the substance in sera from patients with bronchial asthma is not identical to the CDF found in sera from CF homozygotes or heterozygotes or to CFP, a specific marker for CF detected by electrofocusing.

Screening of all sera for CFP and bands B, C, and D by isoelectric focusing indicated the following: (a) all sera from CF patients and heterozygotes for CF were found to contain CFP; (b) serum from CDF-positive siblings of CF patients and serum from the normal control that was CDF positive also contained CFP; (c) normal samples that were CFP-negative were nonreactive in the rabbit tracheal bioassay; and (d) as in our previous study (22), most obligate heterozygote carriers for CF, as well as suspected heterozygotes (e.g., CFP and CDF positive siblings) were found to lack band B, C, or D. In contrast, sera from patients with bronchial asthma were negative for CFP. In addition, when asthmatic sera are fractionated by Sephadex G-200 gel filtration or DEAE-cellulose chromatography (26), the ciliary dyskinesia activity elutes in a different fraction from the CDF activity in CF sera. The CF-CDA is found in the same fractions as CFP.

The finding that all CFP-positive sera produce ciliary dyskinesia suggests that CFP and a CF-specific CDF are identical or related metabolically, especially since these "factors" and the ciliotoxic factor of Bowman *et al.* (1,2) are similar with respect to molecular weight, association with IgG, and cationic charge (1,2,10,21,23). No definite conclusions can be drawn, however, from studies using only whole serum. Separation of CFP, CF-specific CDF, and the CDA found in sera of patients with bronchial asthma will be required to determine whether these substances are structurally identical or related metabolically. Our findings concerning their purification and activity are reported in another paper (26).

CONCLUSION

A modified rabbit tracheal bioassay for cystic fibrosis (CF) ciliary dyskinesia factor (CDF) has been perfected in this laboratory to explore the possible biological activity and role of cystic fibrosis protein (CFP) detected by electrofocusing in the pathophysiology of CF. Ciliary dyskinesia activity (CDA) was found in all sera from homozygotes and heterozygotes for CF without isolation or concentration of the euglobulin fraction. Sera from patients with bronchial asthma, however, were also found to produce ciliary dyskinesia. Our results confirm the existence of a CDF in sera from homozygotes and heterozygotes for CF but indicate that ciliary dyskinesia activity is not specific for CF when whole serum is assayed.

The effect of CDF-positive sera on ciliated rabbit tracheal epithelium was documented photographically and found to consist of three consecutive events: (a) an initial increase in the ciliary beat frequency, followed by (b) ciliary dyskinesia, followed 10-40 min later by (c) destruction of the tissue with extrusion of single ciliated cells, mucus, and debris.

Electrofocusing of the same sera tested for CDA indicated complete concordance between CFP and CDA positivity in sera from CF homozygotes and obligate heterozygotes, siblings of CF homozygotes, and normal healthy controls. Patients with bronchial asthma, however, were CFP negative.

It is possible that several factors in human serum can cause ciliary dyskinesia, but that only one of these may be specific for CF. Our results suggest that CFP may be similar or related structurally or metabolically to a CF-specific CDF; however, purification and comparison of CFP and the substances responsible for CDA found in CF sera and in sera from patients with bronchial asthma or related respiratory disorders is required before any definite conclusions can be drawn.

