CHAPTER - VIII

OBSERVATIONS ON NUCLEAR EXTRUSIONS AND VITELLOGENESIS
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It has been observed in many teleosts that towards the end of the primary growth phase, the nucleoli within the nucleus of the oocyte become active, and most of these have a tendency to pass out of the nuclear membrane into the cytoplasm. This is the well known phenomenon of nucleolar extrusion.

This phenomenon is very vividly present in most of the fishes studied in the present thesis. The synchronised occurrence of these nucleolar extrusions with the vitellogenesis favours the view that the former is an important if not absolute factor for bringing about vitellogenesis. The occurrence and mode of nucleolar extrusion has been studied in each fish species and is reported below :-

1. **GREAT-ES PLAGIOTOMUS (HECKEL)**

Very conspicuous loops of nuclear membrane for the emission of nucleoli are formed in the III stage of the ovary of this fish (plate 1). Two loops may be very close to each other, and most of the nucleoli in contact with the nuclear membrane inside the nucleoplasm; while a few are just outside it in the cytoplasm area of the oocyte. The yolk vacuoles are in large numbers. In stage IV (Plate I) most of the nucleoli have extruded out of the nuclear membrane and in stage V (Plate IV) when there is complete vitellogenesis and spawning, no nucleolus is inside the nucleoplasm. Thus in stage I, the nucleoli are present in the mesh of nucleoplasm in large numbers; in stage II they are arranged along the nuclear membrane on the inserside, in stage III and IV nucleolar extrusion takes place and in stage V all nucleoli have been extruded.

2. **SCHICOTHORAX SSOOCINUS HECKEL**

The mode of extrusion of nucleoli is by the formations of a loop like structure in the nuclear membrane, in which a single nucleolus rests. This condition is found in the III and IV stages of the oocytes. In Stage I,
the nucleoli are present in the nucleoplasm as distinct structures lying more or less centrally (Plate III). In Stage II of the oocyte these come to lie along the inner side of the nuclear membrane (Plate II). Distinct, well defined loops of nuclear membrane extruding into the cytoplasm are seen in the IV stage while a wavy margin is seen even in oocytes fully laden with yolk (Plate II). After extrusion distinct nucleoli brick red (with Mallory Trichome-stain) are visible inside the yolk spherules. At this IV Stage the vitellogenesis is complete while in stages II & III it has begun, and is rapidly on the increase.

3. Cyprinus Carpio Comminis Linn

The phenomenon of nucleolar extrusion is extremely well demonstrated by the oocytes of Cyprinus Carpio Comminis. Here in the majority of the oocytes in Stage II, the nuclear membrane is thrown into several loops (Plate III). In Stage III some of the nucleoli along with the containing nuclear membrane loop, appear cutting separate from rest of the membrane (Plate III). In the stage IV the nucleus shifts to one side of the eva and the nucleoli are very small in number inside the nucleoplasm. Some nucleoli are seen in the cytoplasmic area as well. There are present well formed nuclear membrane loops at the mouth of which are present nucleoli, in the process of moving out through them (Plate III). This shows that the loop formation is followed by the movement of the nucleoli inside it. It has also been observed that the nuclear membrane loop also enters the cytoplasm along with the nucleolus as some nucleoli still surrounded by membrane have been observed in the cytoplasm. The membrane then is reformed after nucleolar extrusion. In Stage V there is hardly any nucleoli present and only yolk fills up.

4. Gambusia Affinis Holbrooki (Baird and Girard)

No nucleolar extrusions could be seen in the growing oocytes of this fish species. These were absent in stage I, stage II and stage III of the oocytes. So was the case in stages IV and V (Plate IV).
In this fish species the nucleolar extrusions are not by the formation of the loops although the nuclear membrane may show wavy margin (Plate Vb). But it appears that nuclear extrusion does take place, as there are large numbers of nucleoli along the inner side of the nuclear membrane at III and IV stages (Plate Va). In I and II stages the nucleoli are inside the nucleoplasm (Plate Vb). The number of yolk granules is small and in the process of formations in stages II and III and is maximum at stage IV. In stage V the yolk granules are absent, and the yolk is fully vacuolated.

