may explain the more sustained nucleoside blood levels noted in one reported case after intravenous administration. Plunkett and co-workers have shown rapid transport of Ara-AMP through cell membranes.

The use of subconjunctival, intramuscular, and intravenous delivery by Ara-AMP against rabbit herpes kerato-uveitis is presently under study in this laboratory.

From the Department of Ophthalmology, College of Medicine, University of Florida. Supported in part by Grants EY-00007, EY-00266, EY-00033, and EY-00063-01. Submitted for publication Oct. 9, 1975. Reprint requests: Dr. Jonathan D. Trobe, Department of Ophthalmology, College of Medicine, Box J-284, J. Hillis Miller Health Center, University of Florida, Gainesville, Fla. 32610.

REFERENCES

Immunofluorescent detection of adenovirus antigen in epidemic keratoconjunctivitis.

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An immunofluorescent technique was used to demonstrate soluble adenoviral antigens in epithelial cells on conjunctival scrapings of patients with epidemic keratoconjunctivitis (EKC). Conjunctival scrapings for immunofluorescence and viral isolation studies were performed on 79 patients suspected of having EKC or other acute follicular or papillary conjunctivitides. Of 41 patients with clinical findings consistent with a diagnosis of EKC and three patients with pharyngocconjunctival fever, 43 were positive by immunofluorescence. All 39 patients with adenovirus isolation had positive immunofluorescence studies. The five remaining cases had other documented evidence of EKC. No false-positive responses were encountered in relation to the clinical diagnoses. The fluorescent staining was predominantly found in the cytoplasm. Speckled nuclear fluorescence was also noted. The cytoplasmic fluorescence is compatible with the adenovirus cellular replication cycle. The immunofluorescent technique was found to be a reliable, sensitive, specific, and rapid diagnostic technique for detection of group-reaction adenoviral antigens in conjunctival scrapings.

Although immunofluorescence is an established technique to identify and localize viral antigens in infected cells,1 limited information is available on the application of immunofluorescence to the rapid diagnosis of adenovirus infections of the eye. In 1964, Inoue and coworkers2 successfully used the indirect immunofluorescent technique to label scrapings from patients with epidemic keratoconjunctivitis (EKC) in which intracytoplasmic inclusions were detected in Giemsa-stained conjunctival epithelial cells. In 1967, Uchida and Inoue detected intranuclear fluorescence in conjunctival scrapings using convalescent human serum in 50 per cent of patients with EKC. In a subsequent study, Inoue1 attempted to demonstrate, by immunofluorescence, adenovirus antigen in conjunctival biopsy material from patients with EKC. In both of these studies diagnosis by immunofluorescence was less sensitive than virus isolation. Uchida and Inoue demonstrated the presence of intracytoplasmic and intranuclear adenovirus antigen in HeLa cells infected with adenovirus using the direct immunofluorescent technique. In 1969, Ellison, Kaufman, and Little attempted to use immunofluorescent techniques to diagnose experimentally induced adenovirus type 3 conjunctivitis in human volunteers with little success.
Adenoviruses types 8 and 19 have been well established etiologic agents in typical EKC with the presence of follicular conjunctivitis, regional adenopathy, and the delayed development of subepithelial infiltrates.1-8 During a recent outbreak of EKC caused by adenovirus types 8 and 19 in Chicago, Ill., a large number of patients were referred to two of us of subepithelial infiltrates. An additional 60 patients with EKC and were subsequently diagnosed as infection of adenoviral conjunctivitis. This report describes the successful application of the fluorescent antibody technique to the identification of adenovirus antigens in conjunctival epithelial cells of patients suspected of having EKC or pharyngoconjunctival fever (PCF).

Material and methods.

Patient selection. During the epidemic of keratoconjunctivitis studied at the Illinois Eye and Ear Infirmary, we examined 97 patients with the typical manifestations compatible with the diagnosis of EKC, i.e., acute follicular conjunctivitis, regional lymphadenopathy, and the delayed onset of subepithelial infiltrates. An additional 60 patients with acute conjunctivitis examined by the resident physicians were referred to two of us (DWV and CEW) for examination. These cases were not considered compatible with the diagnosis of EKC and were subsequently diagnosed as inclusion conjunctivitis, bacterial conjunctivitis, nonspecific allergic conjunctivitis, severe postoperative reaction, and non-specific papillary conjunctivitis or traumatic reactions. At the time of this study, 41 patients with EKC, three with PCF, and 35 patients with the other diagnoses listed had conjunctival scrapings performed for comparative immunofluorescent and viral isolation studies. Paired sera were obtained from 45 patients for the detection of a humoral antibody response to adenovirus infection.

