Such an analogy should not be pushed too far, however, given the limitations of sample size. Nevertheless, the cross-species similarity in the pattern of visual deficits and anatomical abnormalities suggests that albino animals could represent a valid model of the human albino condition.

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From the University of Western Ontario, London, Canada. This research was supported by grant MA 7125 from the Medical Research Council of Canada. Submitted for publication May 18, 1981. Reprint requests: Brian Timney, Department of Psychology, The University of Western Ontario, London, Canada N6A 5C2.

Key words: albinism, contrast sensitivity, perimetry, optic decussation

REFERENCES

Photopic and scotopic flicker sensitivity of a rod monochromat. BERNT CHRISTIAN SKOTTUN, KNUD NORDBY, AND SVEIN MAGNUSSEN.

The sensitivity to sine-wave flicker of a rod monochromat was compared with that of a normal subject at photopic and scotopic levels of luminance. The sensitivity of the rod monochromat in the low-frequency region (below 3 Hz at scotopic levels and below 12 to 14 Hz at photopic levels) was found to be superior to that of the control. This superiority was most pronounced at photopic levels, where the rod monochromat frequently showed a two-peak sensitivity curve. (INVEST OPHTHALMOL VIS SCI 21:877-879, 1981.)

In one previous study, the flicker sensitivity (temporal modulation transfer function, MTF) of a rod monochromat has been compared with that of a normal subject.¹ The results suggest a close similarity to normal vision except for an overall reduction in sensitivity. We have performed similar experiments on another rod monochromat and have found that although the high-frequency sensitivity of the rod monochromat is inferior to that of a control with normal vision, low-frequency sensitivity is clearly superior.

Methods

Subject. Our subject (author K. N.) is a 35-year-old man who shows all signs of typical rod monochromacy: low visual acuity (0.1 on both eyes in conventional tests, 0.12 when tested with laser-interference gratings), small central scotoma (Goldman-Projection Perimeter, object I, intensities 2 and 3), achromacy (Nagel anomaloscope) and congenital nystagmus (electro-oculography performed). His dark-adaptation curve shows only one branch typical of rod vision, as previously shown.²
Fig. 1. Photopic MTFs for rod monochromat and normal control subject. Data are expressed as the amplitude of luminance modulation (\(\frac{L_{\text{max}} - L_{\text{min}}}{L_{\text{max}} + L_{\text{min}}}\)), where \(L\) denotes luminance) necessary to detect flicker as a function of temporal modulation frequency, flicker sensitivity being the reciprocal of this quantity. Time-average retinal illuminance was 300 trolands photopic and 650 trolands scotopic. Each data point represents the mean of five threshold determinations. Curves were fitted by eye.

**Apparatus.** Sinusoidal flicker was generated on the face of a Tektronix T 922 oscilloscope with P 31 phosphor. The screen was masked off to present a spatially homogenous, flickering annulus with an inner diameter of 4.8° and an outer diameter of 12° visual angle. This configuration was chosen for two reasons. First, the dark, nonflickering center served as a rough fixation control for the subject. Second, differences between the rod monochromat and the normal subject would less likely reflect differences between central and peripheral vision. The screen was viewed monocularly from a distance of 40 cm, and head and chin rests were used. The rod monochromat was allowed to wear glasses (+6 D) during the experiments. Since no artificial pupil was used (because of the nystagmus), normal pupil size, as listed by Le Grand,\(^3\) was assumed for the conversion of luminance measures into retinal illuminance values. The validity of this assumption was confirmed by photographic assessment of the pupil size under both photopic and scotopic conditions.

Flicker thresholds (criterion, just flickering) were determined by the method of adjustment. Subject K. N. was tested repeatedly over a period of several months, and the results showed a high degree of stability.

**Results and discussion.** Fig. 1 shows a typical photopic MTF for the rod monochromat, together with data for a visually normal control subject. Confirming previous reports,\(^1\) \(^2\) \(^4\) the temporal MTF of the rod monochromat showed a steep decline in the high-frequency region with a cut-off at about 20 Hz, compared with about 50 Hz for the control. Below 10 to 15 Hz, however, the monochromat's sensitivity exceeded that of the control, at the low frequencies by more than half a log unit. Similar differences, although less pronounced, were found at scotopic levels, as shown in Fig. 2.

The photopic results showed a generally higher flicker sensitivity and a more pronounced low-frequency attenuation than those previously obtained from the same subject at comparable luminances.\(^2\) Several experimental differences, however, make a comparison difficult; for example, in
the previous study, we employed a homogeneous flickering field embedded in a large steady surround rather than the annulus used in the present study. The results in Fig. 1 have been reproduced several times, both with the rod monochromat and with different control subjects.

Another characteristic of the rod monochromat's photopic MTF with this stimulus configuration is the depression in sensitivity in the 5 Hz region, found in about two thirds of the 35 curves obtained. We have at present no good explanation of this finding. It is tempting to interpret the double-peak curve as reflecting the presence of a second receptor mechanism, but we hesitate to do so, since the subject's dark-adaptation curve and the ophthalmologic examination failed to reveal any trace of a second system. An alternative theory is that the nystagmus may have created spatiotemporal interference effects. Electro-oculographic recordings under similar conditions gave a nystagmus frequency of about 4 Hz, lending support to this view. We therefore suggest that nystagmus selectively suppresses flicker sensitivity at certain frequencies in a way perhaps similar to that of saccadic suppression.