REFERENCES AND NOTES

- Barnett, D.R., Kurosky, A., Bowman, B.H., Hutchison, H.T., Schmoyer, I., and Carson, S.D.: Cystic Fibrosis: Molecular weight estimation of the ciliary inhibitor. *Tex. Rep. Biol. Med.* 31:703 (1973).
- Bowman B.H., Barnett, D., and Matalon, R.: Detection and Characterization of the cystic fibrosis ciliary inhibitor. In: J.A. Mangos and R.C. Talamo, *Fundamental Problems in Cystic Fibrosis and Related Diseases*, p. 29. (Intercontinental Medical Book Corp., N.Y., 1973).
- Bowman, B.H., Hirschhorn, K., and Bearn, A.G.: Bioassay of cystic fibrosis factor. *Lancet* 1:404 (1974).
- Cherry, J.D., Roden, V.J., Anthony, B.S., Rejent, J., and Dörner, R.W.: The inhibition of ciliary activity in tracheal organ cultures by sera from children with cystic fibrosis and control subjects. *J. Pediatr.* 79:937(1971).
- Cheung, A.T.W., and Jahn, T.L.: High speed cinematographic studies on rabbit tracheal ciliated epithelia: Determination of the beat pattern of tracheal cilia. *Pediatr. Res.* 10:140 (1976).
- Cheung, A.T.W., and Jahn, T.L.: High speed cinematographic studies on rabbit tracheal (ciliated) epithelia: Cytolytic effect of cystic fibrosis serum on tracheal epithelial cells. *Pediatr. Res.* 10:144 (1976).
- Christensen, J., and Norrevang, A.: Studies on the effect of CF serum on fresh tissue form *Dreissena* gills, oyster gills and rabbit tracheal mucosa. In: D. Lawson: *Proceedings of the fifth International Cystic Fibrosis Conference*, P.49 (C.Nicholls and Co., Ltd., Great Britain, 1969).
- Conover, J.H., Bonforte, R.J., Hathaway, P., Pacinc, S., Conod, E.J., Hirschhorn, K., and Kopel, F.B.: Studies on ciliary dyskinesia factor in cystic fibrosis. I. Bioassay and heterozygote detection in serum. *Pediatr. Res.* 7:220 (1973).
- Conover, J.H., Conod, E.J., and Hirschhorn, K.: Ciliary dyskinesia factor in immunological and pulmonary disease. *Lancet* 1:1194 (1973).
- Conover, J.H., Conod, E.J., and Hirschhorn, K.: Studies on ciliary dyskinesia factor in cystic fibrosis. IV. Its possible identification as anaphylatoxin (C3a)-IgG complex. *Life Sci.* 14:253 (1974).
- Lockhart, L.H., and Bowman, B.H.: Assay for detection of the cystic fibrosis gene. *Tex. Rep. Biol. Med.* 31:631 (1973).
- McCombs, M.L.: Research in cystic fibrosis: A review. *Tex. Rep. Biol. Med.* 31:615 (1973).
- Posselt, H.G., and Bender, S.: Heterozygote testing in cystic fibrosis: Experimental studies with cilia of the mussel *Dreissena polymorpha* Z. *Kinderheilk* 110:98 (1971).
- Schwachman, M.E.: Cystic Fibrosis. In: E.L. Kendig, *Disorders of the Respiratory Tract in Children*, p. 541 (W.B. Saunders Co., Philadelphia, 1967).
- Spock, A., Heick, H.M.C., Cress, H., and Logan, W.: *In vitro* study of ciliary motility to detect individuals with active cystic fibrosis and carriers of the disease. *Mod. Probl. Pediatr.* 10:200 (1967).

16. Spock, A., Heick, H.M.C., Cress, H., and Logan, W.S.: Abnormal serum factor in patients with cystic fibrosis of the pancreas. *Pediat. Res.* 1:173 (1967).
17. Wilson, G.B.: Personal observations while at the University of California, Los Angeles (1972-73).
18. Wilson, G.B.: Unpublished observations, 1975.
19. Wilson, G.B., Arnaud, P., Monsher, M.T., Fudenberg, H.H.: Detection of Cystic Fibrosis Protein by Electrofocusing. *Pediat. Res.* 10:(in press)(1976).
20. Wilson, G.B., Burdack, S.M., Arnaud, P., Monsher, M.T., and Fudenberg, H.H.: Carcinoembryonic antigen and cystic fibrosis protein in blood from cystic fibrosis homozygotes and obligate heterozygote carriers. *Scand. J. Immunol.* (in press)(1976).
21. Wilson, G.B., and Fudenberg, H.H.: Further purification and characterization of serum proteins used to detect cystic fibrosis genotypes by isoelectric focusing. *Tex. Rep. Biol. Med.* 34: (in press) (1976).
22. Wilson, G.B., Fudenberg, H.H., and Jahn T.L.: Studies on cystic fibrosis using isoelectric focusing. I. An assay for detection of cystic fibrosis homozygotes and heterozygote carriers from serum. *Pediat. Res.* 9:635 (1975).
23. Wilson, G.B., Jahn, T.L., and Fonseca, J.R.: Demonstration of serum protein differences in cystic fibrosis by isoelectric focusing in thin-layer polyacrylamide gels. *Clin. Chim. Acta.* 49:79 (1973).
24. Wilson, G.B., Monsher, M.T., and Fudenberg, H.H.: Cystic fibrosis protein factor detected by isoelectric focusing and ciliary dyskinesia. *Clin. Res.* 24:295 (1976).
25. Wilson, G.B., Monsher, M.T., and Fudenberg, H.H.: Purification of cystic fibrosis protein factor and examination of its ciliary dyskinesia activity. *Cystic Fibrosis Club Abstracts* 17:18 (1976).
26. Wilson, G.B., Monsher, M.T., and Fudenberg, H.H.: Studies on cystic fibrosis using isoelectric focusing. IV. Distinction between ciliary dyskinesia activity in cystic fibrosis (CF) and asthmatic sera and association of CF protein with the activity in cystic fibrosis serum. *Pediat. Res.*, submitted for publication (1976).
27. The authors gratefully acknowledge the assistance of Norma Lewiston, M.D., Margaret Jenkins, M.D., and John Whalen, M.D., in obtaining some of the blood specimens used in this investigation, and the editorial assistance of Charles L. Smith.
28. Dr. G.B. Wilson was a Postdoctoral Research Fellow of the Cystic Fibrosis Research Foundation during the early phases of this investigation.
29. The research was supported in part by USPHS Grants HD-09938 and AI-13484 and by the South Carolina State Appropriations for Research HR524 and A705.
30. This is publication no. 61 from the Department of Basic and Clinical Immunology and Microbiology of the Medical University of South Carolina, Charleston, South Carolina (USA).
31. Presented at the 17th Annual Meeting of the Cystic Fibrosis Club, April 27, 1976, in St. Louis, Missouri. Preliminary reports have been published in abstract form (23,24).
32. Requests for reprints should be addressed to: G.B. Wilson, Ph.D., Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina, 80 Barre Street, Charleston, S.C. 29401 (USA).
33. Accepted for publication October 20, 1976.

