Studies on the Fish Oogenesis. I. Histomorphological and cytochemical changes in the oocyte nucleus of *Amphipnous cuchia* (Ham.)

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Introduction

Reviewing the literature regarding the architecture and behaviour of the oocyte nucleus and its contents in various animal groups, it is apparent that the teleost oocytes have received little attention concerning the behaviour of nuclear components, and that the results may vary somewhat from one genus to another. Papers by Yamamoto (1956) on *Liopsetta*, Bara (1960) on *Scomber* and Arndt (1960) on certain fresh water cyprinoids constitute the only adequate descriptions of various aspects of the nuclear structure of fish oocytes. More recently Chouinard (1963) has studied in *Salvelinus* the sites of formation of extra nucleoli during early oocyte growth with interesting results and Hisaoka and Firlit (1962) have observed the localization of nucleic acids in developing oocytes of *Brachydanio*. Brief comments on the subject have been made for *Syngnathus*, etc. (Cunningham 1898), and several fish species by many workers.

This investigation aims at adding further to the existing knowledge of the related subject, chiefly on the behaviour of chromosomes, modes and sites of formation of extra nucleoli, the extrusion of nucleoli and the cytochemical features of these structures. It also seeks to bring together and discuss many controversial ideas held by light microscopists.

Material and methods

Amphipnous cuchia, the common Indian mud-eel, carries a single elongated ovary. Small pieces of the ovary taken from alive specimens were fixed in various fixatives and processed for the cytological and cytochemical procedures. Fluids after Bouin, Helly, Zenker, Regaud, Elftmann, Smith and Baker were employed as fixatives. For the routine microscopic examination, staining was done with haematoxylin after Heidenhain, Harris and Delafield with and without eosin. For the demonstration of nucleic acids Kurnick's methyl green/pyronin Y and Korson's toluidine blue/methyl green/orange G methods (Pearse 1960) were used. Localization of DNA was examined by Feulgen's technique. As a control unhydrolyzed sections were kept in distilled water at 60°C for the optimum time before staining with Schiff's reagent. For basic proteins (arginine and lysine) of nuclei, Alfert and Geschwind's alkaline fast green method (Pearse 1960) was applied.

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Yasuma and Ichikawa's ninhydrin/Schiff method was employed to demonstrate the protein bound NH_2 groups.

Observations

In accordance with Raven (1961) the whole period of growth and development of an oocyte from the earliest immature to mature stage has been designated into three phases and the results obtained have been enumerated under these phases.

1. *Phase of premeiotic changes*: The oocytes in this phase correspond to the chromatin-nucleolus stage and are characterized by a relatively large nucleus filled with distinct chromatin threads, and very little cytoplasm (Figs. 2, 3). The nucleus of oogonium occupies the greater part of the cell. Within each nucleus is observable a fine network of delicate threads on which are suspended more than one strongly basophilic nucleoli of varying sizes, one being usually strikingly bigger than the others. The chromatin elements and the nuclear membrane of this nucleus stain deep purple with Feulgen's stain. During the transformation of the oogonium into the earliest oocyte the nucleoli other than the biggest one, which may be termed the original nucleolus, disappear (Fig. 4). It is usually situated in the centre but subsequently moves towards one pole of the nuclear cavity, the other pole being occupied by the weakly basophilic chromosomal bunch. The free ends of the chromosomal threads tend to reach the vicinity of the original nucleolus (Fig. 2). This stage is variously referred to as the bouquet stage, synaptic stage or leptotene. The original nucleolus stains deeply with haematoxylin and ranges between 1-5 micra in diameter. It is not Feulgen-positive but exhibits little affinity to toluidine blue and pyronin Y. The chromosomes are strongly basophilic and stain fairly well with methyl green and Feulgen's technique (Fig. 22). The original nucleolus seems to be intimately connected with many a chromosomal bivalents (Fig. 4). The chromosomal bunch undergoes rearrangement and soon the individuality and stainability of the chromosomes increase (Figs. 3, 5). Soon afterwards, the threads become comparatively thin and gradually move to spread in the nuclear space (Fig. 5). Some of them are in close contact with the original nucleolus, which is hardly pressed against the nuclear membrane bearing a somewhat irregular appearance and measuring up to 10 micra in diameter (Fig. 5). At the points of contact there appear small basophilic bodies and a weakly positive reaction is shown by methyl green and Feulgen's technique at these sites. These small basophilic and Feulgen-positive bodies later on detach as a whole or in parts and form the extra nucleoli. These newly formed extra nucleoli show presence of small amount of protein by ninhydrin Schiff's technique. During this stage along the axis and ends of the long chromosomal threads are seen basophilic and Feulgen-positive swellings with pyroninophilic margins (Fig. 5). These swellings grow gradually and look spherical or spindle-shaped and later seem to resemble the nucleoli already present in the nucleus. These swellings, representing the heterochromatic centres, either detach as a whole or shed spherical

bodies, which act as the extra nucleoli. During this phase the nucleus increases nearly four times, whereas the oocyte seven times in volume (Fig. 1).