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**Key words:** rod monochromat, flicker sensitivity, temporal modulation transfer function

**REFERENCES**


**Distribution of pemphigus and pemphigoid antigens, laminin, and type IV collagen in corneal epithelium.** Sheldon J. Cowen, Grant J. Anhalt, Max S. Wicha, Alan Sugar, Ramsy S. Labib, and Luis A. Diaz.

Dog corneas were studied by indirect immunofluorescence with antibodies specific for pemphigus and pemphigoid antigens, laminin, and type IV collagen. The distribution of these antigens was similar to that observed in other squamous epithelia and skin. This study provides further evidence for close immunohistochemical similarities between these tissues. (Invest Ophthal Mol Vis Sci 21:879-882, 1981.)

The corneal epithelium and the epidermis are protective tissues derived from embryonic ectoderm. They have many structural and biochemical similarities. It is the purpose of this investigation to further study some immunohistochemical characteristics of these tissues. We have used antibodies specific for certain epidermal cell surface and extracellular components and studied their in-vitro binding in corneal epithelium. The antibodies used were specific for pemphigus vulgaris and bullous pemphigoid antigens, laminin, and type IV collagen. The binding pattern of these antibodies was identical in corneal epithelium and in epidermis. This study provides further evidence of close immunohistochemical similarities between these tissues.

**Materials and methods**

**Corneal specimens.** Dog corneas were dissected from the globes and snap frozen in liquid nitrogen. Four-micron thick cryostat sections were used as substrate for immunofluorescent (IF) techniques.

**IF techniques.** These techniques were performed as previously reported. Corneal cryostat sections were incubated with dilutions of test sera and IgG fractions. Control sections were incubated with similar dilutions of a normal human IgG fraction and normal rabbit and sheep sera. After a washing step, fluorescein isothiocyanate (FITC)--conjugated antisera were applied (rabbit FITC--anti-human IgG: fluorescein-protein ratio ([F/P] = 3.39/µg/ml, total protein [TP] = 20 mg/ml, goat FITC--anti-rabbit IgG: F/P = 2.4 µg/ml, TP = 24 mg/ml, rabbit FITC--anti-sheep IgG: F/P = 2.64 µg/ml, TP = 15 mg/ml). The treated sections were studied under a Zeiss
Fig. 1. A, Normal dog cornea. (hematoxylin and eosin; ×344). B, Dog cornea, indirect IF with IgG fraction from pemphigus vulgaris serum and FITC-conjugated goat–anti-human IgG. Pemphigus antigen is demonstrated in the intercellular space of the epithelium. (×344.) C, Dog cornea, indirect IF with IgG fraction from bullous pemphigoid serum and FITC-conjugated goat–anti-human IgG. Pemphigoid antigen is demonstrated at the epithelial–stromal junction. (×344.)

Antisera. The IgG fractions from the sera of patients with pemphigus and pemphigoid were obtained by ammonium sulfate precipitation and ion exchange chromatography. A 1 mg/ml solution of the IgG fractions showed an indirect IF titer of 1:160 for pemphigus and 1:2560 for pemphigoid antibodies when tested against rat tongue epithelium substrate. Rabbit anti–type IV collagen and sheep anti-laminin sera were kindly supplied by Dr. L. A. Liotta from the laboratories of Pathophysiology of the National Institutes of Health, Bethesda, Md. The preparation and specificity of these antisera have been fully described in previous communications.

Absorption of antisera. An aliquot of sheep anti-laminin serum (200 μl) was incubated with 50 μg of laminin purified by previously published methods, and an aliquot of rabbit anti–type IV collagen serum (100 μl) was incubated with 20 μg of purified type IV collagen at 37° for 30 min. After centrifugation, the supernatant was tested by immunofluorescence as described.

Results. This study shows that the surface of corneal epithelial cells from dog contains antigen(s) that react with pemphigus antibodies, producing a similar IF pattern to that observed in other squamous epithelia and skin (Fig. 1, B). The epithelial–stromal junction of these corneas contained antigen(s) that reacted with pemphigoid antibodies and with antibodies specific for laminin and type IV collagen. A linear IF pattern similar to that described in skin and other epithelia was produced by all three antisera in the junctional zone (Figs. 1, C, and 2, A and B). Control IgG and normal rabbit and sheep serum produced no staining of the cornea. Sheep anti-laminin serum that had been preincubated with purified laminin and rabbit anti–type IV collagen serum preincubated with type IV collagen both showed absence of basement membrane zone fluorescence, demonstrating the specificity of these reactions.

