To The Graduate Council:

I am submitting herewith a thesis written by Patricia L. Allen entitled "The Effects of Normal and Cystic Fibrosis Blood Serum on Mucus Secretion and Epithelial Ultrastructure in Rabbit Trachea." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Zoology.

John R. Kennedy, Major Professor

We have read this thesis
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Vice Chancellor
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THE EFFECTS OF NORMAL AND CYSTIC FIBROSIS BLOOD SERUM ON MUCUS SECRETION AND EPITHELIAL ULTRASTRUCTURE IN RABBIT TRACHEA

A Thesis

Presented for the

Master of Science

Degree

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Patricia L. Allen

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DEDICATION

This thesis is dedicated to the only three people who never doubted my abilities through the entire ordeal of my graduate career: John R. Kennedy, and my Mom and Dad. A special thanks is extended to my brother, Tom, who helped keep the midnight oil burning and my sister, Margaret, who was always good for a laugh.

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To Mr. Dick Williams for his expertise in scanning electron microscopy.

To my committee: Drs. J.R. Kennedy, M.A. Handel and G.L. Vaughan, I express thanks for their critical review of this thesis.

ABSTRACT

The effects of cystic fibrosis serum and normal serum on fresh tracheal rings was examined using scanning and transmission electron microscopy. No damage was observed when epithelium was exposed for 60 minutes to normal serum but when epithelium was exposed to cystic fibrosis serum, well-defined cytotoxic changes occured.

As observed by scanning electron microscopy, mucus production was stimulated by homozygote serum after only 3 minutes exposure. With increased length of exposure, eptihelial crevices filled with mucus, creating a net-like structure. Further mucus secretion and accumulation produced thick strands on the cell surface that eventually fused. By 60 minutes exposure, the epithelial surface was covered with a thick mucus net and many areas showed a high columnar cell loss with some exposure of the basement membrane. Differences between effects of sera from heterozygotes and the homozygotes were not in degree of damage, but in the length of exposure required to produce given effects.

Ultrastructural alterations visible with transmission electron microscopy were also observed. The homozygote and the heterozygote sera produced the same changes during the earlier exposures, but they were more numerous with utilization of sera from homozygotes. During the later exposures, the homozygote sera produced much more extensive cell loss and mucus accumulations than did the heterozygote sera. Major changes included a variety of ciliary alterations, extreme dilation of the granular endoplasmic reticulum, mucus

consistency alterations, swollen and contorted mitochondria, and cell loss from the basement membrane.

These changes are discussed in terms of their relationship with the membrane permeability and the possibilities of their use as a screening mechanism for cystic fibrosis heterozygote detection in the population.

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CHAPTER I

INTRODUCTION

Cystic fibrosis is the most common lethal genetic disease occurring in Caucasian populations. The disease is transmitted as an autosomal recessive trait and has an occurrence of 1 in 2000 (di Sant Agnese and Davis, 1976a). It was first characterized as a disease in 1938 by D.H. Anderson, who correlated the characteristic pancreatic lesions with other concurrent symptoms (Magid et al., 1967). Cystic fibrosis affects primarily the exocrine glands and is characterized by elevated sweat electrolytes, pancreatic insufficiency, and chronic pulmonary disease.

The two major factors in the disease are the elevated salt content and excess mucus secretion. Elevated levels of sodium, potassium, and chloride are present in all cases of the disease. The condition is always present at birth and is detected by the sweat test. Anyone producing sweat with a chloride concentration of over 60 mEq of chloride per liter is considered positive for the test (Magid et al., 1967). This is usually the confirming test for diagnosis of the disease. Clinically, the disease is expressed as extensive salt depletion, and in turn, decreased water content that causes eventual cardiovascular collapse. However, the excess mucus secretions cause most of the clinical problems associated with the disease. The mucus produced by these individuals is thick, has a decreased water content (Kopito et al., 1973), and tends to stick together. Excess mucus

production occurs in the gastrointestinal tract and the respiratory tract. In the intestines, precipates of salts found in mucus tend to form and cause massive obstructions. These are frequently found at birth (McCombs, 1973) and usually require surgery to correct. The mucus in the lungs is extremely thick, has a decreased water content, and has a typically musky odor. Precipation of salts cause obstruction of the small bronchi and the bronchioles (Boxerbaum et al., 1973). These obstructions and further mucus secretion cause mucus accumulation in the lungs in which two pathogens, Staphlococcus aureus and Pseudomonas aeruginosa, thrive (Huang et al., 1961 and Iacocca et al., 1963). The result is repeated cases of pneumonia which eventually lead to irreversible lung damage. Survival of the individual is usually dependent upon the extent of the involvement of the respiratory system.

In 1967, Spock and co-workers, while studying ciliary motility, discovered that serum from cystic fibrosis patients, but not from control individuals, produced a disorganized ciliary beat (ciliary dyskinesia) in rabbit tracheal explants. Further work by Bowman et al., (1969) produced similar results in oyster gills. This latter assay used cessation of ciliary beat as the endpoint as opposed to ciliary dyskinesia. Besley et al., (1969) noted that serum from both cystic fibrosis and normal individuals caused mucus secretion in freshwater mussels, but the cystic fibrosis serum produced significantly greater amounts.

However, not all reports indicated that cystic fibrosis sera was ciliotoxic. Christensen and Norrevarg (1969), using fresh oysters and rabbit tracheal tissue, were unable to demonstrate dyskinesia

differences between cystic fibrosis sera and normal sera. Posselt and Bender (1971), using freshwater mussels, observed ciliary beat after up to 6 hours exposure, and in several cases, 24 hours exposure, to all types of sera and/or plasma used. Cherry et al. (1971), using rabbit and chicken tracheal explants, were able to demonstrate ciliotoxicity from all sera after 30 minutes exposure. Examinations by light microscopy showed extensive destruction to the epithelial layer, associated with eosinophilic degradation of the cytoplasm. Conover, et al., using a modified rabbit tracheal assay, demonstrated that serum from patients with a variety of immunological and pulmonary diseases produced dyskinesia equal to that produced by the cystic fibrosis serum (1973a,b).

There are inherent fallacies involved with both assays. The rabbit tracheal assay using ciliary dyskinesia is based upon a purely subjective interpretation (di Sant Agnese and Davis, 1976c). The oyster and mussel assays are dependent upon the seasonality (Besley et al., 1969) and maintainence conditions of the oysters (di Sant Agnese and Davis, 1976c). All of the above studies involved light microscopic examinations of dyskinesia. Until recently, no attempts had been made to examine ultrastructural changes that might be associated with dyskinesia or ciliastatsis, and as yet, no studies have involved transmission electron microscopic examination of fresh tissue.

The purpose of this study was to answer some of the previously mentioned contradictions appearing in the literature, and utilizing transmission and scanning electron microscopy, to detect any ultrastructural changes associated with the two phenomena of ciliary

dyskinesia and ciliastatsis. This study was designed to answer three major questions:

1. Does normal serum affect cilia and cause mucus accumulation on the ciliary surface?
2. Does cystic fibrosis serum cause damage to cilia and stimulate mucus accumulation on the ciliary surface?
3. Assuming cystic fibrosis does cause some type of damage, can differences be detected between heterozygotes and homozygotes tested?

CHAPTER II

MATERIALS AND METHODS

I. Serum Collection

Blood samples from cystic fibrosis homozygotes, heterozygotes, and normal individuals were obtained from several sources. Samples of 10cc whole blood were allowed to clot and the clot removed. The remaining fluid was centrifuged and separated from any remaining debris. This procedure yielded 4-5 ml blood serum per sample. The serum samples were used immediately or frozen in sterile plastic tubes at -70°C in a Revco Ultra-Low Freezer until used. In all, 10 homozygote, 7 heterozygote, and 9 normal samples were tested. Horse serum (Grand Island Biological Co.) was tested as an additional control.

II. Epithelial Exposure to Serum

For this study, 218 tracheal rings from 17 New Zealand white rabbits were used. The animals were young adults and weighed from 2.7 to 4.1 kilograms. The animals were killed by either air embolism or a 1 to 1.5 cc injection of T-61 Euthanasia Solution (National Laboratories Corp.), the dosage depending on the weight of the animal. Tracheas were removed and put into sterile 0.9% saline. Excess connective tissue was removed and the tracheas were cut into rings approximately 1-2 mm thick. The rings were washed in two more successive changes of saline for 10 minutes per wash to remove any remaining blood or debris. During the final wash, the rings were

checked for ciliary activity by phase contrast microscopy. Only those rings showing good activity were used. From the remaining rings, a ring was chosen randomly as the control for that experiment and put into fixative.

The serum was slowly thawed and brought to room temperature and 0.8 to 1.0 ml serum was transferred into a depression slide. The tracheal rings were then placed in the serum and exposed for various periods of time at room temperature. Early experiments included time exposures of 15 and 60 minutes. Later experiments were expanded to include 3, 5, 10, 15, 60, and in some cases, 90 and 120 minutes exposure. A presumed normal serum was designated as "the standard serum" which was run in every experiment. This provided a standard constant to each trachea so that samples could be compared between tracheas and evaluated for damage. During the exposure periods, ciliary activity was monitored with a Leitz Ortholux phase contrast microscope and the observations recorded.

III. Electron Microscopy

After exposure to sera, the tracheal rings were fixed in a mixture of one part 2.5% glutaraldehyde in 0.1M cacodylate buffer and two parts 1.0% osmium tetroxide in 0.1M cacodylate buffer (pH 7.4) in ice for one hour. Each ring was cut in half, with half being prepared for transmission electron microscopy (T.E.M.) and the other half for scanning electron microscopy (S.E.M.). After fixation, T.E.M. samples were dehydrated in a graded ethanol series, propylene oxide, and left 24 hours in a 1:1 mixture of propylene oxide and Epon 812. After

infiltration, the epithelium was dissected away from the underlying connective tissue and the cartilagenous ring, and embedded in Epon 812. Sections were cut with diamond knives on a Porter-Blum MT-1 ultramicrotome and stained in a saturated uranyl acetate-methanol solution, followed by lead citrate (Venable and Coggeshall, 1965). The sections were then examined on uncoated 300 and 400 mesh grids in a RCA EMU-3H transmission electron microscope.

The S.E.M. samples were dehydrated in a graded series of acetone and critical-point dried in a Bomar SPC-900/EX critical-point dryer. Dried specimens were mounted on aluminum stubs and coated with gold: palladium (60:40) in a Denton DV-515 SM vacuum evaporator. Coated specimens were then examined in an ETEC Autoscan Scanning electron microscope operated at 20 kV.

CHAPTER III

RESULTS

I. Ultrastructure of Untreated Epithelium

Rabbit trachea is lined by a layer of ciliated pseudostratified columnar epithelium resting on a basement membrane. There are three cell types present: ciliated columnar cells, goblet cells, and basal cells. Ciliated columnar cells extend from the basement membrane to the lumen of the trachea. Characteristically the cells possess cilia and microvilli on the apical ends, which constitute the "brush border" of the trachea. Goblet cells are specialized nonciliated columnar cells that secrete the mucus present in the trachea. Basal cells rest on the basement membrane but do not reach the lumen.

At the beginning of each experiment in this study, a control sample of trachea was examined to establish the condition of the epithelium. Overall, the results were fairly consistent. Examinations with electron microscopy at low magnifications showed the epithelium to have a slightly convoluted surface (Figures 1 - 3). At higher magnifications, cells appear to be lined up in ridges (Figure 4) with distinct crevices between them. The two prominent structures observed were the cilia and the microvilli. Relative amounts of each structure were dependent upon the health of the animal at the time of use. Health was judged on respiratory sounds made by the animal just before termination, the presence or absence of mucus-like material in the trachea while dissecting it into individual rings,

Plate I. Surface morphology and ultrastructure of control tissue.

Figure 1. Scanning electron micrograph of tracheal epithelium. The epithelial surface (E) is clearly shown to be divided by crevices (CR). Tufts of loose cells (LC) and the basement membrane (BM) can also be seen. 65 X.

Figure 2. Transmission electron micrograph of tracheal epithelium. Ciliated columnar cells with the oval nucleus (N), compact mitochondria (M), and constricted endoplasmic reticulum (ER) are seen. Cilia (C) and microvilli (MV) constitute the brush border. A goblet cell (GC) is seen at left secreting mucus. 3300 X. Insert: High magnification of a cross-section of a cilium. 15,000 X.

