*J. Anat.* (1975), **120**, 2, pp. 367–372
*With* 8 *figures*
*Printed in Great Britain*



# Developmental origin and early differentiation of retinal Müller cells in mice

J. BHATTACHARJEE AND S. SANYAL

*Department of Anatomy, Faculty of Medicine, Erasmus University, Rotterdam, The Netherlands*

(*Accepted* 12 *February* 1975)

## INTRODUCTION

Although the literature on the developmental cytology of the retina is considerable, information about the origin and initial differentiation of Müller cells is scanty and often contradictory. Müller identified these cells as the most important, if not the only, glial elements of the retina in 1851. Cajal (1892) noted the first appearance of Müller cells as elements scattered throughout the retina in mouse embryos of about 15 mm length, a stage at which the ganglion cells could also be recognized. The observations of Berkow & Patz (1964) on the developmental histochemistry of oxidative enzymes and of Morest (1970) on the rapid Golgi impregnated retina of the rat indicate relatively late appearance of Müller cells. However, recent electron microscopic studies by Uga & Smelser (1973) in the rabbit and by Kuwabara & Weidman (1974) in the rat have shown that Müller cells are the earliest among the retinal cells to differentiate.

Lack of identifiable characteristics prior to their attaining definitive form and location clearly is an impediment in analysing the early events of Müller cell differentiation. In a histochemical study on the developmental changes of enzymes with esterase activity in the retina of mice, it was observed that the initial differentiation of cells, identifiable as presumptive Müller cells, was characterized by a localized and transient activity of non-specific esterase. This report describes the origin and change of these cells, visualized by the non-specific esterase reaction, during the prenatal development of the retina in mice.

## MATERIAL AND METHODS

Balb/cHeA mice were used throughout this study. Sexually mature females and males were allowed to mate overnight and the presence of vaginal plugs in the morning marked the beginning of pregnancy; the next day was considered as day 1. Starting on day 10 of gestation females were killed at daily intervals until birth. The embryos were removed from the uterus and immediately immersed in physiological saline. The entire heads, or the eye regions, were dissected out and fixed overnight in ice-cold fixative (4 % paraformaldehyde in 0·067 M phosphate buffer at pH 7·2 and 7·5 % sucrose). The material was stored in gum-sucrose (Holt, 1959) for up to 5 days before sectioning in a cryostat at 10 $\mu$m. Non-specific esterase was localized according to the method of Barka & Anderson (1963). $\alpha$-naphthyl acetate (Sigma) was used

as substrate at pH 6·5 with hexazotized pararosanilin as coupler. The sections were incubated at 37 °C for 20 minutes and were counterstained with methyl green. Specific substrates and inhibitors were used according to the directions of Pearse (1972) to confirm the histochemical reaction as due to non-specific esterase activity.

## RESULTS

The optic vesicle is formed in the mouse embryo around day 9 of gestation and by day 10 develops into a two-layered optic cup connected with the diencephalon by the optic stalk. The choroid fissure is then still open (Rugh, 1968; Pei & Rhodin, 1970; Theiler, 1972). At this stage the cells of the inner layer, the presumptive neural retina, do not show any noticeable activity for non-specific esterase or any morphological evidence of differentiation (Fig. 1).

On day 11, the choroid fissure has closed and non-specific esterase activity is now seen in a very few cells located on the vitreal side near the optic stalk (Fig. 2). These cells are elongated with external and internal processes oriented perpendicular to the retinal surface (Fig. 6). They are considered to be Müller cells at the beginning of their differentiation (see discussion). At the same time, esterase activity of moderate intensity has appeared along the vitreal border of the retina close to the inner limiting membrane. Some necrotic cells with clumps of esterase-positive material are also seen in the centre of the retina adjacent to the optic stalk.

On day 13, the number of esterase-positive cells has increased markedly. Such cells are seen as far forward as half of the distance from the centre of the optic cup to the ora (Fig. 3). As cells with esterase activity appear in peripheral regions the area immediately adjacent to the optic nerve, where the emergence of the layer of nerve fibres is now discernible, shows considerably reduced enzyme activity (Fig. 7). Although nearly all cells which are most intensely stained are located on the vitreal

---

Photomicrographs of cryostat sections of developing mouse retina showing changes of non-specific esterase activity in Müller cells.

Fig. 1. Optic cup of day 10 embryo; cells in the neural retina (*nr*) and pigment epithelium (*pe*) do not show any esterase activity. *cf*, choroid fissure. × 100.

Fig. 2. Optic cup at 11 days; note esterase activity in cells near the vitreal border of the central retina. × 100.

Fig. 3. 13 days; section of the optic cup lateral to the point of entry of the optic nerve. Note growth of the optic cup and thickness of the neural retina along with the appearance of esterase-positive cells in the fundus. × 100.

