

The histochemical demonstration of haemoglobin and haemosiderin in the growing oocytes of a teleost fish

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With 2 plates (figs. 1 and 2)

Summary

In a previous investigation, evidence was obtained indicating the presence of erythrocytes in the cytoplasm and germinal vesicle of a teleost fish, *Trichogaster fasciatus*. Histochemical tests for haemoglobin and related iron compounds have been applied to verify the histological and cytological findings. Haemoglobin (both intact in the erythrocytes and in the crystalline state) and pseudoperoxidase granules have been demonstrated histochemically both in the ooplasm and in the nucleus of immature eggs. Haemosiderins have also been detected and histochemically localized in and around the ooplasmic vacuoles.

Introduction

IN the course of an investigation on the nourishment of the growing oocytes of a species of teleost fish, *Trichogaster fasciatus*, the author observed that erythrocytes enter the oocytes from the blood-stream. The evidence for this has been reported elsewhere (Chatterjee, 1963). On the basis of this observation it seemed probable that one could identify the intact red blood-corpuses entering an oocyte by applying a histochemical test for haemoglobin. It is also now known that when a mammalian reticulo-endothelial cell takes up erythrocytes, each of the latter breaks into two or more fragments or undergoes haemolysis, and as a result haemoglobin is liberated into the cytoplasm of the former reticulo-endothelial cell (Bessis, 1961). This liberated haemoglobin then undergoes changes in its molecular structure and gives rise to degradation products, among which haemosiderin may be included. Taking these facts into consideration, it seemed likely that one should be able to demonstrate some of the degradation products of haemoglobin in the oocytes. With these objects in view, the author carried out a series of experiments, the results of which form the substance of this paper.

Material and methods

T. fasciatus occurs locally. The immature oocytes of freshly collected specimens constituted the material for the investigation.

According to both Gomori (1953) and Pearse (1960), certain modifications of the peroxidase reaction are the methods of choice for the histochemical demonstration of haemoglobin and some of its immediate degradation products. The benzidine method of Pickworth (Pearse, 1960) was selected. This

method was first tried on a very thin smear of the fish's blood fixed in the vapour of formalin. A positive reaction was obtained in the form of a dark blue ring around the nucleus, with dark blue granules or rods present in addition.

Perl's acid potassium / ferrocyanide method (Gomori, 1953) was selected for localization of haemosiderin. Since acid is said to dissolve haemosiderin, and chromates interfere in the preservation of iron, 10% neutral formalin was employed as the fixative.

Pieces of ovary were fixed in the formalin solution for 12 h, washed, dehydrated, embedded in paraffin, and sectioned at 8 μ . Some of the sections were mounted unstained in balsam; others were used for the benzidine test and Perle's reaction.

Pieces of ovary were also washed in repeated changes of a physiological saline solution adapted for cold-blooded vertebrates, and teased in a drop of this on a slide smeared with albumen. The eggs were pressed gently by covering them with clean coverglasses. They were fixed by running 10% neutral formalin through with the help of a piece of blotting-paper applied to the other end of the coverglass. Such preparations were left, coverglasses down, for 4 or 5 h in a Petri dish full of the fixative. The coverglasses were then carefully detached. Such preparations were used for the benzidine test and Perl's reactions. After the application of the benzidine test some preparations were counterstained with 1% aqueous neutral red solution, while others were left unstained. All preparations were finally dehydrated, cleared with xylene, and mounted in balsam.

Results

Haemoglobin

The reaction for haemoglobin and its immediate degradation products in the blood-smear (fig. 1, A) was treated as control and used constantly as the standard of reference.

The perinuclear dark blue ring of haemoglobin in intact erythrocytes was seen in both small and large eggs. It was present both in the ooplasm and in the germinal vesicle of the oocyte (fig. 1, B, C). Such rings of haemoglobin were also seen in the cytoplasm of almost mature oocytes (fig. 1, D). Examination of preparations counterstained with neutral red revealed filamentous disintegration-products of haemoglobin, stained dull red (fig. 1, E). Such

FIG. 1 (plate). The figure shows the result of the benzidine test. The scales represent 10 μ . A, part of a blood-smear. Rings of haemoglobin are seen around the colourless nuclei of the erythrocytes.