Fig. 1. Rabbit tracheal ciliated epithelium as observed under phase-contrast microscopy. (A,B) Intact ciliated border (CB) free of mucus observed at a magnification of 100X. The tissue in Figure 1B shows underlying connective tissue (CT), making it unsuitable for bioassay. (C,D) The same two sections viewed at a magnification of 400X. Both sections have uniform, synchronously beating ciliated borders.

Fig. 2. Time required for production of dyskinesia of rabbit tracheal cilia by human serum. Serum samples indicated as taking 60min. or longer showed no activity by the end of the assay (60 min). Solid circles indicate sera positive and open circles indicate sera negative for cystic fibrosis protein as determined by isoelectric focusing (see Materials and Methods).

Fig. 3. Effect of application of sera positive for cystic fibrosis protein and ciliary dyskinesia factor on rabbit tracheal ciliated epithelium as observed by phase-contrast microscopy. (A) Tissue just after application of the sample (5 min.). Note that the ciliated border (CB) is free of mucus and beating synchronously. (B,C) Same tissue section, showing ciliary dyskinesia (CD) 15 min. after application of serum. Magnification 400X (B) and 1000X (C). (D) Tissue 20 min. after the onset of ciliary dyskinesia (1000X). The ciliated border is obscured and the tissue is being destroyed, as evidenced by expulsion of mucus, debris, and of single cells (cell ext.).

Fig. 4. Effect of application of normal control serum negative for cystic fibrosis protein on rabbit tracheal ciliated epithelium as observed by phase-contrast microscopy. (A) 5 min. after application of the sample (400X). (B,C) 15 min. after sample application (400X and 1000X respectively). (D) 60 min. after application of the sample (1000X). Note that the cilia continue to beat synchronously and there is no expulsion of mucus or debris or extrusion of single cells.

TABLE 1. Serum IgG Level, Age, and Sex of Subjects Analyzed for Cystic Fibrosis Protein and for Ciliary Dyskinesia Activity

Serum type (no.)	IgG level ¹ (mg/100 ml)	Age (yr)	Sex ²
Normal healthy control individuals (14)	1043±222 (601-1300)	30±8 (22-43)	7M, 7F
Normal patient controls with bronchial asthma (7)	979±283 (660-1500)	22±8 (10-32)	5M, 2F
Obligate Heterozygote Carriers for CF (15)	884±349 (400-1300)	37±7 (24-57)	4M, 11F
Siblings of cystic fibrosis patient (7)	857±235 (476-2129)	11±4 (8-25)	3M, 4F
Cystic Fibrosis Patients (16)	1168±575 (476-2254)	13±6 (6-25)	9M, 7F

¹Serum concentration of IgG determined by single radial immunodiffusion (ref. 22). Mean ± S.D. and range.

²M, male; F, female.