2. *Phase of previtellogenesis*: The oocyte during this phase correspond to the perinucleolus and yolk vesicle stage and show increase in the relative volume of cytoplasm and nucleus. The yolk nucleus, Da Fano-positive granules and yolk vesicles appear and undergo peripheral migration.

The nucleus, which swells into the germinal vesicle during this phase, is more or less spherical in outline measuring between 2.5 and 15 micra in diameter, contained in the oocyte ranging from 6 to 90 micra in diameter. A number of nucleoli of varying size and shape are lined up along the nuclear membrane. Many of these nucleoli are hardly flattened against the nuclear membrane (Figs. 7, 8) and some of them often show fragmentation or budding (Fig. 6). The extra nucleoli grow up in size and show strong affinity for toluidine blue and pyronin Y, and sometimes weak affinity for Feulgen's test, which indicates the occurrence of both RNA and DNA in them. The chromosomes during this phase further stretch and break all the visible contacts with

the nuclear membrane. They show positivity towards Feulgen's stain and methyl green especially at such points where nucleolar-like bodies are attached to them (Fig. 23). The chromosome Feulgen-positivity gradually decreases and is left only at certain loci (nucleoli organizing centres) where nucleoli are produced. The nucleus gets filled with finely granular nucleoplasm in which are suspended the chromosomes. The nucleoplasm seems to contain some amount of RNA demonstrable by pyronin Y (Fig. 24). The chromosomes unwind and become prominent and they gradually shortening assemble

Fig. 1. Graphical illustration of the numerical and measurement data of the nucleus and oocyte size and nucleoli in a median section of nucleus. The ordinate on left is the maximum size of oocyte in micra (dashed line) and also the maximum number of nucleoli (solid line). Ordinate on right is the maximum size of the nucleus in micra (line with strokes and dots). Abscissa represents the stages of growing oocytes: early chromatin-nucleolus stage, late chromatin-nucleolus stage, early perinucleolus stage, late perinucleolus stage, early yolk vesicle stage, late yolk vesicle stage, primary yolk stage, secondary yolk stage, tertiary yolk stage and mature stage.

Figs. 2-15. Photomicrographs of the nucleus of different stages of oocytes stained with Heidenhain's, Delafield's and Harris' haematoxylin with and without eosin. 2, a cluster of oogonia and earliest oocytes (arrow). $\times 1500$. 3, nucleus of an early oocyte in post-synaptic stage. $\times 1500$. 4, nucleus of an oocyte in early chromatin-nucleolus stage showing the original nucleolus (bottom), a chromosomal bivalent in contact with it and a few extra nucleoli at the periphery (arrows). $\times 1500$. 5, nucleus of a late chromatin-nucleolus stage oocyte showing the original nucleolus (arrow), chromocentres and swellings on the chromosomes (parallel arrows). $\times 1500$. 6, a portion of the nucleus of an early perinucleolus stage oocyte showing budding of nucleoli (arrow). $\times 1000$. 7, nucleus of a late perinucleolus stage oocyte showing the accumulation of chromosomal bivalents in the

towards the centre (Figs. 7, 8). They suddenly undergo bewildering changes and arrange themselves in pairs to exhibit the typical lampbrush stage (Figs. 7, 8). The lampbrush bivalents are arranged either in the form of two U's associated with each other through their ends or the two U's crossing each other in the form of an 'X'. Their joints are clearly seen with big knots. At this stage they stain deeply with haematoxylin and show the characteristic lateral projections and beaded look. These lateral projections of the lampbrush bivalents vary in length and width and are positive to methyl green and Feulgen's stain (Fig. 25). These are the loci, at which nucleoli-like structures are shed. The numerical strength of the extra nucleoli increases (Fig. 1) and most of them attain a roughly spherical shape. They show positivity for pyronin Y and toluidine blue. The lampbrush bivalents exhibit signs of decondensation getting stretched and slender (Figs. 9, 10). Their positivity for the basic dyes does not change and no reaction is shown for methyl green and Feulgen's technique (Fig. 26), which is possibly due to the presence of the nucleic acid in an undemonstrable (unstainable) state except at the nucleoli organizing centres. At the same time strong basophilia is shown by the granular nucleoplasm (Figs. 10, 11). The chromosomes spread widely in the nucleoplasm occupying once again a major part of the nuclear space. The nuclear membrane shows irregular undulations (Figs. 6-9) to render the nucleus lobate in appearance. The undulations may be to increase the nuclear surface. The outgrowth are usually occupied by one or more

