Alteration of cell shape in the early chick embryo mesoderm by neuraminidase

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Summary. Living chick embryo mesoderm cells (stages 3 to 5) were exposed to neuraminidase. The mesoderm cells changed shape losing their long thin processes and become like primitive streak cells, with short flat processes. Ruthenium red staining of such treated embryos shows that the surface coat on the mesoderm cells is reduced in thickness. These results show that cell shape in the chick embryo mesoderm is at least partly determined by the thickness and the composition of the surface coat.

Evidence from a variety of cell system suggests that the shape of a cell and the characteristics of its surface coat are interdependent[2-9]. In the chick embryo for example the mesoderm cells adjacent to the primitive streak are flat with long thin processes and have a rich surface coat, whilst those in the streak itself have short flat processes and are depleted in surface coat[10,11]. The investigations described in this paper were therefore undertaken to see whether the change in cell shape as cells left the primitive streak was associated with the increased thickness of surface coat which they appear to synthesise at this time. Neuraminidase was also used in vivo to alter surface coat and the effects on cell shape were examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Materials and methods. Fertile White Leghorn eggs were incubated at 37.5 °C to Hamburger and Hamilton stages 3 to 5[12]. They were then mounted by New Culture[13] and the area pellucida mesoderm exposed by removing the endoderm. After dissection experimental embryos were re-incubated for 2,3 or 4 h with the area pellucida flooded with a solution of 10 µg neuraminidase (Sigma)[14] in 100 ml Pannet and Compton saline[15]. Control embryos were prepared similarly and incubated under saline alone or fixed immediately. After re-incubation embryos were fixed in Karnovsky's solution[16] and prepared for SEM or TEM by standard methods. Half the embryos for TEM were fixed with 0.5–1.0% Ruthenium red added

1 Acknowledgments. It is a pleasure to thank Professor F. Beck for the facilities of the Department of Anatomy, University of Leicester, and Mr G. L. C. McTurk of the University of Leicester Scanning Electron Microscope Unit for operating the ISI 60 SEM. Mr Jeff Smith and Mrs Doris Duncan gave us valuable technical assistance and Dr A. J. Rowe kindly allowed us to use the facilities of the Electron Microscope Unit, University of Leicester School of Biological Sciences, in specimen preparation.

