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An outbreak of adenovirus keratoconjunctivitis in Bristol

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SUMMARY Nineteen cases of keratoconjunctivitis caused by an adenovirus serologically related to types 10 and 19 are described. Seventeen of the patients presented over a period of 7 weeks and included 4 who were involved in a minor outbreak at a factory. The presentation and clinical features closely resembled those caused by adenoviruses types 8 and 19. Mild to severe follicular conjunctivitis, superficial punctate keratitis, discrete subepithelial opacities, membrane formation, and conjunctival scarring were all observed.

The term epidemic keratoconjunctivitis was introduced by Hogan and Crawford (1942) when they described an outbreak of this disease, noting that epidemics had been reported previously in several European countries under a variety of different names. In 1955 the causative organism was identified as an adenovirus (Jawetz *et al.*, 1955), and since that time several different adenovirus serotypes have been shown to cause conjunctivitis with and without corneal involvement (Grayston *et al.*, 1964). An outbreak in Bristol in 1971 caused by adenovirus type 8 has been described previously (Barnard *et al.*, 1973), and isolated cases and small outbreaks resulting from infections with types 10 and 19 have been diagnosed elsewhere (Heubner and Rowe, 1957; Desmyter *et al.*, 1974; Burns and Potter, 1976; O'Day *et al.*, 1976; Darougar *et al.*, 1977; Zografos, 1977).

In late September 1977 a number of patients with severe follicular conjunctivitis of probable viral aetiology presented themselves to the Bristol Eye Hospital and a member of the staff developed symptoms.

This paper describes briefly a group of patients with follicular conjunctivitis seen in the Casualty Department of the Bristol Eye Hospital between September 1977 and January 1978. It reports in detail on patients in whom the condition was shown to have been caused by an adenovirus related to both types 10 and 19, but distinguishable from the 2 prototypes.

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Materials and methods

All patients were subjected to slit-lamp examination. In patients with keratitis it was found helpful to consider the cornea in quadrants when recording the distribution of the lesions. Conjunctival swabs and blood samples were taken on presentation and on subsequent visits. Virus isolation was attempted on specimens obtained from 175 patients seen between late September 1977 and January 1978. Single or paired sera were obtained from 116 patients. Serum samples received from patients attending antenatal clinics were used to establish the approximate incidence of antibodies to adenovirus type 10/19 in the normal adult population. Bacteriological investigation was also undertaken on approximately 40% of the patients.

VIRUS ISOLATION

All swabs were sent to the laboratory in 3 ml of milk saline transport medium; 0·2 ml aliquots of this medium were routinely inoculated into cultures of Hep_2 and human embryo diploid fibroblasts (MRC 5) cells. Specimens which failed to yield a virus in these cultures were subsequently examined in cultures of human embryo kidney cells, and, if they were still negative, an aliquot of the original specimen was inoculated into organ cultures of human embryonic conjunctiva (Higgins and Scott, 1973). Isolates were identified by neutralisation tests using specific immune sera. TRIC—*Chlamydia trachomatis* was isolated in cytochalasin-treated McCoy cells (Sompolinsky and Richmond, 1974).

SEROLOGY

Paired and single sera were examined by complement fixation (CF) tests against herpes simplex virus and adenovirus antigens by overnight fixation. A haemagglutination inhibition (HAI) test using a current isolate of adenovirus 10/19 was performed on all the sera except a proportion of those from patients from whom another viral pathogen had been isolated.

Results

The commonest demonstrable cause of conjunctivitis at this time was an adenovirus serologically related to both adenovirus type 10 and type 19 (Tullo and Higgins, 1978). Of the 17 proved infections this virus was isolated from 15 of the 16 from whom swabs were obtained, the other 2 cases being diagnosed on serological grounds. A 4-fold or greater rise in CF antibody was demonstrated in 9 of 13 pairs of sera from proved cases, and an identical proportion showed a diagnostic rise in HAI antibodies. Although CF and HAI antibodies generally paralleled each other, in 2 instances a diagnosis of adenovirus infection could be made by CF but not by HAI and in 2 further cases the converse was true. Two patients with an adenovirus 10/19 infection had high ($\geqslant 96$) serum CF antibody titres and 2, a high ($\geqslant 48$) HAI titre in both sera or in a single convalescent serum sample. Only 5 of 99 sera from patients without proof of adenovirus 10/19 infection had an HAI antibody titre greater than 8, which was essentially the same proportion (7/122) as was found in serum samples obtained from patients in the asymptomatic group attending the antenatal clinics.