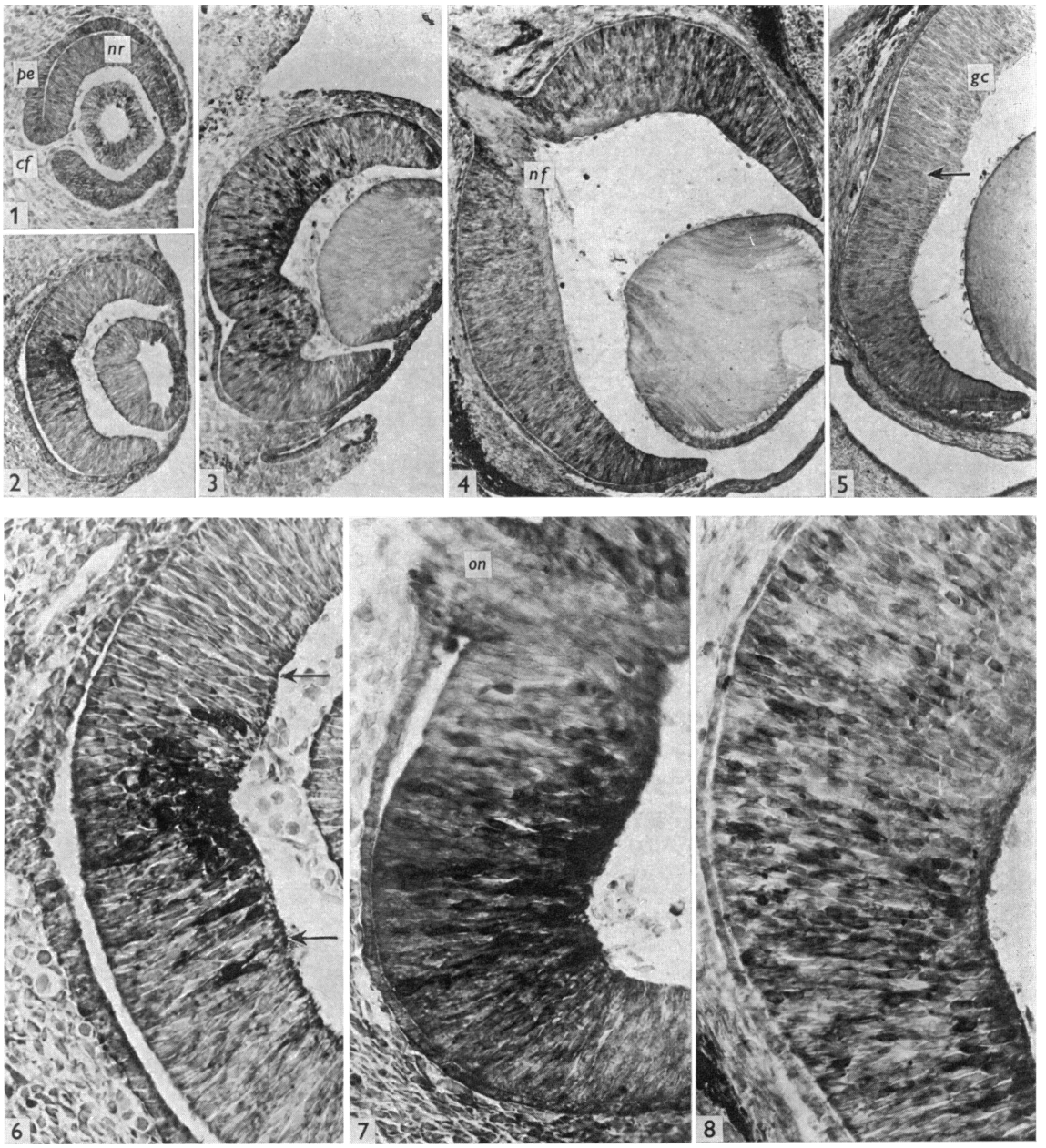
Fig. 4. 15 days; cells with high esterase activity are seen near the ora. Cells with reduced activity are scattered throughout the retina. *nf*, layer of nerve fibres. × 100.

Fig. 5. 16 days; esterase activity is seen near the ora while activity has disappeared from the fundus. Note presence of the layer of ganglion cells (*gc*) and the inner plexiform layer (arrow). × 100.

Fig. 6. Magnified view of 11 day retina (Fig. 2). Note esterase activity along the inner limiting membrane (arrow). × 400.

Fig. 7. 13 days; part of section passing through the optic nerve (*on*). Most intensely stained cells are located along the vitreal border. Cells near the optic nerve show reduced activity. × 400.