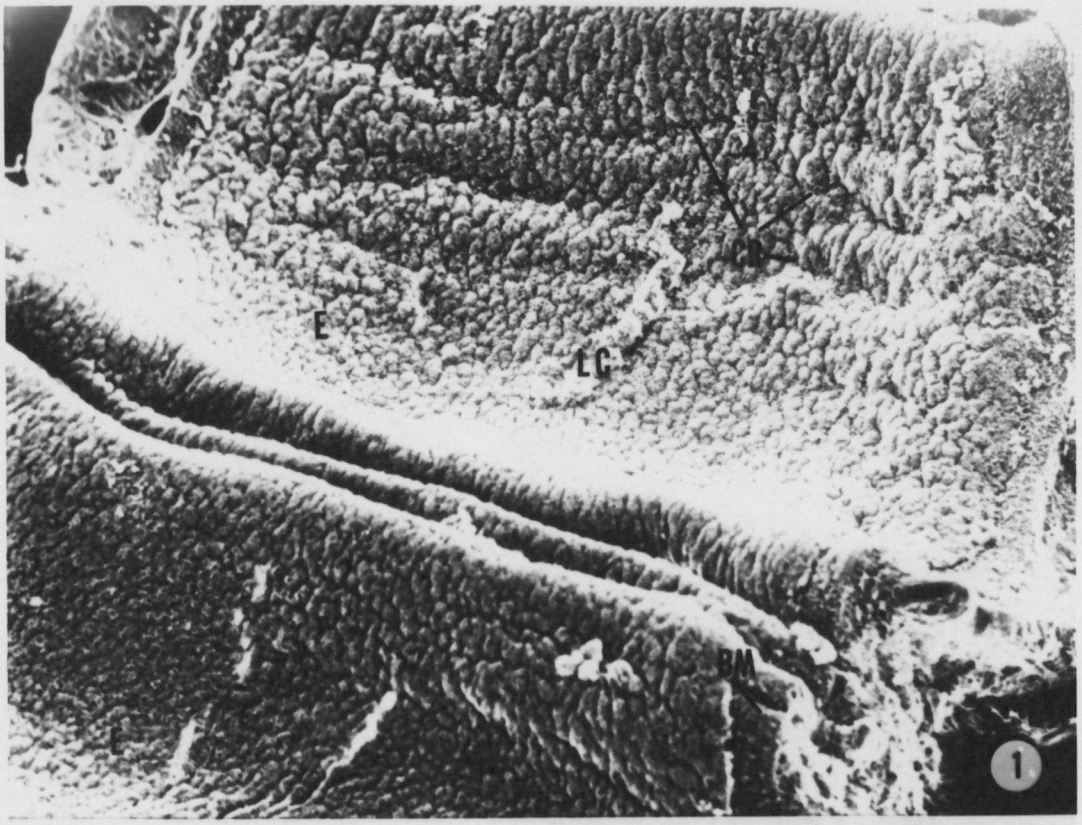




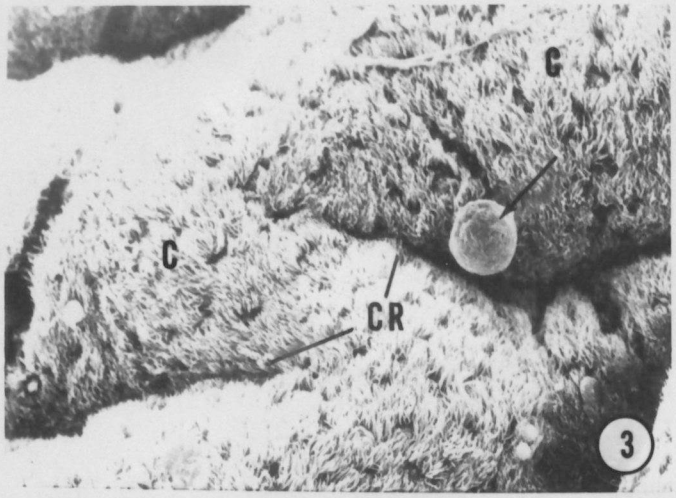
PLATE I

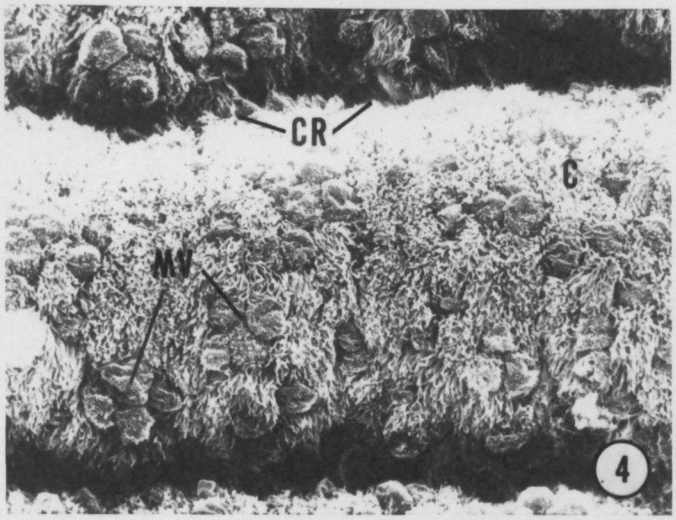
Plate II. Variations in control tissue.

Figure 3. Scanning electron micrograph of tracheal epithelium from very healthy animal. Cilia (C) dominate the cell surface. Crevices (CR) are clear of any material. A single goblet cell (arrow) is about to secrete its contents. 770 X.

Figure 4. Scanning electron micrograph of tracheal epithelium from an animal of average health. An increased number of cells with exposed microvilli (MV) are seen. Cilia (C) are still abundant on the surface. Crevices (CR) are clear of any material. 800 X.

Figure 5. Scanning electron micrograph of tracheal epithelium from an animal whose respiratory health is questionable. A large number of cells with exposed microvilli (MV) are visible. Cilia (C) are not as numerous and are scattered. 800 X.





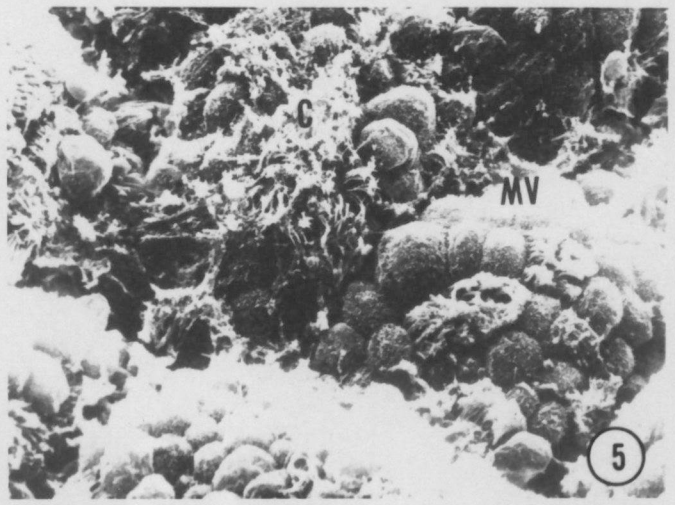


PLATE II

and ciliary activity observed by phase microscopy examination just before use. Healthier animals appeared to have a surface almost covered with cilia and with few exposed microvilli (Figure 3). The majority of the tracheas appeared as in Figure 4. A low to moderate number of cells with exposed microvilli scattered among the ciliated cells was assesed to be the normal condition of the epithelium. Infrequently, a trachea was found to have a large number of cells that had few or no cilia on them (Figure 5). A number of mucus droplets also appeared on such tracheas.

Examination of the epithelium by transmission electron microscopy (Figure 2) revealed features that corresponded with the above scanning microscopy description. Ciliated columnar cells possessed a large oval nucleus that occupied the central portion of the cell. In the supranuclear regions, Golgi bodies were seen. The endoplasmic reticulum appeared throughout the cell. It, along with the Golgi bodies and the perinuclear cisternae, were generally undilated. Mitochondria were seen throughout the cells but were concentrated in the apical regions. They appeared to be rounded to elongated in shape, with varying numbers of cristae. Cilia were normal, possessing the characteristic 9+2 microtubule structure (Figure 2, insert). Goblet cells also possessed the large, centrally located nucleus, but the upper half of the cells were packed with mucus droplets. Many could be found actively secreting these droplets, but the surface remained free of any mucus accumulation. The mucus droplets, either secreted or stored, were membrane bound structures that exhibited a fine grainy texture. Basal cells were generally rounded and possessed large nuclei that

occupied most of the cytoplasm. The cell junctions of all these cells appeared unaltered, even around the secreting goblet cells.

II. Ultrastructure of Epithelium Treated with Normal Serum

In general, treatment of tracheal rings with normal sera, including the standard serum, produced no alterations to the epithelium through 60 minutes exposure. No changes in the structure of the mitochondria, Golgi apparatus, or granular endoplasmic reticulum were observed (Figure 6). Cell junctions remained intact (Figures 6 and 7) and the appearance of the mucus droplets in intact goblet cells remained consistent with that found in the controls (Figure 2). Cilia and microvilli appeared normal, showing no signs of swelling or breakdown (Figures 6-8). A few desquamated cells could be seen on the ciliary surface (Figure 9). However, they were scattered and no accumulations were seen. A few thin strands of mucus were seen on the surface also, but the crevices were clear of debris.

One normal serum sample did cause some damage to the epithelium that was comparable to that seen with some cystic fibrosis samples. The overall effect was similar to that seen in some 15 minutes cystic fibrosis heterozygote samples.

Horse serum produced no surface or ultrastructural changes in the epithelium for up to 60 minutes exposure. A very few cells were sloughed from the epithelial layer and mucus accumulations on the surface appeared minimal. Cilia, microvilli, and the intracellular components appeared normal and cell junctions remained intact.

Plate III. Ultrastructure and surface morphology of tracheal epithelium exposed to normal serum.

Figure 6. Transmission electron micrograph of tracheal epithelium exposed for 1 hour to the standard serum. Mitochondria (M), Golgi apparatus (G), and endoplasmic reticulum (ER) show no signs of swelling or damage. Large centrally located nuclei (N) are seen in the columnar cells. Areas between the cilia (C) and the microvilli (MV) remained clear of debris. A goblet cell (GC) occupies the lower part of the micrograph. 2400 X.

Figure 7. Transmission electron micrograph of tracheal epithelium exposed to normal serum for 1 hour. An empty goblet cell (EC) is about to secrete its minimal contents. The brush border (BB) remains fairly clear of debris. Intact goblet cells (GC) look comparable to that seen in the control samples. The Golgi apparatus (G), mitochondria (M), and endoplasmic reticulum (ER) remain unaffected. 2400 X.

Figure 8. Scanning electron micrograph of tracheal epithelium exposed for 1 hour to standard serum. Cilia (C) and microvilli (MV) have no debris around them. A mucus droplet can be seen coming to the surface (arrow). 3900 X.

Figure 9. Scanning electron micrograph of tracheal epithelium exposed for 120 minutes to a normal serum. Crevices (CR) remain clear of debris even though a few loose cells (LC) have appeared on the surface. A few tiny mucus strands (S) are present on the surface also. 450 X.

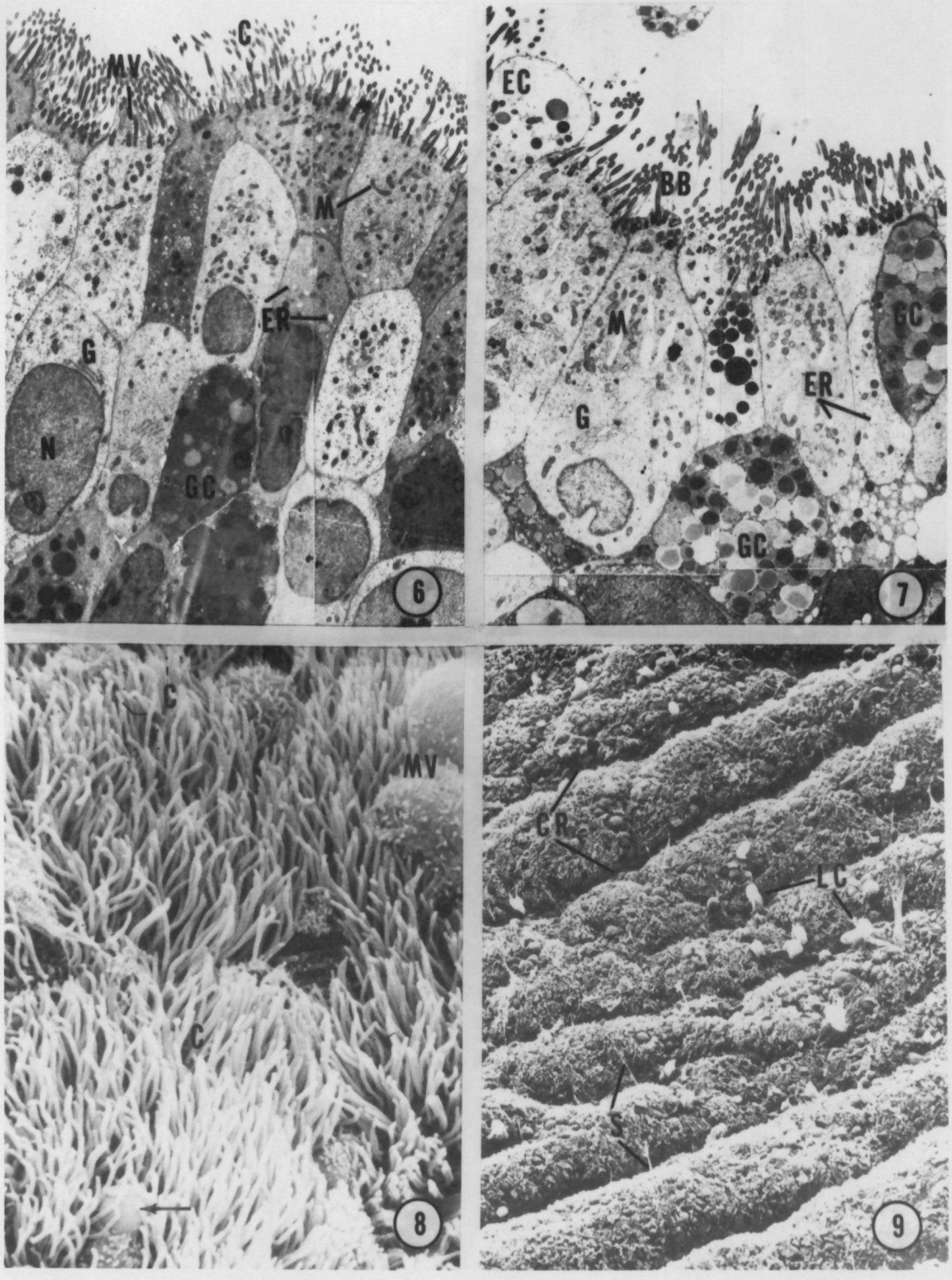


PLATE III

III. Mucus Production Time Sequence

The events of mucus production and surface accumulation resulting from exposure of trachea to cystic fibrosis serum were determined by scanning electron microscopic examination. These events were seen to occur in a definite order regardless of the condition of the trachea. The length of exposure and the extent of the effect was usually consistent with the times presented in this study, but this apparently was dependent upon the amount of cystic fibrosis factor present in the serum sample.

Control tissue and tissue exposed to normal, homozygote, and heterozygote serum samples were examined. The control tissue was evaluated for surface mucus and loose cells before any other samples were examined. Only experimental rings whose controls were similar to those in Figures 3 or 4 were critically examined.

Homozygote-treated Samples

In samples treated with cystic fibrosis homozygote serum for 3 minutes, apical regions of scattered goblet cells could be seen appearing on the surface (Figure 10). Many of these cells could be seen coming out of the crevices described earlier. Few loose cells were seen on the surface at this time.

With 5 minutes exposure, a net-like structure had developed in the crevices (Figures 11 and 12). Further examination of this showed the net to be formed from the breakdown of secreted mucus that had accumulated during the exposure period (Figure 13). The mucus also appeared as thin strands on top of the cilia (Figure 11), but this was not prevalent during the early exposure periods.

Plate IV. Scanning electron micrographs of tracheal epithelium exposed for 3, 5, and 15 minutes to cystic fibrosis homozygote serum.

Figure 10. Tissue exposed for 3 minutes to homozygote serum. Apical regions of scattered goblet (GC) begin to appear on the surface. Crevices (CR) remain clear of any debris. 500 X.

Figure 11. Tissue exposed for 5 minutes to homozygote serum. Crevices have accumulated mucus droplets that have fused in the form of a net-like structure (MN). A few mucus strands (S) are also forming. 760 X.

Figure 12. Tissue exposed for 5 minutes to homozygote serum. Higher mangification reveals the fibrous, branching nature of the mucus net (MN) found in the crevices. 1400 X.

Figure 13. Tissue exposed for 15 minutes to homozygote serum. A mucus droplet (MD) can be seen being incorporated into the fibrous mucus net. This process begins in the 5 minute samples. 1500 X.