The observations on the epidemiology and clinical features of the outbreak of ocular infections with adenovirus 10/19 have been augmented by those made on 2 further cases seen 7 to 8 months previously. The diagnosis was based on isolation of the virus and serology in one case and serology alone in the other. The final total of 19 proved cases consisted of 11 males and 8 females with ages ranging from 19 to 59 years (average 32 years).

Other causes of conjunctivitis during this period included herpes simplex virus in 3 patients and adenoviruses types 3 and 4 in 5 and 1 patient respectively. *Chlamydia trachomatis* was isolated from 1 further patient, but all cultures for bacterial pathogens were negative. Thus a combination of isolation and serological methods enabled a diagnosis to be established for 27 of the patients with follicular conjunctivitis seen during 4 months September 1977 to January 1978.

EPIDEMIOLOGY

Thirteen of the 19 cases infected with adenovirus 10/19 could have acquired the disease from one of the other cases. It was the first patient of the outbreak who was the probable source of infection of a doctor at the Eye Hospital, who first examined him. The only other instance of presumed hospital cross-infection occurred in a 49-year-old woman who was attending the hospital as an outpatient for an anterior uveitis with secondary glaucoma in November 1976. Paired sera, taken in February 1977, when she developed disciform keratitis with central corneal oedema and folds in Descemet's membrane, showed a diagnostic rise in HAI antibodies to adenovirus 10/19. CF antibodies to herpes simplex virus were absent at a dilution of 1/8 on both occasions. This infection occurred at a time when the only other patient with a proved adenovirus 10/19 infection, who was not actually involved in the outbreak, was an inpatient at the hospital. The latter patient had been referred from another eye unit with severe keratoconjunctivitis, and a strain of adenovirus 10/19 was isolated from her conjunctiva.

An important subgroup consists of 4 patients who worked in a factory manufacturing electrical motors. The interval between the onset of symptoms in the first case and the other 3 was 7, 9, and 11 days respectively. One of this group was the factory nurse, who was exposed to infection when one of the other cases, a worker, reported to the first aid centre. This was the only instance of close contact between these patients, as, although all 4 patients knew each other, no other obvious point of contact, for example, common washing facilities, could be elicited.

SIGNS AND SYMPTOMS

The signs and symptoms of the majority of patients were typical of adenovirus keratoconjunctivitis. Patients generally complained of a painful, red, watering eye, sometimes accompanied by a foreign body sensation. Commonly symptoms were first noted on waking and increased in severity to reach a peak usually by the fourth or fifth day. The duration of these symptoms ranged from 10 days to 3 months, lasting 35 days on average. Six patients also complained of malaise and headache. Four patients complained of blurred vision, in each case related to corneal lesions, and 3 of these reported that the degree of visual disturbance varied from day to day. Visual acuity was temporarily reduced to 6/18 (corrected) in the more severely affected eye of each of 2 patients, but no patient has suffered persistent visual impairment.

Of the 19 cases 6 had signs and symptoms in 1 eye only; 4 had uniocular symptoms, but slit-lamp

microscopy revealed evidence of involvement of the other eye. Nine patients had bilateral symptoms and signs, of whom only 1 was affected equally in both eyes. Examination of affected eyes indicated that the cornea and palpebral conjunctiva were characteristically involved (see Table 1).

Conjunctivitis. The papillary reactions was predominantly confined to the upper tarsal conjunctiva while follicles were recorded only in the lower fornix (Figs. 1 and 2). Scarring of the conjunctiva occurred only in those patients who had previously had an inflammatory membrane (Table 1).

