TABLE I. Effect of Underfeeding on Thyroxine Secretion Rate in Female Mice.

Treatment, rate of feeding	No. of animals	Body wt, g Initial	Final	TSR, μg/100 g/day	Decrease, %	Frequency distribution of TSR .25 μg	.5 μg	1 μg	1.5 μg	2.0 μg	2.5 μg
Control *	58			1.25 ± .05			3	30	18	7	
Control 2 †	16	30.3	30.4	1.38 ± .173	100		4	3	4	3	2
¾ 3 ‡	14	28.5	30.1	.93 ± .180	33	2	7		3	1	1
½ 4 §	13	31.0	24.8	.81 ± .142	41	2	6	1	4		

* Wada *et al.*(2). † 2 *vs* 3 $p < .05$. ‡ 2 *vs* 4 $p < .01$. § 3 *vs* 4 not significant. 1 *vs* 2 not significant.

was reduced to 0.80 μg/100 g/day or 41% associated with a 17.3% reduction in body weight. These data suggest that pituitary-thyroid axis is sensitive to slight reduced energy intake by reduction in TSR sufficient to maintain body weight.

1. Amin, A., Chai, C. K., Reineke, E. P., *Am. J. Physiol.*, 1957, v191, 34.

2. Wada, H., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 608.

3. Meites, J., Wolterlink, L. F., *Science*, 1950, v111, 1755.

4. Meites, J., *Iowa State College J. Sci.*, 1953, v28, 19.

5. Blincoe, C., Brody, S., *Mo. Agr. Exp. Sta. Res. Bull.* 576, 1955.

6. Reichlin, S., *Endocrinology*, 1957, v60, 470.

7. Turner, C. W., Pipes, G. W., Premachandra, B. N., Conference on Isotopes in Agr., Okla. TID-7578, 97, 1959.

8. Money, W. L., Kraintz, L., Foger, J., Kirschner, L., Rawson, R. W., *Endocrinology*, 1951, v48, 682.

9. Perry, W. F., *ibid.*, 1951, v49, 284.

10. Albert, A., Tenney, A., Ford, E., *ibid.*, 1952, v50, 324.

11. Myant, N. B., *J. Physiol.*, 1953, v120, 288.

12. Beck, R. N., *Endocrinology*, 1958, v62, 9.

13. Brown-Grant, K., *ibid.*, 1955, v56, 607.

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Ciliocytophthoria in Sputum from Patients with Adenovirus Infections.*† (25886)

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Papanicolaou(1) described morphologic abnormalities in ciliated epithelial cells exfoliated from the respiratory tract of certain patients with acute or chronic pulmonary disease. He designated this phenomenon ciliocytophthoria (CCP). Further observations in our laboratory established an association of CCP with virus infections of the human respiratory tract(2). CCP was found to be present in persons suffering from viral infections but absent in those with bacterial pulmonary disease. Of the viral illnesses studied in which CCP was observed, only those due to influenza were documented by laboratory data (*i.e.* virus isolation or rise in serum antibody). The remainder were classified on the basis of clinical and laboratory findings as diseases of probable viral etiology. The present report presents data derived from screen-

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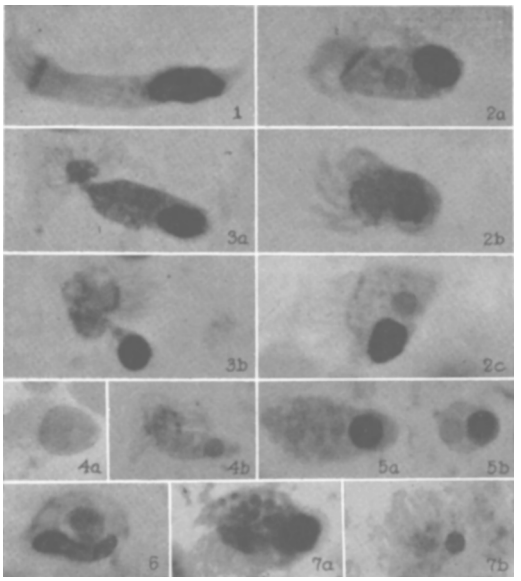

Morphologic changes occurring in Ciliocytophthoria (CCP), magnification × 850, Papanicolaou stain 1-6, Mallory stain 7.

FIG. 1. Normal ciliated epithelial cell.

FIG. 2. Intact ciliated epithelial cells with abnormalities: a. Pyknotic nucleus and cytoplasmic inclusions. b. Nuclear and cytoplasmic inclusions. c. Chromatin marginated at periphery of nucleus. One large and many small cytoplasmic inclusions.

FIG. 3. Pinching off of ciliated portions resulting in: a. Small ciliated tuft and large basal segment containing pyknotic nucleus and cytoplasmic inclusions. b. Large ciliated tuft and small basal segment containing pyknotic nucleus and one small cytoplasmic inclusion.

