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Cell death in the dorsal part of the chick optic cup. Evidence for a new necrotic area

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SUMMARY

The spatiotemporal pattern of morphogenetic cell death during the early development of the chick retina was studied by means of the neutral red vital staining and light microscopy. A modification of the conventional procedure of vital staining, which consisted of the injection of the dye into the neural tube lumen, was used for this purpose. In addition to the two areas of cell death known from previous literature, the first located in the ventral part of the optic cup and the second located in the insertion of the optic stalk with the diencephalon, a new area of cell death was described. This third necrotic area was located in the protruding dorsal part of the optic cup rim and was present throughout the stages 15 to 18. The area consisted of dying cells, fragments and phagocytosed cells.

We suggest that this dorsal area of cell death could stop the intense dorsal growth of the optic cup and/or reshape the optic cup rim. Moreover, this area may influence the production of cell degeneration in the dorsal part of the invaginating lens placode.

INTRODUCTION

Morphogenetic cell death is a basic developmental mechanism (Glücksmann, 1951) which appears during the invagination, fusion, separation or sculpturing of the embryonic rudiments (reviewed by Saunders & Fallon, 1967; Hinchliffe, 1981). This morphogenetic process is not the product of chance. Numerous observations in embryonic processes such as limb-bud morphogenesis (Saunders, Gasseling & Saunders, 1962; Hinchliffe, 1981, 1982), heart development (Ojeda & Hurlé, 1975; Hurlé & Ojeda, 1979), fusion of the secondary palate (Greene & Pratt, 1976) and neural tube closure (Schlüter, 1973) show that cells die at certain times and loci form delimited necrotic areas. There is some experimental evidence suggesting that the necrotic areas in developing tissues are regulated either by intrinsic or extrinsic factors (reviewed by Hinchliffe, 1981; Beaulaton & Lockshin, 1982).

In the early embryogenesis of the vertebrate retina a process of morphogenetic cell death takes place during the complicated series of invaginations which transform the optic vesicle into the optic cup (Glücksmann, 1930, 1951; Källen, 1955, 1965; Silver & Hughes, 1973). In the mammals the strategical orientation of cell death appears to be necessary for the morphogenetic movements that form the

primordium of the retina (Silver, 1978, 1981). Moreover, Silver & Hughes (1973) observe that, during the invagination of the lens placode and the optic vesicle, the degeneration of cells is maximum in the ventral part of these rudiments, suggesting that there is a spatiotemporal relationship between the necrotic loci of lens and retina necessary for the normal eye morphogenesis.

In the systematic study carried out by Schook (1980*a*,*b*) in the developing eye of chick embryos, two areas of cell death were found during the formation of the optic cup and optic fissure (stages 13 to 18 of Hamburger & Hamilton, 1951). One area was located in the ventral part of the invaginating wall of the optic vesicle; the other area was located in the zone of insertion of the optic stalk into the diencephalon. These zones are also present in mammals (Källen, 1955, 1965; Silver & Hughes, 1973). However, in the invaginating chick lens placode the cell death process commences and achieves its maximum intensity in the dorsal part of the placode (García-Porrero, Collado & Ojeda, 1979; Schook, 1980*c*; García-Porrero, Colvée & Ojeda, 1983).

In this work we describe in the chick retina rudiment a new area of cell death located in the dorsal part of the optic cup using the vital staining of necrotic areas and histological sections.

MATERIALS AND METHODS

Fertile White Leghorn eggs were incubated at 38 °C to yield normal embryos ranging from stages 13 to 19 of Hamburger & Hamilton (1951).

Areas of cell death were mapped *in ovo* using the vital staining method for cell death. To ensure that the dye penetrated into the retinal necrotic areas we adopted the following procedure. A portion of the shell was removed to expose the embryo and the extraembryonic membranes were partially dissected.