Preparation of patient slides. Conjunctival scrapings were obtained after anesthetizing the conjunctiva with topical proparacaine hydrochloride (Alcaine) after swab specimens were taken for viral and bacterial culture. The upper eyelid was everted, and the tarsal conjunctiva was scraped off with a platinum-tipped spatula (Storz Instrument Co., St. Louis, Mo.). Multiple slides were made from each patient. The specimens were collected on an average five days after onset of symptoms. The slides were air dried and fixed in absolute methanol or acetone for five minutes and frozen at -70° C. until examination. The slides were stored for two weeks to six months before immunofluorescent examination.

Adenovirus type 2 antisera. Previously prepared adenovirus type 2 antisera was used to detect group-specific hexon adenovirus antigen. The antisera to type 2 adenovirus was prepared by growing prototype adenovirus type 2 in 32 ounce bottles of monolayer human epithelial-2 (HEp-2) cultures. The cultures were observed daily until 3+ cytopathogenic effects (CPE) were detected, at which time the cells were scraped off the bottle with a rubber policeman. The cell suspension was pooled, freeze-thawed rapidly three times, and spun at 1,000 r.p.m. for 10 minutes. The supernatant was then extracted at 4° C. with fluorocarbon (Freon TF) at 1:2 vol./vol. After 30 minutes the top layer was drawn off and spun at 1,000 r.p.m. for 10 minutes. The supernatant was then extracted at 4° C. with phosphate buffer, and aliquoted. This supernatant was used for immunization of New Zealand white rabbits by injecting 5 ml. intravenously on day 1 after pre-immunization bleeding, followed by weekly intraperitoneal injection of the same preparation for four weeks. The rabbits were exsanguinated by cardiac puncture two weeks after the final injection. The serum was separated and heat-inactivated at 56° C. For 30 minutes in a water bath and absorbed on a monolayer culture of methanol-fixed HEp-2 cells at 37° C. For 30 minutes. After centrifugation at 1,200 r.p.m. for 10 minutes, the serum was separated at 0.2 ml. volumes in one dram vials and frozen. The antisera specificity was checked by indirect immunofluorescence against herpes simplex virus types 1 and 2 and chlamydia (lymphogranuloma venereum)-infected cells and was found to be nonreactive toward these antigens.

Goat anti-rabbit globulin conjugated with fluorescein isothiocyanate. Commercially prepared goat anti-rabbit globulin conjugated with fluorescein isothiocyanate (FITC) was obtained from Behring Diagnostics, Somerville, N. J. After resuspension of the conjugate in 5 ml. of distilled water, it was absorbed on HEp-2 cells as described above. The antisera as well as the conjugate were titrated for optimal immunofluorescence using adenovirus type 8-infected HEp-2 cells grown on eight-chamber tissue culture slides (Lab-Tek Products, Naperville, Ill.). The highest dilution giving definite (3+ to 4+) fluorescence was found to be 1:80 and was designated as the end-point.

Immunofluorescence study. Immunofluorescent detection of adenovirus antigen was performed by the indirect fluorescent antibody method. The slides of conjunctival scrapings previously stored at -70° C. were removed, coded, and equilibrated with hemagglutination (HA) buffer (Difco) (pH 7.2) by placing 0.5 ml. of buffer on each slide for four minutes. After air drying, 0.5 ml. of adenovirus type 2 antisera, diluted to one dilution below the previously determined end-point (1:40), was placed on each slide. The slides were placed in humidity chambers and incubated at 37° C. For 30 minutes. Following incubation, they were washed four times in HA buffer during
15 minutes and air dried. They were covered with 0.5 ml of goat anti-rabbit globulin FITC conjugate diluted to one dilution below the predetermined end-point. The slides were placed in humidity chambers and reincubated at 37° C. for 30 minutes, again washed four times in HA buffer during 15 minutes, with a final one-minute wash in distilled water. The slides were air dried, and those not counterstained with Evans blue were mounted with fluorescent antibody mounting media (Difco) (pH 8.2).

