Graefe's Arch Clin Exp Ophthalmol (1983) 221:113–121

Graefe's Archive
for Clinical and Experimental
Ophthalmology

The occurrence of cell death during the remodelling of the chamber angle recess in the developing rat eye*

Ch. Remé, U. Urner, and B. Aeberhard
Laboratory of Electron Microscopy, Department of Ophthalmology, University of Zürich, Zürich, Switzerland

Abstract. Prenatal and postnatal stages of the developing chamber angle in the rat eye were analysed qualitatively by light and electron microscopy. One eye of each of five weight-matched littermates was investigated at the following time points: prenatal days 14, 17, 18 and 21; day 0 = day of birth; postnatal days 5, 10, 15, 20, 40, 60, 100 and 200. From postnatal days 5 to 60, conspicuous amounts of necrotic cells were observed within the chamber angle area. At birth and at postnatal day 200, only a few scattered necroses were observed. Necrotic cells were found within the trabecular area, the region of Fontana's spaces and beams and the peripheral iris, the iris root, the ciliary body and the adjoining choroid. Large macrophages containing lysosomal dense bodies were found within the previously described areas and were frequently in close contact with necrotic cells. It is concluded that cell death within the developing chamber angle of the rat may represent an important event that may contribute to two morphogenetic effects: (1) the opening of large spaces of Fontana and (2) space formation within the trabecular area and uveoscleral outflow routes.

Introduction

The normal development of the chamber angle region has been studied in humans (Wulle 1972; Remé and Lalive 1981), in primates (Smelser and Ozanics 1971) and in other vertebrate species (Aguirre et al. 1972; Bistner et al. 1973). Nevertheless, a question concerning the normal development remained unanswered: by which mechanisms is the chamber angle, which is initially filled with a dense cellular network, opened and reorganized? Several hypotheses have been proposed and these include (1) cleavage within the tissue (Allen et al. 1955; Maumenee 1959), (2) perforation or removal of a membrane (Barkan 1955; Hansson and Jerndal 1971), and (3) tissue rarefaction or atrophy (Smelser and Ozanics 1971). For a review of these concepts see references (O'Rahilly 1975; Lee and Grierson 1982; Mullaney 1982). Among the above-mentioned hypotheses, the atrophy and rarefaction of chamber-angle tissues appear to be the most convincing, even though atrophy due to cellular necroses has not yet been demonstrated in the developing trabecular meshwork. However, it appears to be unlikely that the specific tissue remodelling could occur without cell death at certain developmental stages.

In a preceding paper we demonstrated that the rat is a suitable experimental animal for the study of the normal development of the vertebrate chamber angle (Remé et al. 1983). Thus, in the rat eye a question which is paralleled in other vertebrate eyes emerges–how do the large spaces of Fontana develop within solid cellular tissue? Our study reveals that in the rat eye cell death may be involved in the final formation of the chamber angle area. Not only the spaces of Fontana, but also the distinct spaces within the formerly dense tissues of the iris root, the ciliary body and even the peripheral choroid may be created by cell death.

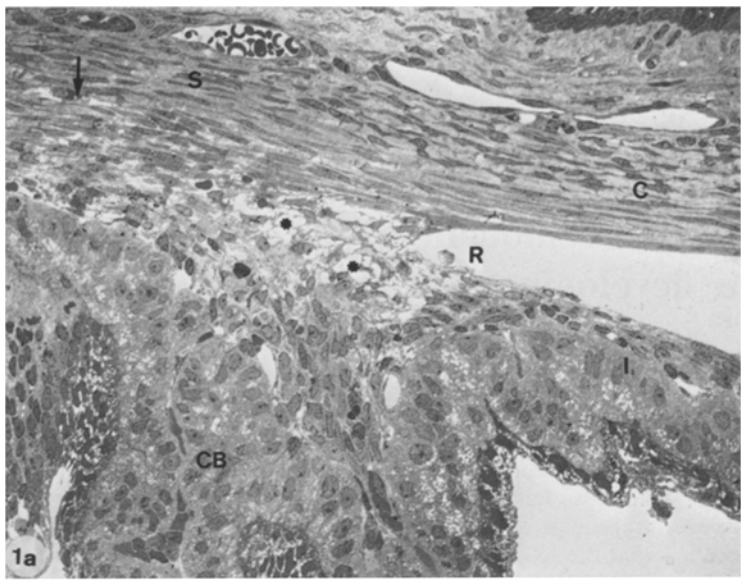
In this context, an obvious problem which arises is the distinction between in vivo cell death that occurs as a remodelling process and post-mortem autolysis in cells which have not been adequately fixed. In our investigations, particular care was taken to achieve well-preserved tissues to reduce the possibility of artefactual autolysis. Qualitative analysis of cell death was performed in material derived from the important stages in the development of the rat chamber angle.