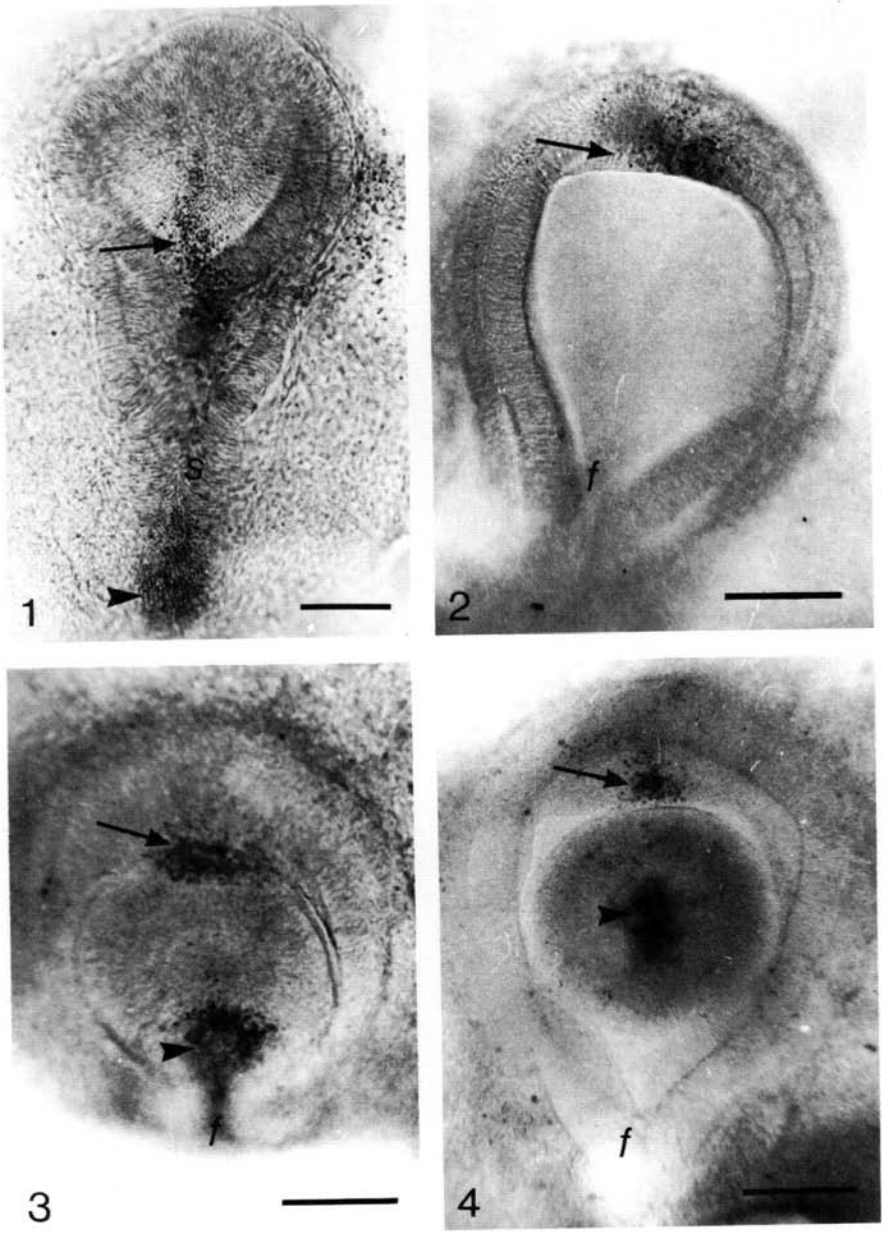
Fig. 1. Ventrolateral view of a vital-stained optic cup rudiment at stage 16. The ectoderm and the lens rudiment have been removed. Note one area of cell death in the ventral part of the optic cup (arrow) and other area of cell death in the zone of insertion of the optic stalk with the diencephalon (arrowhead). Optic stalk (s). Bar = 100 μm.

Fig. 2. Lateral view of a vital-stained optic cup at stage 15 showing a necrotic area (arrow) in the dorsal part of the optic cup rim. The ectoderm and the lens rudiment have been removed. Retinal fissure (f). Bar = 100 μm.