2 L. Weiss and D. L. Kapes, Exp. Cell Res. *41*, 601 (1966).

3 M. S. Steinberg, Science *141*, 401 (1963).

4 C. W. Lloyd, Biol. Rev. *50*, 325 (1976).

5 L. Weiss, J. Cell Biol. *26*, 735 (1965).

6 C. Rosenfeld, M. Paintrand, C. Choquet and A. M. Venant, Exp. Cell Res. *79*, 465 (1973).

7 M. C. Glick, in: Biology and Chemistry of Eucaryotic Cell Surfaces, p. 2. Academic Press 1974.

8 M. S. Nachbar, J. D. Oppenheim and F. Aull, Am. J. med. Sci. *268*, 122 (1974).

9 M. C. Glick, Z. Rabinowitz and L. Sachs, Biochemistry *12*, 4864 (1973).

10 J. Wakely and M. A. England, Differentiation *7*, 181 (1977).

11 P. Mestres and K. Hinrichsen, Anat. Embryol. *146*, 181 (1974).

12 V. Hamburger and H. L. Hamilton, J. Morph. *88*, 49 (1951).

13 D. A. T. New, J. Embryol. exp. Morph. *3*, 325 (1955).

14 G. Gasic and L. Berwick, J. Cell Biol. *19*, 223 (1963).

15 C. A. Pannet and A. Compton, Lancet *102*, 381 (1924).

16 M. J. Karnovsky, J. Cell Biol. *27*, 137A (1965).

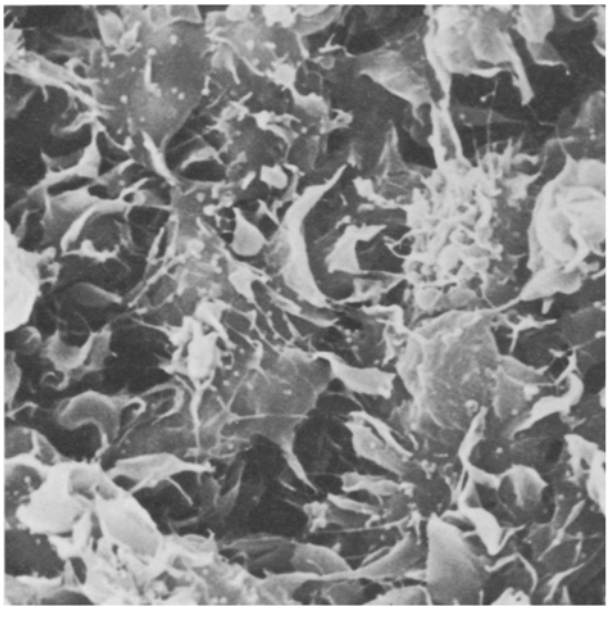

Fig. 1. Cells from the primitive streak of a normal stage 4 chick embryo, viewed from the ventral surface after removing the endoderm. Flat leaflike processes predominate. ×2000.

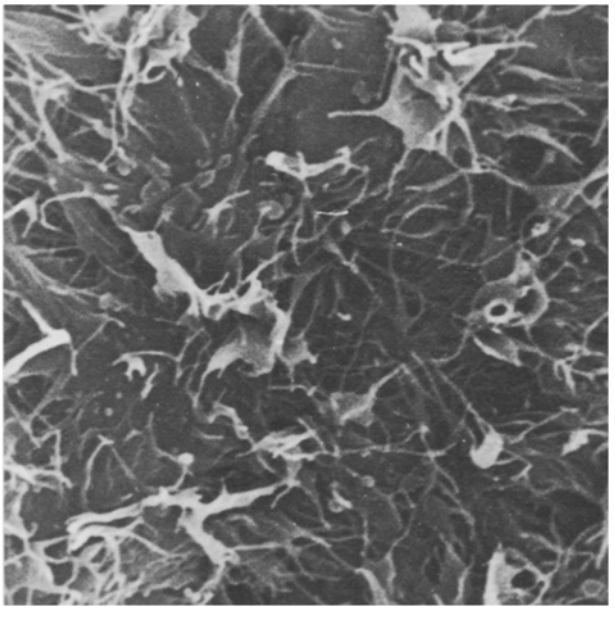

Fig. 2. Mesoderm cells from a normal stage 4 chick embryo viewed from the ventral surface after removing the endoderm. Note the rarity of flat leaflike processes: fine processes predominate. ×1150.

to the fixative[17]. SEM material was examined from the ventral surface in an International Scientific instruments IS160. TEM specimens were sectioned at right angles to the blastoderm on Cambridge or Reichert ultramicrotomes and examined in a JEOLJ 100 S.

Results. 1. Scanning Electron Microscopy. a) Normal Embryos. In the primitive streak the cells were closely packed, with their long axes predominantly at right angles to the underlying ectoderm and most typically had short, flat leaf-like processes (figure 1). At the boundaries of the streak there was a sudden transition to the cells of the mesoderm layer, including its possible endodermal component. These cells were flattened in the place of the ectoderm. Leaf-like processes were rare and the typical cell processes were long thin filopodia (figure 2). The cells at the edges of the mesoderm sheet were flattened onto the ectoderm and had long thin anterior processes (figure 3).

b) Neuraminidase-treated embryos. The primitive streak could still be distinguished from the rest of the mesoderm layer by its midline position and greater cell density but the morphological difference between streak and nonstreak cells was lost. Both in the middle (figure 4) and on the edges (figure 5) of the mesoderm layer the

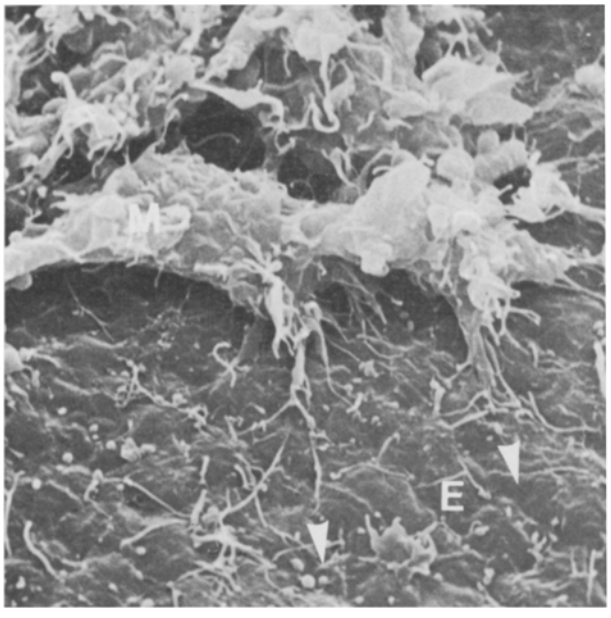

Fig. 3. Part of the leading edge of the mesoderm layer in a normal stage 3 embryo (M = mesoderm, E = ectoderm, arrow shows direction of mesoderm spread). Most of the cell processes are long and thin and anteriorly make contact with the ectoderm. Flat processes are rare. × 2300.