Discussion. This investigation demonstrates that pemphigus and pemphigoid IgG and antisera specific for laminin and type IV collagen react with antigenic moieties of the extracellular matrix of corneal epithelium. The distribution of these antigens in cornea, as demonstrated by IF methods, is similar to that seen in the skin. It is conceivable, therefore, that these corneal antigens are located in the same spaces or structures as in the cutaneous counterpart, i.e., pemphigus antigen in the intercellular spaces of the corneal epithelium, pemphigoid antigen and laminin in the lamina lucida of the epithelial–stromal junction, and type IV collagen in the basal lamina.

Pemphigus and pemphigoid antigens are pres-
Fig. 2. A, Dog cornea, indirect IF with sheep anti-laminin and FITC-conjugated rabbit-anti-sheep IgG. Laminin is demonstrated at the epithelial-stromal junction (arrow). (×352.) B, Dog cornea, indirect IF with rabbit anti-type IV collagen and FITC-conjugated goat-anti-rabbit IgG. Type IV collagen is demonstrated at the epithelial-stromal junction (arrow). (×352.)

ent in normal squamous epithelia from mammals and other vertebrates. Their physiologic role is unknown. Laminin and type IV collagen have been implicated in many cell-matrix interactions, including cell-substrate adhesion. Under normal conditions the immune system is tolerant to these antigens. However, in patients with pemphigus and pemphigoid, altered immune tolerance leads to auto-sensitization to squamous epithelial cell surface antigens and production of autoantibodies. Pemphigus and pemphigoid autoantibodies are detected in the sera and are bound to the target epithelial tissues in these patients.

An increasing body of experimental data implicates the autoantibodies as being primarily pathogenic in the disease. Recently our laboratory has succeeded in inducing an inflammatory corneal disease in rabbits by intrastromal injection of pemphigoid IgG. This corneal model has reproduced in vivo the lesions of pemphigoid. The primary involvement of corneal epithelium in pemphigus and pemphigoid patients is not clinically apparent despite the elevated titers of these circulating autoantibodies in the sera. This clinical observation may result from the relative immunologic isolation of the corneal epithelium.

In summary, this study shows that corneal epithelium contains pemphigus and pemphigoid antigens, laminin, and collagen type IV. It is expected that the corneal epithelium may be used as a source of these molecules in purification studies and as a target tissue for pathogenic autoantibodies (pemphigus and pemphigoid) in experimentally induced corneal epithelial disease.

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Quantitation of herpes simplex virus in vitro and in preocular tear film. Mary T. Green and Edmund C. Dunkel.

Current methods for virus isolation from preocular tear film are not quantitative. This report presents a sensitive method for detection and quantitation of herpes simplex virus type 1 in unknown samples of small size (<50 μl). Serial tenfold dilutions of stock virus were inoculated onto 16 mm vero cell monolayers, which were monitored for the development of cytopathologic evidence (CPE) for presence of virus and were assigned a severity grade. Standard curves were developed on the basis of time interval between inoculation, recognition of CPE, and CPE score. These curves were used to predict virus titers in unknown samples. This method for virus isolation is simple, efficient, and consistently sensitive to virus titers of <10 plaque forming units. (Invest Ophthalmol Vis Sci 21:882-886, 1981.)

Ocular infection with herpes simplex virus (HSV) accounts for more visual morbidity, medical expense, and loss of productivity among otherwise healthy individuals than any other ocular infection in Western countries. Because there is currently no method for quantitation of infectious virus in preocular tear film, a management of herpetic keratitis depends on clinical parameters and the cultural assay for presence or absence of virus. This report describes a quantitative method for virus isolation from preocular tear film, which is applicable to both clinical management of herpetic disease and to efficacy testing of new antiviral agents.

Materials and methods

In vitro study. Vero (CCL81; Flow Laboratories) and primary rabbit kidney (PRK) cell monolayers were grown to confluence in either 16 mm (Costar Clusters; Microbiological Assoc., Inc.) or 5 mm wells (Microtiter Plate; Falcon Plastics). Tenfold dilutions of McKrae strain HSV-1 stock containing 10^4, 10^3, 10^2, 10^1, and 10^0 plaque forming units (PFU) in 50 μl aliquots were inoculated directly onto four 5 mm and four 16 mm wells containing confluent vero cell monolayers. Identical aliquots were inoculated onto PRK cell monolayers. After adsorption for 20 min at 37°C in 5% CO₂ with agitation every 10 min, each monolayer was rehydrated with 500 μl minimal essential medium (MEM) containing 10% fetal calf serum (MEM 10-10; Gibco). All monolayers were observed daily for 10 days via inverted microscopy for cytopathologic evidence (CPE) consistent with HSV-1 infection. If present, CPE was assigned a severity score of 0.5+ if 10% to 15% of the monolayer exhibited CPE, 1+ if 25% of the monolayer exhibited CPE, 2+ for 50% CPE, 3+ for 75% CPE, or 4+ for 100% CPE. Negative cultures were confirmed by blind passage. In the second part of this study, tenfold dilutions of HSV-1 stock containing 10^0 to 1 PFU in 50 μl aliquots were inoculated directly onto cotton-tipped swabs (Scherer Medical Scientific, Inc.). Swabs were processed as if they were ocular tear film cultures.