

CILIOCYTOPHTHORIA (CCP) IN ASTHMATIC CHILDREN, WITH REFERENCES TO VIRAL RESPIRATORY INFECTION AND EXACERBATION OF ASTHMA

Preliminary Report

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ALTHOUGH respiratory infections probably play an important role in some episodes of asthma,^{1, 2} it is difficult to differentiate clinically mild respiratory infections from purely allergic symptoms in asthmatic patients.³⁻⁶ Bacteriologic studies offer only partial information, and virologic investigations which might help in this regard have not been extensively done in relation to asthma.^{7, 8}

In 1950 and 1953, Bryan, applying Papanicolaou's technique, reported the presence of certain types of degenerative ciliated columnar cells in the nasal secretion of the patients during the acute stage of the common cold.^{9, 10, 11} The changes described were formation of acidophilic cytoplasm and inclusion bodies (usually cytoplasmic and acidophilic), nuclear degeneration (pyknosis, karyorrhexis, and total loss of the chromatin material), pinching off of the ciliated portion of the cell, and advanced disintegration. (Figs. 2 to 8.) In 1956, Papanicolaou observed in sputa from patients suffering from acute and chronic inflammatory conditions of the lung degenerative cells similar to those reported by Bryan. He designated the phenomenon ciliocytophthoria (abbreviated CCP). He suggested the possible significance of CCP in the diagnosis of respiratory infections and its correlation with the presence or later development of lung cancer.¹² Pierce and Hirsch, and others, reported a high correlation of CCP in the sputa or nasal secretions from patients with influenza A^{13, 15, 16} and adenovirus¹⁴ infections. Ciliocytophthoria was also reported in some fatal cases of measles and varicella pneumonia.¹³ Bryan, Pierce, and Hirsch felt that the presence of CCP indicates a viral respiratory infection and that the identification of CCP in the sputum or nasal secretion might be a simple method for diagnosis of viral respiratory infections.^{10, 13} Three of the patients studied by Pierce and Hirsch were asthmatic, and exacerbation of asthma in them was associated with positive CCP.¹³ This preliminary communication reports our experience with CCP in forty-five asthmatic children at Children's Asthma Research Institute and Hospital (CARIH).

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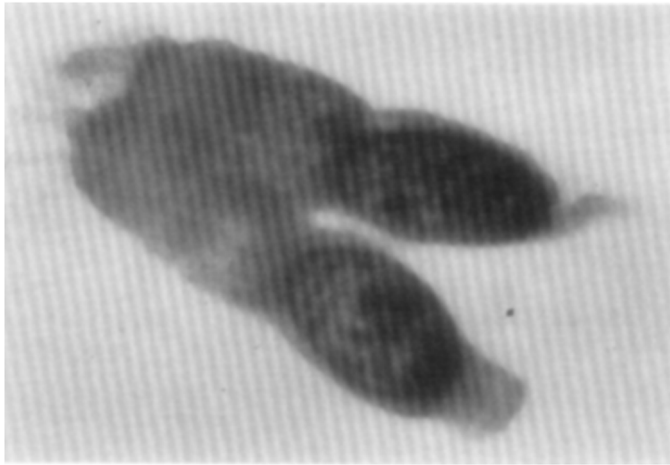


Fig. 1.—Normal ciliated columnar epithelial cells.

METHODS

The patients chosen ranged in age from 7 to 16 years. One hundred sixty-two samples of sputa and ninety-two nasal smears taken by cotton swab from forty-five children were studied for CCP from December, 1961, through March, 1963 (Table I). Some children were studied on more than one occasion. Sputa were usually collected in the morning, and expectoration was assisted by intermittent positive pressure breathing and postural drainage in some of the symptom-free patients. The samples were stored in a refrigerator for not more than a few hours when smears were not made immediately following expectoration. In some cases, sputa were frozen and stored for longer periods, but there was little or no change of the ciliated cell structure. The smears were immediately