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centre and peripheral arrangement of the nucleoli. The nuclear membrane shows low undulations (top arrow). Chiasmata are indicated with parallel arrows. $\times 700$. 8, nucleus of a late perinucleolus stage oocyte showing the lampbrush bivalents at the height of their organization. Note their beaded look and swollen joints (arrows). Many peripheral nucleoli are flattened against the nuclear membrane (bottom arrows). $\times 700$. 9, nucleus of an early yolk vesicle stage oocyte showing the wide distribution of decondensating lampbrush bivalents in the nucleoplasm. Note the many nucleoli-like bodies (arrows) in contact with the chromosomes and free in the nucleoplasm. Extruded nucleolus is shown with the uppermost arrow. $\times 400$. 10, nucleus of a yolk vesicle stage oocyte showing further decondensation of chromosomes. The nucleoplasm is strongly basophilic. The nuclear membrane projecting into the ooplasm, usually carrying a nucleolus. Number of nucleoli is considerably less. $\times 400$. 11, nucleus of a late yolk vesicle stage oocyte showing lampbrush bivalents in late phase of decondensation. Euchromatin of most of the bivalents has disappeared by loss of stainability. A thick lampbrush bivalent is shown in the centre (arrow). $\times 280$. 12, the lampbrush bivalent arrowed above under high magnification. Lateral projections (heterochromatic centres) are prominently shown (arrows). Dark sheddings in the nucleoplasm are the heterochromatic centres of disappeared bivalents. Chiasmata are seen. $\times 700$. 13, nucleus of a late yolk vesicle stage oocyte. Almost all the bivalents have disappeared from view leaving behind heterochromatic nucleoli organizing centres (arrows). Note the large number of nucleoli in the process of extrusion with some amount of nucleoplasm into the ooplasm. Nuclear membrane is broken at places. $\times 280$. 14, nucleoli organizing centres showing budding after being shed to form extra nucleoli (arrows). An extruded nucleolus is seen at the bottom. $\times 1000$. 15, nucleus of an oocyte in the same stage as above showing basophilic extra nucleoli left in place of disappeared chromosomes (arrow). The number of peripheral nucleoli is much less. $\times 280$.

nucleoli and are possibly the sites of nucleolar extrusions. Sometimes the nuclear membrane extends into the cytoplasm in the form of long irregularly-shaped processes (Fig. 10). The chromosomes after having occupied a greater part of the nucleoplasmic field stain deeply with the haematoxylin (Fig. 10) and are negative for the Feulgen's reaction except the nucleoli organizing centres (Fig. 26), where seem to be active during this stage producing nucleoli-like bodies, which move to the periphery and act as the extra peripheral

Figs. 16-27. Photomicrographs of the nucleus of developing oocytes. 16-21, are stained with Heidenhain's and Regaud's haematoxylin with and without eosin. 16, nucleus of a late yolk vesicle stage oocyte showing peripheral migration of centrally left minute

nucleoli. The nucleoli exhibit weak positivity for the Feulgen's technique at certain loci (Figs. 26, 27), the smaller ones sometimes show no affinity to this test, while pyroninophilia is exhibited by them. Frequent nucleolar extrusions occur during this phase, which results in a considerable reduction in the numerical strength of nucleoli (Fig. 10). The nucleoli destined to be extruded into the ooplasm break through the nuclear membrane bodily. During this process some amount of nucleoplasm is also passed into the ooplasm (Fig. 13). The extruded nucleoli remain in the vicinity of the nucleus (Fig. 17) for sometime and then losing their stainability disappear from view. It was often noted that the nuclear membrane breaks at such places where nucleolar extrusions take place. The lampbrush bivalents contract gradually becoming thick and smaller and one by one become obscure from view by losing their staining intensity (Figs. 11, 12). During this disappearance their nucleoli forming activity appears to have raised because the nucleoli organizing centres are very prominently shown and lie free in the nucleoplasm (Fig. 13). They actively produce nucleoli-like bodies by the process of budding (Fig. 14) for sometime and soon after their disappearance a large number of spherical and basophilic bodies resembling peripheral nucleoli are seen in their place (Fig. 15). Among them are also seen Feulgen-positive granules, which represent the remnants of disappearing heterochromatic nucleoli organizing centres. The basophilic bodies migrate towards the periphery and form a part of the population of extra nucleoli (Fig. 16). The process of nucleolar extrusion is at its height during this stage (Fig. 13). These lately born extra nucleoli, however, do not swell up to equal the size of already present nucleoli and show the presence of RNA only with pyronin Y. The presence of protein is indicated with ninhydrin/Schiff's technique.