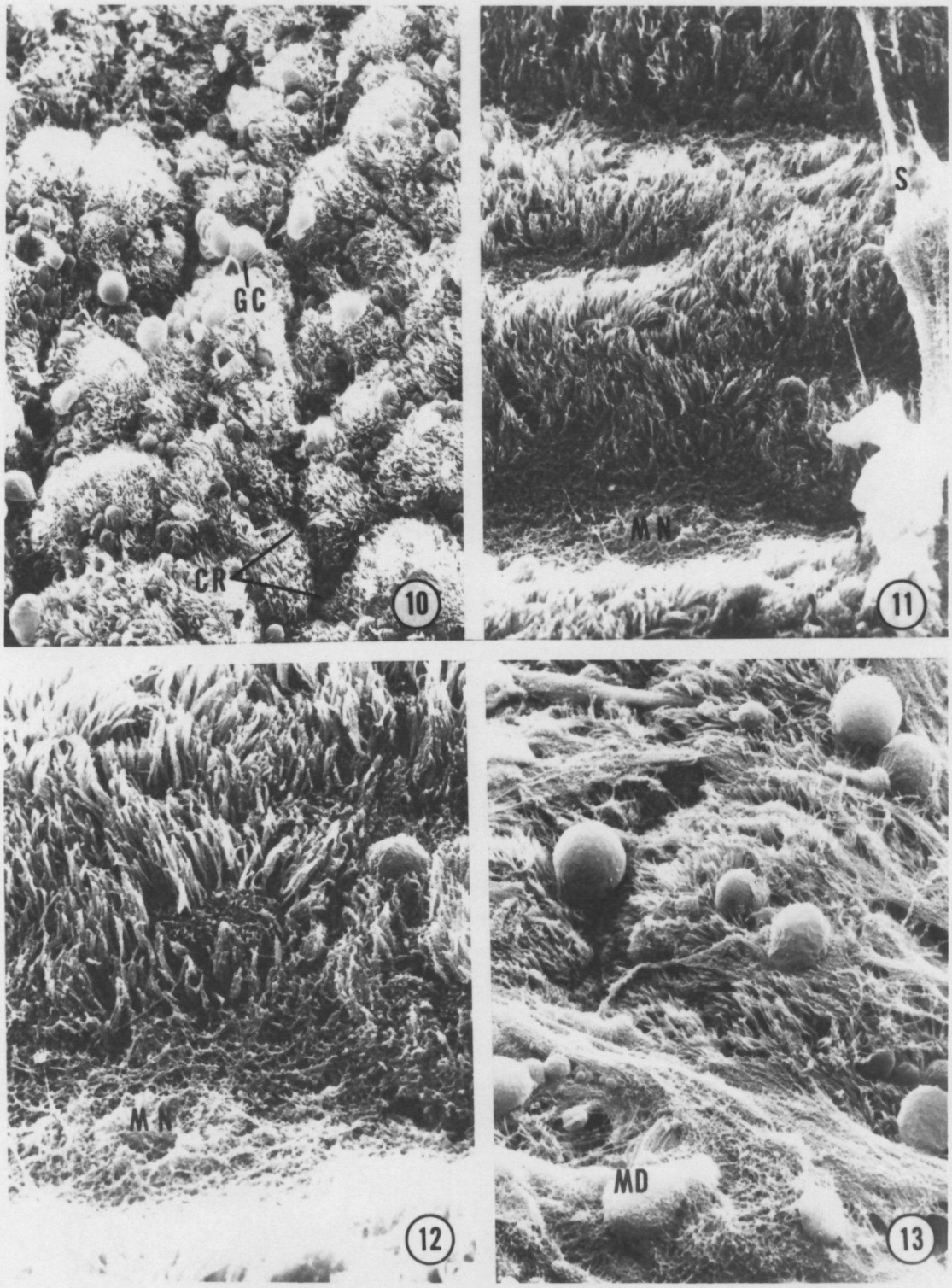


PLATE IV

Ten minutes exposure produced an increased number of loose cells (Figure 14). Mucus strands continued to form also. "Patching," the fusion of the mucus on top of the cell ridges, also had begun to occur.

The 15 minute exposure interval produced some varied results. Some serum samples produced isolated areas of concentrated mucus droplet accumulation. This was interpreted as locations of large numbers of goblet cells actively secreting in response to serum exposure. Other samples produced non-localized mucus secretion (Figure 15). Differences in the amount of strength of a cystic fibrosis factor present could account for this. Mucus strands and loose cells continued to appear in greater numbers.

Thirty minutes exposure produced the same effects as seen in the 15 minutes sample (Figure 16), but to a greater extent. The strands seen in the 15 minute sample (Figure 15) had fused with other strands, patches, and free mucus droplets to produce the "mucus net" that appeared over approximately 50% of the ciliary surface (Figure 16). Loose cells attached to the mucus strands and were incorporated in net formation also (Figure 17).

Sixty minutes exposure generally produced massive columnar cell loss and exposure of the basal cells (Figure 18). In some areas exposure of the basement membrane had occured also (Figure 18), with 90 minutes exposure causing basal cell loss and further exposure of the basal membrane. Two hour exposure times produced either a massive mucus net with no or few cilia exposed or extreme degradation of the epithelial layer with extensive exposure of the basement membrane.

Plate V. Scanning electron micrographs of tracheal epithelium exposed for 10, 15, and 30 minutes to cystic fibrosis homozygote serum.

Figure 14. Tissue exposed for 10 minutes to homozygote serum. Patching (P) can be seen occurring on the cell ridges. Mucus strands (S) can be seen getting thicker. An increased number of mucus droplets (MD) can be seen on the surface. A few loose cells (LC) are beginning to appear also. 870 X.

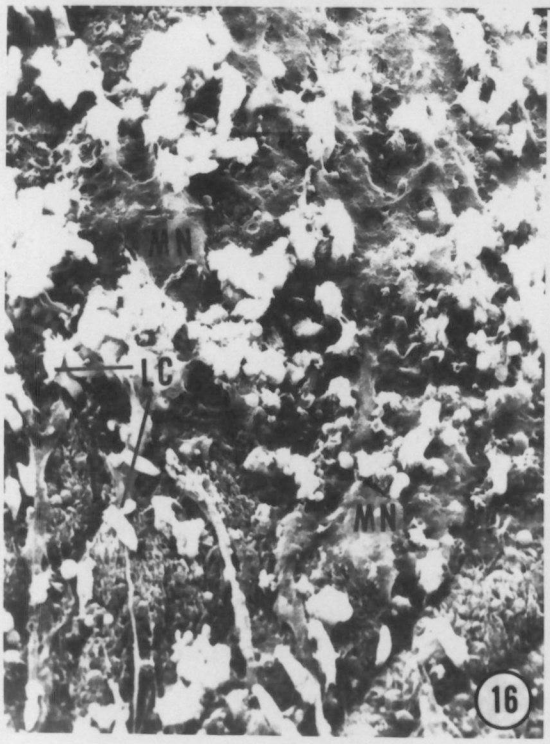
Figure 15. Tissue exposed for 15 minutes to homozygote serum. An area of non-localized mucus secretion is shown. A large number of mucus droplets (MD) occupy the surface. Note the increase in the number mucus strands (S) that are on the surface as opposed to the 10 minute sample (Figure 14). 600 X.

Figure 16. Tissue exposed for 30 minutes to homozygote serum. Patches have now fused to form the mucus net (MN). An increase in loose cells (LC) is noted and many of these become incorporated into the net. 400 X.

Figure 17. Tissue exposed for 30 minutes to homozygote serum. Higher magnification shows crevices (CR) full of material (arrow). Thick mucus strands (S) are present in an area that has not patched over. Note the loose cells (LC) and the small mucus droplets (MD) becoming part of the larger strand. 720 X.







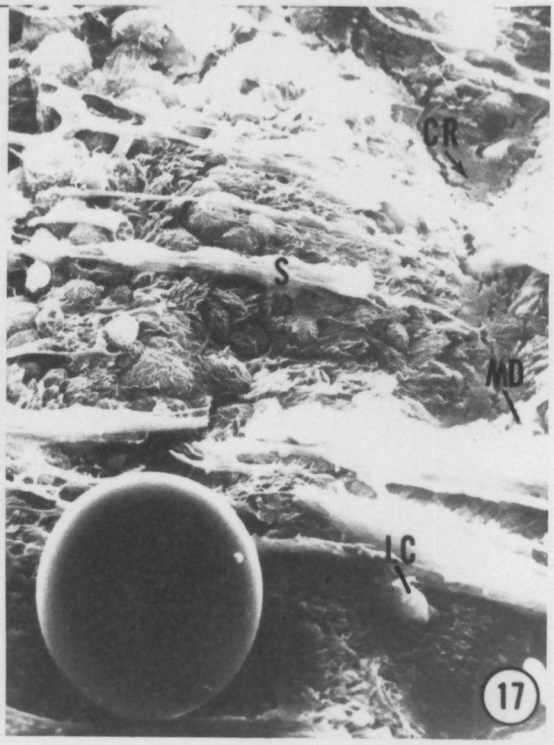


PLATE V

Heterozygote-treated Samples

The tissue treated with cystic fibrosis heterozygote sera followed the same general pattern of effects although the times at which effects were observed differed from those using homozygote sera. The mucus production that occurred in the 3 and 5 minute homozygote samples did not usually occur until 10 minutes in the heterozygotes. At this time, moderate amounts of goblet cells and mucus droplets began appearing in the crevices (Figure 19). A few mucus strands and patches were also evident on some ridges.

The 15 minute heterozygote samples varied also. Some had changed very little from the 10 minute samples while others had a thin but extensive mucus net on the surface (Figure 20). Cilia could be seen protruding the net at this time. Loose cells were not prevalant but they were present. Many columnar cells of both the goblet and ciliated type could be seen with swollen apical ends ballooning over the ciliary surface.

No further significant changes occurred until 60 minutes exposure. Again, results were variable but all tissue showed a thick mucus net over the ciliary surface (Figure 21) with an increased number of loose cells either incorporated into the net or laying on top of it (Figure 21). Cilia could be seen protruding through the net, thus making it appear less thick than the one produced by the homozygote sera.

Exposures longer than 60 minutes produced no more significant effects. An increase in cell loss was observed but it was not as extensive as seen in the 90 and 120 minute exposure periods with the homozygote sera.

Plate VI. Scanning electron micrographs of tracheal epithelium exposed for 60 minutes to homozygote serum and for 10, 15, and 60 minutes to heterozygote serum.

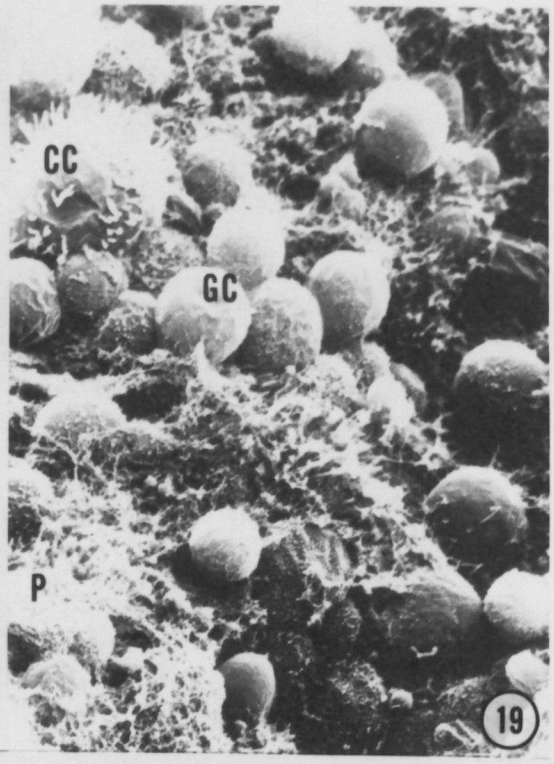
Figure 18. Tissue exposed for 60 minutes to homozygote serum. Extensive columnar cell loss has exposed the basal cell (BC) layer. The basement membrane is exposed in some small areas (BM). 800 X.

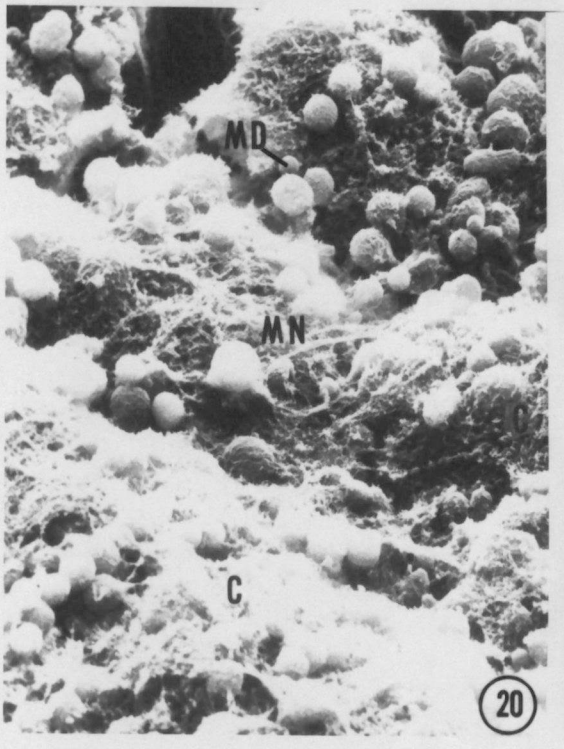
Figure 19. Tissue exposed for 10 minutes to heterozygote serum. Numerous goblet cells (GC) can be seen ballooning over the surface. A single patch of mucus can also be seen (P). Note the large swollen columnar cell (CC) with its extended cilia. 1700 X.

Figure 20. Tissue exposed for 15 minutes to heterozygote serum. A thin extensive mucus net (MN) can be seen extending over the ciliary surface. Cilia (C) can still be seen through it. Numerous loose mucus droplets (MD) can be seen. 1700 X.

Figure 21. Tissue exposed for 60 minutes to heterozygote serum. An extensive mucus net (MN) covers the cell surface. A few cilia (C) can be seen protruding through the layer. Note the loose cells (LC) and underlying cilia (UC) associated with the net. 1700 X.







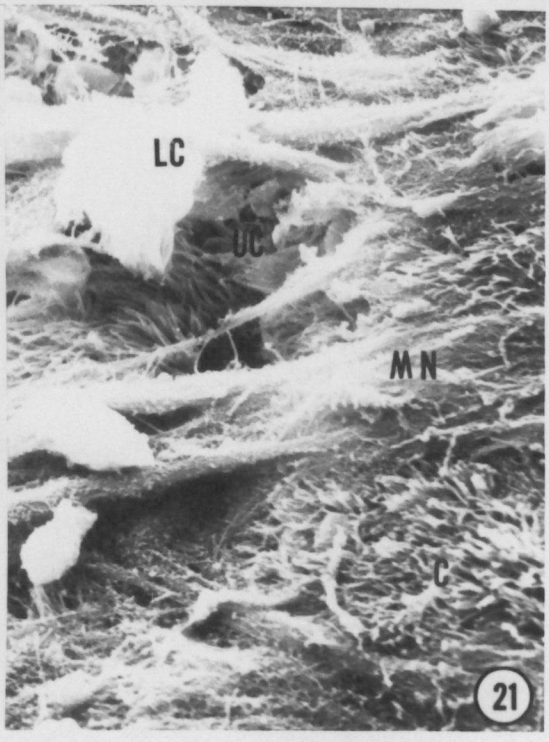


PLATE VI

Normal-treated Samples

Normal sera, including the standard, produced no significant changes on the epithelial layer (Figures 8 and 9, page 15) through 120 minutes exposure. The crevices remained clear of any material and only a few loose cells and strands were observed on the surface. Since mucus is a normal constituent of tracheal epithelium, these strands were considered not significant.