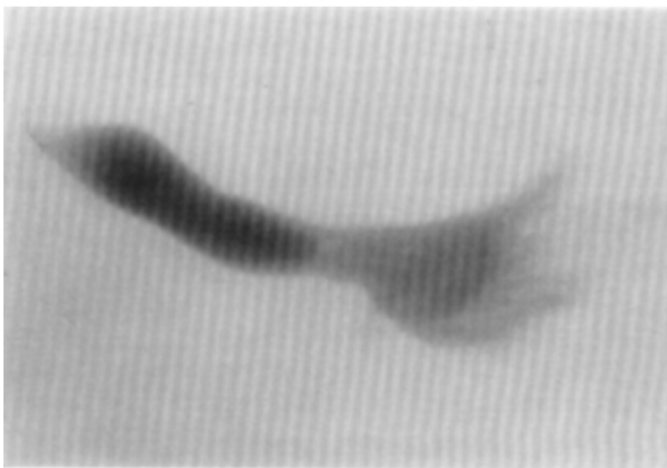


Fig. 2.—Early degeneration with pyknotic nucleus and acidophilic cytoplasm.

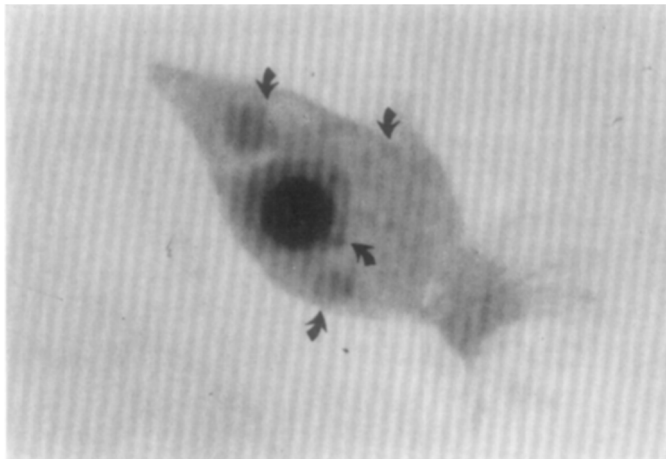


Fig. 3.—Advanced degeneration: Note partially detached cilia. Various sized acidophilic inclusion bodies (arrows), pyknotic nucleus. Possibly two nuclear inclusions.

fixed in a solution with equal parts of ether and alcohol for 15 to 30 minutes or more and later stained according to Papanicolaou's technique.

When a specimen was taken from a patient during the exacerbation of asthma or clinically evident acute respiratory infections, the follow-up specimens were usually taken 2 or more weeks after the onset of the acute episode as the control samples. All the slides were screened and described by one of us (I.K.) without knowledge of the clinical status at the time of study. A sputum specimen was considered satisfactory only when its origin was assured by the presence of ciliated columnal cells and alveolar dust cells.^{13, 18} Since enumeration of the total numbers of CCP cells in the slides is extremely time consuming and impractical, frequency of the cells was estimated and described as many, mod-

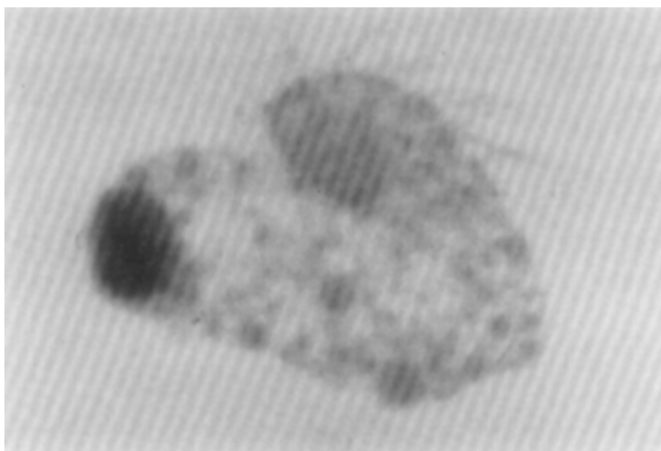


Fig. 4.—Markedly swollen cytoplasm presenting scattered inclusion bodies.