Slides counterstained with Evans blue were prepared by adding 0.5 ml. Evans blue (2 per cent vol./vol.) diluted 1:10 in HA buffer for one minute. They were washed four times with HA buffer in 15 minutes, air dried, and mounted as previously described.

Quench reaction. Rabbit antiserum against adenovirus type 11 was titered for indirect immunofluorescence using adenovirus types 8 and 19 infected HEp-2 cells grown in eight-chamber tissue culture slides. The highest dilution of antiserum giving definite fluorescence was selected as the end-point (1:80). Adenovirus type 11 antiserum was diluted to one dilution below the end-point, and then was used for indirect immunofluorescence on conjunctival scrapings of patients known to be infected with adenovirus types 8 and 19. This antiserum was also used to “quench” the immunofluorescent reaction of known adenovirus types 8 and 19 antigens in the epithelial cells of the conjunctival scrapings of patients in whom these particular serotypes were isolated. The pretreated slides were recoded and evaluated with the other test slides in a double-blind fashion in the indirect fluorescent antibody technique, using adenovirus type 2 antiserum.

Virus isolation. Conjunctival swabs were collected in 2 ml of Hanks’ balanced salt solution containing 0.5 per cent gelatin (Bacto) and 500 units per milliliter of penicillin, 500 μg per milliliter of streptomycin, and 5 μg per milliliter of amphotericin B (Fungizone). Specimens were inoculated into duplicate tube monolayer cultures each of primary human embryonic kidney (HEK) and the human embryonic WI-38 lung cell strain after 30 minutes of antibiotic treatment at room temperature. Each culture tube received 0.2 ml of inoculum and was maintained at 36° C. in a humidified CO2 incubator with Eagle’s basal medium containing 2 per cent fetal calf serum. Cultures were examined microscopically at one- to three-day intervals for a period of three weeks. Cultures exhibiting 2+ to 3+ viral CPE were harvested, and the viral isolate was identified by neutralization in HEK cells employing approximately 100 TCID50 of virus and adenovirus typespecific antisera.

Complement fixation test. Complement-fixing (CF) antibody titrations were done by micro-
titer technique,10 using adenovirus CF antigen obtained from Microbiological Associates, Bethesda, Md. Paired sera obtained during the acute and recovery stages of the disease were titrated in parallel, and the CF titer was recorded as the highest dilution of patient’s serum exhibiting 3+ to 4+ fixation (no hemolysis in the indicator system). The CF test for determination of group-specific adenovirus antibody employed 2 units of complement and 4 units of adenovirus antigen.

Examination of slides. Conjunctival scrapings from staff personnel without clinical evidence of adenoviral infection and demonstrated to be normal by virus isolation and seroconversion studies were used as negative controls. The HEp-2 cells, infected with adenovirus types 8 or 19 grown on eight-chamber tissue culture slides, were used as positive controls. A normal rabbit serum and buffer conjugate control were included. Positive, negative, and conjugate controls were processed with each batch of patient slides examined by immunofluorescence. The coded slides were examined in a double-blind fashion by three independent observers on three fluorescence microscopes: an American Optical microscope with 100 W. mercury vapor lamp with a BG-12 excitation filter and an OC-1 barrier filter, Zeiss microscope with a 100 W. mercury vapor lamp with a BG-12 excitation filter and an OC-1 barrier filter; a Zeiss fluorescent microscope with a 50 W. halogen quartz lamp with an FITC excitation filter and a 530 nm barrier filter. Photomicrographs were taken on the Zeiss fluorescent microscope with a Zeiss camera using Kodak Tri-X 35-mm. film, ASA 400, exposed for 30 to 90 seconds.

Immunofluorescence. All patient slides were examined in a double-blind fashion. Each observer recorded his findings and only after all three observations were made were the data collated and the code broken.

A positive slide was one in which intense fluorescence was demonstrable in either the nucleus or the cytoplasm of an epithelial cell. When one positive field or isolated cell (Fig. 1) was found, another distinctive cell or group of cells (Fig. 2) was sought until two positive fields were found. A negative slide was one in which no fluorescence was localized to an individual epithelial cell, but diffuse nonspecific background fluorescence was present (Fig. 3). A common characteristic of infected cells was a rounding of the cellular cytoplasm (Figs. 1 and 4). In some fields, infected cells were seen adjacent to noninfected epithelial cells (Fig. 5).

