II. MOLLUSCA

Introduction

Studies in molluscan oogenesis are facilitated by the fact that all the developmental stages of the oocyte, especially in slugs (Bridgeford and Pelluet (1952) and Cowden (1958, 1962a)), can be found side by side in the hermaphrodite gonad. In addition to this, certain special structural aspects of the oocytes of some of the gastropods and slugs have attracted particular attention. The oocyte nucleolus for example of the gastropod Patella coerulea (Jörgensen (1913), Ludford (1921), Bolognari (1959a,b)) and the slugs, Deroceros reticulatum (Bridgeford and Pelluet (1952), Cowden (1958, 1962a)), Arion (Cowden (1962a)), Vaginulus (Gupta (1968b)) is of two types, viz. (1) primary nucleolus and (2) secondary nucleolus or amphi-nucleolus, which have different behaviour and chemistry during the oocyte growth.

Rebhun (1956a,b) in a snail and a clam has described special basophilic membranes with electron microscope, his 'periodic lamellae', which he erroneously called yolk nuclei, as the periodic lamellae really correspond to the annulate lamellae of Swift (1956) which are pinched off from the nuclear membrane. Cowden (1962a) in the young oocyte of the slugs has also demonstrated ribonucleoprotein-rich fibres or membranes oriented parallel to the nuclear membrane by the cytochemical techniques with light microscopy.
There are conflicting claims regarding the origin and Woodger of proteid yolk in molluscs. Gatenby (1920) in Helix, Limnaea and Patella derived proteid yolk from the Golgi bodies. Later Ludford (1921) in Patella and Fahmy (1949) in Eremina have also arrived at the same conclusions. Brambell (1924) derived proteid yolk from mitochondria in Patella and Helix. With electron microscopy Carasso and Favard (1950) describe yolk formation in the mitochondria in Planorbis while Yasuzumi and Tanaka (1957) derive yolk from Golgi dictyosomes in Cipangopaludina.

Again, Rebhun (1956a,b), on the grounds of cytochemical and electron microscopical studies, derived proteid yolk from his basophilic periodic lamellae, erroneously named as yolk nuclei in the snail and the clam. But, later in 1961 he withdrew his previous statements.

Cowden (1962a) is rightly of the opinion that the fibrous or membranous components certainly play an important role in proteid yolk synthesis by virtue of their basophilic nature though they may not have any direct connection with yolk bodies.

Ludford (1921) in Patella, Fahmy (1949) in Eremina and Bretschneider and Raven (1951) in Limnaea have also reported nucleolar extrusions.

I have recorded my observations on the role of RNA-containing cell organelles in yolk formation during the oogenesis of the slug, Vaginulus sp.
Observations

The youngest oocytes of *Vaginulus* continue to remain in the vicinity of the germinal epithelium throughout their growth period. The oocytes can be distinguished from the germinal epithelium and spermatocytes by their slightly larger size and by the presence of a distinct basophilic nucleolus. To begin with, there is a large single nucleolus in a big rounded nucleus (Pl.XIII, Fig.1). Later, as growth proceeds, there are two nucleoli present in the nucleus, one being larger (secondary) than the other (primary) (Pl.XIII, Figs.2,3). The staining behaviour of these two nucleoli differs very considerably. The primary nucleolus, which is always smaller in size, stains more deeply, with pyronin G, azure B and toluidine blue for RNA, Sudan black B (in acetone) for lipoproteins and mercuric bromphenol blue for general proteins than the secondary nucleolus (Pl.XIII, Figs.2-5). In a few sections the secondary nucleolus is observed piercing the nuclear membrane giving the appearance of its migration into the ooplasm (Pl.XIII, Fig.4) but such an appearance may be a mechanical artifact.

Initially the primary and secondary nucleoli are stained homogeneously with haematoxylin, azure B, toluidine blue, pyronin G, Sudan black B and bromphenol blue dyes (Pl.XIII, Figs.2,3), but, as growth proceeds, the secondary nucleolus shows vacuolization (Pl.XIII, Fig.2). Both the nucleoli are negative for carbohydrates in PAS and in Himes and Moriber techniques.
The nucleoplasm is negative for basic dyes like azure B, pyronin G, etc. in the initial growth phase (Pl. XIII, Figs. 1, 3) but in the late growth phase it starts showing colouration in the RNA-staining techniques (Pl. XIII, Figs. 2, 4) and with Berenbaum's technique for lipoproteins (Pl. XIII, Fig. 6). In Himes and Moriber's reaction the nucleoplasm of the oocytes stains yellow throughout oogenesis for proteins. In the vitellogenic oocytes besides the nucleolus in the nucleoplasm some small RNA- and protein-rich granules are also observed (Pl. XIII, Figs. 7, 8). There is no visible evidence that during oogenesis of Vaginulus the nucleolar material passes out to the ooplasm except for the secondary nucleolus which seems to migrate as such in the ooplasm in some oocytes.

Throughout the ooplasm of the young oocyte there are present large distinct RNA-rich granules (Pl. XIII, Fig. 1). However, in a slightly advanced oocyte the ooplasm does not show any granulation, the concentration of ooplasmic RNA decreasing progressively. This decrease seems to be due to the fact that the rate of synthesis of RNA is much less than the synthesis of ooplasm and also to the fact that the ooplasmic RNA is being rapidly utilised in protein synthesis.