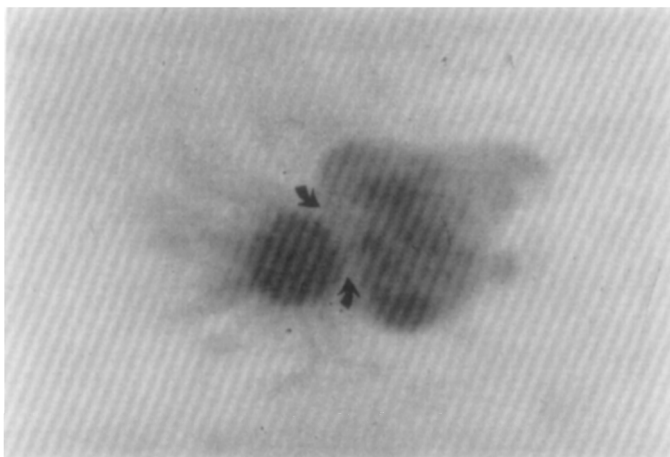


Fig. 5.—Ciliated portion is ready to pinch off (arrows). Anucleated cytoplasm with inclusions.

erate, or few. The cytologic findings and clinical data were transferred to McBee Keysort punch cards, and efforts were made to find correlations between them.

RESULTS

A. The Criteria for Positive CCP.—Shortly after the beginning of this study, it became apparent that the columnar cells exhibiting CCP may be present in relatively small quantities in the sputa and nasal secretions from asymptomatic children as well as from children with exacerbation of asthma or clinically apparent respiratory infections. Although the number of such cells appeared to increase with infections or exacerbations of asthma and diminish with



Fig. 6.—Nuclear inclusion body (arrow).

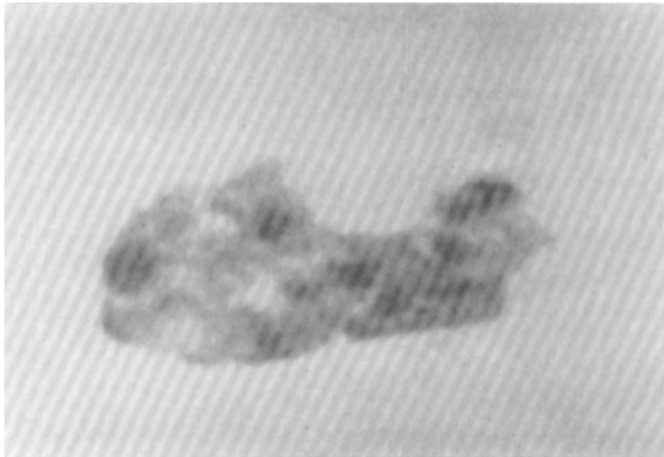


Fig. 7.—Far advanced degeneration: Cilia and nucleus are not seen. Note inclusions.

subsidence of the symptoms in general, the pinching off of the ciliated tuft seemed to be an exception, since this was present in large numbers in some quiescent patients as well as in the symptomatic patients. Therefore, a slide was classified as CCP "positive" only when large numbers of CCP cells (Figs. 2 to 8) were present exclusive of the pinched-off ciliated tufts. The possible explanation for this exception will be discussed later. In nasal smears, the amount of the secretion obtained and smeared on slides was usually small, and normal ciliated columnar cells were found relatively frequently. These differences were counted in the description of the nasal CCP.