Keratitis. There was corneal involvement in 13 of the 19 patients. One patient had a disciform keratitis. Eleven developed discrete stromal corneal opacities, of whom 7 had a previous or concurrent superficial punctate keratitis (Figs. 3 and 4). Four

Table 1 *Distribution and frequency of signs during the course of adenovirus 10/19 infection in 19 patients*

Site	*Sign*	*Number of patients in whom present*	(%)
	Follicles	18	(95)
	Papillae	18	(95)
Conjunctiva	Chemosis	5	(26)
	Inflammatory membrane	7	(37)
	Scarring	7	(37)
	Superficial punctate keratitis	8	(42)
Cornea	Subepithelial lesions	11	(58)
	Disciform keratitis	1	(5)
Regional Glands	Lymphadenopathy	7	(37)

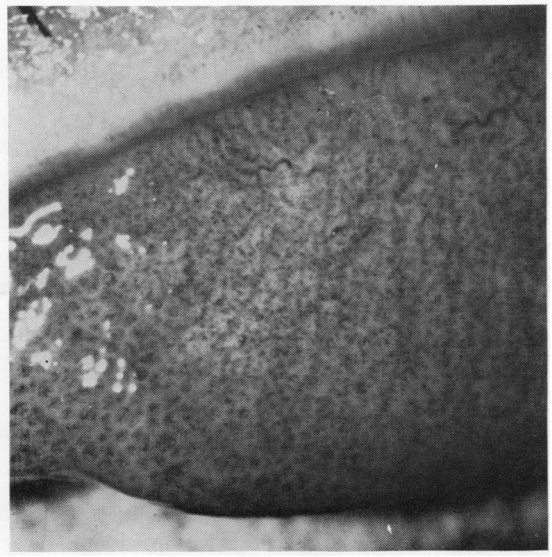

Fig. 1 *Everted upper lid showing papillary reaction*

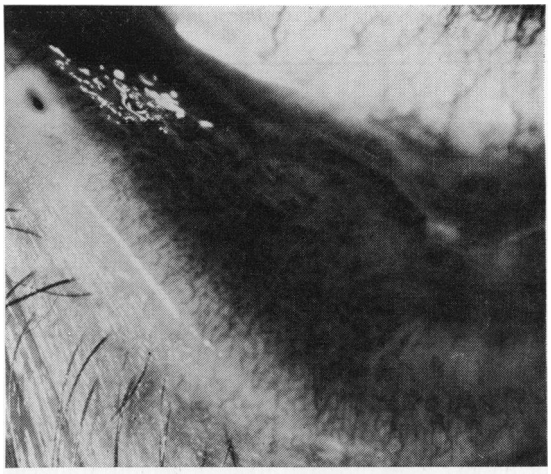

Fig. 2 *Lower fornix showing follicular reaction and conjunctival scarring*

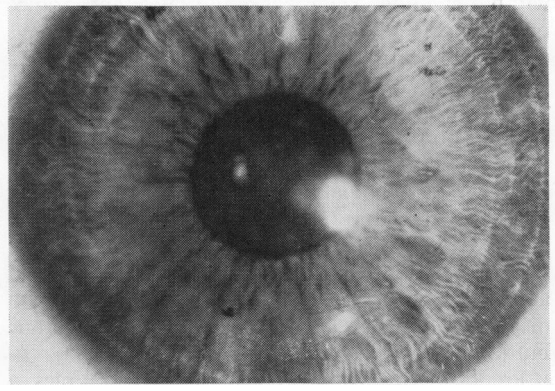

Fig. 3 *Stromal opacities seen against the pupil*

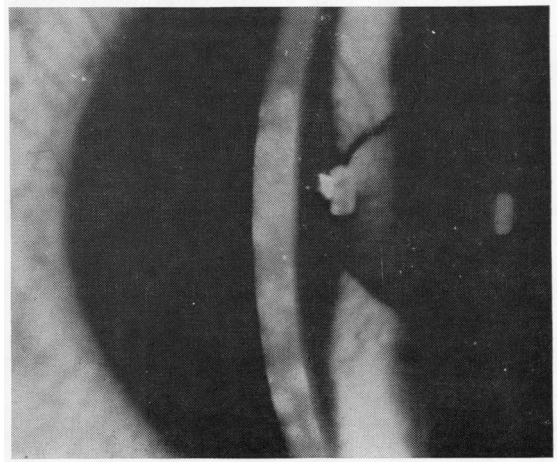

Fig. 4 *Numerous corneal subepithelial lesions seen in slit-lamp photograph*

Table 2 *The response to treatment in each eye of one patient, as measured by the number of stromal lesions and visual acuity*