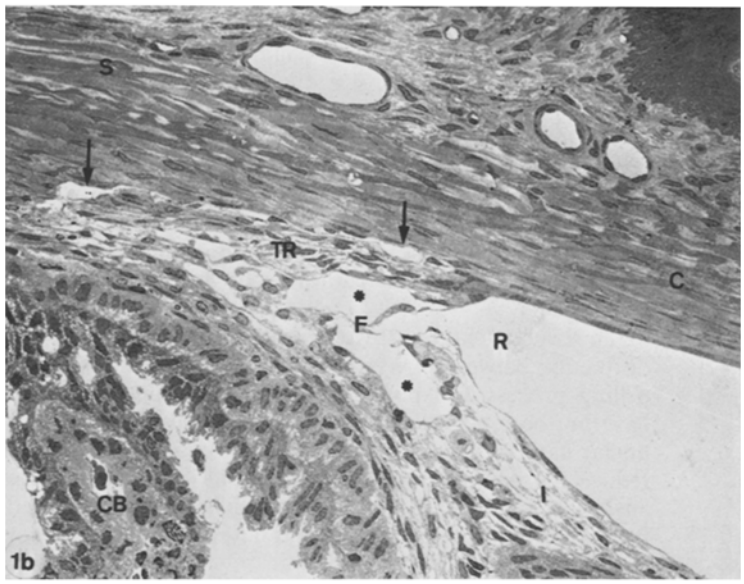
Materials and methods

Experimental procedures. Pregnant Sprague-Dawley females were obtained from the animal breeding institution of Tierspital Zürich. Pregnancy, delivery and postnatal care of the rats was performed under controlled conditions with an 12:12 light-dark cycle with water and food ad libitum. Various pre- and postnatal stages were investigated. At each time point of the investigation five weight-matched littermates were used, and male and female animals were selected randomly. The animals were always killed between 10 a.m. and 12 noon, at the following time points: day 14, 17, 18 and 21 during gestation, day 0 = day of birth, and days 5, 10, 15, 20, 40, 60, 100 and 200 after birth. In order to obtain specimens from prenatal stages, pregnant females were anaesthetized with intramuscular injections of 0.2 ml fluanison. A Caesarean section was performd and the heads of the foetuses removed and immediately placed into fixative. Determination of the foetal age was performed in the following manner: after mating, a vaginal smear was taken

* This study was supported in part by EMDO – Foundation for Medical Research, and by a grant from Hartmann Müller Foundation

Offprint requests to: PD Dr. med. Charlotte Remé, Dept. of Ophthalmology, University of Zürich, CH-8091 Zürich, Switzerland





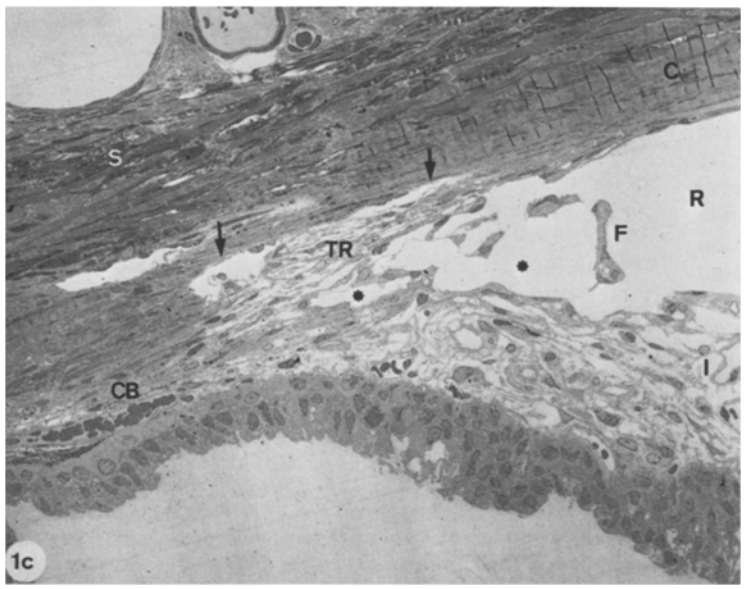


Fig. 1 a–c. Low-power electron micrographs depicting the chamber-angle area at different developmental stages. **a** Postnatal day 5: the chamber angle is filled with a loose cellular network (*). Vascular channels constituting Schlemm's canal appear at the scleral sulcus (↑). *C*, cornea; *S*, sclera; *I*, iris; *CB*, ciliary body; *R*, chamber angle recess. ×460. **b** Postnatal day 20: conspicuous spaces (*) appear among the tissues that fill the chamber angle. Schlemm's canal (↑) is seen within the corneoscleral trabecular meshwork (*TR*). A beam of Fontana (*F*) is apparent. *C*, cornea; *S*, sclera; *I*, iris; *CB*, ciliary body; *R*, chamber angle recess. ×460. **c** Postnatal day 60: large spaces (*) appear within the chamber angle tissue, the corneoscleral trabecular meshwork (*TR*) and the iris root (*I*). Fontana's beams (*F*) extend from the corneoscleral region towards the iris root. Schlemm's canal (↑). *C*, cornea; *S*, sclera; *CB*, ciliary body; *R*, chamber angle recess. ×460. All pictures: uranyl acetate and lead citrate