To show that we were dealing with a specific group reaction, tissue culture slides infected with reference types 8 and 19 viruses were reacted with adenovirus type 11 antiserum. A known positive clinical diagnostic conjunctival scraping was reacted with type 11 antiserum. In both cases
the group-specific antibody was able to quench the reaction indicated by a negative immunofluorescent preparation after reaction with adenovirus type 2 antisera.

**Results.**

*Virus isolation.* Virus isolation studies and conjunctival smear preparations for immunofluorescent examination were performed on 79 of the 157 patients seen. The conjunctival swab was inoculated into WI-38 and HEK cells as described. Of the 79 isolation attempts, 39 adenovirus isolates were obtained. Detection of CPE occurred from 2 to 17 days, with the mean being 7.2 days. The remaining 40 patients had their cultures observed for three weeks without evidence of any CPE. Of the 41 patients with diagnostic scrapings designated as EKC by clinical assessment, 36 had adenovirus isolated. Three patients designated as having PCF by clinical observation had adenovirus isolated. Adenovirus types associated with EKC and PCF and the relationship of clinical diagnosis to virus isolation and immunofluorescent detections are shown in Table I. Of the five cases of EKC in which no virus was isolated the clinical diagnosis...
Fig. 2. A cluster of epithelial cells from a conjunctival smear with cytoplasmic fluorescence (outlined with small arrows). The nuclei show stippled specific fluorescence (large arrows). x200.

Fig. 3. Dull nonspecific fluorescence of an epithelial cell (short arrow) and several polymorphonuclear leukocytes with nonspecific staining (long arrows). Note the presence of nonspecific background staining. The poor focus is due to the long exposure time with light diffusion causing blurring. x200.

was established by the appearance of typical subepithelial lesions after long-term follow-up. None of the remaining 35 cases in which neither clinical suspicion nor virus isolation indicated an adeno viral disease had positive immunofluorescence.

Serology. Paired sera were available on 48 of the 79 patients on whom virus isolation and immunofluorescence studies were attempted. Of the remaining 31 patients, seven refused diagnostic serologic studies, and 14 were lost to follow-up; ten patients were seen in very late stages.
Fig. 4. Cluster of epithelial cells with brightly staining cytoplasm. One cell has some moderate nuclear fluorescence (arrow). The deeper texture is due to counterstaining with Evans blue. Note the tendency for the infected cells to round the cytoplasm. ×200.

Fig. 5. A patch of positive staining cells with adjacent noninfected epithelial cell (circumscribed with arrows). ×200.

of the disease, and their sera were designated as "convalescent." The acute sera were obtained within two to six days after the onset of symptoms. Convalescent specimens were obtained between five and eight weeks after the onset of symptoms.

Reciprocal titers determined by CF on these 49 patients ranged from 8 to 512 with the average titer being 64. Seroconversion was designated whenever a fourfold or greater rise in antibody titer was determined. Of the 48 patients with paired sera, 32 were designated as clinical EKC and PCF with the remainder falling into the other diagnostic categories. The relationship of seroconversion to virus detection is shown in
Table I. Relationship of clinical diagnosis and virus isolation to immunofluorescent detection of adenovirus antigen in epithelial cells on conjunctival smears

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>No. of patients</th>
<th>No. of virus isolation</th>
<th>Adenovirus type</th>
<th>Positive immunofluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidemic keratoconjunctivitis</td>
<td>41</td>
<td>36</td>
<td>28 6 2</td>
<td>40</td>
</tr>
<tr>
<td>Pharyngoconjunctival fever</td>
<td>3</td>
<td>3</td>
<td>1 — 2</td>
<td>3</td>
</tr>
<tr>
<td>Inclusion conjunctivitis</td>
<td>6</td>
<td>0</td>
<td>— — —</td>
<td>0</td>
</tr>
<tr>
<td>Suspect viral conjunctivitis</td>
<td>5</td>
<td>0</td>
<td>— — —</td>
<td>0</td>
</tr>
<tr>
<td>Bacterial conjunctivitis</td>
<td>6</td>
<td>0</td>
<td>— — —</td>
<td>0</td>
</tr>
<tr>
<td>Allergic conjunctivitis</td>
<td>8</td>
<td>0</td>
<td>— — —</td>
<td>0</td>
</tr>
<tr>
<td>Others*</td>
<td>10</td>
<td>0</td>
<td>— — — —</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>39</td>
<td>29 6 4</td>
<td>43</td>
</tr>
</tbody>
</table>

*Includes five cases of nonspecific conjunctival reaction lost to follow-up; trauma (two), uveitis (one), and postoperative reactions (two) account for the other five cases.