Fig. 3. Lateral view of a vital-stained optic rudiment at stage 16 showing a necrotic area (arrow) in the dorsal part of the optic cup rim. Note that this retinal necrotic area is located in a small protrusion of the retinal rim. The area of cell death in the lens cup appears surrounding the lens pore (arrowhead). Retinal fissure (f). Bar = 100 μm.

Fig. 4. Lateral view of a vital-stained optic rudiment at stage 17 showing a necrotic area (arrow) in the protruding dorsal part of the optic cup. Note that this necrotic area is more reduced than the area showed in Fig. 3. The focal plane chosen does not allow the lens necrotic area located in the zone of closure of the lens pore (arrowhead) to be seen clearly. Retinal fissure (f). Bar = 100 μm.

Approximately 10 μl of a 1: 40 000 solution of Neutral red in Ringer's solution was injected with a micropipette into the neural tube lumen through the rhombencephalic vesicle. The micropipette was connected to a microsyringe by polyethylene tube. The injection was controlled under a binocular microscope. This procedure was considerably more accurate for the staining of the ocular necrotic areas than the conventional embryonic surface administration of the



Figs. 1–4

dye. The embryos were reincubated for an additional time of half an hour; fixed in formol–calcium at 4 °C, cleared and mounted *in toto* following the method of Hinchliffe & Ede (1973). In some embryos, during the fixation, the superficial head ectoderm and the lens rudiment were carefully removed with tungsten microneedles under a binocular microscope to expose the optic cup.

For light microscopy a series of embryos was fixed with Carnoy's solution, dehydrated and embedded in paraffin. Serial frontal and sagittal sections were stained using the Feulgen method. Another series of embryos was fixed with 3 % glutaraldehyde in 0·1 M-cacodylate buffer at pH 7·3 for two hours. The head region was dissected free and dehydrated in acetone and propylene oxide, and embedded in Araldite. Serial semithin frontal and sagittal sections were cut and stained with toluidine blue.