Fig. 8. 15 days; part of retina shown in Fig. 5. Note cells with reduced esterase activity scattered throughout the depth of the retina. × 400.


pe
nr
cf
1
2
3
nf
4
gc
5
6
on
7
8

border, many cells with a varying intensity of esterase activity are seen more externally with a few close to the scleral border of the neural retina, thus producing a characteristic radial orientation (Figs. 3, 7). It appears that the perikarya of these cells acquire the esterase activity while located in the vitreal position, and migrate in the scleral direction, losing some of the activity on the way. By day 15, the most intensely stained cells are located in the more peripheral part of the retina, close to the ora, and cells with esterase activity of varying but reduced intensity are scattered uniformly throughout the fundus (Figs. 4, 8). The layer of nerve fibres has now expanded into the peripheral area, but no further progress of development is recognizable. Along with the growth of the optic cup from 11 days onwards, a sudden increase in the thickness of the neural retina is recorded and is most pronounced in that part of the central retina where esterase-positive cells have appeared.

On day 16, the esterase activity of cells has considerably declined (Fig. 5). In the fundus, towards the optic nerve, the cells appear uniformly negative, but some cells with faint activity are still found scattered in the peripheral part, including the ora. Besides the extension of the layer of nerve fibres throughout the central part of the retina, further advance in the histogenesis of the retinal layers is seen in the separation of the layer of ganglion cells along with the emergence of the inner plexiform layer. Gradually the changes hitherto observed in the fundus extend into the ora, and the esterase activity in the remaining scattered population of cells disappears around the time of birth. The margin of the optic cup which develops into the iris and ciliary body continues to show a high level of esterase activity.

## DISCUSSION

The identification of the esterase-positive cells as Müller cells is based on two previous findings. Müller cells are the first type of cells to differentiate in the rabbit retina; this has been demonstrated by the early appearance of cells with smooth endoplasmic reticulum, characteristic of mature Müller cells (Uga & Smelser, 1973). The earliest cells with these organelles appeared in the vitreal border on the 14th prenatal day which is developmentally equivalent to the 11th prenatal day of mice, when the esterase-positive cells first appear at the same site. The Müller cells subsequently migrate with the growth of the retina, and this leads to their scattered distribution (which Cajal (1892) observed with the silver method in 15 mm mouse embryo, approximately corresponding to the 15 or 16 day old embryos used in our study).

Migration presumably involves only the perikarya, including the outer ends of the cells, while the inner ends remain fixed to the vitreal surface, since the inner limiting membrane is considered to be formed by the Müller cell endfeet and has been shown to be a basement membrane continuously present from the optic vesicle stage (Cohen, 1961). It is tempting to speculate, in this context, whether position along the vitreal border predetermines the presumptive Müller cell population of the retina in the way positional information has been proposed to control cellular differentiation in many instances (Wolpert, 1969).

The functions generally attributed to Müller cells in the adult retina are the provision of mechanical support in the form of a framework to the retinal neurons

(Cajal, 1904; Polyak, 1941), of nutritive and metabolic support as evidenced by high concentration of glycogen (Shimizu & Maeda, 1953; Kuwabara & Cogan, 1961) and several enzymes (Cogan & Kuwabara, 1959; Kuwabara & Cogan, 1960) and of insulation of the neuronal networks (Polyak, 1956). Early detection of Müller cells at the light microscopic level provides an opportunity to observe their appearance in relation to the progress of development in the whole retina. The initial phase of differentiation of Müller cells, marked by transient esterase activity, advances as a concentric wave from the centre to the periphery. A rapid growth of the neural retina occurs almost immediately and is followed by differentiation and separation of the inner retinal layers as the perikarya of the Müller cells migrate towards more outer levels. The sequence of these changes suggests that early acquisition of some of the metabolic functions might be important in promoting growth of the retinal cells. Further, outward migration of the perikarya is a part of the process that results in formation of the radial fibres which constitute the glial framework. The early development of this structure suggests that it might act as a substratum for the neuronal precursor cells which show repeated to and fro movement along the scleral–vitreal axis in the course of the cell cycle and subsequent differentiation (Sidman, 1961). Furthermore, active participation of Müller cells in the separation of the receptor and the bipolar cells has been suggested by Meller (1964) and Meller & Glees (1965) on the basis of electron microscopic observation on 9 day old chick embryos. It has been earlier reported (Coulombre, 1955) that, in the chick retina, Müller cells differentiate around 4–5 days of incubation, which is developmentally comparable to the 11–12 day stage in mice. Thus a spatio-temporal relationship between differentiation of Müller cells and histogenetic separation of the neuronal layers appears to be a regular feature of retinal development. Experiments with other enzyme markers and autoradiography are in progress to observe retinal histogenesis during the postnatal period when the outer retinal layers differentiate, and the Müller cell perikarya become finally located in the inner nuclear layer.