FIG. 4. Free anuclear ciliated tufts: a. Homogeneous cytoplasm with one weakly stained inclusion. b. Vacuolated cytoplasm with one large deeply stained inclusion.

FIG. 5. Basal nucleated segments: a. Pyknotic nucleus and several cytoplasmic inclusions. b. Nuclear inclusion and large cytoplasmic inclusion.

FIG. 6. Phagocytosis by macrophage of an anuclear segment containing large inclusion.

FIG. 7. Mallory stained preparations: a. Intact ciliated epithelial cell with pyknotic nucleus and many cytoplasmic inclusions. (Nuclear inclusion out of focus, visible only upon refocus.) b. Anuclear ciliated tuft containing large inclusion.

ing sputum specimens obtained from armed forces personnel at Fort Dix. The results demonstrate the presence of CCP in persons suffering from documented adenovirus infections and also confirm occurrence of CCP during viral influenza.

Methods. Through the courtesy of Col. Joseph W. Cooch, Health Center, Fort Dix, N. J., single sputum specimens were obtained from 375 recruits exhibiting symptoms of respiratory disease during March 4 to Sept. 9, 1958. Each specimen was stained according to the Papanicolaou technic(3) and examined microscopically for presence of CCP using criteria previously reported(2). In a small series of CCP positive specimens duplicate smears were made and stained with Mallory's aniline blue collagen stain(4) (acid fuchsin 30 seconds, aniline blue solution 50 seconds, followed by gradual dehydration). Paired acute and convalescent sera obtained from 86 of these patients were titrated for adenovirus antibodies in complement fixation (CF) tests. In addition, sera from 32 of the 86 patients were similarly examined for antibodies against influenza A. The prevalence of adenoviral infection among recruits during the period under study was determined by assaying complement fixing antibodies in individually paired serum specimens from a total of 578 patients. Immunologic tests were done with minor modifications of a previously described technic(5). A 4-fold or greater increase in titer of the convalescent serum as compared with that of the acute phase specimen was used as criterion of infection.

Results. The accompanying photomicrographs demonstrate the abnormal morphologic changes characteristic of CCP. The typical orange-pink color of the acidophilic inclusions obtained with the Papanicolaou stain can be seen in photomicrographs previously published(2). In Mallory-stained smears, cytoplasmic inclusions are a brilliant cherry red and nuclear inclusions are orange. Although the Mallory technic of staining as employed in our laboratory does not allow as good preservation of cells as does the Papanicolaou method, the rapidity and simplicity of the former procedure make it valuable in certain instances.

Among a total of 357 expectorated samples, 282 were deep cough specimens suitable for examination. CCP was observed in 59% of the specimens (Table I). No evidence of CCP was found in 30%, and in 11% there were abnormal elements suggestive of CCP, but not conclusive. In such instances small red-staining spherical bodies and mononuclear cells predominated in the sputum smear. It

TABLE I. Occurrence of CCP in Single Deep Cough Specimens from 282 Military Recruits Suffering from Acute Respiratory Disease.

CCP	No. of specimens	% of total
Positive	167	59
Negative	85	30
Abnormal	30	11

is uncertain whether or not this pattern represents a transition phase between the early stage of viral infection, when CCP is readily recognized, and the later stages of infection, or convalescent period, when large multinucleated histiocytes with deeply staining orange cytoplasm are the principal cellular elements.

During CCP observation period, 578 paired serum specimens were examined for presence of adenovirus antibodies by complement fixation (CF). Incidence of patients with CCP was compared with the prevalence of adenovirus infection at Fort Dix during monthly intervals throughout the survey. The results shown in Table II demonstrate that a relatively high percentage of sputum samples were positive for CCP during each month, and that concomitantly there was a high incidence of adenovirus infection, as demonstrated serologically.

The direct comparison of cytologic findings with adenovirus antibody studies in 86 patients from whom sputum specimens as well as acute and convalescent sera were obtained is shown in Table III. Sera from 32 of these individuals (Group A) also were examined for increased titers against influenza A virus. Among this group were 27 positive CCP specimens. Twenty-two of the CCP positive cases occurred in persons who had a rise in adenovirus antibody titer, 3 showed increases in influenza antibody, and one revealed serologic evidence of both adenovirus and influenza infection. Only one case was CCP positive without a demonstrable rise in antibody titer. Single sputum specimens from 5 individuals with serum antibody rises against adenovirus failed to show CCP. Among the 54 patients comprising group B, CCP was observed in 33, 25 of whom showed significant rises in adenovirus antibodies. All 6 persons whose sputum smears were abnormal, but not classified as CCP, had adenovirus infection as documented serologically.

