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Diagnosis of herpes virus hominis keratitis by immunofluorescence

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The high prevalence and destructive effect on the human cornea of herpes virus hominis (HVH) makes its detection desirable at an early stage in eye involvement (Duke-Elder, 1965).

The action of the virus in the human cornea, at least when associated with active corneal disease, can produce a keratopathy with certain well-known clinical signs. The presence of one or more of these clinical signs may be pathognomonic for the existence of HVH in the cornea; but where clinical doubt exists as to the real presence of the virus in a diseased cornea the virologist may demonstrate the virus in the cornea by examination of infected corneal epithelium, usually on tissue culture. Many cell lines, such as human amnion (Scott, McNair, Coriell, Blank, and Gray, 1953) or human diploid cell lines (Scherer and Syverton, 1954) show cytopathic effects suggestive of HVH in 1 or 2 days, but this still needs confirmation by a neutralization test. Also, isolation may be difficult when the virus is passing through an "eclipse" phase (Kaufman, 1960).

Quicker and probably simpler ways of establishing the existence of HVH in infected material are available in the laboratory and might fill a gap in the present range of diagnostic procedures for this virus. Biegeleisen, Scott, and Lewis (1959), first showed that it was possible to detect HVH in skin cells by immunofluorescence. Then Kaufman (1960) used immunofluorescence to show HVH in corneal cells from patients with "dendritic ulcers". Pettit, Kimura, and Peters (1964) used similar methods in their investigation of corneal disease. Gardner, McQuillin, Black, and Richardson (1968) have referred to the use of a fluorescent antibody technique in investigating skin and corneal HVH infections.

Immunofluorescence (IFL) seems to be a technique which is applicable as a valuable and rapid diagnostic test in the diagnosis of herpetic infections of the cornea and the study to be described is an attempt to confirm the usefulness of IFL in this context.

Material and methods

Thirty patients with keratopathy were included in the study. The cases were divided into two groups: twenty with "dendritic ulcers" (*i.e.* presumed HVH keratitis) and a heterogeneous group of ten presumed non-herpetic keratopathies. In each case corneal epithelium was removed and examined as described below.

After the cornea had been anaesthetized with Novesine the specimens for examination were taken by scraping off the corneal epithelium with a sterile scalpel blade. The fragments were placed in two spots of phosphate buffered saline on a slide and large fragments were broken up by teasing with needles. The spots were allowed to dry in air and then immediately fixed for 10 minutes in acetone at 4°C.

The scalpel blade was thoroughly rinsed in Hanks medium containing 0·2 per cent. bovine albumin, and a swab was also taken from the area and broken into the same media, which was then used for the culture of the virus; specimens were transported to the laboratory on ice.

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ANTI-HERPETIC RABBIT SERUM

This was prepared by inoculating rabbits with an egg-culture virus. The antiserum was prepared and supplied to us by Winthrop Biologicals Limited.

FLUORESCEIN-CONJUGATED ANTI-RABBIT GLOBULIN (FLUOROSCAN PLUS)

This has been fully described in previous communications and was again supplied to us by Winthrop Biologicals Limited.

VIRUS ISOLATION TECHNIQUES AND NEUTRALIZATION TESTS

The cell lines used for cultures were HEp2, HeLa, WI 38. Virus isolates were grown in duplicate tissue culture tubes, one of which was used to confirm the identity of the virus by a routine neutralization test, based on the method previously described (Andrew and Gardner, 1963). Cells from the second tube were scraped off and examined by fluorescent antibody technique, the slides being prepared by the same method as previously described for the examination of tissue culture cells infected with respiratory syncytial virus (RSV) (McQuillin and Gardner, 1968; Gardner and McQuillin, 1968).

PREPARATION OF SLIDES FOR STAINING AND EXAMINATION

Slides from tissue culture material contained three spots—one containing infected cells for treatment with normal rabbit serum, another containing infected cells for treatment with herpes virus antiserum, and the last having uninfected cells of the same batch for treatment with herpes virus antiserum. These spots were then stained with Fluoroscan Plus (FP). Slides of corneal scrapings contained two spots only, one for treatment with herpes virus antiserum and the other with normal rabbit serum, both were then stained with FP. For full details of this method consult Gardner and others (1968).

FLUORESCENT MICROSCOPY

Specimens were examined under dark ground illumination by blue light using a Wild M20 fluorescent microscope with an Osram HBO 200 mercury vapour lamp, a blue BG 12/4 mm. exciter filter, and an OG 1 barrier filter. Photographs were taken on the same microscope, exposures were all 180 sec. duration using an Ilford Mark V film.

Results

(1) SPECIFICITY OF HERPES VIRUS ANTISERUM

(*a*) Uninfected tissue culture cells and also monkey kidney tissue culture cells were tested with HVH antiserum on innumerable occasions and showed no evidence of non-specific staining.