Summary of Data

Thus, the data allow three conclusions: first, exposure to normal serum causes no significant damage to healthy tracheal epithelium; second, there is a definite sequence of events to account for the mucus net that forms on the surface of the tissue-

1. The cystic fibrosis serum stimulates mucus production and/or secretion,
2. The crevices fill with mucus,
3. Further production and accumulation forms mucus strands which form patches of surface net; these patches incorporate loose cells and cilia of intact cells,
4. The net covers the entire surface,
5. Eventually, there is cell loss and exposure of the basement membrane,

and third, despite effects showing up at different times, both homozygote and heterozygote sera produce the above pattern of mucus production. Differences in the amount of factor present, which would account for some heterozygotes showing comparable effects to some homozygotes. But in a given homozygote/heterozygote pair from the

family, the homozygote always produced more cell loss or more extensive damage to the tissue than did the heterozygote.

IV. Ultrastructure of Epithelium Treated with Cystic Fibrosis Serum

Once it was confirmed that cystic fibrosis serum did affect mucus production and secretion and that normal serum did not, ultrastructural studies were undertaken to determine if any cellular alterations were occurring that coincided with the excess mucus accumulations.

Heterozygote-treated Samples

As early as 3 minutes after exposure to heterozygote serum, some definite changes in the ultrastructure of the cells were produced. The most striking changes were effects on the cilia. Three different changes were noted. The most common was ciliary resorption by the columnar cells (Figures 22 and 23). This resorption occurred in the apical region of the cell resulting in ciliary microtubule configurations devoid of ciliary membranes (Figure 23). Fusion of the cilia (Figure 25) also occurred, but was not as prevalent as resorption. The fused cilia retained their individual 9+2 microtubule structures at this time. A few cilia had lost their central pair of tubules (Figure 24). Intact cilia were not excessively swollen and retained their characteristic 9+2 microtubule structure. In addition to these changes in cilia, some goblet cells were beginning to secrete mucus (Figures 26 and 27), observed as a spontaneous discharge from the cell. Granular endoplasmic reticulum was slightly dilated in the secreting goblet cells.

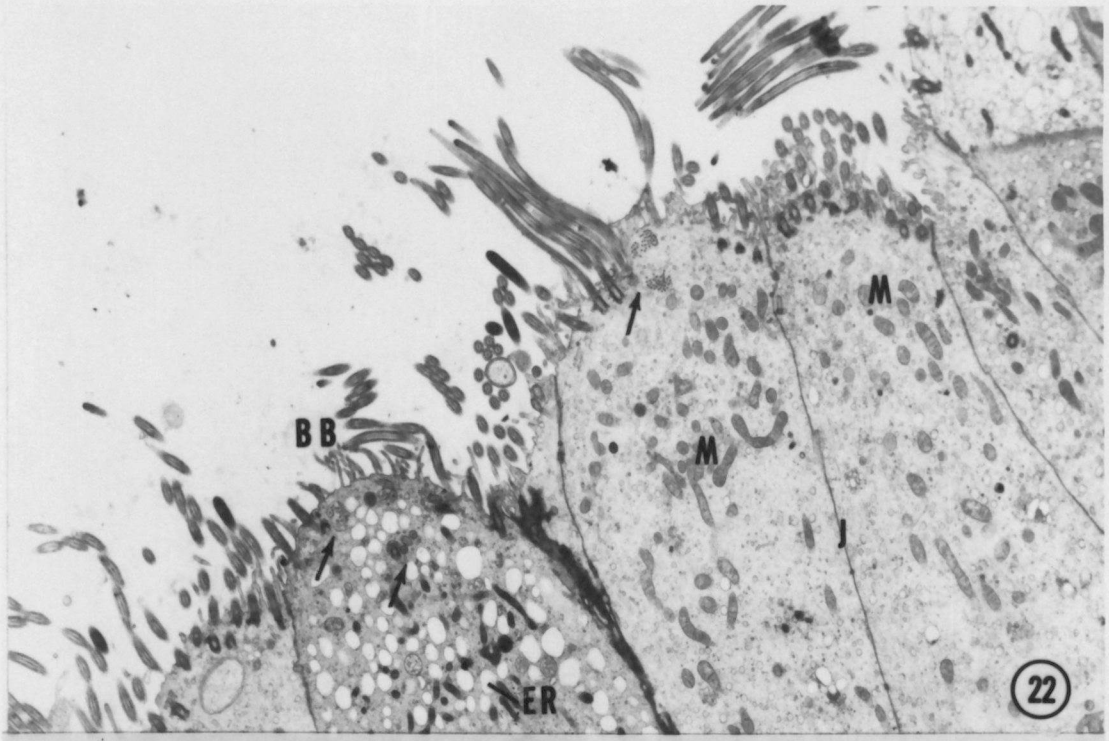
Plate VII. Ciliary alterations after 3 minutes exposure to cystic fibrosis heterozygote serum.

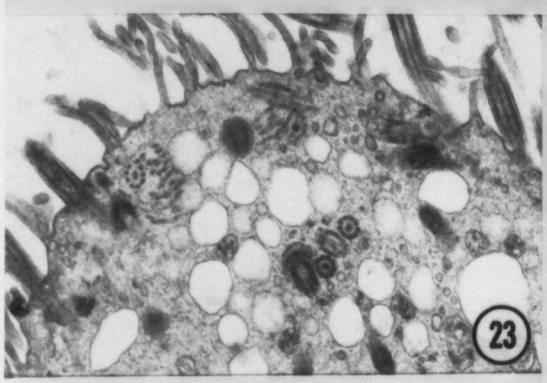
Figure 22. Many columnar cells have resorbed cilia into the cytoplasm (arrows). Most retain their 9+2 microtubule structure but an outer membrane is absent. Mitochondria (M) remain normal. Endoplasmic reticulum (ER) in some cells is dilated to some extent. Cell junctions (J) remain intact and the brush border (BB) is free of debris. 4800 X.

Figure 23. Higher magnification of a columnar cell's apical region showing resorbed cilia. 14,000 X.

Figure 24. A cilium (arrow) with no central pair of mictrotubules. 18,000 X.

Figure 25. A pair of fused cilia. Both retain their characteristic 9+2 microtubule arrangement but they share the same outer membrane and cytoplasm. 22,000 X.







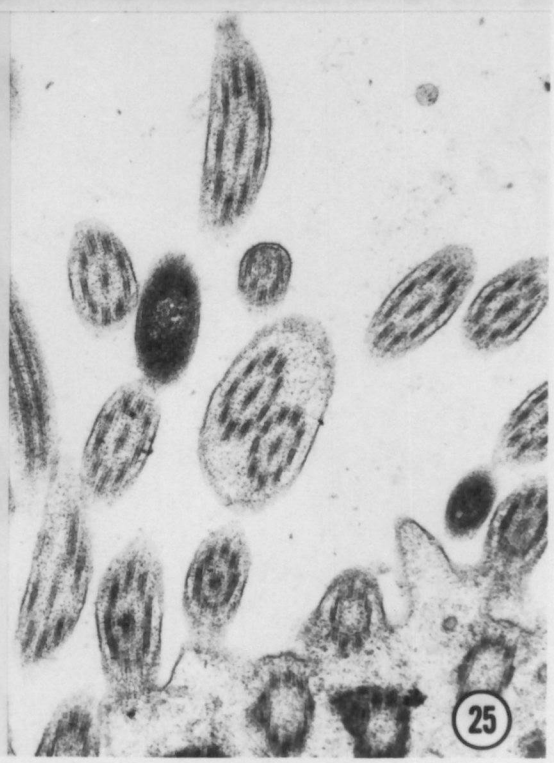


PLATE VII

Plate VIII. Goblet cell secretion.

Figure 26. Transmission electron micrograph of epithelium exposed for 3 minutes to heterozygote serum. Only a few goblet cells (GC) are secreting at this time. Granular endoplasmic reticulum (GER) is somewhat dilated in some cells. 5400 X.

Figure 27. Scanning electron micrograph of tracheal epithelium exposed for 15 minutes to heterozygote serum. Mucus is being secreted from a goblet cell and individual droplets (MD) can still be distinguished. 4500 X.



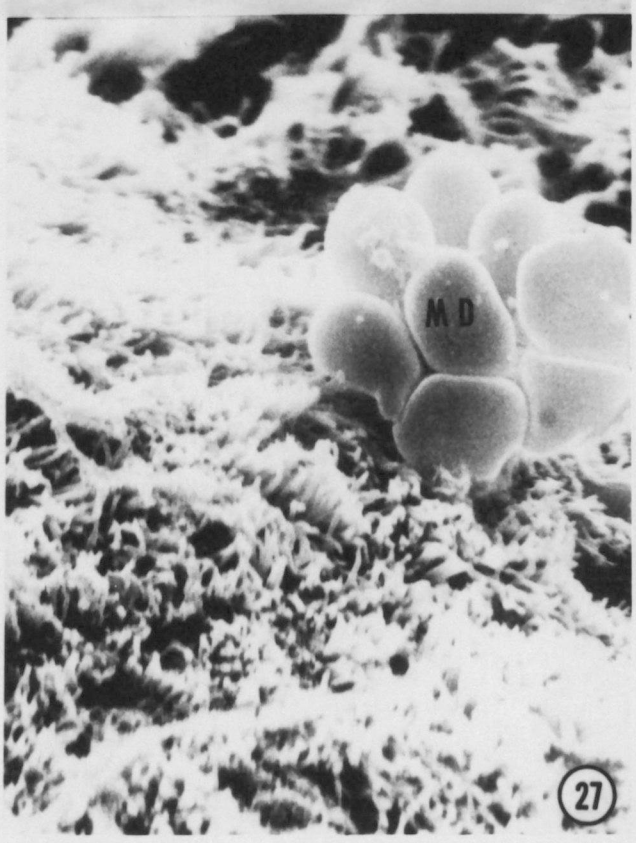


PLATE VIII

Mitochondria remained normal (Figures 22 and 26) and cell junctions remained intact, even around the secreting goblet cells.

These changes continued through the 5 and 10 minute samples with progressively greater frequency. A higher number of goblet cells were secreting mucus (Figure 28) and more ciliary resorption had occurred (Figures 28 and 29; Figure 30). At 10 minutes, many resorbed cilia had lost all of their 9+2 microtubule structure (Figure 31). Many other extended cilia were swollen also (Figure 32). Microvilli appeared to be unaffected except where resorption was occurring. Here, they seemed to be incorporated into the process (Figure 30), often containing ciliary remnants.

Another prominent feature was that of a single large vesicle in the apical end of the ciliated columnar cells just below the basal bodies (Figure 33). It usually contained 2 to 4 lamellar structures. The entire structure was bound by a unit membrane structure. These were not seen in the controls and only a few were seen in any of the normal serum samples. When they were present, they did not contain the lamellar structures.

Mitochondria showed signs of swelling and displayed some variation in shape. Some cells showed an increased dilation of the granular endoplasmic reticulum (Figure 28) and the Golgi apparatus. The basal cells remained relatively unaffected through the 10 minute exposure period.

At 15 minutes, the effects varied. Up until this point, the ciliary surface had remained fairly free of mucus accumulation around the cilia or any cellular debris. At this time, two changes were observed. The lesser effect was that the goblet cells continued

Plage IX. Transmission electron micrographs of tracheal epithelium exposed to cystic fibrosis heterozygote serum for 5 minutes.

Figure 28. Goblet cells (GC) can be seen secreting mucus on the epithelial surface. Note the breaking up of the cell membrane (arrows) to allow the droplets to be released. Extended cilia (C) and microvilli (MV) appear unaffected. A resorbed cilium (RC) can also be seen. Mitochondria (M) show slight polymorphism and a few show swelling. Golgi apparatus (G) and extensive granular endoplasmic reticulum (GER) is seen also. 6,000 X.

Figure 29. Higher magnification of a resorbed cilium similar to that seen in Figure 28. The lower half of the shaft still retains some structure. 16,000 X.



PLATE IX

Plate X. Transmission electron micrographs of epithelium exposed for 5 and 10 minutes to cystic fibrosis heterozygote serum.

Figure 30. Tissue exposed for 5 minutes to heterozygote serum. A microvillus (MV) has become the site or resorption of several cilia. 14,000 X.

Figure 31. Tissue exposed for 10 minutes to heterozygote serum. Many of the resorbed cilia have lost their 9+2 microtubule arrangement. Mitochondria (M) have few cristae. 15,000 X.

Figure 32. Tissue exposed for 10 minutes to heterozygote serum. A number of swollen cilia (SC) can be found extending into the lumen. Note the ballooning cell (BC) with swollen mitochondria (arrows) and dilated granular endoplasmic reticulum (GER). 5,500 X.

Figure 33. Tissue exposed for 5 minutes to heterozygote serum. A ciliated columnar cell with a large apical vesicle containing 1 predominant lamellar structure (arrow). A swollen microvillus (MV) is present. 9,700 X.

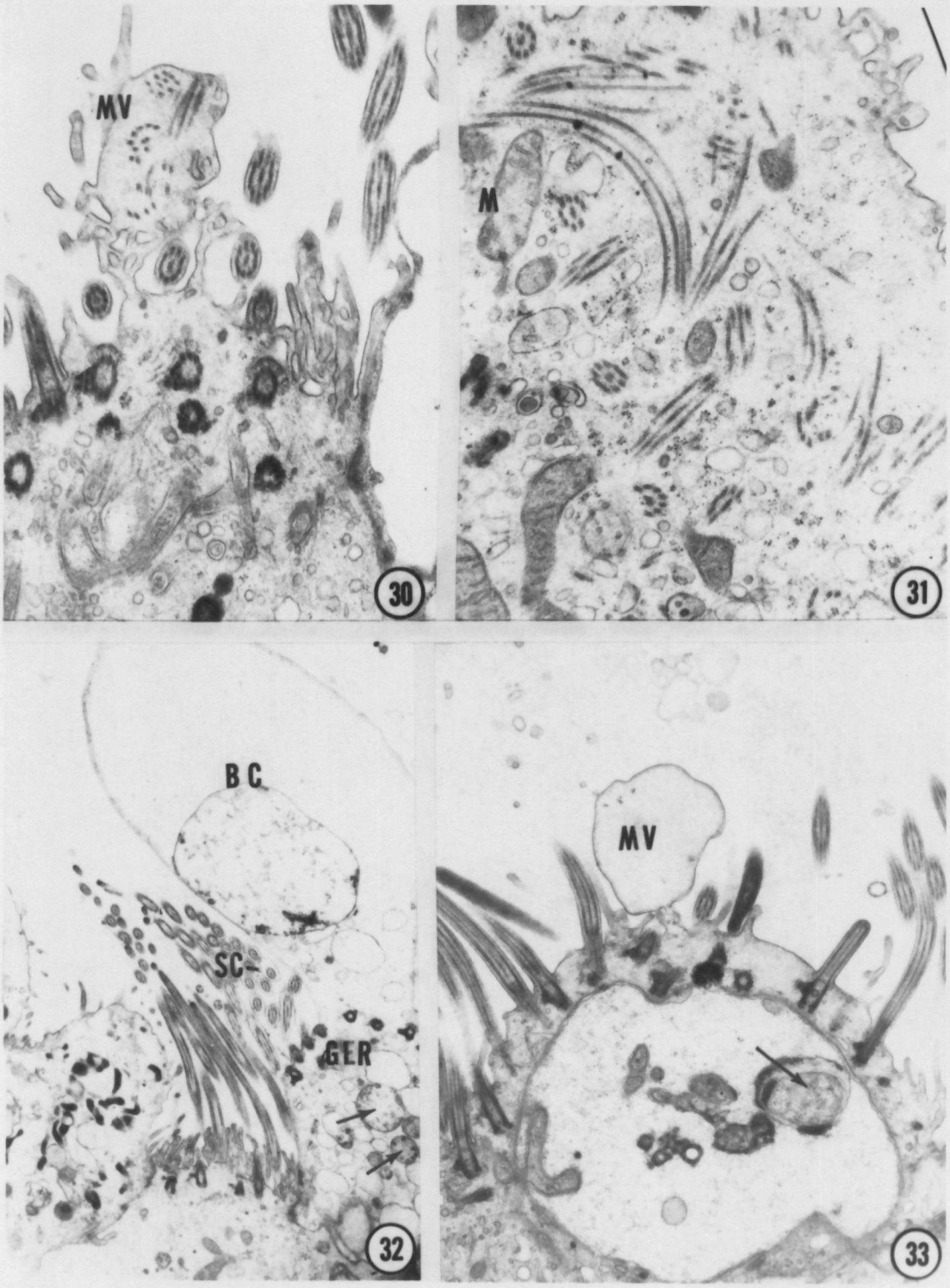


PLATE X

secretion of mucus, causing matting of the cilia to occur on the ciliary surface and in the crevices (Figure 34). Most mucus droplets were unfused at this time. Intact goblet cells were extremely full. In ciliated columnar cells, large numbers of contorted mitochondria also appeared, with the more highly effected ones in the cells' apical regions (Figure 34).