An examination of the ooplasm of a slightly grown oocyte of Vaginulus with light microscopy has revealed unexpectedly highly basophilic parallel membranes in close vicinity of the nuclear membrane (Pl. XIII, Figs. 2, 5, 7),
corresponding clearly to the 'periodic lamellae' of Hebhun (1956a,b) or the 'annulate lamellae' of Swift (1956). These membranes are also rich in lipoproteins (Pl.XIII, Fig.5). Cowden (1962a) in the slugs, Arion and Deroceros, also describe with light microscopy some basophilic parallel membranes but he does not relate them to the periodic lamellae or the annulate lamellae.

There can hardly be any doubt that the RNA of these membranes plays an important part in the synthesis of proteid yolk in Vaginulus.

As the oocyte enters into the vitellogenic phase, the tinctorial behaviour of the ooplasm changes. There is a gradual increase in the intensity of staining for protein (Pl.XIII, Fig.8) and carbohydrate. In RNA-staining methods the ooplasm is stained poorly. Small, irregular and protein-rich bodies are differentiated in the ooplasm (Pl.XIII, Fig.8). These are the yolk granules.

Discussion
Perhaps the most interesting feature of the molluscan oocyte (particularly slugs) is the unusual presence of two types of nucleoli in its growth, which differ both morphologically and cytochemically. This condition was first reported by Jörgensen (1913) in Patella coerulea. Later Ludford (1921) in Patella sp., Bolognari (1959a,b) in Patella coerulea and Cowden (1962a) in Arion sp. and Deroceros reticulatum described the morphology and cytochemistry of both the types of nucleoli with light and electron microscopy.
Two nucleoli have also been observed by the author in the oocytes of the slug, *Vaginulus*. Initially these nucleoli have a similar size, morphology and tinctorial behaviour. This is confirmed by Bolognari (1959b) who has also shown a similar ultrastructure of these nucleoli at the initial stages in *Patella*. But with growth the size, morphology and tinctorial behaviour of the two nucleoli differ markedly from each other. Whereas the primary nucleolus now is smaller and stains homogeneously and deeply with basic dyes, the secondary nucleolus is large, vacuolated and stains lightly. In *Arion* the number of secondary nucleoli reaches up to five (Cowden, 1962a) but in the slug, *Vaginulus*, there is only one secondary nucleolus.

The primary nucleolus in *Vaginulus* is rich in RNA, proteins and lipoproteins. The secondary nucleolus is comparatively poor in RNA and lipoproteins but rich in proteins inasmuch as it stains in Hg-BPB with the same intensity as the primary nucleolus. These observations suggest that both the nucleoli differ in their ribonucleoprotein constituents. Both the types of nucleoli are devoid of carbohydrates as indicated by their negative reaction in PAS and yellow colouration in Himes and Moriber. It may, however, be stated that Cowden (1962a) in *Arion* and *Deroceros* has described the presence of carbohydrates in both kinds of nucleoli.

With electron microscope Bolognari (1959b) in the slightly grown oocytes of *Patella* has shown two types of
nucleoli, one composed of dark granules (primary nucleolus) and the other (secondary nucleolus) of rather thick and dark granules surrounding cavities of varying size (= intranucleolar vacuoles).

It is interesting to note that two types of nucleoli have also been recently reported in animal groups other than the Mollusca with electron microscopy (Brown and Ris (1959) in Triturus and Kessel (1966) in Thyone).

Another very interesting and unusual feature of growing oocytes of Vaginulus is the presence of parallel membranes rich in RNA, lipoproteins and proteins, corresponding to the 'periodic lamellae' of Rebhun (1956a,b) in Spisula and Otala, and the 'annulate lamellae' of Swift (1956) except that the author could not possibly observe 'annuli' with the resolution available to him.

Rebhun (1956a,b) derived his periodic lamellae from blebs of the nuclear membrane of the germinal vesicle. But later in 1961 this author has advocated that the nuclear membrane acts only as a 'mold' for the formation of this cytoplasmic structure from the endoplasmic reticulum and is not its precursor. The close association of this component with the nuclear membrane and the cytochemical studies of the author also suggest that the basophilic membranes originate from the basophilic ground substance (endoplasmic reticulum or ergastoplasm) and not from the nuclear membrane.
Cohen (1966) has shown that DNA is transcribed to give ribosomal, soluble and messenger RNA. The ribosomal RNA combines with proteins to form ribosomes, which can then attach to messenger RNA to form polysomes. In the polysomes the information contained in the messenger RNA is translated so as to govern the formation of specific polypeptides. The oocyte filled with yolk material in Vaginulus is characterised by the disappearance of ooplasmic RNA which might have been converted into polypeptides through an intermediate stage of polysomes.

In Vaginulus the quantity of ooplasmic RNA is closely related to the synthesis of proteins inasmuch as when vitellogenesis starts the concentration of RNA markedly decreases in the ooplasm and the concentration of proteins correspondingly increases.

The basophilic membranes in the ooplasm gradually disappear as vitellogenesis advances. Thus, there is no doubt that the RNA of these membranes also plays an important role in proteid yolk synthesis in the oocytes of the slug, Vaginulus (cf. Caspersson (1950), Brachet (1958), Raven (1961), and Nath (1968)). These observations are in conformity with Cowden (1962a) in the slugs Arion and Deroceros.

Yasuzumi and Tanaka (1957) state that the 'chromidial bodies' in the oocytes of the snail Cipangopaludina, which are the precursor substance for the proteid yolk, come in association with Golgi bodies and form such yolk. These authors did not examine the cytochemistry of the
chromidial bodies but, keeping in view the observations of Caspersson (1950) that the chromidial bodies are of ribonucleoprotein nature, it is likely that in Cipangopaludina oocytes the Golgi bodies play no role in the formation of proteid yolk, especially when they are known to be generally devoid of RNA.