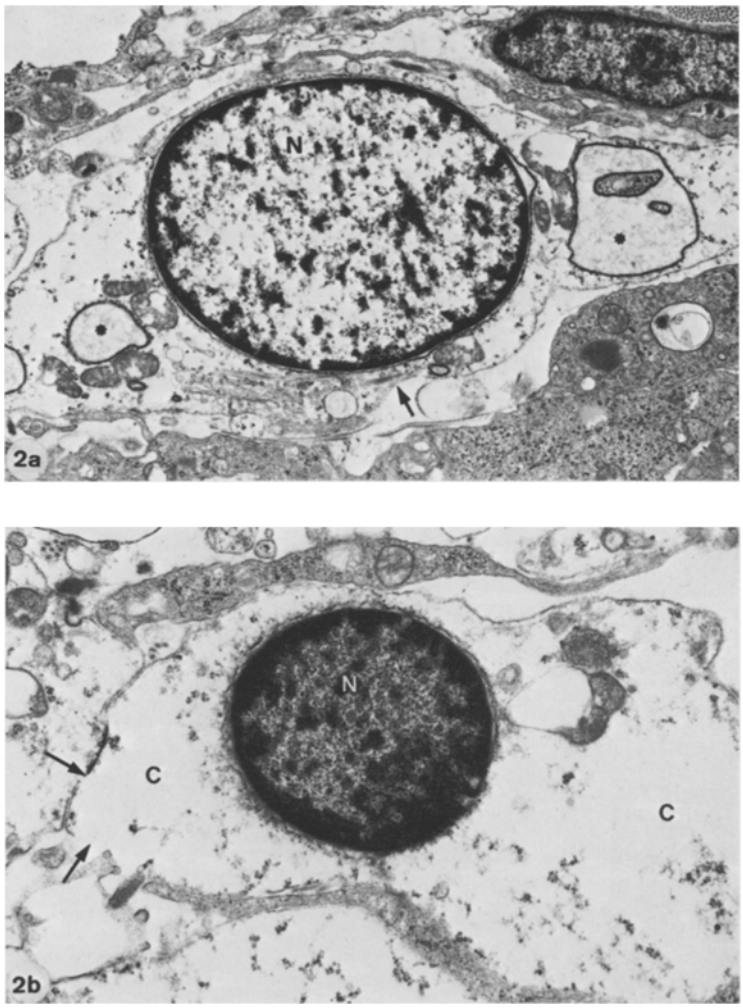


Fig. 2a, b. Electron micrographs showing the appearance of cell necrosis. **a** The nucleus (*N*) is swollen and reveals a patchy distribution of the chromatin. Cisternae of the rough endoplasmic reticulum show a vacuolar distension (*), the cytoplasm is electron lucent and contains a few organelles, the plasma membrane reveals disruptions (↑). ×12,900. **b** The nucleus (*N*) is pyknotic, the chromatin electron dense. The cytoplasm (*C*) is electron lucent and almost entirely devoid of organelles; the plasma membrane shows conspicuous disruptins (↑). ×22,900. Both pictures: uranyl acetate and lead citrate

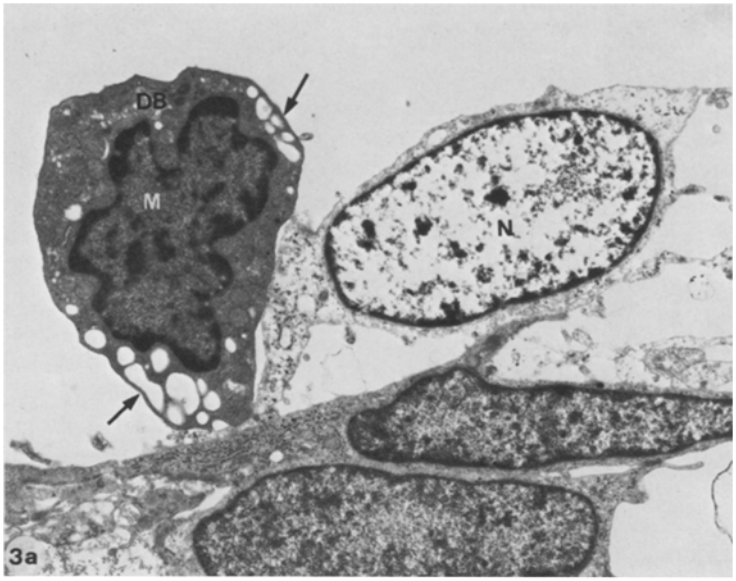
from the female animals within 24–36 h; the day the positive smear was obtained was counted as day 1 of pregnancy.

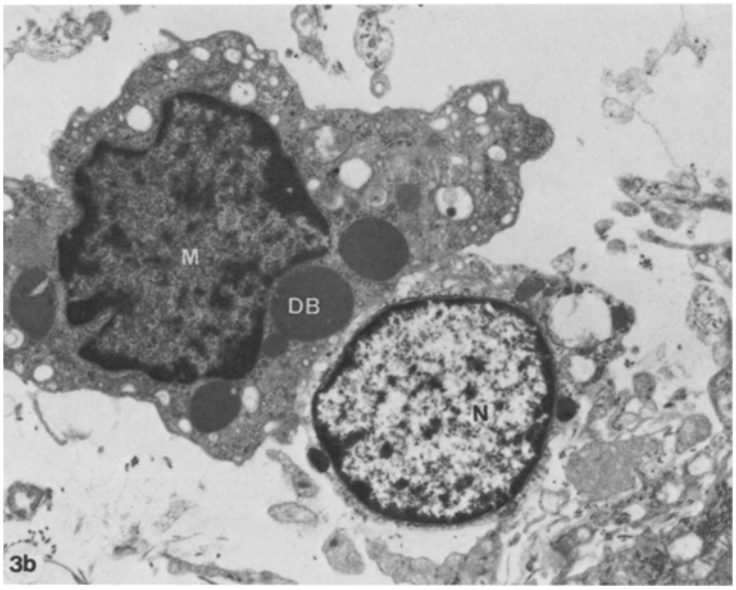
Preparation for morphological investigation. At the prenatal and postnatal stages of development the heads of the animals were removed and immediately immersed in fixative. The eyes were enucleated under a dissecting microscope while immersed in a dish containing fixative. The eyes were then cut into halves or quarters and left in the fixative for 18–20 h. The fixative was 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, 480 mosmol. Tissues were postfixed in OSO_4 for 1 h, dehydrated and embedded in Epon 812. Semithin sections were stained with toluidine blue. Thin sections were mounted individually on foil-covered one hole grids and stained with uranyl acetate and lead citrate. At each designated period, one eye of each of five littermates was investigated, and from each eye three different tissue blocks were selected from the anterior segment.

Criteria of cell death. In our material, the distinction of cellular necroses was unequivocal only at the electron microscopic level. Therefore, the analysis was performed by electron microscopy and cells displaying the following fine structural criteria were defined as necrotic cells: clumping and densification of nuclear chromatin, reduction in cytoplasmic organelles, vacuolar dilation of the rough endoplasmic reticulum, discontinuities of the plasma membrane.

