
Cytological changes in the conjunctiva in the megaloblastic anemias. J. D. Brodrick, I. M. Strachan, and T. Speed.

An attempt has been made to demonstrate increased nuclear size of the conjunctival cells in the megaloblastic anemias. Conjunctival scrapings were taken, wet fixation employed, and nuclear diameters measured under the microscope. The resulting data was subjected to statistical analysis. Two approximate tests of the null hypothesis that the affected group has the same nuclear size as the control group have been performed. Both tests reject this hypothesis at the 5 to 10 per cent level.

Cytologic changes in the mucous membrane of the mouth and vagina are well recognized in patients suffering from megaloblastic anemia. The nature of these changes is similar irrespective of whether folic acid or vitamin B12 deficiency is the underlying cause and it would be somewhat surprising if similar cytologic changes did not occur in the conjunctiva. As far as we are able to ascertain, the only attempt made to find these expected changes led to the conclusion that there was no alteration of the conjunctival cytology in megaloblastic anemia.1

This communication deals with the results of our investigations into the conjunctival cytology of proved cases of megaloblastic anemia.

Material and methods. Patients were obtained from the Department of Hematology, having been referred there for sternal marrow examination on the basis of symptoms, clinical examination, and demonstration of the characteristic changes in the peripheral blood film. Before treatment was commenced, conjunctival scrapings were taken by the same observer following instillation of 0.5 per cent Ophthaine. The specimens were spread onto the center of a slide, immersed immediately in 50:50 absolute alcohol and ether, and fixed for 24 hours. They were then air dried, taken down through 70 per cent and 50 per cent alcohol to distilled water, washed in distilled water, and stained with 1 per cent Cresyl fast fiolet acetate. The stained sections were then taken through 95 per cent absolute alcohol and xylol and mounted in Canada Balsam. Scrapings were taken from a series of clinically normal control specimens and the whole series was randomized so that the subsequent microscopic observations were carried out "blind."

When the affected patients were ultimately identified, the hematology record was consulted for details of the blood picture, sternal marrow, hemoglobin, serum vitamin B12, and serum folate.

The smears were viewed monocularly with a microscope using the x100 oil immersion objective and an eyepiece graticule. Certain criteria were adopted regarding the nuclei to be measured. In general, the largest, most regular nuclei in the field were selected and those with overlapping or contiguous borders and shrunken, irregular, or abnormal nuclei due to artifacts of preparation were excluded.

One-hundred nuclei were selected on each slide and two measurements were made on each nucleus at 90° to each other. The measurements were made in arbitrary scale division to the nearest half-unit, so that the effect of magnification and eyepiece distance can be discounted.

The results obtained were submitted for statistical analysis.

Results. Data. The data available is in the form of measurement of areas (in arbitrary units) of nuclear cross-sections.

A Mann-Whitney Test was performed with the null hypothesis being that the control and affected groups were the same (as far as the nuclear area was concerned) and (one-sided) alternative that the affected group had larger nuclear areas than the control group. The test statistic was significant at the 2.5 per cent level, indicating an upward shift in the area of nuclei of affected individuals in comparison with the control group (Fig. 1).

Another more crude analysis was performed as follows: The averages were classified as high (H) or low (L) according to whether they were

<table>
<thead>
<tr>
<th></th>
<th>High</th>
<th>Low</th>
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<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Affected</td>
<td>9</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>8</td>
<td>21</td>
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Table I. Table showing high and low classification in control and affected specimens
greater or less than 46, respectively. This gave the following 2 by 2 table (Table I).
Under the hypothesis of no association between the average (H/L) and the classification C/A (C = control, A = affected), the probability of getting a configuration like that above, or more extreme, is about 0.08, so this hypothesis would be rejected at about the 10 per cent level.

No correlation was found between nuclear size and the hemoglobin, serum vitamin B₆, or serum folate.

Discussion. Extensive reviews of conjunctival cytology³–⁴ do not mention any changes thought to be characteristic of the megaloblastic anemias and we have been unable to find any reference to such changes in a search of the literature. The failure of Boddington and Spriggs¹ to find any changes may have been due to their method of dry fixation which inevitably leads to considerable shrinkage and distortion. Atypical conjunctival cells have, however, been described in other conditions. Sekino⁵ described extensive deformation of the corneal epithelial cells with loss of glycogen, RNA, and DNA in rats fed on a folic acid-deficient diet.