The more severe of the two effects was the beginning of cell loss from the epithelial layer. Entire cells were desquamated (Figure 35), the anterior portion often appearing in thin sectioned material (Figure 36). Swollen mitochondria and distended granular endoplasmic reticulum were found in all of these cells.

Many intact columnar cells appeared to be degenerating (Figure 37). These cells had very distended granular endoplasmic reticulum, more so than any sample yet examined. Mitochondria were contorted and frequently, cristae could not be seen.

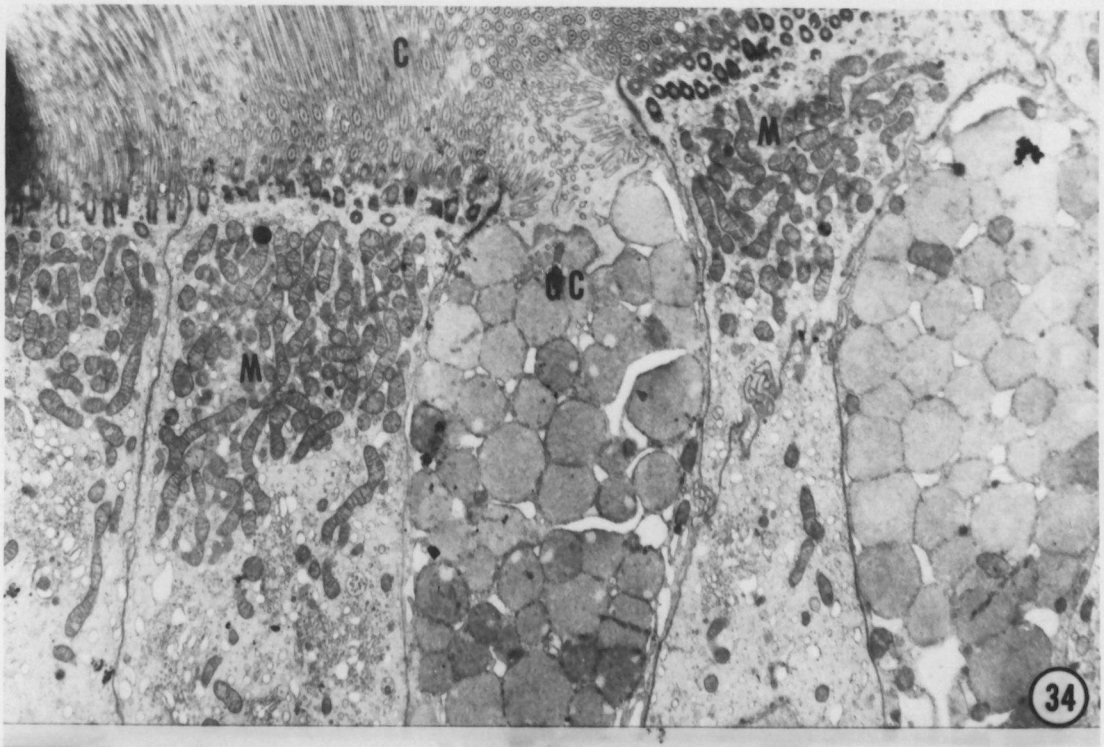
Fused, disrupted, and/or resorbed cilia were found in both types of damaged tissue (Figure 38). The less affected tissue contained areas that had scattered masses of large numbers of fused cilia (Figure 38). In these masses, ciliary microtubule structures were mixed, with most retaining their 9+2 structure. Several had begun breaking up and others assumed a 9+0 structure, losing only their central pair. In samples where the tissue was more severely altered, showing more cell loss, there was a more generalized effect on the cilia. The fusion was only of two or three cilia and was more widely scattered. In both samples, the incidence of ciliary damage was more than the previous time intervals studied.

Plate XI. Tracheal epithelium exposed for 15 minutes to cystic fibrosis heterozygote serum.

Figure 34. Transmission electron micrograph of slightly altered epithelium. It shows increased goblet cell (GC) secretion, causing the cilia (C) to clump together. Mitochondria (M) continue to vary in shape, particularly in the apical regions of the cells. 6,000 X.

Figure 35. Scanning electron micrograph of some ciliated columnar cells that have been sloughed from the epithelial layer. 4,000 X.

Figure 36. Transmission electron micrograph of more severely affected epithelium. It showed whole cells or cell fragments (CF) appearing on the cell surface. Goblet cells (GC) continue to secrete mucus, and contain large amounts of dilated endoplasmic reticulum (ER). The Golgi apparatus (G) appears slightly dilated. 2,300 X.





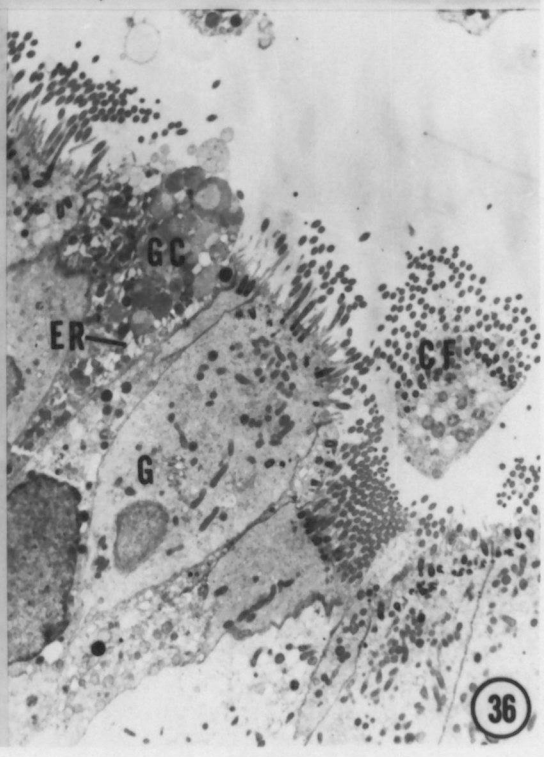


PLATE XI

Plate XII. Transmission electron micrograph of epithelium exposed for 15 minutes to cystic fibrosis heterozygote serum.

Figure 37. Swollen cilia (SC) are seen on many cells that also have highly dilated endoplasmic reticulum (ER). Mitochondria (M) are also seen. 10,400 X.

Figure 38. A large mass of fused cilia is seen. All have lost their outer membrane and many have started to lose their 9+2 microtubule structure. A cilium that has lost its central pair (arrow) has fused with the mass also. 11,200 X.

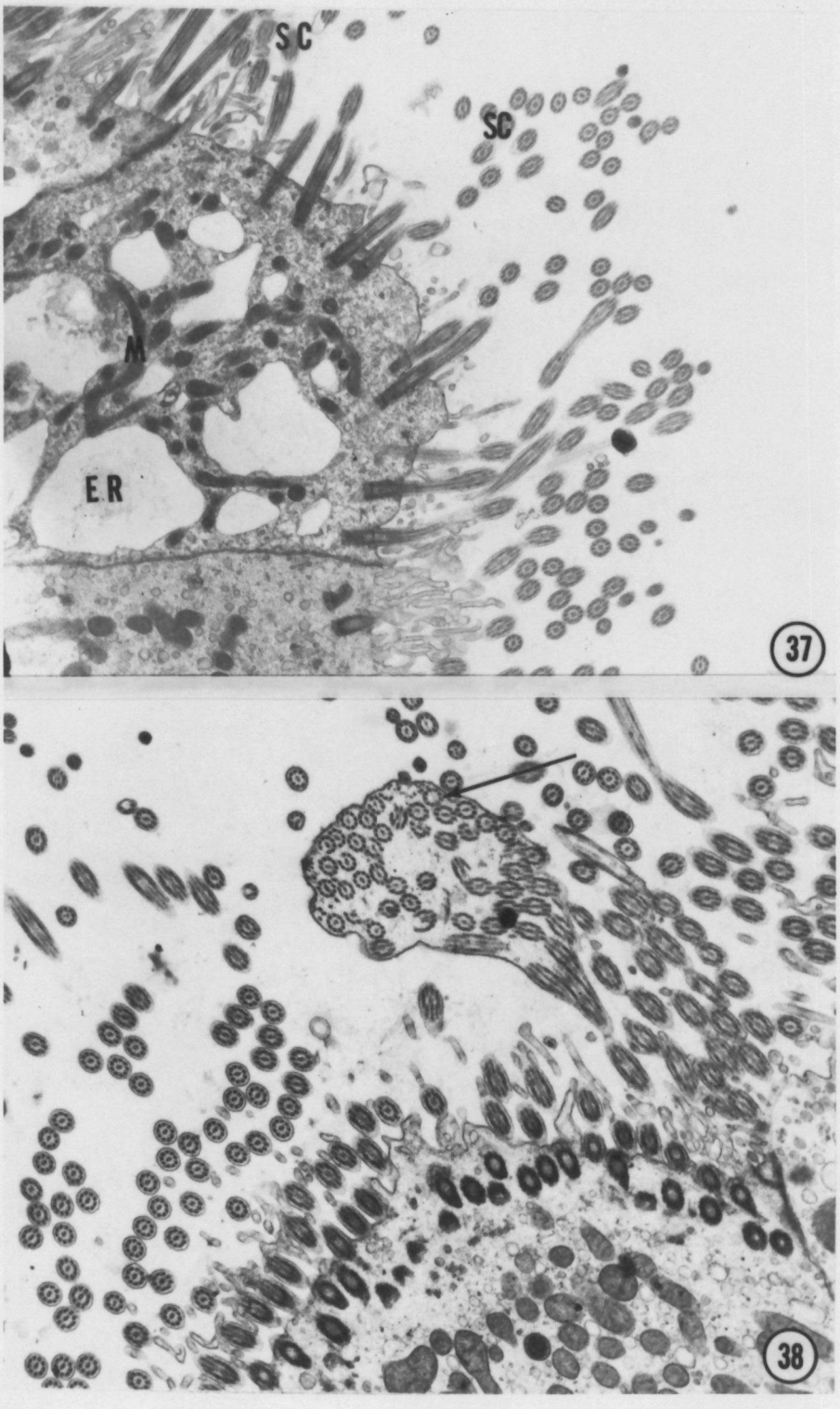


PLATE XII

Examination of samples exposed for 60 minutes to heterozygote serum revealed further mucus accumulation on the ciliary surface. Enough mucus had been secreted to trap cilia in bundles (Figure 21, page 23, and Figure 39). A large number of cilia were disrupted (Figure 40). This disruption took the form of alteration of the peripheral tubules. Many of the ciliated columnar cells had granular endoplasmic reticulum that was greatly distended throughout the cytoplasm of the cell. These cells appeared to be flared in the basal region and tapered in the apical region (Figure 41). Characteristically, they contained dark granules scattered throughout the cytoplasm, 2 to 4 small lamellar structures (Figure 42) in the basal regions with a few appearing apically, and dark opaque mitochondria with little internal structure visible. A large round nucleus located basally was also present (Figure 41).

Other columnar cells showed increasing numbers of vesicles, as previously described in cells after 5 minutes exposure (Figure 33, page 34). Resorbed cilia were not as common at this time perhaps due to increased cell loss or to internal degradation. Cilia continued to fuse, and appeared in larger fusion groups than in many earlier samples.

Homozygote-treated Samples

All of the changes associated with the 3 minute heterozygote sample were found in the 3 minute homozygote sample, but damage was much more extensive. Many columnar cells contained a highly dilated endoplasmic reticulum network (Figures 43-45). This network was much more extensive than the comparable heterozygote. The Golgi apparatus remained relatively unaffected at this time. Cilia on these dilated

Plate XIII. Transmission electron micrographs of tracheal epithelium exposed for 60 minutes to cystic fibrosis serum.

Figure 39. Accumulations of mucus (MU) cause cilia (C) to bunch together. Goblet cells (GC) can be still found secreting mucus. Many mitochondria (M) present are swollen to varying degrees. 1,900 X.

Figure 40. An area where many cilia are trapped in the mucus net. Many have an altered 9+2 microtubule structure, this resulting mostly from a disruption of the outer doublets. 10,000 X.

Figure 41. Ciliated columnar cells displaying altered shape and distended granular endoplasmic reticulum (GER). Mitochondria (M) are quite dark and no internal cristae are visible. Dark granules (DG) and scattered lamellar structures (arrows) are present. Nucleus (N) appears rounded instead of the characteristic oval shape. 2,700 X.

Figure 42. Two basally located lamellar structures. These do not contain the vesicle core found in the apical vesicles. 14,600 X.