(*b*) The following viruses growing on tissue culture were treated with herpes virus antiserum and subsequently stained with FP:

Influenza A	Echoviruses 9, 12, and 30	Parainfluenza virus type 3
Poliovirus types 1, 2, and 3	M type rhinovirus	Adenoviruses 1, 2, and 5
	Coxsackie viruses types B_3 and B_5	

None showed any non-specific fluorescence with this antiserum and FP.

(2) CONFIRMATION OF HERPES VIRUS HOMINIS INFECTION BY IFL

Twenty cases of presumed herpetic keratitis (all "dendritic ulcers") were subjected to corneal scraping as described above and the material tested in the manner outlined. In addition ten cases of presumed non-herpetic keratitis were subjected to corneal scraping and the material tested as described above.

Table I summarizes the results of the investigation of the twenty cases of "dendritic ulcer".

Table I *Results in 20 cases of presumed herpes virus hominis infections*

Case no.	*Age (yrs)*	*Sex*	*Clinical diagnosis of dendritic ulcer*	*Steroid treatment*	*Corneal disease*	*Result of fluorescent antibody test*	*Virus isolation*
1	59	M	Typical	No	F	+	+
2	60	F	Atypical (after herpes zoster keratitis)	Yes	F	+	−ve
3	55	M	Typical	NK	F	+	+
4	59	M	geographic	No	F	+	
5	30	F	Typical	No	F	+	+
6	65	M	Typical	NK	R	+	+
7	68	M	Typical	Yes	F	+	+
8	18	F	Typical	NK	F	+	+
9	58	F	Typical	Yes	F	+	+
10	66	M	+	NK	F	−ve	−ve
11	54	F	Typical	Yes	F	+	+
12	55	F	Atypical	NK	F	+	+
13	43	M	+	No	R	+	+
14	34	M	+	No	R	+	+
15	46	M	+	Yes	F	+	+
16	7	M	+	No	R	+	+
17	59	M	+	NK	R	+	+
18	25	M	+	No	F	+	+
19	50	M	+	NK	R	+	+
20	50	F	+	NK	NK	+	+

F = First attack of corneal disease
R = Recurrent corneal disease
NK = Not known
+ = Positive
−ve = Negative (no intracellular fluorescence)

Scrapings from nineteen of the twenty cases of "dendritic ulcer" showed cells stained specifically with antiserum and fluorescein labelled anti-rabbit globulin. Fig. 1 shows this characteristic appearance in stained scrapings. One case was negative and the diagnosis was subsequently changed to recurrent erosion. Culture was positive in all but two cases, the latter including the case of recurrent erosion.

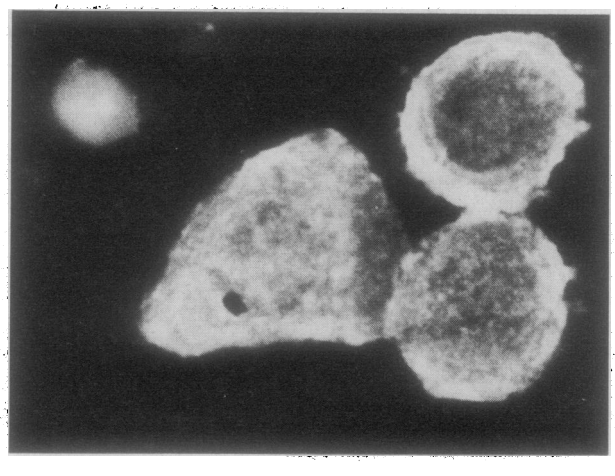

FIG. 1 *Characteristic appearance of herpes virus hominis in scrapings stained by the fluorescent antibody technique*

Table II summarizes the results of examining scrapings from ten patients with presumed non-herpetic keratitis; all were negative both on fluorescence and culture.

Table II *Results in* 10 *cases of presumed non-herpetic keratitis*

Case no.	*Clinical diagnosis*	*Result of fluorescent antibody test*	*Virus isolation*
1	Marginal ulcer	−ve	−ve
2	Disciform keratopathy	−ve*	−ve*
3	Corneal ulcer	−ve	−ve
4	Recurrent corneal ulcer (? dendritic)	−ve	−ve
5	Corneal ulcer	−ve	−ve
6	Superficial punctate keratitis	−ve	−ve
7	Recurrent corneal ulcer	−ve	−ve
8	Superficial punctate keratitis	−ve	−ve
9	Contact lens abrasion (dendriform pattern)	−ve	−ve
10	Corneal ulcer with blepharo-conjunctivitis	−ve	−ve

* Scrape and anterior chamber puncture

Fig. 2 shows a negative staining pattern and Fig. 3 shows cells from a tissue culture infected with HVH and stained by the fluorescent antibody technique. In all cases a report on the immunofluorescence is possible in about 3 hours.