B, a small oocyte showing 4 rings of haemoglobin (marked with arrows) in the cytoplasm.

C, a small oocyte showing one clear ring of haemoglobin inside the nucleus (marked with an arrow) and two brownish yellow clumps of haemosiderin (black in the photomicrograph) in the cytoplasm.

D, a part of a large oocyte, showing large and small cytoplasmic vacuoles, *ov*, rings of haemoglobin, and two large clumps (deep black in the photomicrograph) of haemosiderin.

E, a part of an oocyte showing filamentous structures, stained with neutral red, in and around the cytoplasmic vacuoles, *ov*.

F, an aggregate of 4 crystals of haemoglobin deposited near a cytoplasmic vacuole.

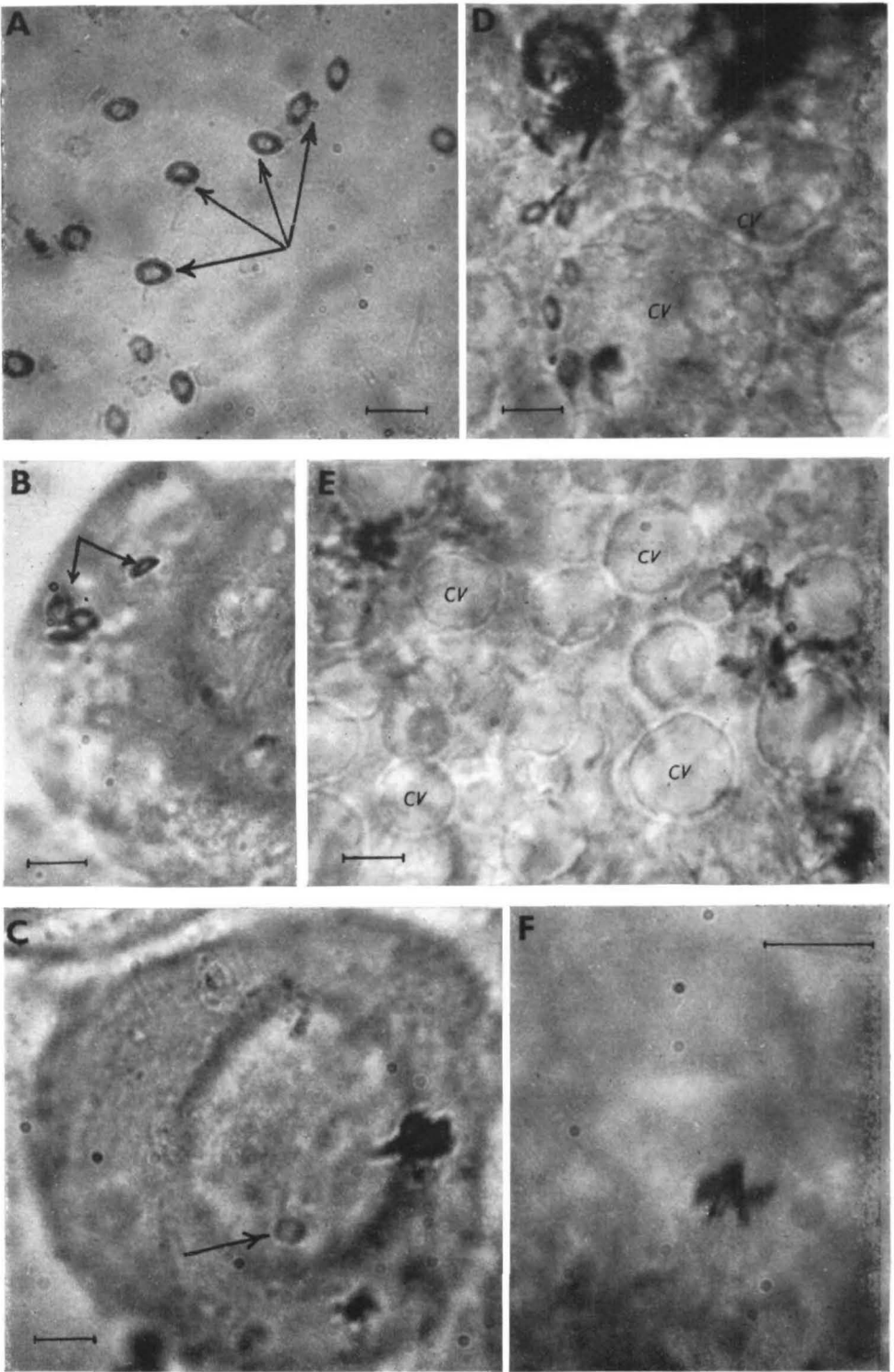


FIG. 1

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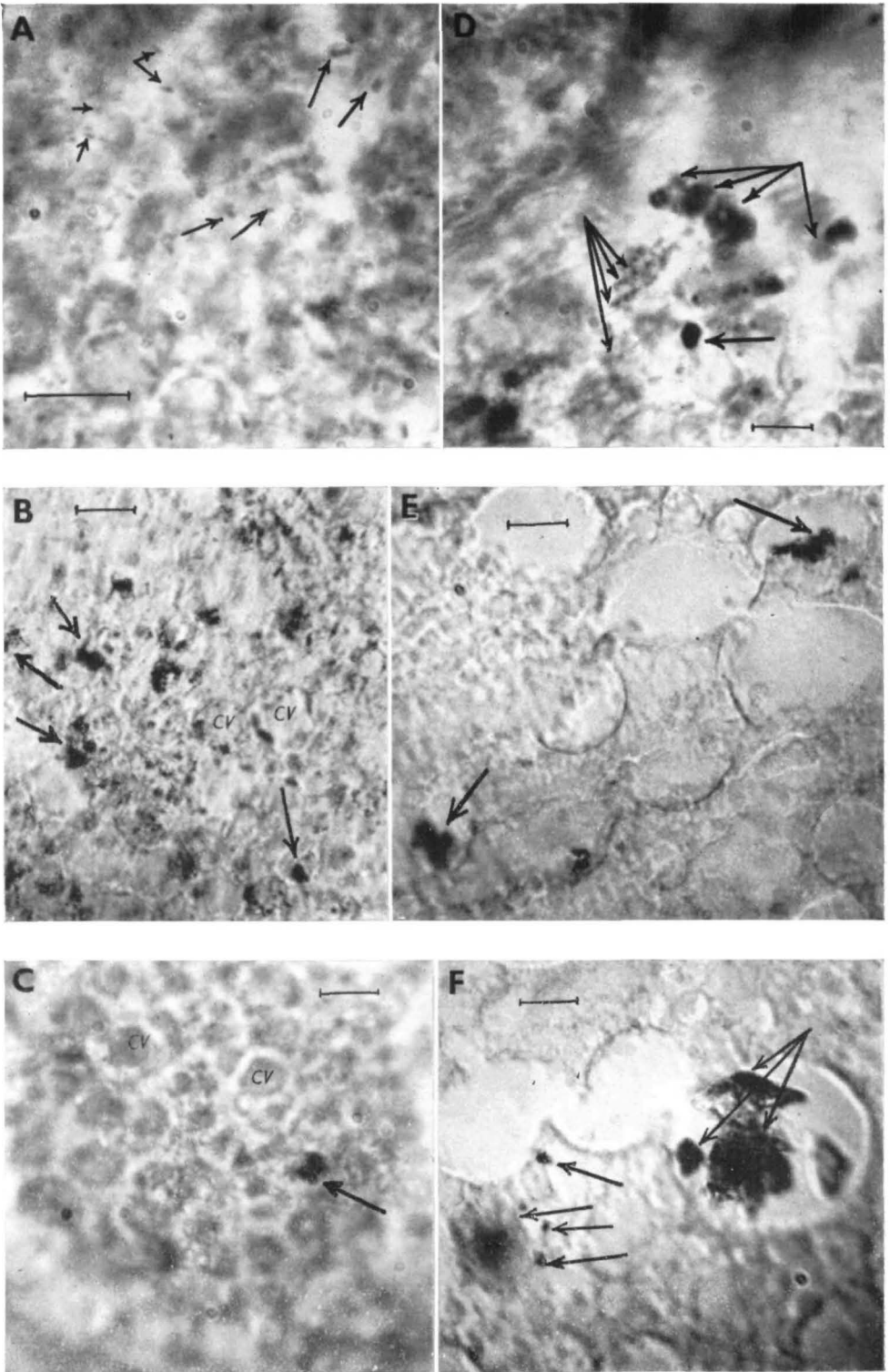