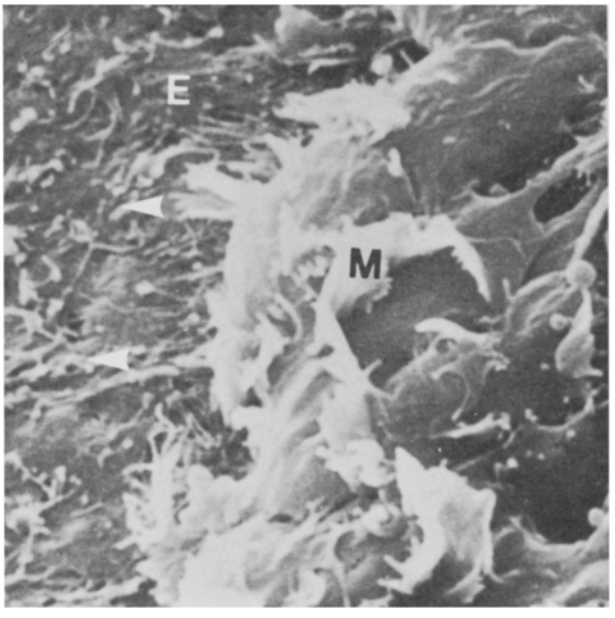

Fig. 5. Part of the leading edge of the mesoderm layer in a stage 4 embryo neuraminidase treated 4 h. Many of the cells have flat processes and long thin processes are rare. × 5000.

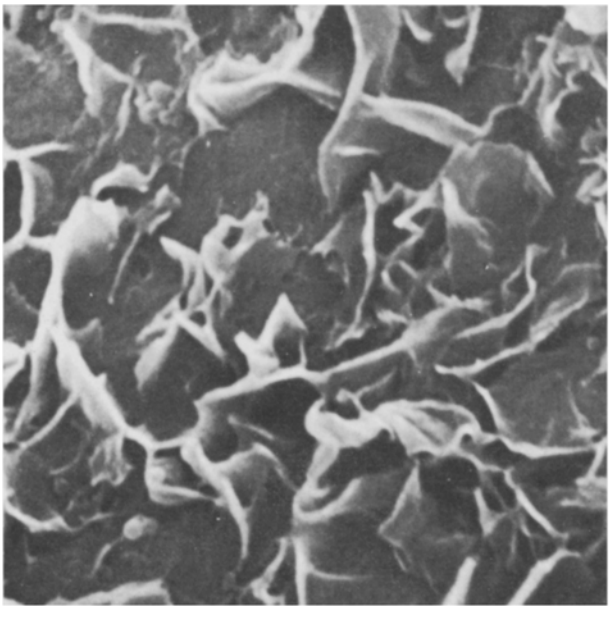

Fig. 4. Mesoderm cells of a stage 4 embryo neuraminidase treated 4 h. Note the large number of short flat processes. × 5000.

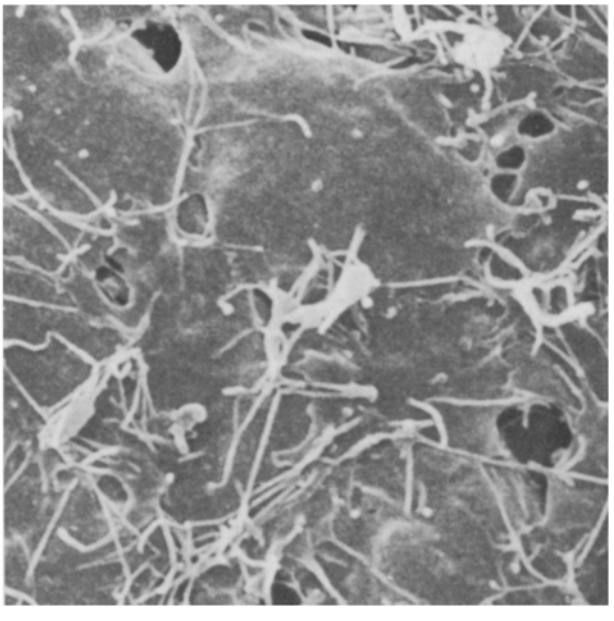

Fig. 6. Mesoderm cells of a stage 5 embryo incubated 4 h under saline. The cells have long thin processes and are very flat. × 5000.