Day	Right eye Treatment (guttae)	Number of lesions	VA	Left eye Treatment	Number of lesions	VA
0	Prednisone 0·25%	10	6/9	Nil	10	6/9
7	Betamethasone	10		Nil	13	
14	Betamethasone	8	6/5	Guttae betamethasone	20	6/6
21	Betamethasone	3	6/6	Guttae betamethasone	11	6/6
	Betamethasone continued for a further 20 days, then stopped. Patient reviewed after 14 days of no treatment					
55	Betamethasone	24	6/18	Nil	11	6/6
71	Betamethasone	13		Nil	6	
78	Betamethasone	7	6/6	Nil	3	6/6
	Treatment discontinued					

of these 7 were observed to have a more transient intermediate lesion which was immediately subepithelial and caused elevation of the epithelium without staining. The earliest and latest appearance of either subepithelial lesion was 10 and 35 days respectively with an average of 19 days.

TREATMENT

There is no proved treatment for adenovirus keratoconjunctivitis in the early stages, but antibiotics may be of value in palliating symptoms and preventing secondary infection (Dudgeon *et al.*, 1969; Dawson *et al.*, 1970). All patients in this outbreak received topical chloramphenicol initially. One patient had additional treatment with acetyl cysteine 10%, with dispersion of a developing inflammatory membrane but failure to prevent conjunctival scarring.

Prednisolone drops (Predsol) 0·25%, 4 times a day, were given to all patients who developed subepithelial lesions. With an uncomplicated response and persisting lesions the dose of steroids was increased by giving betamethasone (Betnesol) drops 4 times a day. Partial or complete resolution of the corneal lesions was noted in all patients thus treated.

Three patients merit further mention of their response to treatment. One, the brother of a patient seen during the acute stage, presented 6 weeks after the onset of symptoms because he had developed blurring of vision. Examination of the affected eye revealed a 'snow-storm'-like cornea with profuse stromal lesions (approximately 120). He was treated with betamethasone drops for 16 days and his vision improved from 6/9 to 6/5, by which time the lesions were scarcely discernible. He has suffered no further episodes of blurred vision.

The second patient was equally affected in both eyes, and 38 days after onset of symptoms he was left with 10 subepithelial opacities in each. Initially steroid therapy was restricted to 1 eye, the untreated eye serving as a control, but when the treated eye improved and the untreated deteriorated steroids were instilled into both eyes (Table 2).

A third patient, first seen in January 1977, had developed subepithelial opacities by 3 weeks after the onset of symptoms and the visual acuity had fallen to 6/18 in each eye. With steroid therapy she improved, and when she was followed up 9 months later 8 stromal opacities were noted to have persisted in the central cornea of the right eye. The visual acuity was 6/9 and 6/5 in the right eye and left eye respectively. At this time she was experiencing intermittent blurring of vision in the right eye. She was last seen 18 months after the initial infection and still complained of blurring of vision which gave rise to difficulties with her clerical job. Examination revealed a white eye with approximately 30 faint large opacities immediately subepithelially, mostly in the upper marginal cornea of the right eye. She had a Hudson-Stahli line in the right eye and a small central area of pigmentation in the left, neither of which was previously present.

Discussion

The epidemiological and clinical features of these adenovirus 10/19 infections were very similar to those found in 'classical' adenovirus keratoconjunctivitis caused by type 8 and more recently by type 19 (O'Day *et al.*, 1976). Spread of the 10/19 virus within the community was uncommon, with only 5% of the adults having antibodies, a similar proportion to that found previously in Bristol for type 8 adenovirus (Barnard *et al.*, 1973). This might be expected, as adenoviruses types 8, 10, and 19 all belong to the same subdivision, group II, 1 of 4 groups into which adenoviruses are divided on their haemagglutination properties (Rosen, 1960). The term 'epidemic keratoconjunctivitis' perhaps now

justifies the inclusion of these serotypes rather than specifically referring to infections caused by serotype 8. Of other adenoviruses commonly associated with conjunctivitis types 3 and 7 belong to group I and type 4 to group III. None the less, the variation in severity of the symptoms and the lack of pathognomonic signs makes the accurate diagnosis of adenovirus follicular conjunctivitis difficult. Eleven such cases in this study were shown to be infections with agents other than the adenovirus type 10/19. The close correlation between the results of virus isolation and serology suggests that very few, if any, of the cases in which a diagnosis was not established were in fact infections with adenovirus 10/19.