At the end of this study, slides of twenty-one sputa and seven nasal secretions were reexamined to see if the classification of positive or negative CCP

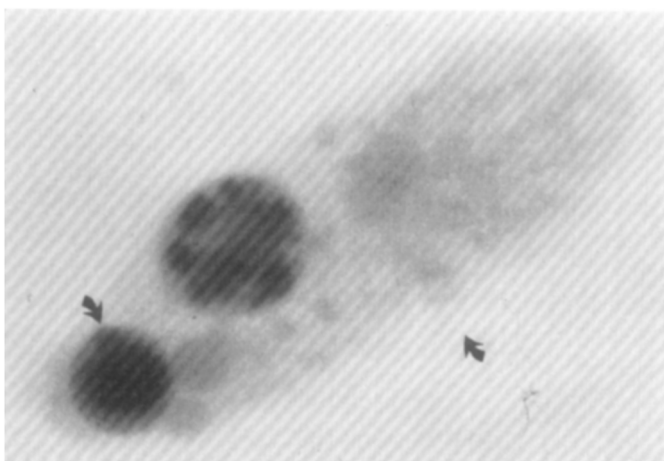


Fig. 8.—Double nuclei: One showing karyorrhexis and the other pyknosis with an inclusion (arrow). Scattered cytoplasmic inclusions are seen. Note degenerated cilia (arrow).

TABLE I. MATERIAL OF THE CCP STUDY

CLASSIFICATION OF PATIENTS	NO. PATIENTS	NO. SPUTA	NO. NASAL SWABS
Group I	8	25	22
Group II A	6	17	11
Group II B	12	58	34
Group III	19	62	25
Total	45	162	92

Note: group I—No or little asthma after admission to CARIH; group IIA—Moderately frequent, but mild asthma responding to bronchodilators, no steroids given; group IIB—Requires intermittent steroid therapy; group III—Severe asthma, requires continuous steroid therapy.

TABLE II. CILIOCYTOPHTHORIA WITHIN 10 DAYS OF FEBRILE RESPIRATORY INFECTIONS
WITHOUT EVIDENCE OF BACTERIAL CAUSE (GROUP I)

NO. SPUTA COLLECTED FROM INDIVIDUAL PATIENTS	PATIENTS			SPUTA		
	TOTAL	POSITIVES		TOTAL	POSITIVES	
		NO.	%		NO.	%
1	15	9	60	15	9	60
2	5	4	80	10	6	60
3	3	3	100	9	6	67
Total	23	16	70	34	21	62

could be defined by the ratio of estimated CCP cells to total columnar cells, but such a ratio was not applicable. Another series of slides of thirty-two sputa and sixteen nasal secretions were randomly selected from the file and rescreened in order to test reproducibility of the classification of CCP positive and negative. In the sputum slides, twenty-eight of thirty-two readings (87.5 per cent) were found to be reproducible. In nasal smears, twelve of sixteen (75 per cent) interpretations were considered correct by the reexamination. This relatively good but rather limited reproducibility appears to result from the nature of this technique, in which the interpretation had to depend upon the frequency of the CCP cells in the slides rather than upon the count.

B. The Correlation Between CCP and Clinical Symptoms.—In an attempt to test the value of CCP in the diagnosis of viral respiratory infections, thirty-four sputa from twenty-three patients with acute, febrile respiratory infections without evidence of bacterial cause were studied for CCP (Table II). The highest rectal temperatures ranged from 100.4° to 104° F. Patients with beta-hemolytic streptococcal infection, pneumonia, or leukocytosis with shift to the left were excluded from this group. One to three sputa were collected from each patient during the first 10 days of the illnesses. Sixteen patients (70 per cent), or twenty-one (62 per cent) sputa, were CCP positive. When two or three sputa were studied from an individual patient, the rate of detecting positive CCP per patient increased. It appears that a high correlation exists between febrile respiratory infections with probable viral cause and positive CCP.

Forty-seven sputa from thirty-three patients without symptoms of infections and without increase of asthma were selected as the controls (Table III). Nine patients were completely asthma free, and twenty-four had some asthma that was relatively quiescent. Only one of thirty-three cases, or one of forty-seven sputa, was positive. The high incidence of positives in the infected group (Table II) and the low incidence in the control group (Table II) appears to suggest that CCP may be useful in the identification of viral respiratory infections in asthmatic children. Thirty-seven sputa from twenty-four patients having unexplained, increased asthma without clinically apparent infections were studied (Table IV, group II). Thirteen patients (54 per cent) or seventeen sputa (46 per cent) were positive. This indicates rather high incidence of CCP with exacerbations of asthma, suggesting the important role of mild viral respiratory infections in asthma. The incidence of sputum CCP among the three groups of patients is summarized in Table IV. The incidence of positive nasal CCP was much less compared with the sputum CCP (Table V).