cells showed short flat leaf-like or cup-shaped processes closely resembling those on the normal streak and the long thin processes were rare or missing. The mesoderm cells in some specimens retained their flattened cell bodies, as in figure 4, in others they were rounded. Lengthening or shortening the incubation time in the enzyme did not noticeably alter its effects on cell shape.

c) Control embryos incubated with and without saline. The SEM appearance of control embryos was more variable than that of the neuraminidase-treated specimens. The variations could not be attributed to either incubation time after endoderm removal or to the presence or absence of saline on the exposed mesoderm during the reincubation period. Results obtained ranged from embryos in which the mesoderm cells were normal in appearance (figure 6) to those in which there was some formation of flat leaf-like processes as in the neuraminidase-treated specimens along with the normal mesodermal filopodia. This was most often seen close to the margins of the primitive streak.

Assessment of both control and enzyme treated embryos, especially those incubated for 4 h was frequently complicated by the simultaneous occurrence of endoderm regeneration. Regenerated endoderm varied in appearance from a complete fenestrated epithelium covering the mesoderm layer so that it was possible to see the mesoderm only through the holes in this layer, to plaques of flattened cells over and near the primitive streak.

2. Transmission Electron Microscopy. Examination of a control specimen by TEM showed no evidence of cell damage due either to the removal of the endoderm or to the reincubation. Processes of various types were seen on all parts of the mesoderm cells (figure 7). In control specimens stained with Ruthenium Red the surface of the mesoderm cells exposed by endoderm removal was very heavily stained (figure 8) demonstrating a well developed surface coat. Neuraminidase treatment resulted in sparse staining of the exposed surface, though where stain had penetrated sufficiently the deep surfaces of the mesoderm cells were heavily stained (figure 9).

Discussion. Our results show that cell shape in the chick embryo mesoderm is altered by treatment with neuraminidase. Because of the low specificity of commercial neuraminidase[18], however, we cannot claim that the change which we have seen is due to the removal of sialic acid alone from the cell surfaces. However, the loss of Ruthenium red staining from the treated samples does demonstrate that we have eliminated a significant amount of the surface coat carbohydrates. The changes observed are the reverse of those normally seen at the boundaries of the primitive streak: normally the cells within the streak cells have broad flat short processes and those outside have long thin processes[10]. Whilst after neuraminidase treatment all the mesoderm cells take on an appearance more like that of streak cells.

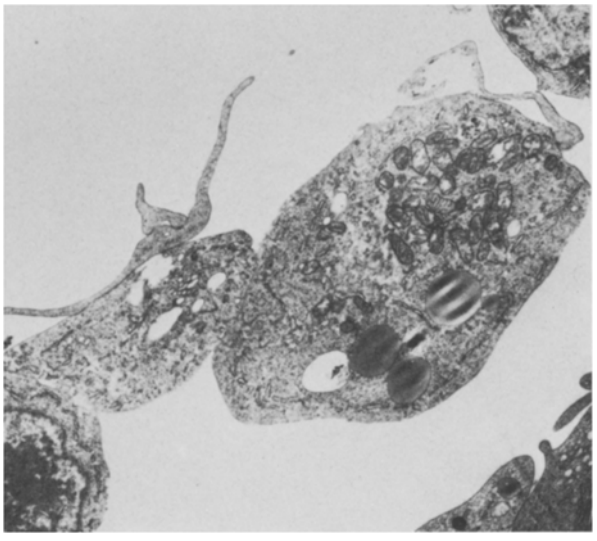

Fig. 7. Transmission electron micrograph of a group of normal mesoderm cells after removing the endoderm. Note the cytoplasmic processes. ×6000.