Results

In low-power electron micrographs from specimens of postnatal day 5, small spaces appear within the tissue filling the chamber-angle recess (Fig. 1a). Those spaces are distinctly enlarged and Fontana's beams are formed until postnatal day 60 (Fig. 1b, c). From postnatal days 20 to 60, a tissue rarefaction occurs within the iris root and the ciliary body (Figs. 1b and c). At higher magnifications, cellular necroses are observed in the chamber angle area concomitant with the creation of spaces. One type of necrotic cells reveals a swollen nucleus with a patchy distribution of the chromatin, vacuolar transformation of the cisternae of the rough endoplasmic reticulum, a washed-out appearance of the cytoplasm with a reduction in organelles, disruptions of the plasma membrane (Fig. 2a). Another type of necrosis consists of a pyknotic nucleus with heavily condensed chromatin, an almost empty, electron lucent cytoplasm devoid of organelles and lysis of the plasma membrane (Fig. 2b). The necrotic cells are seen in groups within one area, or they are scattered among regular extracellular tissue components and cells of normal cytoarchitecture. At early devel-





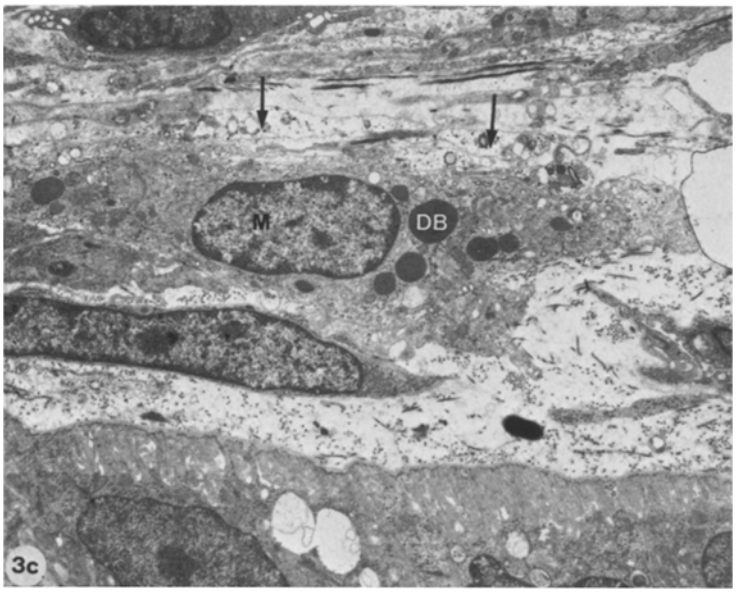


Fig. 3a–c. Electron micrographs showing phagocytic cells in different chamber angle regions. **a** A macrophage (*M*) next to a necrotic cell (*N*) in the trabecular meshwork. Small lysosomal dense bodies (*DB*) and thin cytoplasmic processes (↑) are seen. ×9,200. **b** A macrophage (*M*) next to a necrotic cell (*N*) in the iris stroma. Large lysosomal dense bodies (*DB*) appear in the cytoplasm. ×10,300. **c** A macrophage (*M*) in the ciliary body. Large lysosomal dense bodies (*DB*) are seen in the cytoplasm. Parts of lytic cells appear close to the macrophage (↑). ×5,400. All pictures: uranyl acetate and lead citrate

opmental stages, cells in mitosis occasionally appear next to necrotic cells. Macrophages with conspicuous cytoplasmic processes and numerous lysosomal dense bodies within their cytoplasm are found in the chamber angle area, frequently in close contact with necrotic cells (Fig. 3a–c).

Necrotic cells occur in the stroma of the peripheral iris (Fig. 4a) and the iris root (Fig. 4b). Tissues that line the chamber angle recess at early stages and Fontana's beams at later stages reveal necrotic cells (Fig. 4c). Cell necroses also appear in the developing trabecular meshwork (Fig. 4d) and within the inner wall region of Schlemm's canal (Fig. 4e). In the growing pars plana ciliaris and the most peripheral choroid, groups of necrotic cells appear and create electron lucent, confluent spaces (Fig. 4f). Since cellular necroses cannot be visualized in low-power overview pictures, a schematic drawing representing an intermediate stage (day 20) summarizes the localisations of cell death within the chamber angle region (Fig. 5a and b).

Cellular necroses are not observed within the chamber angle at prenatal stages, a few lytic cells are first seen at day 0 (average of 1–3 per section), increased numbers occur from postnatal days 5 to 100 (average of 10–20 per section), and small numbers are still present at postnatal day 200 (average of 1–3 per section).

Discussion

Methodological considerations. An obvious question arising is whether the necrotic cells represent a true morphological phenomenon or whether they are an artefact. For the following reasons we do not consider the changes we have described as an artefact. Cell necroses are only observed in certain regions of the developing eye. Furthermore, necrotic cells are most commonly observed next to well-preserved cells of the same histological type. Finally, in many instances macrophages containing phagolysosomes are seen in close contact with lytic cells, which points towards an intravital process of phagocytosis of necrotic material.

Morphology of cell death. The morphology of cell death appears to display heterogeneous aspects in different developing tissues. At the light microscopic level, cell death in the retina is observed as pyknosis (Glücksmann 1965) or as a necrotic locus (Silver 1981). At the electron microscopic level, large secondary phagolysosomes within phagocytic cells are described in the leg bud or the wing region of the chick embryo (Saunders 1966; Schoenwolf 1981). Cellular and nuclear decay (Vogel 1978) and pyknotic nuclei and fragments of degenerating cytoplasm within the cytoplasm of neighbouring cells (Theiler et al. 1976) are noted in the developing retina. In our study, cell necroses are not identified by light microcopy. Under the electron microscope, lytic cells reveal condensation of the nuclear chromatin, vacuolisation of the rough endoplasmic reticulum and cytoplasmic rarefaction. The reason for this apparent discrepancy may be that mesenchymal tissues of the developing chamber angle reveal a different morphological aspect of cell death than other developing tissues, particularly neural tissues.