VITELLOGENESIS

This is the phenomenon of yolk formation in the oocyte and is discussed in the light of observations made in the present investigations made on the maturing oocytes of five Kashmiri fish species, viz. Oreinumplagiostomus, Schizothorax appendiculatus, Cynodon carpiceps, Gambusia affinis holbrooki (BAIRD AND GIRARD) and Nemachilus kashmiensis.

Some workers believe that the yolk nucleolus (BALBIANI) due to its golgi bodies, mitochondria and other cellular components, takes part in the formation of yolk or transformed into yolk. Balfour and Gegenbaur (CIT. SCHARFF 1887) were the first to suggest the internal origin of yolk without attaching any importance to the nuclear extrusions.

Cunningham (1897) states the presence of a yolk nucleus in vicinity of the yolk layer. Gatensby and Woodger (1920) in frogs, Bhattacharya (1925) and in tortoise Brambell (1925) and Das (1931) in birds, and Lanius and Doorae (1908) in mammals, have attributed the formations of yolk to MITOCHONDRIA. While Losz (1906) described direct transformation of sarcoplasm mitochondria into yolk. Narain (1950, 1957) for Ophiocephalus and Heteropneustes fossilis, and Sclarias batrach as and Ambass scandinia, has attributed yolk formation to the golgi bodies of the yolk nucleus.
NATH and NAIGIA (1951) also derive fatty yolk from the golgi bodies. WHEELER (1924), while describing the egg of the dab (LIMANDRA) has given a more compromising view, in as much as the golgi bodies do not actually transform into yolk but are only connected with its formation. CHAUDHRY (1949) has advocated that the golgi and mitochondria granules of the pallial layer, which is derivation of yolk nucleus, get transformed into fatty and protein yolk; but according to Gurava (1962, 1965 and 1964) for various groups of vertebrates, there is a definite association of yolk formation.

The present author fails to support the above mentioned views in the cases of the oocytes of the Kashmir fishes studies, because a yolk nucleus of Balbani is conspicuously absent in these fishes; and the dark zone of cytoplasm which according to CHAUDHRY (1949) is the pallial layer derived from yolk nucleus is not a regular feature preceding vitellogenesis as observed in these fishes. BALINSKY (1961) suggestion that nucleic acids are essential pre-requisites of vitellogenesis is now widely accepted.

That RNA is an important factor for yolk formation has also been proved by Hisaka and PELLET (1962) for Zebra fish. BALINSKY (1961) states that the nucleoli consist mainly of RNA. The penetration of nuclear material into the cytoplasm may account, at least in part, for the increase in cytoplasmic RNA in oocytes.

Hisaka and PELLET (1962) have suggested that the decrease in the number of intra-nuclear extravesicular yolk vigorously increases in the perinuclear zone. In ORCHIS spicatus and Schistothorax escinimus the nucleolar activity starts at a very early state and they pass out in large numbers into the cytoplasm.
Bit nuclear extrusions alone as a single factor cannot be responsible for the control of this very important physiological process in the oocyte. Since increase in the size of the oocyte is enormous in *Schizothorax asocinus* and *Orinimus pleistomus*, synthesis of yolk must be extra-ovarian, the nucleoli only act as an *environer* for vitellogenesis.

The role of follicular epithelium in yolk formation is advocated by Beddard (1886) and Schrader and Leuchtenberger (1952), although some workers like Scharff (1887) and Wallaie (1905) failed to ascribe any function associated with yolk formation to the cells of the follicular epithelium. But, *Schizothorax asocinus* and *Orinimus pleistomus*, and the consensus of arguments is in the favour of the opinion that follicular epithelium plays an important part in yolk formation because (i) follicular epithelium around the oocyte appear either before or along with the nuclear extrusions, but definitely much before vacuolation and yolk formation; (ii) the yolk formation takes place where the nucleoli have distintegrated but its formation in this region is also due to the nearness of the follicular epithelial cells which convey the raw material; (iii) the vitelline membrane, even when thick is perforated by channels through which the food material from the cells of follicular epithelium is conveyed to the oocyte.