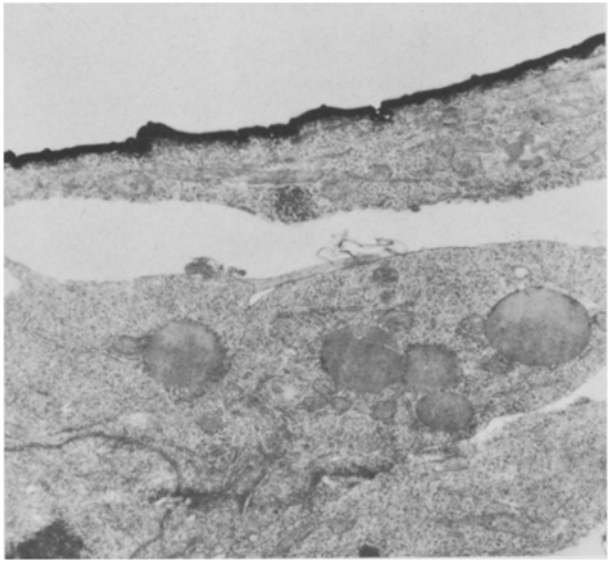

Fig. 8. Saline incubated control embryo stained with Ruthenium red. The exposed mesoderm surface at the top of the picture shows heavy electrondense deposits of Ruthenium red. The stain has not penetrated further, but appears to be trapped on the outer surface. ×11,500.

17 N. Ashton and R. Tripathi, Expl Eye Res. *14*, 49 (1972).

18 R. L. Winzler, Int. Rev. Cytol. *29*, 77 (1970).

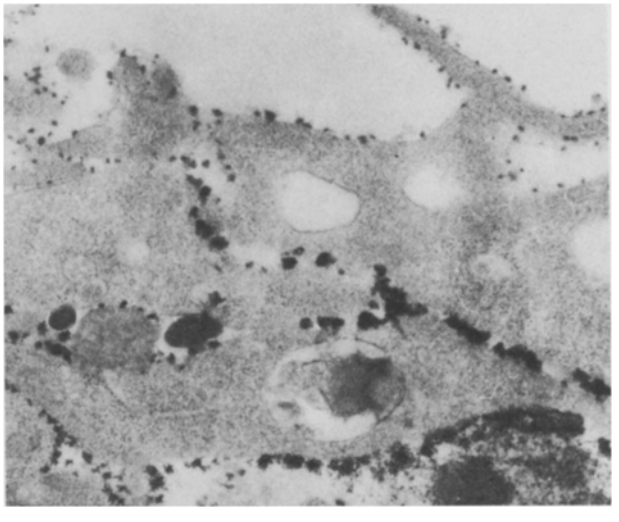

Fig. 9. Exposed mesoderm from embryo incubated 4 h with neuraminidase, treated with Ruthenium red. The basal surfaces of the cells have stained; the surface exposed to the enzyme (top of picture) shows only a little staining. ×22,800.

The same change is seen to a lesser degree in control embryos and this is possibly related to endoderm regeneration since previous studies[19,20] and our own unpublished observations show that endoderm regeneration begins in the primitive streak. It is possible that before the rest of the mesoderm can begin to regenerate endoderm its cells may have to lose some of their more specialised mesoderm features and revert to a condition more like that of the primitive streak. Neuraminidase appears to exaggerate this normal response. Ruthenium red staining is not a sufficiently sensitive method to detect any loss of surface coat in the untreated specimens. In other cell types the surface coat is constantly renewed to replace losses during cell activities[21,22]. However, during development it is possible that the rate of synthesis may be varied by external stimuli or internal genetic factors, leading to altered surface properties of the cell concerned. These may then be demonstrated histochemically[11] or as changes in cell behaviour or shape[23].

Changes in cell shape, are produced by the action on the cell membrane of an internal cytoskeleton of protein microfilaments[24–27]. These filaments have insertions on the plasma membrane and may even attach to the intracytoplasmic protein end of surface coat glycoproteins[4,27]. By cross linking membrane glycoproteins Rees et al.[27] stabilised the shape of cultured cells. Thus they demonstrated a functional link between the relationships of adjacent glycoproteins in the surface coat and the activities of the cytoskeleton by altering the composition of the surface coat we may similarly have altered the arrangement or degree of contraction of cytoskeletal filaments in the mesoderm cells to one characteristic of the primitive streak, thereby changing the cells' shape and reversing the normal developmental process.