FIG. 2

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filamentous structures, though found in between and around the dark blue haemoglobin rings, were not seen in the unstained preparations. There were also haemoglobin crystals (fig. 1, F) attached to the rim of the ooplasmic vacuoles, especially in large oocytes. The cytoplasm of both small and large oocytes contained a considerable number of scattered dark blue granular and rod-shaped bodies (fig. 2, A). These bodies were also present in the nuclei of the small oocytes. On the whole, the test for haemoglobin and its immediate breakdown-products was more satisfactory in preparations of the intact eggs than in sections of material embedded in paraffin.

Haemosiderin

Sections that had been mounted unstained revealed many aggregations of crystalline structures in the cytoplasm of the oocytes. The number increased progressively with the increase in the egg size of the oocyte. They were yellow, yellowish brown, or deep brown (fig. 2, B). These crystalline deposits were always associated with the ooplasmic vacuoles and were not seen inside the germinal vesicle. Even after the application of the benzidine test, they remained as brown clumps (fig. 1, C, D). These aggregates were insoluble in formalin, alcohols, and alkaline solutions, and were visible in material fixed in Carnoy's solution, but they were dissolved by strong mineral acids. Besides these, there was also a light brownish tinge at the periphery of some ooplasmic vacuoles adjacent to such aggregates.

After the sections and intact oocytes had been treated according to Perl's method, these yellow and brown crystalline aggregates disappeared and in their places there appeared intensely blue aggregated or filamentous structures (fig. 2, C to F). There were also some very small blue granules near the blue aggregates (fig. 2, D, F). All these coloured structures were distributed in the cytoplasm of the oocytes. Their location appeared to be in and around the cytoplasmic vacuoles, which are very prominent in these eggs. The walls of the cytoplasmic vacuoles at many places were tinged with faint blue. In no case was there any positive reaction in the germinal vesicles.

Discussion

The foregoing observations, taken together, testify to the fact that haemoglobin, pseudoperoxidase granules, and haemosiderin are located in the

FIG. 2 (plate). The scales represent 10 μ .

A, dark blue granules and rods (marked with arrows) in the cytoplasm of an oocyte. Benzidine test.

B, part of a section of an oocyte showing haemosiderin crystals (marked with arrows) in and around the cytoplasmic vacuoles, *cv*.

C, a Prussian blue aggregate (marked with an arrow) in a cytoplasmic vacuole. Part of an intact oocyte.

D, a part of an oocyte showing aggregates and granules coloured with Prussian blue (marked with arrows).

E, F, parts of sections of large oocytes showing aggregates coloured with Prussian blue in the vacuoles; also granules.

growing oocytes of this fish. The germinal vesicles contain both haemoglobin and pseudoperoxidase granules but no haemosiderin. The nuclear haemoglobin and granules are more conspicuous in the smaller oocytes, at the stage when no cytoplasmic vacuoles have been formed or only a few are beginning to appear. At later stages of growth they become more conspicuous in the cytoplasm. Haemosiderin appears in the form of yellow to brown crystalline aggregates in the cytoplasm. When tested for the Prussian blue reaction, it is seen only in the cytoplasm of the oocytes in the form of aggregates, filaments, and small granules. It is therefore reasonable to infer that haemosiderin was localized only in the ooplasm, while haemoglobin and pseudoperoxidase granules are found both in the ooplasm and in the germinal vesicle.

Studies with neutral red suggest that haemoglobin probably undergoes degradation to give rise to filamentous structures that are capable of giving a positive reaction with the Prussian blue test. On the basis of the evidence presented, it may be assumed that these filamentous structures may become aggregated as clumps and finally may become granular, in both cases retaining the ability to give a strong Prussian blue reaction. Haemosiderin, in the form of clumps, granules, or filaments, shows a very close relationship with the cytoplasmic vacuoles.

Since the growing oocytes have no source of these iron compounds other than the red blood-cells, results of the histochemical tests corroborate the histological and cytological findings that nutrient material passes from the blood-stream into the immature oocytes. The results also provide evidence indicating that haemolysis or fragmentation of erythrocytes inside the germinal vesicle and later also in the ooplasmic vacuoles leads to the setting free of iron compounds.

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