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CYTOCHEMISTRY OF THE OOCYTE IN THE POLYCLAD *PSEUDOCEROS* SP.*

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ABSTRACT

The cytochemistry of turbellarians has not received the attention it deserves. During a study in this direction it has been found that the cytochemical aspects of the growth stages and the fully formed oocyte in invertebrates in general and turbellaria in particular have been neglected. In the present study the cytochemistry of the polyclad *Pseudoceros* sp. which is available in abundance in the intertidal region of Waltair Coast has been studied. It is well known that the egg-shell of polyclads pertains to a quinoe-tanned protein and the precursors are synthesised in the oocyte. In the beginning stages the shell precursors fill the cytoplasm and there is little amount of yolk. With growth the shell precursors gradually move to the periphery and ultimately come to lie there. Coincidence with these changes yolk bodies begin to appear and become progressively bigger. The most prominent type of yolk bodies which are oval to spherical are strongly PAS positive a reaction which is fast to saliva. They are positive to BPB and Congo red techniques but negative to Sudan black B. In the light of these tests it has been possible to say that the yolk bodies are carbohydrate-protein in nature. Details of cytochemical procedures are given and discussed.

INTRODUCTION

IN HIS compendium on animal gametes (female) Vishwa Nath (1968) did not include the turbellarians. It is clear that histochemical and cytochemical studies in turbellarians are scanty. In an attempt to provide comprehensive information on some cytochemical aspects of some invertebrate groups, opportunities were provided to investigate the turbellarians of Waltair Coast. This paper is devoted to the oocyte of the Polyclad *Pseudoceros* sp.

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MATERIAL AND METHODS

Specimens of *Pseudoceros* sp. were collected from the shingles of Waltair intertidal region and brought to the laboratory as soon as possible. It usually measures 15 mm and is violet in colour. They were usually fixed in hot Susa or Alcoholic Bouin or Formol calcium. Dehydration and embedding were done according to

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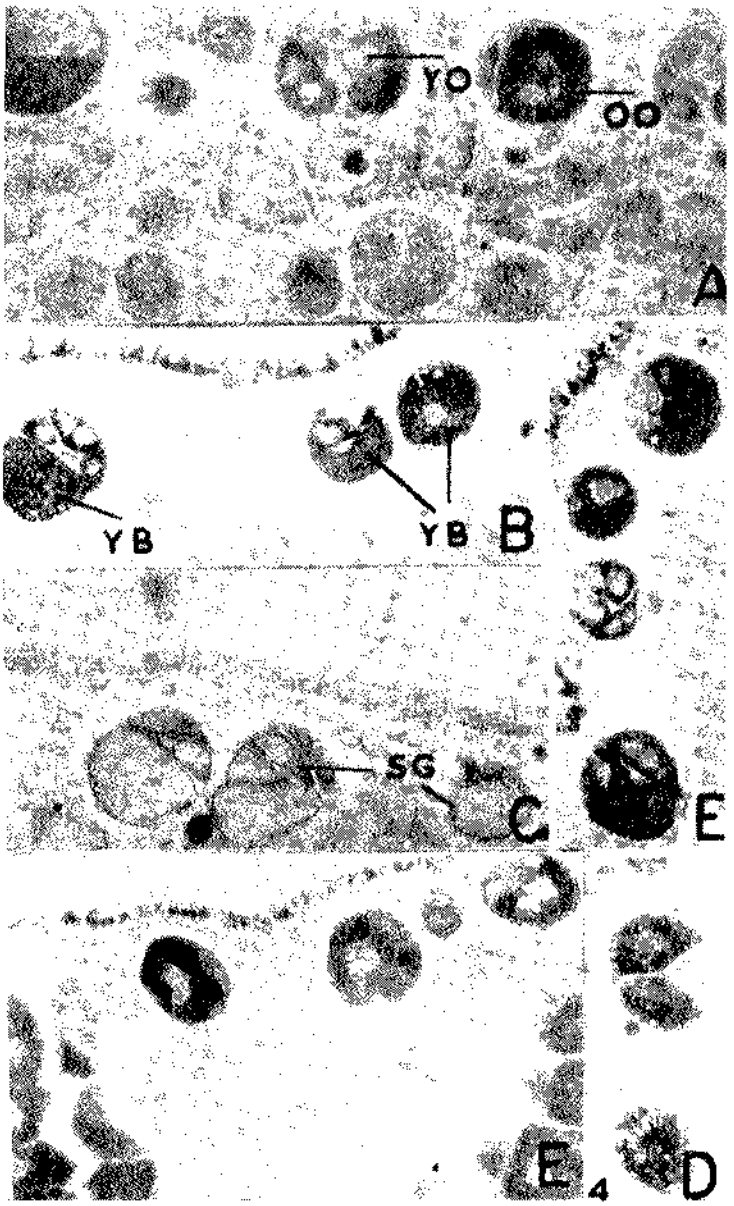

PLATE I Sections of *Pseudorcinis* sp. showing oocytes in follicles — A. Azan : Note young oocytes (YO) with shell precursors and older oocytes (OO) packed with yolk, B. PAS-LG : Yolk bodies (YB) in older oocytes strongly PAS positive, C. PAS after acetylation : Note yolk bodies are PAS negative but shell globules (SG) in younger oocytes slightly PAS positive, D. Bromophenol blue : Yolk bodies are positive, E. Iron Haematoxylin : Yolk bodies and shell precursors are stained, and F. Congo red : Yolk bodies alone are positive

the usual procedures and sections 8-12 μ thick were stained according to the histological and histochemical procedures given in Carleton and Drury (1957) and Pearse (1960). Details of the staining reactions are mentioned in the text at appropriate places.