The ease with which conjunctivitis caused by adenovirus type 8 can be spread, especially by medical personnel, is notorious (Thygeson, 1957; Dawson *et al.*, 1972). The relatively few secondary cases in this outbreak would indicate that the organism was of low virulence or, more probably, that preventive measures recommended previously (Clarke *et al.*, 1972), such as hand washing between examining patients, were effective.

While the constituent features of patients with this infection were typical of group II keratoconjunctivitis, the incidence and degree of chemosis was notably less than that sometimes seen in outbreaks caused by other serotypes (Hart *et al.*, 1972). The proportion of patients who developed conjunctival scarring was high (Table 1).

In this study the average interval from the onset of symptoms to the appearance of subepithelial opacities was 1 to 2 weeks. However, the interval to opacity-related symptoms may be considerably longer, as shown by the patient who presented for the first time 6 weeks after the initial infection complaining of blurred vision. Subepithelial opacities were always more pronounced in the eye in which symptoms developed first, and these lesions are liable to become more pronounced and numerous after the abrupt stoppage of steroids (Table 2). The incidence of subepithelial lesions (58%) was lower than that described in infections caused by adenovirus type 8 (82%) (Barnard *et al.*, 1973).

It is thought that punctate subepithelial lesions result from the interaction of viral antigen and antibody in the anterior stroma, the cornea acting as a blotter (Jones, 1958). At the time of infection the virus becomes established in the epithelium, producing a superficial punctate keratitis. Infective virus is seldom demonstrable after 10 to 14 days, but this does not exclude the continued production of viral antigens (Pereira, 1972). That the epithelium is important in facilitating this antigenic stimulus is suggested by the fact that in patients with epithelial erosions the disease rarely progresses to the stage of typical subepithelial infiltrates (Laibson, 1975).

The patient who developed a disciform keratitis is of considerable interest. She was the only patient to have neither follicles nor papillae. Not only are these unusual features of adenovirus infection, but she was the only patient to have had previous ocular disease. Although anterior uveitis occurs in adenovirus disease, the diagnostic rise in CF antibody titre to adenoviruses from $<1/8$ during the development of the disciform keratitis suggests that the preceding uveitis had a different cause. Other investigations were negative. In the disciform keratitis of herpes simplex virus there is evidence of delayed hypersensitivity (Swyers *et al.*, 1967). However, it is not clear how the mechanisms producing discrete punctate stromal lesions and disciform keratitis differ; indeed, both may occur together (Hogan and Crawford, 1942). On clinical grounds this patient was treated as a case of herpetic keratitis and given topical idoxuridine and low-dosage steroids. The diagnosis was made in retrospect on a rise in HAI antibodies. Gradual resolution took place over a period of 8 months, and she was left with a Hudson-Stahli line in the affected eye, but the cornea was otherwise normal. She was thus the second patient observed to develop such pigmentation during the late follow-up.

The response observed to the application of topical steroids substantiates previous reports of their usefulness in resolving subepithelial lesions (Dawson *et al.*, 1972). As it is rare for stromal opacities to occur without preceding superficial punctate keratitis, it is possible that many stromal lesions are preventable, although the use of prophylactic steroids with its attendant risks is debatable (Laibson *et al.*, 1970), and withdrawal of steroids may precipitate a recurrence of stromal opacities (Table 2).

Further investigations are proceeding to establish the precise relationship between the adenovirus involved in this outbreak and the type 10 and 19 prototype viruses. Meanwhile, it is interesting to note that an adenovirus showing similar antigenic cross-reactions has been isolated in Holland in association with small outbreaks of keratoconjunctivitis resembling the one reported here (de Jong *et al.*, 1978). An awareness that such variants are circulating will serve to prevent confusion in the typing of strains isolated from future outbreaks of epidemic keratoconjunctivitis.

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