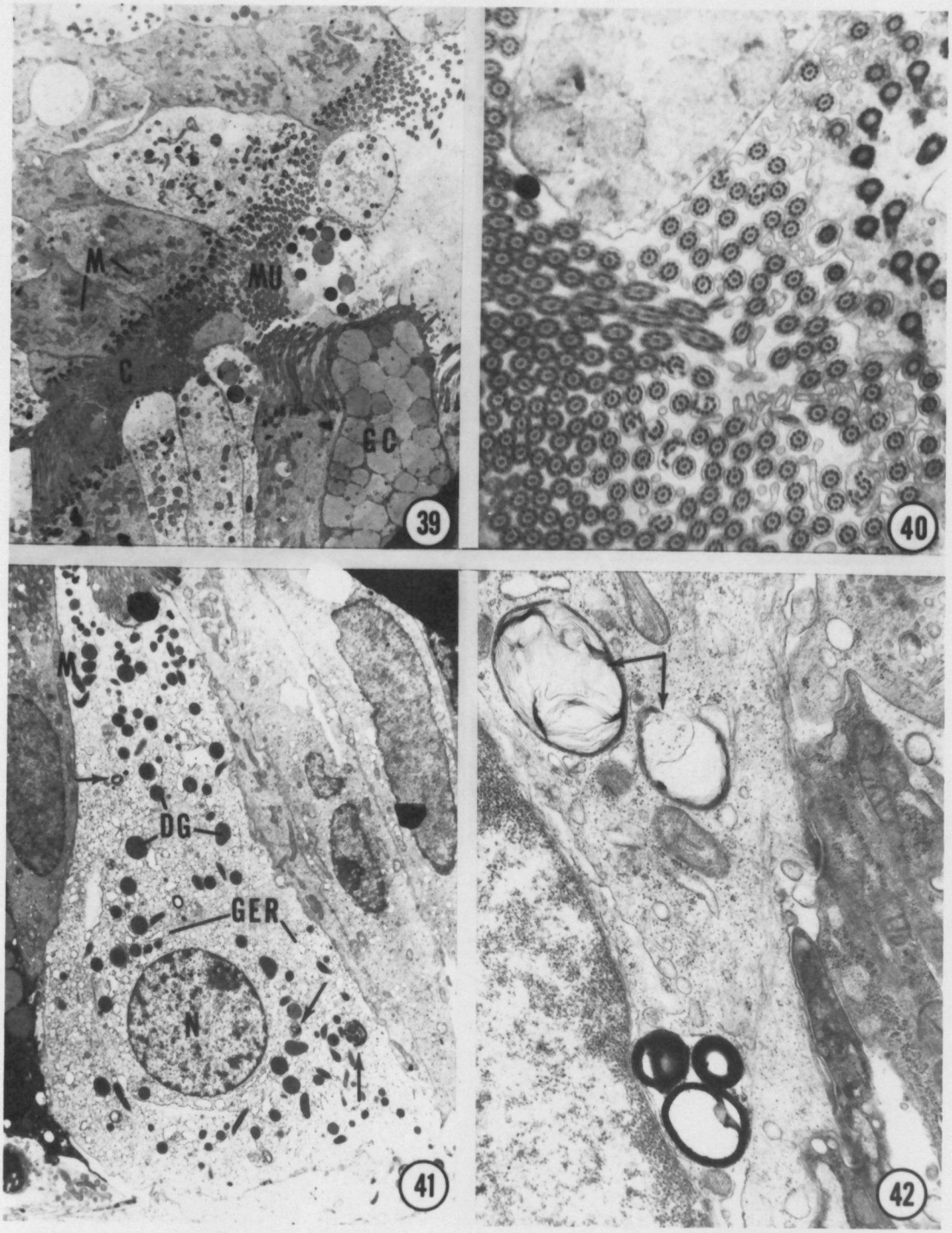


PLATE XIII

Plate XIV. Transmission electron micrographs showing distended granular endoplasmic reticulum after 3 minutes exposure to cystic fibrosis homozygote serum.

Figure 43. Columnar cells at this time characteristically exhibit highly dilated granular endoplasmic reticulum (GER) particularly near the nucleus (N) and apically in swelling columnar cells (CC). 2,300 X.

Figure 44. Some dilated columnar cells have greatly distended granular endoplasmic reticulum (GER). Mitochondria in these cells appear dark and contorted (DM). The cell at the left (GC) is a goblet cell with little mucus remaining. 5,100 X.

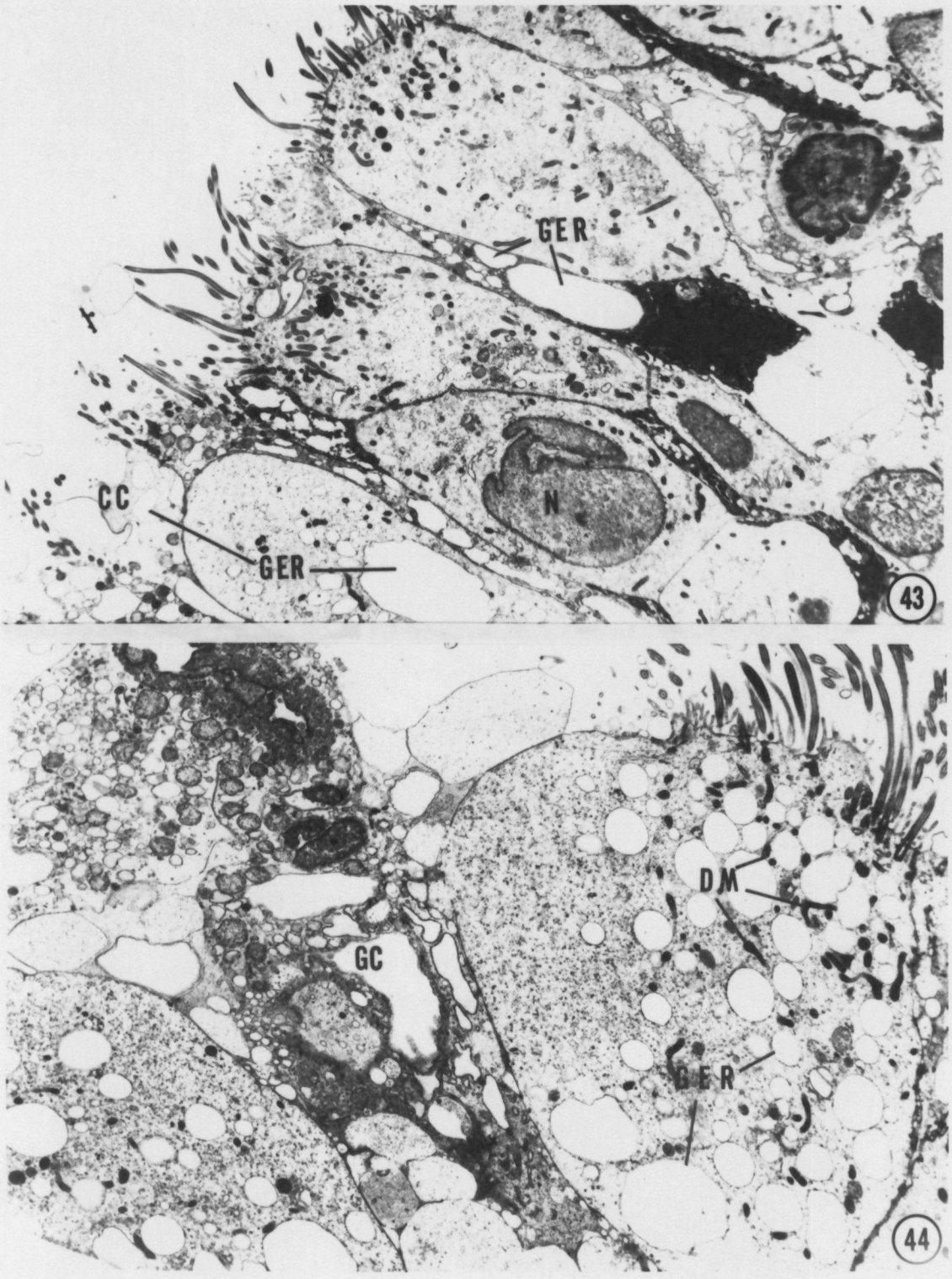


PLATE XIV

cells appeared swollen, but retained their 9+2 microtubule structure (Figure 45). Resorption and fusion were not as prevalent as in the 3 minute heterozygote samples. Mitochondria had begun to take on slightly contorted shapes, particularly in the dilated cells (Figure 45). Many of these were quite dense, displaying little internal structure.

Basal cells showed significant changes as opposed to the 3 minute heterozygote. While some seemed unaffected (Figure 46), others had swollen mitochondria, extreme distension of the perinuclear cisternae, and possessed a large vacoule containing dark flocculent material (Figure 47).

Goblet cells were secreting mucus at this time, although no surface accumulations were detectible. The droplets appeared to be diffusing into the lumen and not fusing on the ciliary surface.

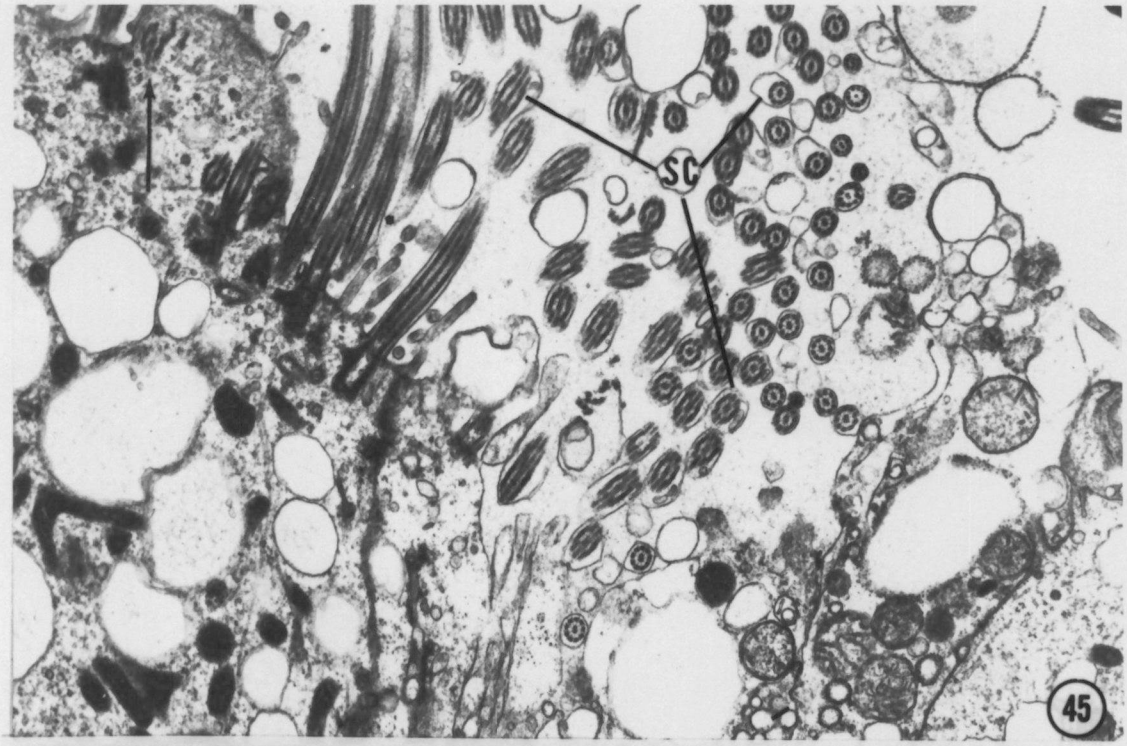
The 3 minute effects carried over through the 5 minute samples showing increased mucus and some cell loss (Figure 48). Much of the mucus seen had a different structure, appearing as a coarse, two-toned granule (Figure 48). (This was also seen infrequently in the later heterozygote samples.) Mitochondria continued to become swollen and polymorphic. Ten minutes exposure produced some additional changes. Accumulation of mucus and an increased number of cell fragments appeared on the ciliary surface (Figure 48). However, in some areas, adjacent cells appeared to have the ciliary surface totally free of mucus or cell debris. This observation, in conjunction with the 10 minute exposure from the mucus production sequence, suggested that areas such as this was where patching was beginning to occur.

Plate XV. Transmission electron micrographs of tracheal epithelium exposed for 3 minutes with cystic fibrosis homozygote serum.

Figure 45. Swollen cilia (SC) on scattered columnar cells can be seen. These cells exhibit all the characteristics described in Figures 44 and 45. 13,700 X.

Figure 46. Some areas reveal basal cells that appear unaffected by the exposure. A large round nucleus (N) occupies the majority of the cell's inner space. 2,600 X.

Figure 47. Other areas reveal basal cells that are severely affected by 3 minutes exposure. Perinuclear cisternae (PC) is extremely dilated. Mitochondria (M) are swollen and have no internal structure visible. A large vacoule (F) containing flocculent material is observed in these cells. 3,000 X.





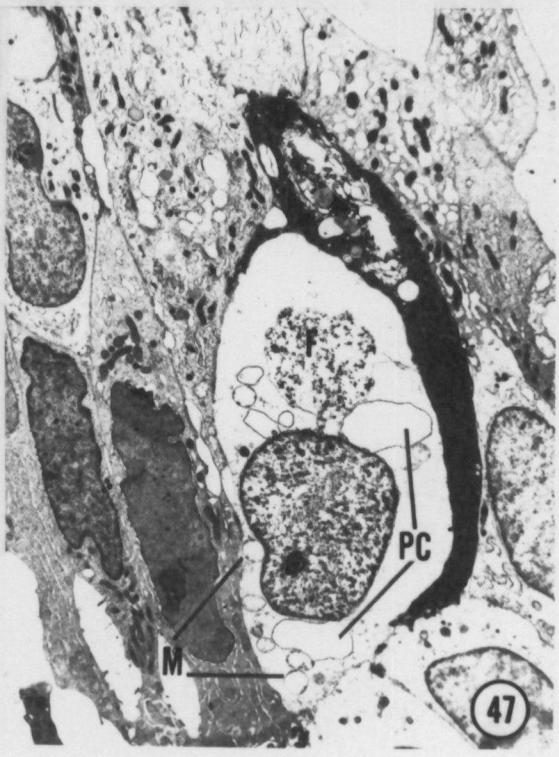


PLATE XV

Fifteen minutes exposure increased alterations to the epithelium similar to that observed in the 15 minute heterozygote sample. In the less damaged tissue, mucus had started to accumulate over most of the epithelial surface. Goblet cells usually displayed their apical ends ballooning over the ciliary surface (Figure 49) instead of lysing within the cell layer. Mitochondria were very swollen in these cells. The endoplasmic reticulum was highly distended in most cells. Other columnar cells showed extensive damage to the cilia, more than in any previous sample studied. Resorbed cilia were quite common (Figure 50). Many of these had already lost their 9+2 microtubule structure. The cells that resorbed cilia usually had few cilia left extending into the lumen (Figure 50). Large numbers of cilia on these, and other cells, had lost their central pair of tubules (Figure 50) had fused outer membranes but retained their individual 9+2 structures (Figure 51), had ciliary membranes lysed in the lumen (Figure 52), or had their 9+2 microtubule structure disorganized while extending into the lumen (Figure 53).