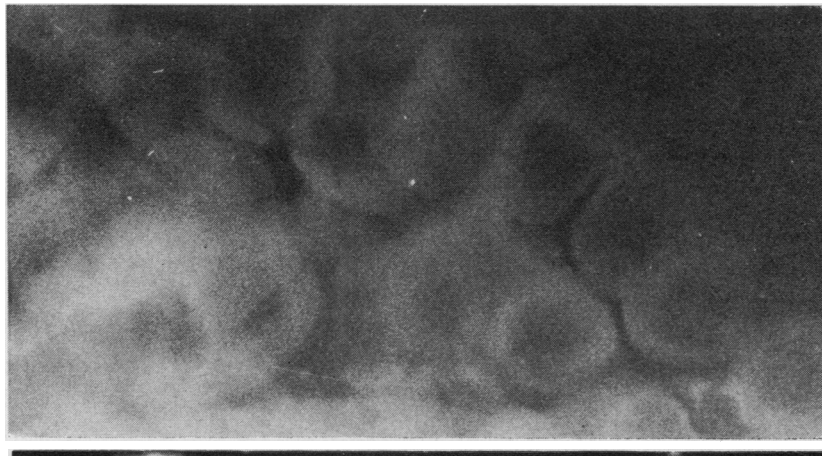

FIG. 2 *Negative staining pattern in scrapings*

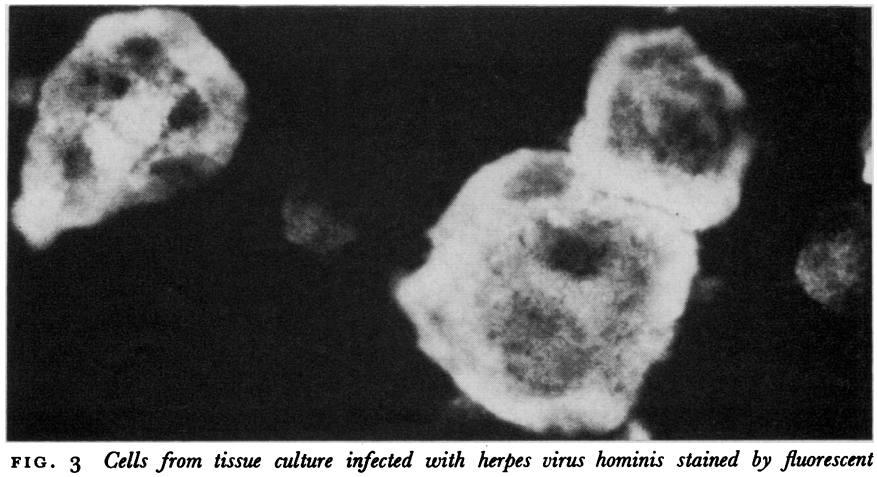

FIG. 3 *Cells from tissue culture infected with herpes virus hominis stained by fluorescent antibody technique*

Discussion

The study has confirmed that, by immunofluorescence, rapid identification of HVH in corneal epithelium is readily possible. It is a test on which the clinician can place great reliance and thus has a definite place in the clinical management of corneal disease. Previous administration of steroids does not appear to affect the immunofluorescent response and the test is equally effective in primary or recurrent herpetic keratopathy. In this clinic the test is used as a routine in all cases of corneal disease in which epithelial debridement is required at any stage, and when clinical doubt exists as to the nature of a keratopathy, corneal scraping for immunofluorescent testing is undertaken in addition.

Its apparent specificity and speed makes the test acceptable as a routine measure and casts doubt on the need for the older, more time-consuming methods of culture, isolation, and neutralization.

Summary

An immunofluorescent method of establishing the presence of herpes virus hominis in corneal epithelium is described. Results are given of its use in thirty patients.

References

ANDREW, J. D., and GARDNER, P. S. (1963) *Brit. med. J.*, **2**, 1447
BIEGELEISEN, J. Z., JR., SCOTT, L. V., and LEWIS, V., JR. (1959) *Science*, **129**, 640
DUKE-ELDER, S. (1965) "System of Ophthalmology", vol. 8, pt 1, p. 307. Kimpton, London
GARDNER, P. S., and MCQUILLIN, J. (1968) *Brit. med. J.*, **3**, 340
————, ————, BLACK, M. M., and RICHARDSON, J. (1968) *Ibid.*, **4**, 89
KAUFMAN, H. E. (1960) *Arch. Ophthal. (Chicago)*, **64**, 382
MCQUILLIN, J., and GARDNER, P. S. (1968) *Brit. med. J.*, **1**, 602
PETTIT, T. H., KIMURA, S. J., and PETERS, H. (1964) *Arch. Ophthal. (Chicago)*, **72**, 86
SCHERER, W. F., and SYVERTON, J. T. (1954) *Amer. J. Path.*, **30**, 1057
SCOTT, T. F. MCNAIR, CORIELL, L. L., BLANK, H., and GRAY, A. (1953) *J. Immunol.*, **71**, 134