TABLE III. CILIOCYTOPHTHORIA IN PATIENTS WITH NO SYMPTOMS OF RESPIRATORY INFECTIONS AND NO INCREASE OF ASTHMA (GROUP III)

	PATIENTS		SPUTA	
	TOTAL	POSITIVES	TOTAL	POSITIVES
Asthma free	9	0 (0%)	11	0 (0%)
Some asthma, but relatively quiescent	24	1 (4%)	36	1 (3%)
Total	33	1 (3%)	47	1 (2%)

TABLE IV. SUMMARY OF THE INCIDENCE OF CCP AMONG THE THREE GROUPS

GROUPS	PATIENTS		SPUTA	
	TOTAL	POSITIVES	TOTAL	POSITIVES
I. Without evidence of bacterial cause	23	16 (70%)	34	21 (62%)
II. Increased asthma without clinically apparent infection	24	13 (54%)	37	17 (46%)
III. Asthma-free or quiescent	33	1 (3%)	47	1 (2%)

TABLE V. THE INCIDENCE OF NASAL CCP AMONG THE THREE GROUPS

	PATIENTS		NASAL SWABS	
	TOTAL	POSITIVES	TOTAL	POSITIVES
I. Without evidence of bacterial cause	13	5 (38%)	19	6 (32%)
II. Increased asthma without clinically apparent infection	18	6 (33%)	19	6 (32%)
III. Asthma-free or quiescent	15	0 (0%)	18	0 (0%)

DISCUSSION

The cellular components of the sputum normally include pulmonary macrophages from the alveoli; squamous epithelial cells exfoliated from the oral, laryngopharyngeal, and sometimes esophageal epithelium; ciliated and goblet cells from the trachea, bronchi, and the bronchioles; and leukocytes and bacteria.^{17, 18} In sputum voluntarily expectorated by a normal person, ciliated cells are seen infrequently and appear normal (Fig. 1). In sputum obtained by artificial stimulation of the cough reflex, normal ciliated cells may be found with fair frequency.¹⁸ Clusters of relatively normal bronchial mucosal cells may be seen in the sputum from patients with bronchiectasis or asthma,¹⁸ especially during attacks,²⁶ but the CCP cells usually do not form such clusters. Bronchial smears made from fatal cases of cystic fibrosis of the pancreas, congenital heart failure, and meningococcemia have not revealed CCP.¹⁹ Ciliocytophthoria has also not been observed in bacterial pulmonary infections studied by others.¹³ Nasal and tracheobronchial smears from guinea pigs sensitized with ovalbumin and repeatedly challenged with aerosolized ovalbumin showed slight swelling and irritation of the ciliated epithelial cells, but CCP was not identified.²⁰ Histopathologic examination of the tracheal mucosa of rats after protracted exposure to relatively low concentration of sulfur oxide showed proliferation of the ciliated cells, submucosal edema, capillary dilatation, and considerably increased mucous secretion, but changes suggestive of CCP were not reported by the author.²¹ Such evidence seems to suggest that CCP is a specific phenomenon associated with viral respiratory infections in general.

As summarized by Pinkerton,²² pathologic changes in virus-infected cells may include staining alterations, assumption of spherical shape, inclusion body formation,²³ hypertrophy, giant cell formation, squamous metaplasia, and necrosis. In addition to these changes, pinching off of the ciliated portion of the columnar cell has been encountered in human and experimental respiratory virus infections.⁹⁻¹⁵ According to Pigarevski,¹⁵ the main portion of the ciliated cell may remain after detachment of the ciliated portion and continue to protect submucosal tissue in mild influenzal infection of experimental animals. In severe infections, however, the lining mucosal cells may be lost extensively and the submucosal tissue exposed without protection. Accordingly, it seems reasonable to expect that pinched-off ciliated tufts represent relatively mild viral infection, while the other types of CCP represent severe infection at the cellular level.