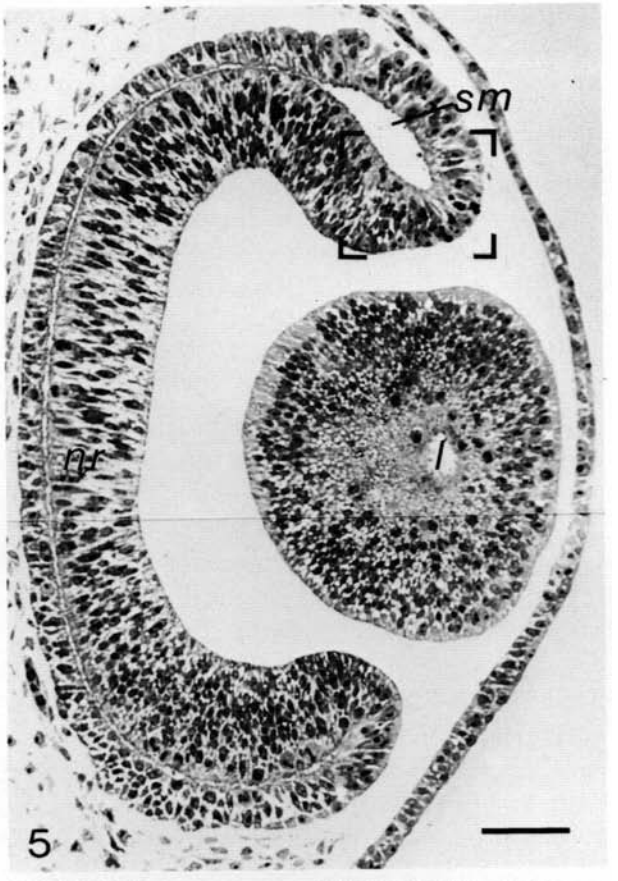
RESULTS

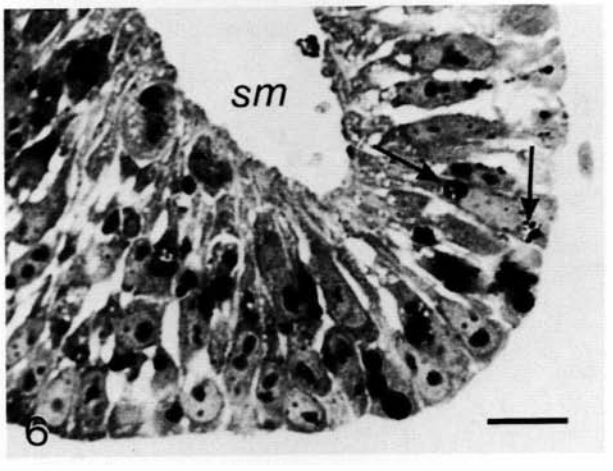
During the stages studied here (stages 13 to 19 of Hamburger & Hamilton, 1951) the optic vesicle and the optic stalk which connects it with the diencephalon invaginate forming the double-layered optic cup and the optic fissure. Using the *in ovo* vital staining method, three morphogenetic areas of cell death can be detected. The first area (Fig. 1) is located ventral to the insertion of the optic stalk with the diencephalon and is present throughout stages 15 to 19. The second area (Fig. 1) is observed throughout stages 14 to 19 in the ventral part of the back of the optic cup; the area lies immediately dorsal to the optic stalk lumen. The third area of cell death (Figs 2, 3, and 4) is located in the dorsal part of the optic cup rim. This area begins to be observed at stage 15. It achieves its maximum intensity at stage 16, and by stage 19 it is no longer identified. The Feulgen method and semithin sections (Figs 5 and 6) confirm the presence of these necrotic areas. In the dorsal part of the optic cup numerous dying cells appear surrounding the sinus marginalis both in the retinal layer and in its pigment layer. The dying cells are identified as pycnotic nuclei and dark-staining cell material which are present both scattered among apparently healthy neighbouring cells and sequestrated into large phagosomes. The morphological characteristics of this process appear similar to those observed in other epithelial necrotic areas where the dying cells and fragments are phagocytosed by the neighbouring healthy cells (García-Porrero *et al.* 1979; García-Porrero & Ojeda, 1979; Beaulaton & Lockshin, 1982; García-Porrero *et al.* 1983). At stages 16 and 17, concomitant with the presence of dead cells, the dorsal part of the optic cup rim appeared prominent in relation

Fig. 5. Light micrograph composition of a frontal semithin section of the eye rudiment at stage 17. The dorsal necrotic area of the optic cup has been outlined. Lens (l). Neural retina (nr). Sinus marginalis (sm). Bar = 50 μm.

Fig. 6. Detailed view of the necrotic area showed in Fig. 5. Note the presence of numerous pycnotic nuclei and phagosomes (arrows). Sinus marginalis (sm). Bar = 10 μm.

to other parts of the optic cup rim (Figs 3 and 4). This prominence of the rim is orientated toward the lens rudiment. At stage 19 this prominence is not identified and the optic cup rim appears uniform.





Figs. 5–6

DISCUSSION

The present study reports, using the *in ovo* vital staining method, a spatiotemporal mapping of the morphogenetic zones of cell death during the early chick retina morphogenesis. In contrast with preceding observations (Schook, 1980*a*,*b*) which reveal two areas of cell death, this work shows clearly that during the formation of the optic cup three zones of cell death are present.

Dead cells in the ventral part of the inner wall of the optic cup and at the level of the insertion of the optic stalk in the diencephalon have been previously described in the chick embryo (Källen, 1965; García-Porrero & Ojeda, 1979; Schook, 1980*a*,*b*) and in some mammals (Ernst, 1926; Källen, 1955, 1965; Silver & Hughes, 1973). Studies on the variations in the normal pattern of cell death in a number of different mouse mutants suggest that these areas play a functional role in the regulation of ocular size and shape (Silver & Hughes, 1974; Theiler *et al.* 1976; Silver, 1978).

In addition to the two well-known necrotic areas, our observations show a zone of cell death located in the dorsal part of the optic cup which has not been described until now. The exact reason why some dorsal rim cells of the retinal rudiment undergo degeneration during a relatively short period of time is not clear and, as in other necrotic areas, an experimental approach is necessary. However, some suggestions about the possible role of this cell death can be made in relation to the morphological characteristics of the chick eye development.