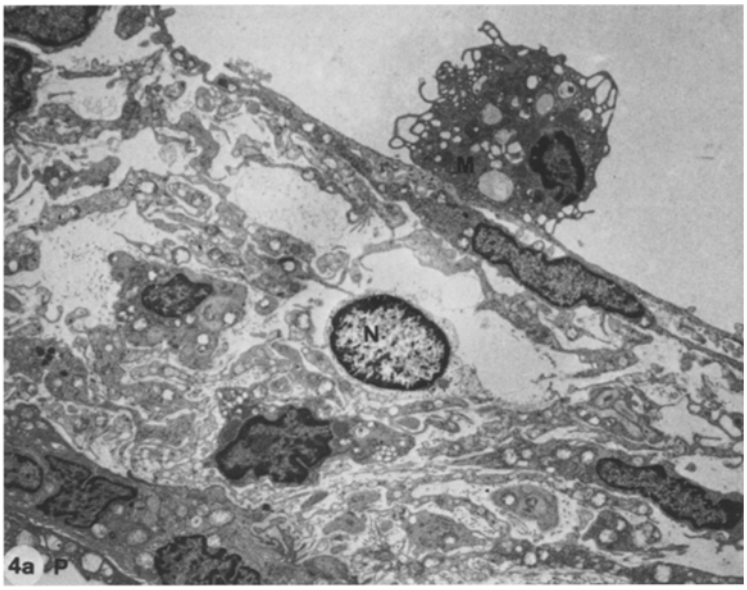
Another possibility implies that the appearance of cell death found in our material merely represents stages wich precede those found in other developing tissues. All of our experiments were performed between 10 a.m. and 12 noon. It cannot be excluded that cell death may follow a diurnal rhythm with a peak period during those hours. In the developing rat retina, indications of a rhythmicity of cell necroses have been observed (Vogel and Möller 1980).

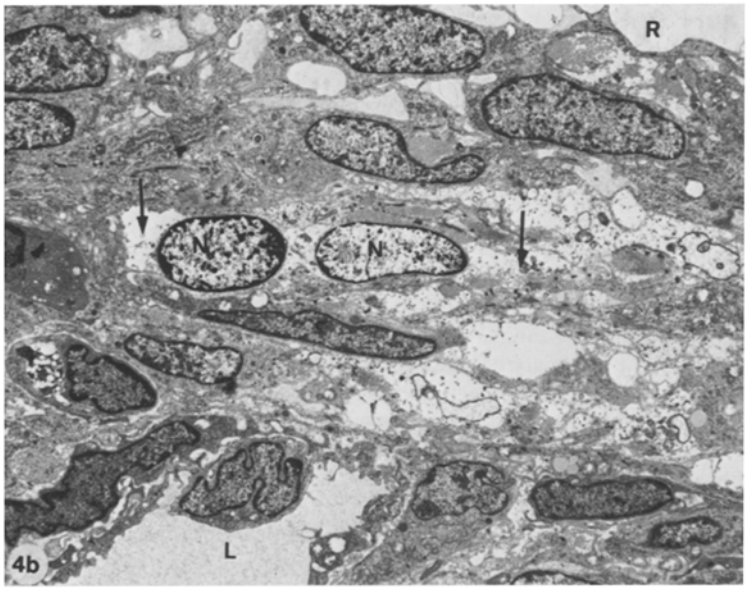
Generally, two modes of cell death may be distinguished: cell necrosis and apoptosis. Whereas cell necrosis is considered to be almost exclusively confined to pathological conditions in mature tissues, apoptosis is part of the physiological turnover of tissue components or of developmental morphogenesis (Wyllie 1981). In our material, cell necroses are most commonly observed. However, no pathological conditions are present such as hypoxia or exposure to various toxins that are considered to cause cell necroses. Instead, cell death occurs within apparently physiological developmental stages of the chamber angle region. Thus it appears that cell necroses may also occur under the physiological condition of developing tissue.

Mechanisms of removal of cellular components following cell death during development appear to be different in different tissues. In the retina, exogenous macrophages have not been observed; nevertheless, cellular debris is removed (Theiler et al. 1976). Other possibilities include phagocytosis by Müller cells (Kuwabara and Weidman 1974) or phagocytosis by intact neighbouring cells, as is observed in many cases of apoptosis (Wyllie 1981). Moreover, breakdown products from cellular decay may be recycled within growing tissues (Silver 1976). In our material, macrophages are observed in relatively large amounts at all stages investigated. At the electron microscopic level, recognizable cell particles are not observed within macrophages, but large lysosomal dense bodies are distinct. Thus we may assume that macrophages engulf and degrade remnants of cell necroses in the developing rat chamber angle. From our studies, however, no indications emerge as to whether the macrophages are of local or of hematogeneous origin and where the macrophages finally migrate to.

In addition to phagocytosis, other functions may be ascribed to macrophages found in our material. In general, a wide variety of functions has been elucidated for macrophages (Diegelmann et al. 1981). In our material, macrophages may modulate fibroplasia and/or collagen degradation. Reduced activity of macrophages may lead to an increased tissue density within the chamber-angle region.