Since some areas of the mucous membrane may be severely affected while other areas are only mildly affected in actual viral infection, the pinched-off ciliated tufts may be mixed with other CCP cells during the acute stage, and the former may predominate during convalescence. In this study it appeared that, although the amount of CCP generally reflects the amount of the mucosal damage caused by the viral respiratory infection, the pinched-off ciliated tufts were present in relatively large numbers in sputa from some patients long after subsidence of acute infections or from those without apparent clinical symptoms. This persistence of CCP may indicate latent viral respiratory infections such as are observed in other situations.²⁴ Papanicolaou also stated that the

CCP correlation was more clearly defined in acute infections, and there was, as a rule, an abatement of CCP coincident with clinical improvement.¹² This phenomenon of latency made us doubt the value of CCP in the diagnosis of viral respiratory infections during the early stage of this study. However, with the application of the criteria previously described in the interpretation, sputum CCP correlated with the clinical impression very well (Tables II and III). The nasal CCP appeared to be less valuable than the sputum CCP (Table V).

In the twenty-four patients having unexplained, increased asthma without clinically apparent infections, cytologic evidence of viral infection was identified in thirteen cases (54 per cent) by the technique discussed (Table IV). These nonfebrile and mild infections are very difficult to diagnose clinically in asthmatic patients,³⁻⁶ but they probably triggered the exacerbations of asthma.

It has been reported that Salisbury and coryza virus are of major importance in cases of the common cold and perhaps in other clinical syndromes in both children and adults, and that respiratory, syncytial, adenovirus, and para-influenzal viruses figure prominently in childhood respiratory disease, but play only a small role in adult life.²⁵ On the other hand, it may be possible that certain viruses trigger asthma more frequently than others.⁷ Therefore, further exploration into the relationship between viral respiratory infection and asthma by simultaneous virologic and cytologic studies appears to be justified, since such an approach may provide specific virologic information as well as cytologic evidence of infection. Leukocyte morphology was studied with parallel Wright's staining in some cases in order to investigate its relationship to CCP, but the results were inconclusive.

SUMMARY

One hundred sixty-two sputa and ninety-two nasal secretions from forty-five asthmatic children were studied by Papanicolaou's technique during the acute stage of respiratory infections, exacerbations of asthma, and quiescent or symptom-free periods. With application of the criteria described, the incidence of CCP positive sputa was high (62 per cent) in patients with acute febrile respiratory infections without evidence of bacterial cause. The incidence was also high (46 per cent) in patients with unexplained increases of asthma without clinically apparent respiratory infections. The incidence was very low (2 per cent) during quiescent or symptom-free periods. The incidences of CCP positive nasal smears were relatively low among the symptomatic groups. The results seem to indicate high correlation between viral respiratory infection and sputum CCP, and frequent association of the mild viral infection with exacerbation of asthma. Further exploration into the relationship between viral respiratory infection and asthma by simultaneous virologic and cytologic approaches appears to be justified.

REFERENCES

1. Feingold, F. B.: Infection in Bronchial Allergic Diseases; Bronchial Asthma, Allergic Bronchitis, and Asthmatic Bronchitis, *Pediat. Clin. North America* (Pediat. Allergy) 6: 703, 1959.