SUMMARY

During the prenatal development of the retina in mice Müller cells at the initial stage of differentiation show a high level of histochemically detectable non-specific esterase activity. These are the first of the retinal cell types to differentiate and appear at the 11th day of gestation along the vitreal border in the central retina. As development proceeds they appear in more peripheral areas and their perikarya migrate outwardly and become scattered throughout the depth of the retina. Appearance of presumptive Müller cells is followed by rapid growth of the retina and differentiation of the inner retinal layers. With the progress of histogenesis from the central to the peripheral areas the esterase activity in the Müller cells gradually diminishes. The possible significance of early differentiation of Müller cells in promoting growth and histogenesis of retina is discussed.

We thank Professor J. Moll for discussion, Miss A. de Ruiter and Mr R. K. Hawkins for expert technical assistance and Mr W. van den Oudenalder and Miss P. C. Delfos for preparing the photographs. The award of a fellowship from the Medical Faculty funds to one of us (J.B.) is gratefully acknowledged.

## REFERENCES


BARKA, T. & ANDERSON, P. J. (1963). *Histochemistry, Theory, Practice and Bibliography*. New York: Hoeber.

BERKOW, J. W. & PATZ, A. (1964). Developmental histochemistry of the rat eye. *Investigative Ophthalmology* **3**, 22–33.

CAJAL, S. RAMON Y. (1892). *The Structure of the Retina* (English translation, 1972). Springfield: C. C. Thomas.

CAJAL, S. RAMON Y. (1904). *Histologie du Système Nerveux de l'Homme et des Vertébrés* (French edition, 1955). Madrid: Instituto Ramon Y. Cajal.

COGAN, D. G. & KUWABARA, T. (1959). Tetrazolium studies on the retina. II. Substrate dependent patterns. *Journal of Histochemistry and Cytochemistry* **7**, 334–341.

COHEN, A. I. (1961). Electron microscopic observations of the developing mouse eye. I. Basement membrane during early development and lens formation. *Developmental Biology* **3**, 22–33.

COULOMBRE, A. J. (1955). Correlation of structural and biochemical changes in the developing retina of the chick. *American Journal of Anatomy* **96**, 153–189.

HOLT, S. J. (1959). Factors governing the validity of staining methods for enzymes, and their bearing upon the Gomori acid phosphatase technique. *Experimental Cell Research* Suppl. 7, 1–27.

KUWABARA, T. & COGAN, D. G. (1960). Tetrazolium studies on the retina. III. Activity of metabolic intermediates and miscellaneous substrates. *Journal of Histochemistry and Cytochemistry* **7**, 214–223.

KUWABARA, T. & COGAN, D. G. (1961). Retinal glycogen. *Archives of Ophthalmology* **66**, 680–688.

KUWABARA, T. & WEIDMAN, T. A. (1974). Development of the prenatal rat retina. *Investigative Ophthalmology* **13**, 725–739.

MELLER, K. (1964). Elektronenmikroskopische Befunde zur Differenzierung der Rezeptorzellen und Bipolarzellen der Retina und ihrer synaptischen Verbindungen. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **64**, 733–750.

MELLER, K. & GLEES, P. (1965). The differentiation of neuroglia–Müller-cells in the retina of chick. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **66**, 321–332.

MOREST, D. K. (1970). The pattern of neurogenesis in the retina of the rat. *Zeitschrift für Anatomie und Entwickelungsgeschichte* **131**, 45–67.

PEARSE, A. G. E. (1972). *Histochemistry, Theoretical and Applied*, 3rd ed., vol. 2. London: Churchill Livingstone.

PEI, Y. F. & RHODIN, J. A. G. (1970). The prenatal development of the mouse eye. *Anatomical Record* **168**, 105–126.

POLYAK, S. L. (1941). *The Retina*. Chicago: The University Press.

POLYAK, S. L. (1956). *The Vertebrate Visual System*. Chicago: The University Press.

RUGH, R. (1968). *The Mouse–its Reproduction and Development*. Minneapolis: Burgess Publishing Co.

SHIMIZU, N. & MAEDA, S. (1953). Histochemical studies on glycogen of the retina. *Anatomical Record* **116**, 427–434.

SIDMAN, R. L. (1961). Histogenesis of mouse retina studied with thymidine-$H^3$. In *The Structure of the Eye* (Ed. G. K. Smelser), pp. 487–506. New York: Academic Press.

THEILER, K. (1972). *The House Mouse*. Berlin: Springer Verlag.

UGA, S. & SMELSER, G. K. (1973). Electron microscopic study of the development of retinal Müllerian cells. *Investigative Ophthalmology* **12**, 295–307.

WOLPERT, L. (1969). Positional information and the spatial pattern of cellular differentiation. *Journal of Theoretical Biology*, **25**, 1–47.