Cell death and remodelling of the chamber angle region. Two possible functional effects may be ascribed to cell death which occurs in the rat chamber angle. One effect may be to open the chamber-angle recess; the other may be to create space within the trabecular and the uveoscleral outflow routes. Cell necroses occur concomitantly with the opening of conspicuous spaces of Fontana. Parallel with the opening of Fontana's spaces, cell death occurs within the trabecular meshwork. In the rat, the trabecular region is confined to a relatively small area which corresponds topographically to the cribriform layer in the human eye and may not include the main aqueous outflow pathway (Van der Zypen 1977). Moreover, cell necroses parallel the creation of rather conspicuous spaces within the iris root, the ciliary body and even the peripheral choroid. These latter spaces may be important in the rat eye as a uveoscleral drainage route, since the rat has no mechanism of distension of the trabecular meshwork and thus facilitation of aqueous outflow, as is found in the primate eye (Grierson et al. 1978).





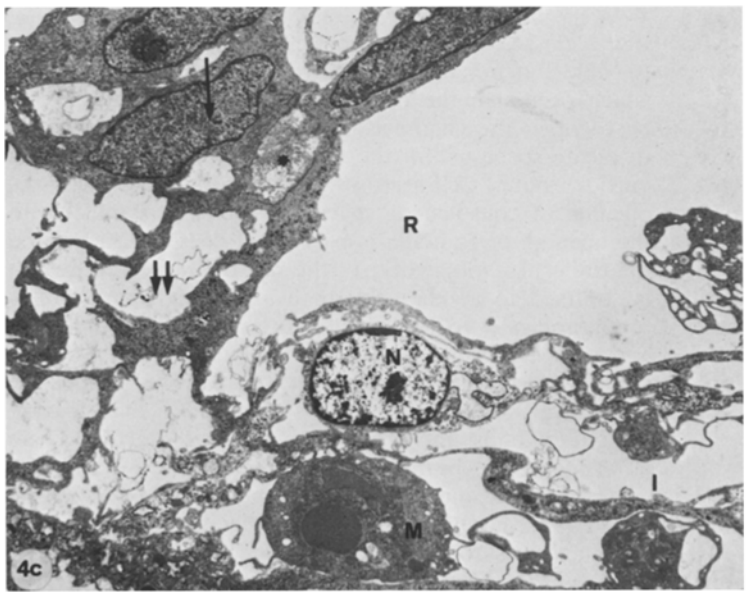
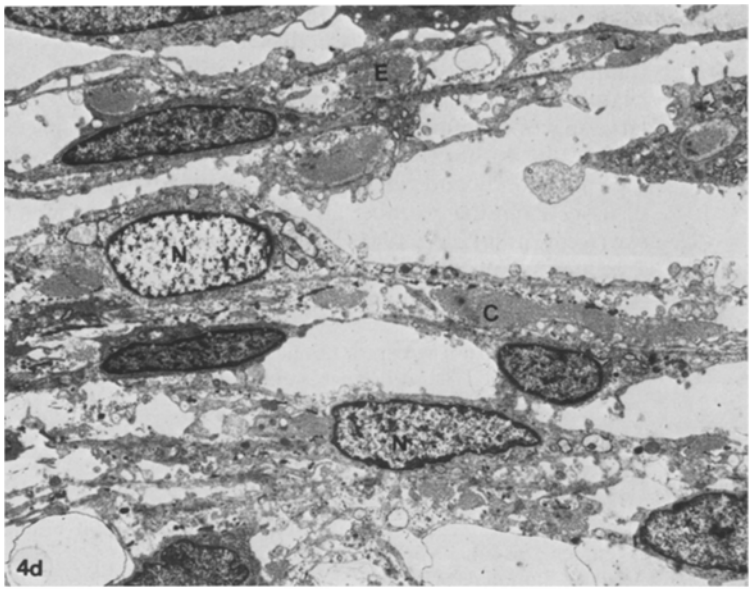
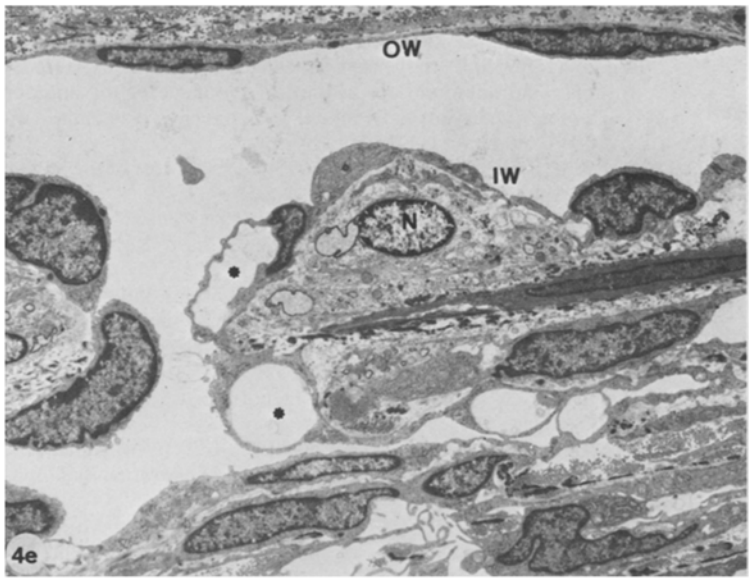
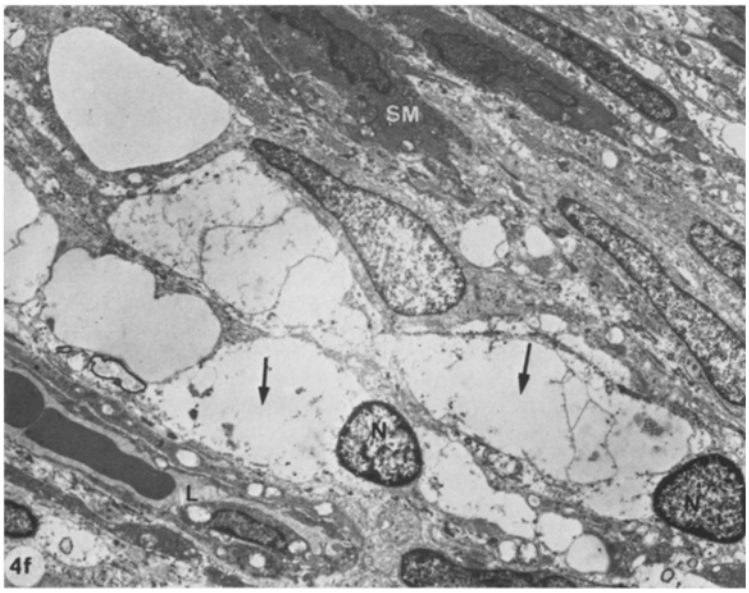