2. Falliers, C. J., Halberstein, S. G., and Bukantz, S. C.: Effect of Rubeola, Rubella, Varicella, and a Viral Upper Respiratory Infection Upon the Severity of Childhood Asthma; With a Note on the Role of Corticosteroids, *J. ALLERGY* **32**: 69, 1961.
3. Glaser, Jerome: Allergy in Childhood, Springfield, Ill., 1956, Charles C Thomas, Publisher, p. 179.
4. Colldahl, H.: Oxygen Uptake, Carbon Dioxide Elimination, and Body Temperature (in Asthma), *in* Jamar, J. M., editor: International Textbook of Allergy, Springfield, Ill., 1959, Charles C Thomas, Publisher, p. 142.
5. Vaughan, W. T., and Black, J. H.: Practice of Allergy, ed. 3, St. Louis, 1954, The C. V. Mosby Company, p. 981.
6. Vaughan, W. T., and Black, J. H.: Practice of Allergy, ed. 3, St. Louis, 1954, The C. V. Mosby Company, p. 225.
7. Freeman, G. L., and Todd, R. H.: The Role of Allergy in Viral Respiratory Tract Infections, *A. M. A. Am. J. Dis. Child.* **104**: 330, 1962.
8. Frankland, A. W.: Allergy of Infancy and Childhood, *in* Jamar, J. M., editor: International Textbook of Allergy, Springfield, Ill., 1959, Charles C Thomas, Publisher, p. 569.
9. Bryan, W. T. K., and Bryan, M. P.: Structural Changes in the Ciliated Epithelial Cells During Upper Respiratory Infection; Preliminary Report, *Laryngoscope* **60**: 523, 1950.
10. Bryan, W. T. K., and Bryan, M. P.: Structural Changes in the Ciliated Epithelial Cells During the Common Cold, *Trans. Am. Acad. Ophth. & Otolaryng.*, May-June, 1953, p. 297.
11. Bryan, W. T. K., and Bryan, M. P.: Cytologic Diagnosis in Otolaryngology, *Trans. Am. Acad. Ophth. & Otolaryng.*, Sept.-Oct., 1959, p. 597.
12. Papanicolaou, G. N.: Degenerative Changes in Ciliated Cells Exfoliating From the Bronchial Epithelium as a Cytologic Criterion in the Dagnosis of Diseases of the Lung, *New York State J. Med.* **56**: 2647, 1956.
13. Pierce, C. H., and Hirsch, J. G.: Ciliocytophthoria; Relationship to Viral Respiratory infections of Humans, *Proc. Soc. Exper. Biol. & Med.* **98**: 489, 1958.
14. Pierce, C. H., and Knox, A. W.: Ciliocytophthoria in Sputum From Patients With Adenovirus Infections, *Proc. Soc. Exper. Biol. & Med.* **104**: 492, 1960.
15. Pigarevski, V. E.: Cellular Factor in the Defense of the Host in Influenza, *Acta virol.* **1**: 30, 1957. (English ed.)
16. Hsu, Chen-Wu: A Study on Rapid Diagnosis of Influenza, *Chinese M. J.* **81**: 394, 1962.
17. Bertalanffy, F. D.: The Modern Concept of Respiratory Tissue Structure, *Acta cytol.* **5**: 385, 1961.
18. Koss, L. G.: Diagnostic Cytology and Its Histopathologic Basis, Philadelphia, 1961, J. B. Lippincott Co., p. 170.
19. Ko, I.: Unpublished data.
20. Kim, C. J., and Ko, I.: Personal experience.
21. Dalhamn, T.: Mucous Flow and Ciliary Activity in the Trachea of Healthy Rats and Rats Exposed to Respiratory Irritant Gasses, *Acta physiol. Scandinav.* **36**: (Suppl. 123) 109, 1956.
22. Pinkerton, H.: Relation of Viruses to Inclusion Bodies, *Ann. New York Acad. Sc.* **81**: (article I) 207, 1959.
23. Liu, Chien: Relationship of Viral Antigens to Inclusion Bodies, *Ann. New York Acad. Sc.* **81**: (article I) 193, 1959.
24. Huebner, R. J.: Considerations of Natural "Latency" Exhibited by Certain "Inclusion-Body" Viruses, *in* Walker, D. L., Hanson, R. P., and Evans, A. S., editors: Symposium on Latency and Masking in Viral and Rickettsial Infections, Minneapolis, 1958, Burgess Publishing Company, p. 51.
25. Hilleman, M. R., et al.: Acute Respiratory Illnesses Among Children and Adults, *J. A. M. A.* **180**: 101, 1962.
26. Naylor, B.: The Shedding of the Mucosa of the Bronchial Tree in Asthma, *Thorax* **17**: 69, 1962.