OBSERVATIONS

The follicles of the ovary are packed with oocytes to be seen at various stages of development. The youngest oocytes abutt the wall of the follicle while the older ones lie towards the follicular duct leading to the oviduct. Youngest oocytes measure 0.012 mm and the oldest oocytes about to be released into the oviduct measure, 0. 15 mm.

In the early oocyte the cytoplasm is of a homogeneous texture and no inclusions of any appreciable dimensions are discernable. But with growth, minute refringent spherules begin to appear. These spherules are Azur Schiff positive. They stain deeply with Iron Haematoxylin. In PAS-LG preparation they take up light green and appear as green globules. They are PAS negative. These globules appear as minute birefringent spherules which fill the entire cytoplasm in the early oocyte but with growth they become slightly bigger and come to lie at the periphery only. Obviously they represent the shell precursors.

At the time when the oocyte reaches the size of 0.105 mm the shell globules begin to be displaced to the periphery coincident with the origin of the yolk bodies. In the fully grown oocyte ready to be released into the oviduct all the shell globules lie at the periphery although their coalescence into the complete egg shell is not at accomplished. In Azan preparations the shell precursors appear as bright red globules while the yolk bodies are blue (Pl. I A).

Chemistry of the yolk bodies

One of the chief characteristics of the main types of yolk bodies which fill the cytoplasm is their strong PAS positive nature (Pl. I B). It is well known that the following five groups of substances are PAS positive (Pearse, 1960) :

Polysaccharides like Glycogen, neutral Mucopolysaccharides, Mucoprotein, Glycoprotein, Glycolipids and unsaturated Lipids and Phospholipids.

To find out the precise nature of the yolk bodies the following tests have been conducted. The PAS positive nature could not be abolished by pretreatment of sections with saliva (saliva 10 ml + distilled water 40 cc + 1 cc 0.1 Na Cl). Sections were incubated in this mixture for an hour at 37°C. Control sections were left in distilled water for the same period. Acetylation (sections were treated at room temperature for 24 hours with 16 ml acetic anhydrids + 24 ml dry pyridine) abolished the PAS reactivity (Pl. I C) which however could be restored by deacetylation (carried out by treatment of sections with 0.1 N Potassium hydroxide for 45 minutes at room temperature or with 20% ammonia in 70% alcohol at 37°C for 24 hours). Thus the presence of 1 : 2 Glycol groups could be established. These globules are Sudan black B negative. They are positive to Bromophenol blue (Pl. I D).

The main histochemical reactions of the oocyte inclusions in *Pseudoceros* sp. are given (Table 1).

TABLE 1

Technique	Yolk bodies	Shell precursors
Iron Haematoxylin	Brownish	+ (Pl. I E)
A. PAS	PAS +	—
B. PAS-LG	PAS + (saliva fast)	—
A. Acetylation	PAS —	—
B. Deacetylation	PAS +	Stained red
Congo Red	++	— (Pl. I F)
BPB	++	Greenish
Sudan Black B	—	—

DISCUSSION

It is evident that the oocyte cytoplasm in the turbellarian *Pseudoceros* sp. displays two chief kinds of substances. Shell precursors which are largely synthesized initially preceding the synthesis of yolk substances. These shell precursors may belong to the class of Quinone-tanned protein. But more work in this connection is however needed. The yolk material which arises at it does in the form of small spherules appears gradually to displace the shell precursors to the periphery during the growth phase of the oocyte. In sections of the oocyte which has reached the optimum size of 0.15 mm the shell precursors could be seen as a string of beads at the circumference while the entire cytoplasm is densely packed with the yolk bodies.

The yolk bodies are strongly PAS positive. Among the various groups of PAS positive substances by a battery of reactions it could be concluded that the spherules belong to group III which includes Glyco- and Muco-proteins. Pearse (1960) stated that the protein material in the tissue which do not contain lipid (Sudan black B negative) but which contain with diastase fast carbohydrate and do not exhibit gamma metachromacy Toludine blue are to be regarded as Muco- and Glycoprotein complexes. Since at present the distinction between Muco- and Glycoproteins is not possible, histochemically the yolk globules in *Pseudoceros* sp. or for that matter in other animals may be simply referred to as carbohydrate protein complexes. It may be mentioned here that in the oocyte of the amphipod (crustacea) Shyamasundari and Ganapati (1970) have found similar carbohydrate protein bodies.

The positive Congo red reaction is considered to be characteristic of amyloid which is a carbohydrate containing protein (Pearse, 1960). Thus the ensemble of reactions discussed here establish beyond doubt that in the oocyte of these invertebrates the main type of yolk is in the form of carbohydrate protein. It would be of great interest to make a synthetic study of the oocyte of the various invertebrate

groups to explore the uniformity of this pattern. It is well known that the developing systems are in great need of energy and building material. Both these are combined in the carbohydrate protein yolk bodies.

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