Fig. 4a–f. Electron micrographs demonstrating necrotic cells in different areas of the chamber angle. **a** Necrotic cell (*N*) in the iris stroma. A macrophage (*M*) appears on the anterior surface of the iris. *P*, posterior surface of the iris. Postnatal day 60. ×4,800. **b** Necrotic cells (*N*) within the iris root showing an electron lucent cytoplasm (↑). *R*, chamber angle recess; *L*, vascular lumen in the developing ciliary body. Postnatal day 5. ×4,800. **c** Necrotic cell (*N*) and a macrophage (*M*) within the lining cellular layer of the chamber angle recess. Prospective trabecular beams (*), cells (↑) and spaces (↑↑) appear. *I*, iris; *R*, chamber angle recess. Postnatal day 5. ×4,800. **d** Necrotic cells (*N*) within the developing trabecular meshwork. Trabecular beams with collagen fibers (*C*) and patches of elastic like material (*E*) are distinguished. Postnatal day 20. ×4,800. **e** Necrotic cell (*N*) underneath the lining endothelium of the inner wall (*IW*) of Schlemm's canal. Endothelial vacuoles (*) appear in the inner wall. *OW*: outer wall of Schlemm's canal. Postnatal day 60. ×4,800. **f** Necrotic cells (*N*) in the pars plana of the ciliary body. The electron lucent, washed-out cytoplasm (↑) is conspicuous. *SM*, smooth muscle cell; *L*, vascular lumen. Postnatal day 20. ×4,800. All pictures: uranyl acetate and lead citrate


E
N
C
M
4d


OW
IW
N
4e


SM
N
L
N
4f

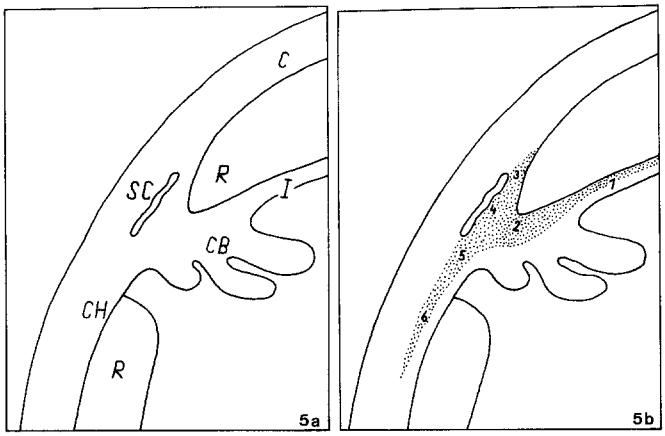


Fig. 5a, b. Schematic drawings indicating the occurence of cell necroses in different chamber-angle areas. An intermediate stage of development (day 20) is depicted. **a** Chamber angle area: *C*, cornea; *I*, iris; *R*, recess; *SC*, Schlemm's canal; *CB*, ciliary body; *CH*, choroid; *R*, retina. **b** The stippled area shows the occurrence of cell necroses in different chamber angle regions: (*1*) iris stroma, (*2*) recess and iris root, (*3*) trabecular meshwork, (*4*) inner wall region of Schlemm's canal, (*5*) ciliary body and (*6*) most peripheral choroid

In our material, conspicuous amounts of necrotic cells are registered. The reason for this observation may be the rapid development of the rat eye in general. Compared with the human or primate eye, similar developmental steps occur within a much shorter time course. Thus, an accumulation of cell death might accomplish the opening processes within a short time period. In primates and the human eye, accumulations of cell necroses may never occur. Instead, a continuous process set at a lower level might within longer periods fulfill the same functional task.

Rats are considered to be mature at 60 days of age, but they continue to increase their body weight until much later in their lives. Necrotic cells are still present in 200-day-old rats. Thus, cell death continues in young adult animals. This indicates that the final moulding of the chamber-angle region extends over a relatively long time period. Finally, single cell necroses might also occur throughout the lifetime of an individual rat, as well as in the human or primate, representing a continuous remodelling and turnover of tissue components.

Cell death as a morphogenetic factor. Observations on cell death during the normal development of vertebrate species have been made by Glücksmann (1965) in the retina and a variety of other tissues. Subsequently, numerous investigations revealed cell necroses during embryological development and ascribed major functions to morphogenetic cell death (Schook 1978; Silver 1978; Hinchliffe 1981). Necroses within retinal nuclear layers and the optic stalk appeared to be the condition for a regular outgrowth of optic nerve fibers and the development of the optic fissure in rats and mice (Theiler et al. 1976; Silver 1978; Silver 1981). Cell death within the developing retina of the cat and rat were considered to contribute to the final thickness of inner and outer nuclear layers (Vogel 1978; Vogel and Möller 1980). During the development of the vitreous body, the hyaloid vessels and the primary vitreous were found to disappear, possibly by cellular necroses, in the human eye (Gloor 1973).