Table II. Relation of serologic conversion to virus isolation and immunofluorescent detection of adenovirus antigen in epithelial cells on conjunctival smears

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Diagnostic laboratory criteria</th>
<th>No. tested</th>
<th>Immunofluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidemic keratoconjunctivitis and pharyngoconjunctival fever (32 patients)</td>
<td>Adenovirus isolated</td>
<td>29†</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Seroconversion*</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Adenovirus isolated; seroconversion</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Adenovirus isolated; no seroconversion</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>No virus isolated; seroconversion</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No virus isolated; no seroconversion</td>
<td>2</td>
<td>1‡</td>
</tr>
<tr>
<td>Other (16 patients)§</td>
<td>No virus isolated; no seroconversion</td>
<td>16</td>
<td>0</td>
</tr>
</tbody>
</table>

*Paired sera exhibiting a fourfold or greater rise in adenovirus CF antibody titer.
†Ten patients had CF titers ≥ 64.
‡This patient had typical disease with epidemiologic isolated contact without virus isolation or seroconversion (CF titers 1:128). The patient had typical juvenile rheumatoid arthritis.
§Includes all diagnostic categories other than epidemic keratoconjunctivitis listed in Table I.

Table II. Positive immunofluorescence was found in all cases where virus was isolated and in all cases of seroconversion with or without virus isolation. The immunofluorescence method was positive in one of two clinically diagnosed cases of EKC with detectable subepithelial lesions which were negative by virus isolation and serologic criteria. This patient with CF titers of 1:128 had active rheumatoid arthritis with epidemiologic relationship to other known cases.

**Immunofluorescence.** All the test slides, internal control slides, and external control slides were evaluated in a double-blind fashion on different microscopes as described. The observers agreed on 74 of 79 (94 per cent) slides examined; they agreed on all 43 positive immunofluorescent slides. Of these 43 patients, 39 had positive virus isolates with immunofluorescence being positive in all 39 cases (Table III). Immunofluorescent-positive smears were obtained in 43 of the 44 patients designated as clinical EKC or PCF (Table III). Diagnostic seroconversion was the least sensitive, with 18 of 32 patients (56 per cent) positive.

**Discussion.** Epidemic keratoconjunctivitis has been commonly associated with adenovirus type 8 and more recently, type 19. Less frequently, adenovirus types 2, 3, 4, 7, 9, 10, 11, 14, 16, and 29 have been implicated in the typical EKC syndrome. Difficulty in differential diagnosis of adenovirus infections of the eye, inclusion conjunctivitis, atypical herpes simplex infections, and other viral conjunctivitides is well known. To date, a rapid diagnostic tool specific for these conditions has not been readily accessible. Virus isolation is a technique which may require at least a week’s time to indicate positivity and as much as three weeks to show negative results. Serologic data are a retrospective tool. The time interval between date of onset and seroconversion is, at the very least, a week or more, and in many cases serologic tests are not routinely available. Patients with difficulty in returning for further treatment prove an additional burden to
the physician attempting a plan of management. A quick, yet sensitive, diagnostic test for adenovirus infection is needed.

This study has shown that the immunofluorescent technique applied to detection of group-specific adenovirus antigen in conjunctival scrapings is such a rapid and sensitive technique. This technique detects antigens to adenovirus types 7, 8, and 19 in epithelial cells of conjunctival scrapings. Although the slides had been frozen from two weeks to six months, the immunofluorescent examination was, nonetheless, diagnostic. This indicates the stability of the antigens within the epithelial cells. However, in the usual circumstance, immunofluorescent study would be done within a day or so after conjunctival scraping. In addition to its diagnostic value, early diagnosis of EKC may have some effect on treatment in the future with newer antiviral agents. A previous study of severe EKC implicated a decrease in infiltrates in patients treated early for severe conjunctival reaction. 13 Further study of the influence of topical steroid is under evaluation.

In conjunctival scrapings the adenovirus type 2 antiserum detected the group-specific hexon antigen common to all adenovirus types. No changes in the fluorescent staining pattern were observed with heterotypic types 2 and 11 antiserum applied to adenovirus types 8 and 19 antigen in epithelial cells on conjunctival scrapings. This indicates that the specificity of the adenovirus antiserum is directed toward the hexon antigen shared by all adenovirus types.