19 L. Vakaet and M. Mareel, C. r. Séanc. Soc. Biol. *158*, 902 (1964).
20 S. P. Modak, Revue suisse Zool. *73*, 877 (1966).
21 C. P. Leblond and G. Bennet, in: The Cell Surface in Development, p. 29. John Wiley and Sons, New York 1974.
22 G. Gasic and T. Gasic, Nature *196*, 170 (1962).
23 A. A. Moscona, in: The Cell Surface in Development, p. 67. John Wiley & Sons, New York 1974.
24 A. C. Allison Locomotion of Tissue Cells. CIBA Foundation Symposium no. 14, p. 109, 1973.
25 R. D. Goldman, G. Berg, A. Bushnell, C. M. Chiang, L. Dickerman, N. Hopkins, M. L. Miller, R. Pollack and E. Wang, Locomotion of Tissue Cells. CIBA Foundation Symposium No. 14, p. 83, 1973.
26 N. K. Wessels, B. S. Spooner and M. A. Luduena, Locomotion of Tissue Cells. CIBA Foundation Symposium no. 14, p. 53, 1973.
27 D. A. Rees, C. W. Lloyd and D. Thom. Nature *267*, 124 (1977).

Structural differences of cone 'oil-droplets' in the light and dark adapted retina of *Poecilia reticulata* P.

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Summary. The vast majority of 'oil-droplets' in the dark and light-adapted retinal twin-cones of *Poecilia reticulata* is of the 'matrix-type'. In bright light (day light + overhead strip light) there occurs in some regions a very pronounced numerical change from 'matrix' to 'cristate-type', whereas other regions remain unaffected. The functional significance of these differences is discussed.

Oil-droplets are abundant in the retinal cones of amphibia, reptiles and birds. They are thought to constitute intraocular filters which may serve to increase contrast, reduce glare and lessen chromatic aberration[1–3]. Oil-droplets were, for a long time, considered to be absent in teleosts, until Berger[4] described globular structures, which he called 'oil-droplets', in the twin-cones of *Poecilia reticulata* (*Lebistes reticulatus*). Subsequently, 'oil-droplets' were observed in the cones of other teleosts, all members of the Cyprinodontoidei and the closely related Exocoetoedei[5–7].

In teleosts, with 'oil-droplets', the ellipsoidal mitochondria mature in a vitreoscleral direction, and the 'oil-droplets' are considered modified scleral-end mitochondria[5–8]. *P. reticulata* has 4 types of cones arranged in tiers, which, in accordance with their distance from the membrana limitans externa, are called outer, middle and inner cones. The outer tier is made up of twin-cones and the 2 other tiers contain single cones[9]. The 'oil-droplet' is observed only in the twin-cones, and only in their shorter accessory member. There are 2 types of 'oil-droplets': a) 'matrix', with the cristae limited to the periphery and the lumen filled with a densely staining granular material; b) 'cristate', which contains vesicular membranes, clumps of fibrous material and a matrix similar to that of a mitochondrion[4,8]. The principal member of the twin-cones and the single middle cones also show a maturation of mitochondria, following a vitreo-scleral gradient to become progressively denser[4,8]. The single, inner cones display a mitochondrial size gradient from the periphery to the centre[8]. However, none of these cones develop an 'oil-droplet'. Various classical histochemical tests failed to reveal lipids in the lumen of the 'oil-droplets' of *P. reticulata*[10]. It appears that none of the 'oil-droplets' of other teleosts have been tested histochemically.

Between cristate and matrix type 'oil-droplets', intermediate stages have been observed. This would suggest that 'cristate' and 'matrix', rather than being 2 different types of droplets, may in fact merely represent different metabolic states of the cones. To test this hypothesis, eyes of fish kept in the dark and in the light were compared.

Material and methods. Adult fish (eye diameter > 1.6 mm) were used. Group 1 was dark adapted for 3 h. Group 2 consisted of daylight adapted fish taken from stock aquaria.

1 G. L. Walls, The Vertebrate Eye and its Adaptive Radiation. Broomfield Hills, Mich., Cranbrook 1942.
2 S. Duke-Elder, System of Ophthalmology, vol. 1. Kimpton, London 1958.
3 C. Pedler and M. Boyle, Vision Res. *9*, 525 (1969).
4 E. R. Berger, J. Ultrastruct. Res. *14*, 143 (1966).
5 M. Anctil and M. A. Ali, Copeia *1*, 43 (1970).
6 B. Borwein and J. J. Hollenberg, J. Morph. *140*, 405 (1973).
7 M. Anctil and M. A. Ali, Zoomorphologie *84*, 103 (1976).
8 Y. W. Kunz and C. Wise, Revue suisse Zool. *80*, 694 (1973).
9 H. Müller, Zool. Jb. Physiol. *63*, 275 (1952).
10 Y. W. Kunz and C. Regan, Revue suisse Zool. *80*, 699 (1973).