In conclusion, our investigations on the rat chamber angle development reveal selective cell death as a process that may contribute to the final configuration of the chamber angle. Parallel with the occurrence of cell death, large spaces of Fontana are opened and conspicuous spaces are created within the trabecular meshwork, the iris root, the ciliary body and the anterior choroid. Re-examination of tissue from the chamber angle of the developing human eye (Remé and Lalive 1981) revealed cell necroses in several specimens. Thus, it is conceivable that cell death may contribute to the remodelling of the human chamber angle.

Acknowledgements. The expert technical assistance of Mrs. Silvia Hofmann and Ms. Astrid Rhyner is gratefully acknowledged.

References

Aguirre GD, Rubin LF, Bistner SI (1972) Development of the canine eye. Am J Vet Res 33:2399–2414

Allen L, Burian HM, Braley AE (1955) A new concept of the development of the anterior chamber angle. Its relationship to developmental glaucoma and other structural anomalies. Arch Ophthalmol 53:783–798

Barkan O (1955) Pathogenesis of congenital glaucoma. Gonioscopic and anatomic observation of the angle of the anterior chamber in the normal eye and in congenital glaucoma. Am J Ophthalmol 40:1–11

Bistner SI, Rubin L, Aguirre GD (1973) Development of the bovine eye. Am J Vet Res 34:7–12

Diegelmann RF, Cohen IK, Kaplan AM (1981) The role of macrophages in wound repair: a review. Plast Reconstr Surg 68:107–113

Gloor BP (1973) Zur Entwicklung des Glaskörpers und der Zonula. II Glaskörperzellen während Entwicklung und Rückbildung der Vasa hyaloidea und der Tunica vasculosa lentis. Graefe's Arch Clin Exp Ophthalmol 186:311–328

Glücksmann A (1965) Cell death in normal development. Arch Biol (Liège) 76:419–437

Grierson I, Lee WR, Abraham S (1978) Effects of pilocarpine on the morphology of the human outflow apparatus. Br J Ophthalmol 62:302–313

Hansson HA, Jerndal T (1971) Scanning electron microscopic studies on the development of the iridocorneal angle in human eyes. Invest Ophthalmol Vis Sci 10:252–265

Hinchliffe JD (1981) Cell death in embryogenesis. In: Bowen ID, Lockshin R (eds), Cell death in biology and pathology, Chapman Hall, London, pp 35–70

Kuwabara T, Weidman TA (1974) Development of the prenatal rat retina. Invest Ophthalmol Vis Scin 13:725–739

Lee WR, Grierson I (1982) Anterior segment changes in glaucoma. In: Garner A, Klintworth GK (eds) Pathobiology of ocular disease, Part A. Marcel Dekker, New York Basel, pp 525–551

Maumenee AE (1959) The pathogenesis of congenital glaucoma: a new theory. Am J Ophthalmol 47:827–859

Mullaney J (1982) Normal development and developmental anomalies of the eye. In: Garner A, Klintworth GK (eds), Pathobiology of ocular disease, Part A. Marcel Dekker, New York Basel, pp 443–522

O'Rahilly R (1975) The prenatal development of the human eye. Exp Eye Res 21:93–112

Remé Ch, Lalive S (1981) Periods of development of the normal human chamber angle. Doc Ophthalmol 51:241–268

Remé Ch, Urner U, Aeberhard B (1983) The development of the chamber angle in the rat eye. I Morphological characteristics of developmental stages. Graefe's Arch Clin Exp Ophthalmol 220:139–153

Saunders JW jr (1966) Death in embryonic systems. Science 154:604–612

Schoenwolf GC (1981) Morphogenetic processes involved in the remodelling of the tail region of the chick embryo. Anat Embryol (Berl) 162:183–197

Schook P (1978) A review of data on cell actions and cell interactions during the morphogenesis of the embryonic eye. Acta Morphol Neerl Scand 16:267–286

Silver J (1976) A study of ocular morphogenesis in the rat using 3H-thymidine autoradiography: evidence for thymidine recycling in the developing retina. Dev Biol 49:487–495

Silver J (1978) Cell death during development of the nervous system. In: Jacobson M (ed), Development of sensory systems, vol 9. Springer-Verlag, Berlin Heidelberg New York, pp 419–436

Silver J (1981) The role of cell death and related phenomena during formation of the optic pathway. In: Hilfer SR, Sheffield JB (eds), Ocular size and shape. Springer-Verlag, New York Heidelberg Basel, pp 1–23

Smelser GK, Ozanics V (1971) The development of the trabecular meshwork in primate eyes. Am J Ophthalmol 71:366–385

Theiler K, Varnum DS, Nadeau JH, Stevens LC, Cagianut B (1976) A new allele of ocular retardation: early development and morphogenetic cell death. Anat Embryol (Berl) 150:85–97

Vogel M (1978) Postnatal development of the cat's retina: a concept of maturation obtained by qualitative and quantitative examinations. Graefe's Arch Clin Exp Ophthalmol 208:93–107

Vogel M, Möller K (1980) Cellular decay in the rat retina during normal post-natal development: a preliminary quantiative analysis of the basic endogenous rhythm. Graefe's Arch Clin Exp Ophthalmol 212:243–260

Wulle KG (1972) The development of the productive and draining system of the aqueous humor in the human eye. Adv Ophthalmol 26:296–355

Wyllie AH (1981) Cell death: a new classification separating apoptosis from necrosis. In: Bowen ID, Lockshin R (eds) Cell death in biology and pathology. Chapman Hall, London, pp 9–29

Zypen E van der (1977) Experimental morphological study on structure and function of the filtration angle of the rat eye. Ophthalmologica 174:285–298

Received July 18, 1983