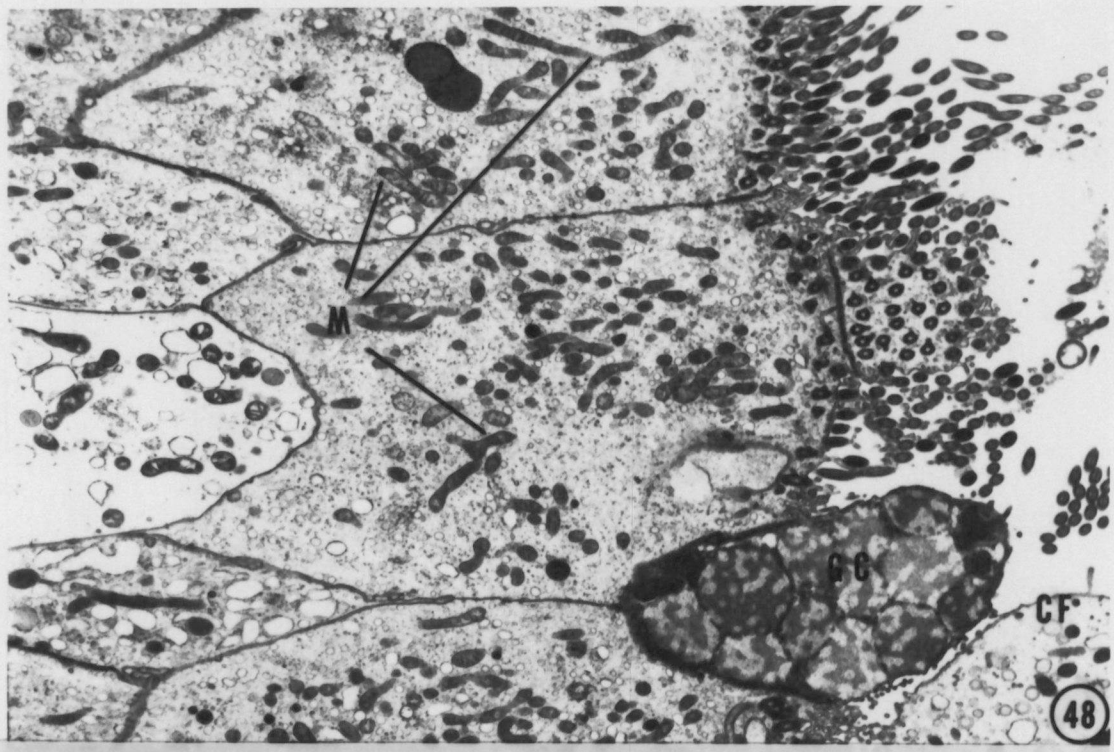
The more severely affected tissue was characterized by many lysed cells. Numerous cell fragments would be seen above the basal cell layer (Figure 54). The vast majority of the cells were lysed and spilling out their contents. Others that had not lysed contained highly distened endoplasmic reticulum or perinuclear cisternae. Mitochondria were darker than usual and tended to be swollen. In some cases, no columnar cells were attached to the basement membrane. In these cases, the basal cells appeared quite swollen as in Figure 47.

With 60 minutes exposure most cells were either lysed or severely damaged. A thick layer of mucus had been deposited on the top of the cell

Plate XVI. Tracheal epithelium exposed for 5 and 10 minutes to cystic fibrosis homozygote serum.

Figure 48. Tissue treated for 5 minutes. Mitochondria (M) continue to swell and vary in shape. Goblet cells (GC) are beginning to secrete mucus with an altered texture and appearance to it. Note the cell fragment (CF) in the lower right corner. 7,000 X.

Figure 49. The lesser affected tissue contains an increased number of cells that appear to be ballooning (BC) over the ciliary surface. A portion of a goblet cell (GC) is also seen. Arrows indicate debris accumulation on the cell surface. 3,900 X.



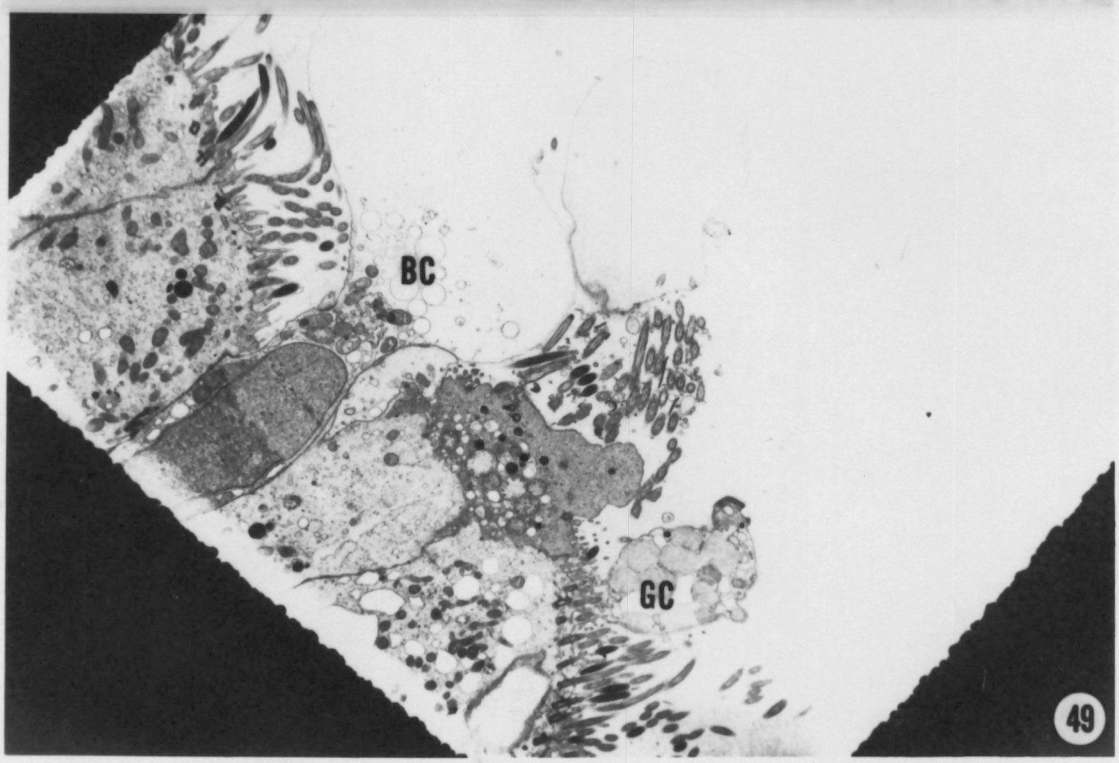


PLATE XVI

Plate XVII. Tracheal epithelium exposed for 15 minutes to cystic fibrosis homozygote serum.

Figure 50. An area of resorbed cilia. Many have lost their axonemal structure. Note the debris on the cell surface. 8,600 X.

Figure 51. Fusion of two cilia into one shaft. Both still retain their individual 9+2 arrangements. 16,000 X.

Figure 52. A cilium has lysed while extended into the lumen (arrow). Note the swollen mitochondria (M) and the highly distended granular endoplasmic reticulum (GER). 10,200 X.

Figure 53. Cilia extending into the lumen. Many areas display cilia that appear normal (long arrow) with swollen, disarranged cilia (short arrows) close by. 12,400 X.

Figure 54. Tissue exposed to serum for 15 minutes that has been totally destroyed. Most of the cells appear lysed (LC). Only a few basal cells (BC) still rest on the basal membrane (BM). 5,100 X.

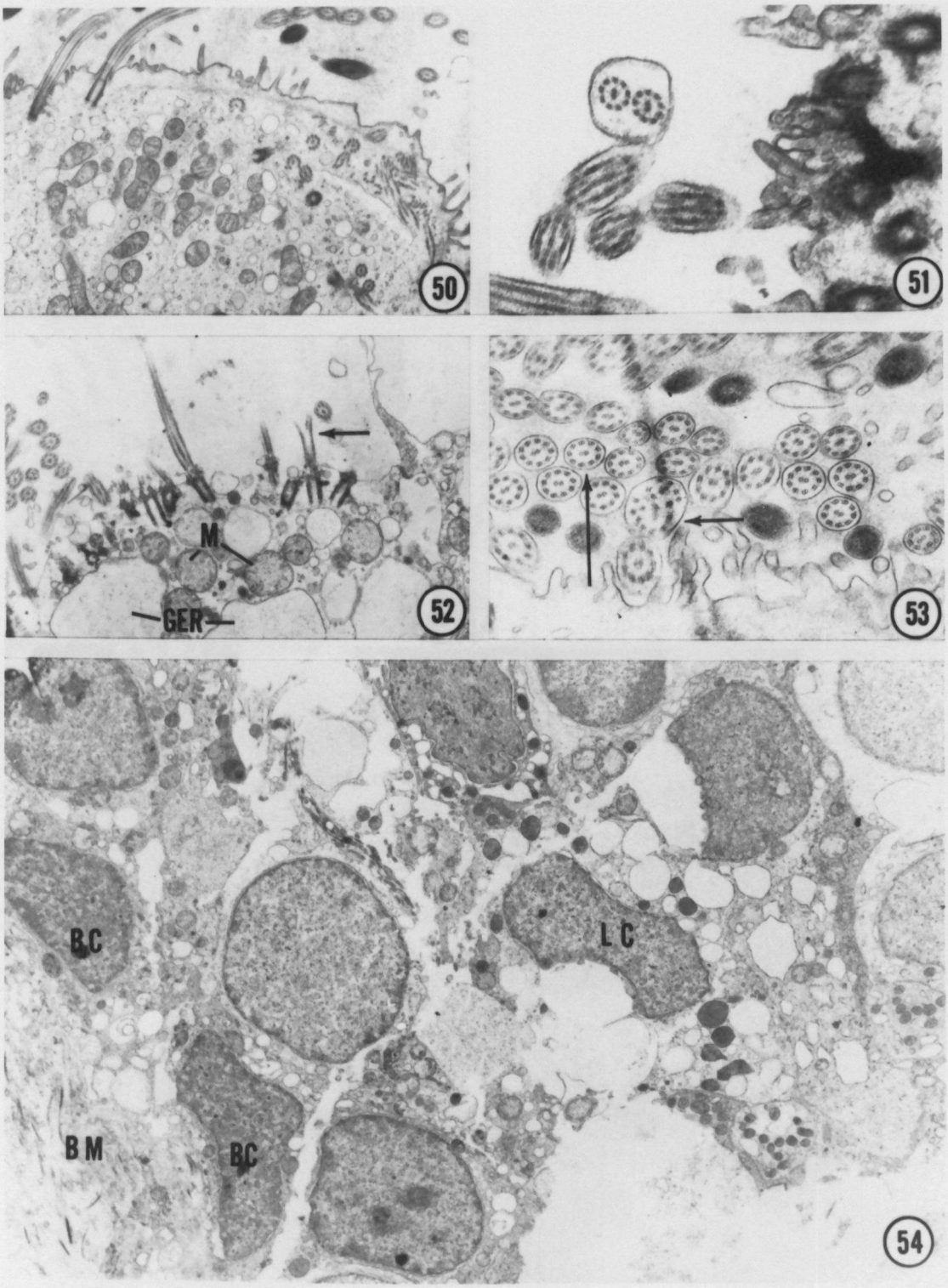


PLATE XVII

layer enclosing all of the cilia (Figure 18, page 23, and Figure 55). Large areas of cilia trapped in the mucus layer were in various stages of disarrangement (Figure 56). Mitochondria in these areas were extremely swollen (Figure 57) and in many, few cristae were visible. Only a few goblet cells could be found with any mucus still left in them.

In areas where the mucus layer was not as thick, bundles of cilia could be seen fusing (Figure 58). Microvilli were relatively unaffected except in these areas, where they became incorporated into the fused mass. Mucus still contained in goblet cells continued to show the coarse two-toned texture described in the 15 minute sample. Most of the cilia had swollen considerably, as much as 3 times their normal diameter (Figure 59). These swollen membranes in longitutional section appeared very irregular (Figure 60).

One of the constitutents of both mucus coated and uncoated cells was that of lamellar bodies developing in the apical ends of the cells (Figure 61). These were found predominately in cells with few or no cilia on them. These bodies were different from the large vacoules reported earlier in that they appeared as sets of concentric membranes with a central core of very small vesicles. These bodies were not bound by a double membrane as were the large vesicles with the lamellar structures in them (Figure 33, page 34).

Plate XVIII. Transmission electron micrographs of tracheal epithelium exposed for 60 minutes to cystic fibrosis homozygote serum.

Figure 55. A thick mucus net (MN) surrounds all the cilia extending into the lumen. Large areas of cilia in the net appear disarranged (insert). Mitochondria (M) are usually swollen at this time. 6,000 X.

Figure 56. Higher magnification of trapped cilia in Figure 55 insert. All still retain their outer membranes but the internal structure is disrupted to varying degrees. 19,200 X. Insert: a cross-section of a disarranged cilium. The doublets still appear in pairs and only the organization is altered. 26,300 X.

Figure 57. High magnification of mitochondria after 60 minutes exposure. Most are swollen and contorted (M). Microvilli (MV) are unaffected where resorption is not occuring, despite thick mucus coat. 14,000 X.

Figure 58. An area where mucus droplets (MD) have not yet fused. Cilia (C) can be seen fused into bundles. Goblet cells (GC) are still full but have a two-toned texture to them. Mitochondria (M) vary from cell to cell. 1,900 X.