Kimura and Thygeson⁶ demonstrated large multinucleate epithelial cells which they regarded as pathognomonic of viral infection and, in addition, large epithelial cells have been observed in Bowen’s disease.

Lirot and Iris⁷ in a study of normal and pathologic conjunctival epithelium using mainly Pappenheim, Folpeff, and Toluidine Blue stains, demonstrated some cells in which, although the nuclei were of normal size, the nuclear pattern was rather coarse, suggesting that the cells were undergoing chromatolysis. In addition, they noted large cells with pyknotic nuclei and considered both these types to be separate stages in the normal degeneration of conjunctival cells.

The nuclear chromatin in the former type and the large cell size of the latter might be considered a source of confusion in differentiating normal cells from those seen in megaloblastic anemia. In fact, however, the large nuclei seen in the conjunctiva in megaloblastic anemia appear to stain less intensely than apparently normal ones and the chromatin pattern in these large nuclei is more “open and lattice-like” (Fig. 2). These changes appeared to be so striking to us that we felt they alone might be indicative of an affected individual by comparison with a normal control specimen (Fig. 3).

In this study, superficial conjunctival scrapings have demonstrated the expected changes in the conjunctiva of patients with megaloblastic anemia.

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Genesis of light-induced avian glaucoma.
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Light-induced avian glaucoma is characterized by eye enlargement, high intraocular pressure (IOP), low outflow facility (C), and reduced aqueous space volume. In this study we have identified several lesions occurring in the early pathologic process, from five to 28 days of age. At seven days, corneal lactic dehydrogenase (LDH) is low and aqueous LDH is high. By 28 days, aqueous LDH is 3.5 times normal levels and corneal LDH is reduced by 10 per cent. By nine days, aqueous space volume is reduced, and eye enlargement is evident by three weeks. IOP and C remain normal during this period, although C is later impaired (at six weeks) and homeostatic control of IOP breaks down at approximately 16 weeks. Though the primary cause(s) for the dimensional changes in cornea and vitreous body have not been identified, the difference in the time of their appearance indicates that these two lesions may be independent of one another. The corneal LDH change suggests early alteration in endothelial permeability, allowing excessive enzyme loss to the aqueous humor.

Domestic chicks reared under continuous light (24L/0D) develop severe morphologic and physiologic ocular lesions which culminate in glaucoma and blindness. We have called the condition light-induced avian glaucoma. Although human open-angle glaucoma is a serious clinical problem, few experimental animals develop such a disease and in no others, to our knowledge, can the condition be precipitated at the will of the investigator.

Light-induced avian glaucoma is associated with increased eye weight and diameter, reduced corneal curvature, impaired outflow facility, and elevated intraocular (IOP) pressure. Eye enlargement and refractive error are detectable several weeks before outflow facility is impaired, and IOP is elevated still later in the disease process. Although the iridocorneal angle is narrow, iridectomy of chicks, subsequently reared under 24L/0D, failed to alter the course of the developing glaucoma, as might be expected if pupillary block had contributed to iris bombé.

A systemic rather than a local etiology was suggested by the finding that eyes of 24L/0D birds, when covered by an opaque vision occluder, showed no less enlargement than those not so covered. However, these positive findings have provided few clues about the etiology of light-induced avian glaucoma. Improvements in our techniques have now made it possible to monitor aqueous fluid dynamics in chicks as young as seven days after hatching; here we detail these early pre-glaucomatous changes. We have also measured lactic dehydrogenase levels during the same period. This enzyme is thought to be essential for normal corneal metabolism and its relative activity in cornea and aqueous has been used as an indication of the viability of the corneal endothelium.

**Materials and methods.** White Rock (broiler type) chicks were reared from hatching in either continuous incandescent light (24L/0D) or a diurnal photoperiod of 14 hours of light per day (14L/10D). Birds were maintained in temperature-controlled environment chambers and were supplied with food (commercial chick starter crumbles) and water ad libitum. Brooder heat without light was supplied by heating the inflow air of the positive-pressure ventilation system.

Eye weight, corneal height, corneal diameter, and aqueous space volume were determined as previously described. To separate the effect of body growth from eye enlargement, a number of chick pairs were selected, one bird from each lighting treatment, but having equivalent body weights. Eye weights of these birds were compared by paired t-test. In evaluation of all other data, Student’s t-test was applied.

Under Combuthal anesthesia, IOP was monitored by closed manometry and aqueous outflow facility (C) was estimated after constant-rate perfusion, as previously described.