According to Glücksmann (1951), cell death allows the normal infolding of the optic vesicle to form the optic cup. There is some evidence which supports the idea that invagination of the optic vesicle takes place in the absence of cell death (Silver & Hughes, 1974; Silver & Robb, 1979), and therefore the relationship between cell death and the invagination of the optic vesicle is not clearly established. In any case, since this dorsal necrotic area begins to be observed at stage 15, when the invagination of the optic vesicle is almost complete (Hilfer, Brady & Yang, 1981), a possible relationship with the infolding process could be ignored.

Our observations suggest that this necrotic area could be related to the characteristic morphology of the optic cup rim in the chick embryo. After and at the time of the invagination of the optic vesicle, the retinal disc undergoes a remarkable growth due to the intense mitotic activity, and it is assumed that persistent mitotic activity at the margin of the cup accounts for the increase in the area of the retina (reviewed by Coulombre, 1965). In the chick embryo this growth of the optic cup rim does not appear to be uniform in some developmental stages. Romanoff (1960) describes the primordial optic cup as asymetric in shape, and he suggests that this is due to the fact that the cells in the dorsal part of the optic cup multiply most rapidly. Furthermore, our results show that at stages 16 and 17 a protruding zone is present at the dorsal part of the optic cup rim. This marginal protrusion is also identifiable in previous pictures reported by us

(García-Porrero *et al.* 1979) and in the reconstructions of the optic rudiment made by Schook (1980*b*). It is worth mentioning that the protrusion of the rim and the dead cells disappear concomitantly. It strongly suggests that cell death stops the growth of the dorsal part of the optic cup and/or removes tissue surplus in this zone. There are numerous examples of the role of cell death in the remodelling of embryonic organ shape (Saunders & Fallon, 1967). If this dorsal protrusion does not exist in mammals, as can be ascertained from the reconstructions showed by Mann (1928) and Duke-Elder & Cook (1963), it may also explain the absence of cell death in this zone of the mammalian optic cup (Silver & Hughes, 1973).

It is intriguing that in the chick embryo the necrotic area in the dorsal part of the optic cup is coincident with the onset of cell death in the lens rudiment which takes place in the dorsal part of the lens cup (García-Porrero *et al.* 1979; Schook, 1980*c*), and that these areas are absent in mammals (Silver & Hughes, 1973). In the early morphogenesis of the mammalian lens and retina, cell death appears to be located in the ventral parts of the optic stalk, optic cup and lens cup (Silver & Hughes, 1973). The different distribution of the lens cell death between mammals and chicks could be related to the different form of invagination of the lens placode (García-Porrero *et al.* 1979). The characteristic arrangement of cell death in the mammalian eye has led to a claim that there is an axis of degeneration which passes ventrally through the eye rudiment suggesting the existence of a spatiotemporal relationship between these degenerating loci (Silver & Hughes, 1973) although the nature of this relationship is unknown. However, in the chick eye development this ventral axis is not present, but the location of two neighbouring zones of cell death in the dorsal parts of the lens placode and the optic cup suggests a possible relationship between them. This fact strengthen the hypothesis that degenerating cells might induce necrosis in the neighbouring tissues (Stockenberg, 1936), either by the liberation of necrotizing factors (Silver & Hughes, 1973) or by the cessation in the production of hypothetic trophic factors (Beaulaton & Lockshin, 1982). In this way some experiments suggest that some zones of the developing limb bud can regulate the extension of morphogenetic areas of cell death (Fallon & Saunders, 1968; Hinchliffe & Gumpel-Pinot, 1981; Hinchliffe, García-Porrero & Gumpel-Pinot, 1981). The retina has a trophic effect on the lens development (reviewed by Coulombre, 1965). The decrease of such a trophic effect might trigger off the onset of cell death in the lens cup (García-Porrero *et al.* 1979). The cell necrosis in the dorsal retinal rim might well account for such a decrease and eventual cessation in the production of the trophic factor. If this is true, the cessation of a trophic influence on the dorsal side of the invaginating lens placode might contribute to its asymmetric shape.

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