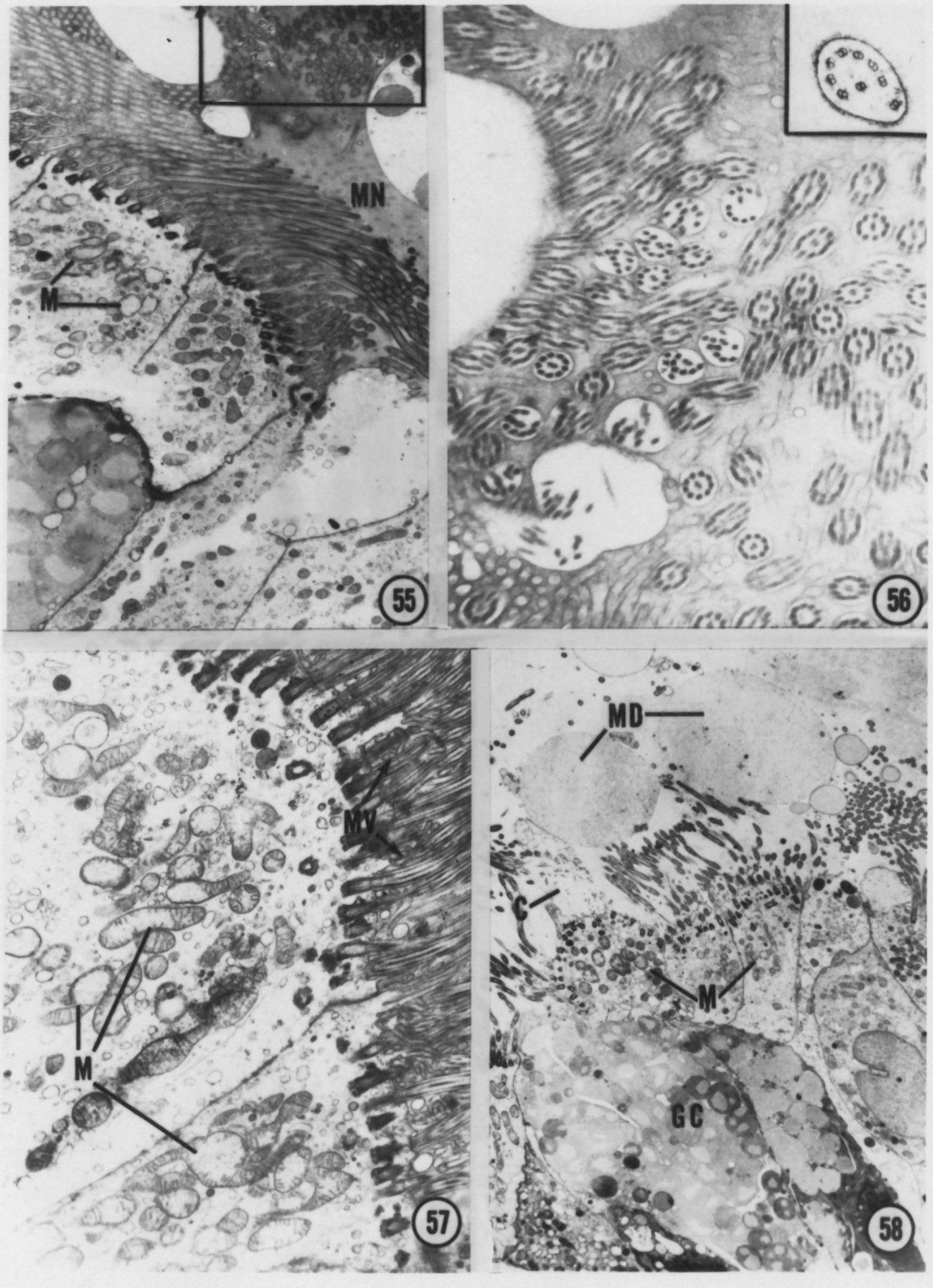


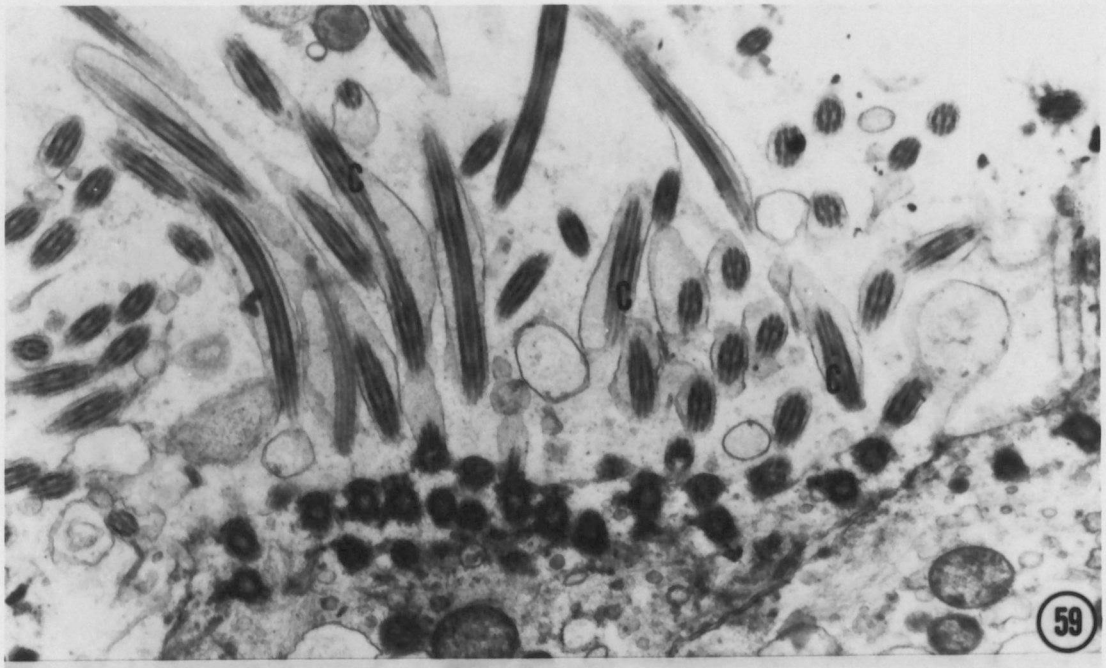
PLATE XVIII

Plate XIX. Ultrastructural changes associated with 60 minutes exposure to cystic fibrosis homozygote serum.

Figure 59. Cilia extending into the lumen. In areas not covered with the thick mucus coat, cilia appear extensively swollen (C). 12,200 X.

Figure 60. A longnititudinal (LS) and cross-section (XS) of swollen cilia are seen. Note the irregular contour of the outer membrane. 9,200 X.

Figure 61. Lamellar bodies (LB) develop in many cells that are devoid of or had few cilia. These bodies consist of a set of concentric lamellae surrounding a core of small vesicles (arrows). 7,600 X.





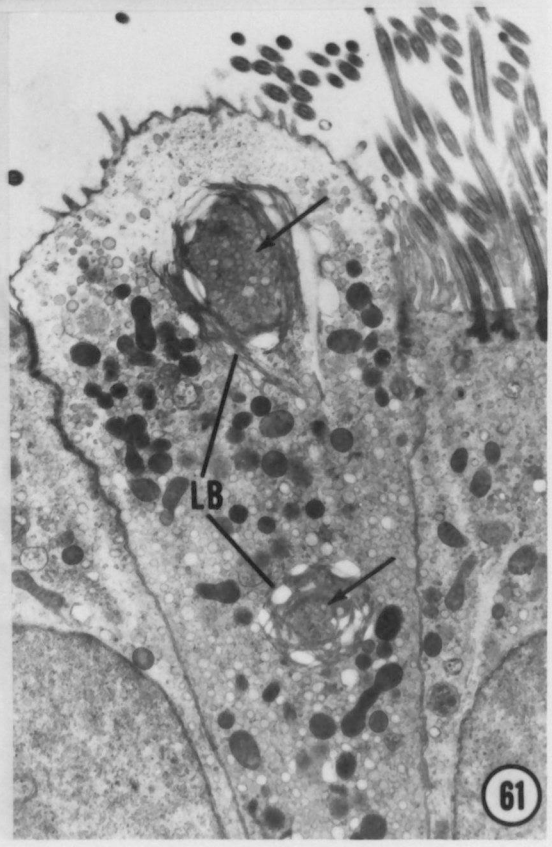


PLATE XIX

CHAPTER IV

DISCUSSION

The effects of normal serum and cystic fibrosis serum on mucus production and the epithelial ultrastructure of fresh rabbit trachea was examined by scanning and transmission electron microscopy. Nomral sera, including the standard serum, produced no ultrastructural alterations to the epithelium through 60 minutes exposure. Cytoplasmic organelles appeared comparable to those observed in control tissue. Mucus secretion was minimal through 120 minutes exposure. Epithelial crevices remained clear of any material and only a few loose cells and thin mucus strands appeared on the surface. Horse serum was also utilized as an added control. This material produced no alterations through 60 minutes exposure and the tissue appeared comparable to control tissue when examined with both scanning and transmission electron microscopy.

Exposure of tracheal epithelium to cystic fibrosis serum produced a variety of alterations to the ciliary surface and to the ultrastructure of the epithelial cells. Mucus secretion and its accumulation on the ciliary surface began as early as 3 minutes after exposure to homozygote serum; the mucus appeared as droplets in the crevices and fused to form a net-like structure. Further secretion over the ciliary surface produced areas that appeared as patches and strands of mucus. By 60 minutes exposure, the patches and mucus strands had fused to form a mucus net that covered the ciliary surface. Desquamation of columnar cells occurred where the mucus net was not thick or had not covered the surface of the columnar cells. Both the homozygote and heterozygote sera produced the same sequence of mucus accumulation. However, the

exposure times required to attain a particular effect were different. Also, some heterozygote samples produced damage equal to that of some homozygote samples; however when a given homozygote/heterozygote serum pair (from the same family) was tested, the homozygote serum always produced more extensive cell loss and/or a thicker mucus net than did the heterozygote serum sample.

Mucus accumulation has long been associated with cystic fibrosis. Bowman et al. (1969) cited debris accumulation, presumedly mucus, in conjunction with ciliastasis in oyster gills. Mucus accumulation was extensively studied in oyster gills by Baur et al. (1976) and in rabbit tracheal explants by Czegledy-Nagy and Sturgess (1976). Both found stimulation and accumulation of mucus to be a prime factor influencing ciliary beat. The present study supports these observations. The latter two investigators and this study show early accumulation of mucus developing into a net over the ciliary surface. Baur et al. (1976) suggest no mechanism for net formation and state that homozygote and heterozygote sera are indistinguishable. This study shows that both sera do follow the same pattern of net formation, and furthermore, that a distinction can be made between the two on the basis of damage present after a given time of treatment. Czegledy-Nagy and Sturgess (1976), using 6 to 10 day old tracheal explants, suggest that the real action of cystic fibrosis factor is on the goblet cells, and that ciliary dyskinesia is secondary. If this is the case, then expants should show only minimal mucus production since rabbit tracheal epithelium left in tissue culture for 24 hours looses a significant amount of its goblet cell mucus (Kennedy and Allen, 1978). This study

indicates that with fresh tracheal rings, a much thicker and more extensive mucus layer is formed than with tracheal explants. The use of fresh tissue presumably more closely approximates the condition found in vivo, giving greater clinical validity to the results.

Another finding of this study was the alterations in the mucus composition within goblet cells. Baur et al. (1976) while observing no ciliary alterations in oyster gill cilia, did describe mucus droplet alterations. In Baur's study, examinations of goblet cells showed that they become enlarged and swollen with typically electron-dense mucus granules which changed to a swollen light colored granule with a fibrous internal composition. Differences in Baur's observation and those presented here could be due to differences in mucus composition between rabbits and oysters. It is interesting that changes in mucus granule structure were noted in both cases where it was critically examined.

This study shows several ultrastructural alterations to cells, the most dramatic being displayed by the cilia. Fused, resorbed, lysed, and disarranged cilia were all seen during various exposure periods to cystic fibrosis sera. Resorption and fusion were the most common, occurring as early as 3 minutes and 5 minutes after exposure. Resorption occurred in the apical region of ciliated columnar cells and cilia could be seen in various stages of apparent degradation. Cilia were observed with and without the outer membrane and were also seen with and without an intact 9+2 microtubule arrangement. Disorganization of the 9+2 microtubule structure was also apparent in many extended cilia. After short treatment periods, alterations of peripherial tubule pairs occurred frequently, but several cilia displayed loss of the central pair while

the outer doublets remained intact. Fusion of cilia was common and most retained their individual 9+2 microtubule arrangements. Lysed cilia were seen predominantly in tissue that had been exposed to serum for 60 minutes.

Bardele (1972) reported that flagellate to heliozoan transformation in the protozoa Clathrulina elegans occurs in about 3 minutes. Therefore, 3 minutes is not an unrealistic time for ciliary resorption to occur. Adshead et al. (1975), examining freshwater mussel gill cilia, reported that cystic fibrosis serum produced two effects on the cilia. Shock-stopped cilia quit beating immediately, retained their normal diameters, but lost all signs of any internal microtubule structure. Slow-stopped cilia appeared shortened and retained their axonemenal structure. Differences for the two are not discussed but the protein content of the cystic fibrosis serum is suggested to be a factor in the microtubule disappearance. In the present study, however, the 9+2 microtubule structure was altered significantly but tubules were always visible within the ciliary membrane. Differences in microtubule composition between the outer and inner pairs have been suggested to account for variability of microtubule stability (Adshead, 1975). Behnke and Forer (1967), using crane-fly spermatids, rat sperm, and rat trachea cilia, showed a variety of results that suggested that there might be several classes of microtubules. On the basis of varying treatments of pepsin, temperature, and colchine they concluded that there are 4 classes of microtubules: cytoplasmic microtubules, accessory tubules and the central tubules of the 9+2 complex, B-tubules of the 9+2 complex,

and A-tubules of the 9+2 complex. Kennedy and Zimmerman (1970), using Tetrahymena, showed that increasing pressure changes caused degradation of the central ciliary tubules with little or no effect on the peripheral tubules. Similar results were shown using Paramecium exposed to chloral hydrate (Kennedy and Brittingham, 1968). Shelanski and Taylor (1967) treated sea urchin sperm with colchicine and observed that after treatment the central pair was more sensitive to degradation during dialysis than the peripheral doublets. However, Behnke and Forer (1967) showed that the crane-fly spermatids responded differently after colchine treatment, showing no damage after exposure.

Thus it appears that the central ciliary microtubules are less stable and therefore are more likely to disappear than the peripheral tubule doublets. The peripheral tubules, on the other hand, while separating from the adjacent pairs tend to retain their structure longer, first losing their organization.

Studies with calcium ionophore A23187 have produced evidence which suggests that a membrane permeability dysfunction could cause the ultrastructural effects presented in this study. Ionophore A23187 is an antibiotic that forms complexes with cations, has a high calcium specificity, and transports calcium ions across membranes (Bogart et al., 1978). Satir (1975) noted that ionophore - mediated calcium entry into mussel gil cilia caused ciliary arrest, and Weisenburg (1972) noted that the stability and function of microtubules could be impaired by changes in cytoplasmic calcium levels. Bogart et al. (1978) treated tracheal explants with cystic fibrosis serum, ionophore, and normal serum. Normal serum produced no increase in the size or number of secondary lysosomes, while the cystic fibrosis serum and the ionophore induced a

swelling or an increase in the size of secondary lysosomes. The lysosomes appeared as vacoules containing dense bodies and multivesicular structures; the same appearance as the apical vesicles that appeared in this study. Bogart's lysosomes appeared after only 6 minutes and seemed to fuse into larger vacuoles with only 30 minutes exposure. He also reported that in the larger vacuoles, cytoplasmic organelles could be seen. Bogart et al. (1978) reported no ciliary alterations, but this study suggests that the large vacoules form in response to the microtubule breakdown. Such breakdown may be induced by calcium with resorption of damaged cilia stimulating the lysosomal activity. Kirchner et al. (1975) observed that low temperature or calcium caused dissociation of the interactions between parallel ciliary protofilaments, which could account for the above mentioned ciliary damage. Swollen mitochondria that have been affected could also be some of the cytoplasmic structures found in the vacuoles. It has also been suggested that calcium plays a role in the precipitation of glycoproteins, particulary mucus (di Sant Agnese and Davis, 1976). It has been demonstrated by Gibson et al. (1971) that the levels of calcium in certain secretions increases with progression of the disease. This increase could account for some of the varibility of the samples tested in this and other studies.

The lamellar bodies located in the basal regions of the cells could possibly also be explained by membrane dysfunction. Gallagher et al. (1974), studying sodium-potassium active transport, demonstrated that cells in culture medium with 1mM concentration ouabain show extensive cytoplasmic vesiculation, which suggested and accumulation of

secretory material within the cells. The vesicles that Gallagher et al. (1971) described were identical to the lamellar bodies seen in this study.

As a final point, this study clearly shows that contrary to the observations of Cherry et al. (1971), normal sera causes little if any damage to the epithelial layer. Some mucus and a few loose cells were seen, but only in one case, was extensive damage evident. This abnormal sample produced some mucus accumulation, swollen cilia and some cell loss, equivalent to damage seen in 15 minute exposed heterozygote serum tissue. A point to consider is that the occurance of the heterozygote in the population is 1 in 25, therefore the chances of obtaining one of these individuals as a test subject is not entirely out of the question.

Thus, this study results in three major conclusions: first, normal serum does not cause any damage to the epithelial surface. Mucus accumulation is minimal and the cells show no ultrastructural damage. Second, cystic fibrosis sera do cause damage to the epithelium, in part by stimulating mucus secretion to the point of covering the entire ciliary surface. During this process, a variety of ciliary alterations are observed. Third, the data suggest that this type of study could be modified to become a bioassay for heterozygote screening. Since the ultrastructural alterations are confined only to the cystic fibrosis serum-treated samples, this type of screen, along with current biochemical test, such as the sweat test, is certainly feasible.

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