A summary of the foregoing data with respect to adenovirus infection shows that from 60 patients having positive CCP specimens, 48 had significant adenovirus antibody rises as did all 6 individuals presenting abnormal specimens.

Discussion. The finding of CCP in 59% of 282 single sputum specimens indicates that this phenomenon is by no means uncommon among military recruits exhibiting symptoms of respiratory disease. Whether other population groups would yield such a large percentage of CCP positive specimens cannot be determined from the present study. Data from the immunologic studies serve to establish that CCP is associated with adenovirus infection as well as with influenza. In addition, the results confirm the high incidence of adenovirus disease among military personnel at Fort Dix as previously reported(6).

Since the cytologic observations here reported were obtained by examining only a single deep cough specimen from each patient, the negative findings associated with positive serologic evidence of adenovirus infection might have been reversed had several sputum

TABLE II. Incidence of CCP and Adenovirus Infection during Survey Period.

Test periods during 1958	CCP					Significant adenovirus antibody rise		
	No. patients	Pos.	Abn.	Neg.	% pos.	No. patients	No. pos.	% pos.
March	76	48	4	24	63	279	220	79
April	75	45	6	24	60	84	72	86
May	41	23	8	10	56	53	48	91
June	47	25	4	18	53	42	38	91
July	16	7	3	6	44	31	25	81
August	17	13	1	3	76	43	22	51
September	10	6	4	0	60	46	32	70

TABLE III. Comparison of CCP and CF Antibody Tests.

	Total No. individuals	Presence of CCP		Significant antibody rise: Adenovirus +	Adenovirus 0	Influenza A +	Influenza A 0
A.	32	Positive	22	22	0	0	22
			3	0	3	3	0
			1	1	0	1	0
			1	0	1	0	1
		Negative	5	5	0	0	5
B.	54	Positive	25	25	0		
			8	0	8		
		Abnormal	6	6	0	Not done	
		Negative	9	9	0		
			6	0	6		

samples from each individual been analyzed. Moreover, the possibility of a superimposed bacterial infection cannot be excluded in such instances, since it has already been observed that influenza patients with secondary bacterial infection no longer produce positive CCP specimens(2).

Examination of sputum specimens for CCP is a rapid laboratory test to distinguish viral infections of the respiratory tract from bacterial disease when used in conjunction with other laboratory procedures(2). The Papanicolaou stain, however, has not yet revealed significant differences between viral species in respect to their effect upon ciliated epithelium exfoliated from the respiratory tract. Other workers have shown that the finding of abnormal changes in exfoliated respiratory epithelium is an aid in establishing a tentative diagnosis of influenza(7,8). In addition morphologic changes in respiratory epithelium *in situ* have been observed upon examination of autopsy material obtained from fatal cases of influenza(7) and adenovirus infection(9,10).

The present results demonstrate the association of CCP with both adenovirus and influenza disease and support the concept that CCP may occur in a variety of viral respiratory infections.

Summary. A total number of 282 single sputum specimens from patients with acute respiratory disease were stained by Papanicolaou technic and examined for presence of certain abnormalities, known as ciliocytophthoria (CCP), in exfoliated ciliated epithelial cells. Fifty-nine % yielded positive results, 30% were negative, and 11% inconclusive. During this period complement fixation (CF) tests carried out on paired acute and convalescent sera from 578 patients demonstrated high incidence (77%) of adenovirus infection. The direct association of CCP with adenovirus infection was established by study of patients from whom sputum as well as serum specimens were obtained. Significant adenovirus antibody rises occurred in 48 (80%) of 60 individuals whose sputum specimens were positive for CCP.

1. Papanicolaou, G. N., *N. Y. State J. Med.,* 1956, v56, 2647.

2. Pierce, C. H., Hirsch, J. G., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 489.

3. Papanicolaou, G. N., *Atlas of Exfoliative Cytology,* Harvard Univ. Press, 1954.

4. Kolmer, J. A., Boerner, F., *Approved Laboratory Technic,* D. Appleton-Century Co., New York, 1945.

5. Lennette, E. H., Clark, W., Dean, B. H., *Am. J. Trop. Med.,* 1949, v29, 527.

6. Hilleman, M. R., Anderson, S. A., Levinson, D. J., Luecking, M. L., *Am. J. Hyg.,* 1958, v67, 179.

7. Hers, J. F. Ph., *Histopathology of the Respiratory Tract in Human Influenza,* H. E. Stenfert Kroese, N. V., Leiden, 1955.

8. Pigarevski, V. E., *Acta Virol.* (English Edition), 1957, v1, 30.

9. Chany, C., Lépine, P., LeLong, M., Le-Tan-Vinh, Satgé, P., Virat, J., *Am. J. Hyg.,* 1958, v67, 367.

10. Kusano, N., Kawai, K., Aoyama, Y., *Jap. J. Exp. Med.,* 1958, v28, 301.

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