The cytoplasmic pattern of fluorescence noted throughout our study is explainable. Electron microscopic work has shown complete viral particles residing in the nucleus of cells infected with adenovirus. No comment is made concerning viral particles in the cytoplasm, but photographs appear to demonstrate incomplete viral particles in the cytoplasm. 15 According to the replication cycle of adenovirus, maturation occurs in the nucleus and the soluble antigens or viral-specific proteins are made in the cytoplasm on cytoplasmic ribosomes. 14 The adenovirus antiserum is interacting with these viral-directed soluble protein antigens. The fluorescence pattern noted is consistent with the tagging of these antigens in the infected cell cytoplasm. Although the virus matures in the nucleus, its viral proteins are available within the cytoplasm. 15 Although nuclear fluorescence was observed, the preponderance of cytoplasmic fluorescence for adenovirus antigen in infected tissue has been shown in another recent study of immunofluorescence in oropharyngeal scrapings. 16

It appears that either one or both factors may play a role in the presence of cytoplasmic fluorescence in infected cells. First, the other IF studies, 2-11 with adenovirus used antisera prepared against the entire virion. The heterotypic antisera used in this study are directed mainly against the capsid-hexon specific adenoviral component. The second factor is that with the known life cycle of the adenovirus in the cell, the accumulation of complete viral antigen in the nucleus occurs late in the virus life cycle. In the clinical situation, the older cells are, for the most part, exfoliated or discharge the virus from the nucleus with cell lysis before being sampled by conjunctival scrapings. The technique of scraping presumably samples the younger epithelial cells which represent an earlier stage of infection. These cells may have virus in the cytoplasm and the IF technique stains these particles and the site of production of the hexon specific antigen of the capsid.

The successful IF identification of adenovirus antigen in our study, compared to that of Ellison, Kaufman, and Little, 4 may be related to the severity of the infection or the response of the human host to different adenovirus infections. The isolation of adenovirus type 3 in the human volunteers in Ellison's study is also very low and may in part explain the very low rate of detection of adenovirus antigens by immunofluorescence. In addition, this was an experimentally induced infection in volunteers and not a naturally acquired infection of a known epidemic ocular pathogen.

The studies by Imre and co-workers 5 showed the presence of cytoplasmic immunofluorescence in conjunctival cells in which cytoplasmic inclusions were noted on Giemsa smears. They found
this pathogen using only homotypic adenovirus antiserum. No mention is made of nuclear fluorescence. The studies by Uchida and Inoue:3

used human convalescent sera with antibody of low titer which may explain the lower rate of positive results in clinical EKC. They observed both nuclear and cytoplasmic fluorescence. The presence of nuclear and cytoplasmic fluorescence may be related to the life cycle of the organism or the use of homologous or heterologous antisera. Further studies are in progress to define the role of antisera specificity in the pattern of cellular immunofluorescence.

In this study the immunofluorescent technique was positive in 43 of 44 (98 per cent) patients with the clinical diagnoses of EKC or PCF. No false-positive tests were found. In only one of 44 cases (2.2 per cent) were the results not confirmed in relation to the clinical diagnosis. On the other hand, virus isolation was less sensitive, revealing five less adenoviral infections. Serology was the least sensitive with only 18 of 32 (56 per cent) seroconversions (Table III). Thus, the immunofluorescent technique was more rapid, sensitive, and reliable than the other diagnostic laboratory procedures currently available.

Addendum. Subsequent to this controlled study additional cases were studied by virus isolation and immunofluorescent detection, and were reported as part of the epidemic to the American Academy of Ophthalmology and Otolaryngology, September, 1975, Dallas, Texas.

Dr. N. Khoobyarian, Department of Microbiology, University of Illinois College of Medicine, provided the rabbit antisera against adenovirus type 2; Dr. M. Mufson, Department of Medicine, University of Illinois College of Medicine, provided rabbit antisera against adenovirus type 11. We acknowledge the assistance of Susan Guth in collection of clinical material, Donna D. Saxtan for technical assistance, Roberta Smith for virus laboratory for assistance in typing adenovirus isolates.

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Key words: adenovirus, epidemic keratoconjunctivitis, pharyngoconjunctival fever, immunofluorescence, viral isolation, laboratory diagnosis.

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