3. *Phase of vitellogenesis*: During this phase the oocytes experience depositions of yolk and migration of the nucleus to the animal pole. In the

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nucleoli (shown in last figure). $\times 280$. 17, nucleus of a primary yolk stage oocyte. Note the homogenous nucleoplasm and indistinct nuclear membrane. Nucleolar extrusions are seen (arrows). $\times 280$. 18, a part of the nucleus in the same state as above showing the extrusion of nucleoli (arrows). $\times 1000$. 19, nucleus of a secondary yolk stage oocyte. Nuclear membrane is not seen and the number of nucleoli is considerably less. $\times 290$. 20, nucleus of a secondary yolk vesicle stage oocyte. $\times 280$. nucleus of a tertiary yolk vesicle stage oocyte. A few nucleoli are gathered in the centre of nucleoplasmic field, where thin and weakly basophilic chromosomes are seen (arrow). $\times 280$. 22, nucleus of a late chromatin-nucleolus stage oocyte showing Feulgen-positivity of the chromosomes. $\times 1500$. 23, nucleus of an early perinucleolus stage oocyte stained with Feulgen's technique showing the positive areas on chromosomes (arrows). $\times 1000$. 24, nucleus of a late perinucleolus stage oocyte stained with methyl green and pyronin Y showing the pyroninophilic nature of chromosomes and nucleoli. $\times 1000$. 25, nucleus of an early yolk vesicle stage oocyte stained with Feulgen's technique. Note the Feulgen-positive heterochromatic regions of chromosomes (arrows). $\times 700$. 26, nucleus of a late yolk vesicle stage oocyte stained with Feulgen's technique. Feulgen-positivity is entirely lost. $\times 400$. 27, a portion of the nucleus (marked in Fig. 26) highly magnified showing the Feulgen-positive areas on the nucleoli (arrow).

early phase the nucleus occupies the central position, but the deposition of yolk changes its outline (Fig. 17); it gradually becomes irregular in shape. No nuclear membrane is visible. Almost all the nucleoli present in the nucleus are situated at the periphery. Their number is much less than in the former stage and they show an affinity for RNA only. Nucleolar extrusion are seen during this stage also, but not very frequently (Fig. 18). The chromosomes are no more visible as distinct structures and the whole nuclear field looks a platform of homogenous consistency (Figs. 17, 19, 20). With the more deposition of yolk and beginning of its peripheral migration the irregularity in the nucleus' outline is further increased. The nucleoli left unextruded are very few in number and tend to move towards the centre of the nucleus, where ultimately the chromosomes reappear as extremely thin, small and weakly basophilic threads (Fig. 21). They tend to form the metaphase picture of the first maturation division and the centrally located nucleoli most probably take some part in the formation of metaphase spindle. At this stage the nuclear membrane disappears totally and the ooplasm and nucleoplasm meet freely (Fig. 17-21). Further observations of chromosomes was impossible, because of the occurrence of heavily deposited yolk material.

Discussion

Sadov (1957) studied and calculated the ratio of the volume of nuclei to the volume of oocytes in the sturgeon, sevryuga and sterlet. According to him, during early growth the ratio of nuclear volume to oocyte volume come to 74.57% in the sturgeon, 37.47% in the sevryuga and 89.96% in the sterlet, during advanced growth 60.15%, 23.97% and 79.9% respectively and during mature stage 11.34%, 7.76% and 11.71% respectively. In the present fish the nucleus increases gradually, its volume nearly 21 times during the full oocyte growth, whereas the oocyte increases nearly 500 times in volume. The relative volume of the nucleus decreases from 80% to 4.5%. This indicates a constant decrease in the nucleo-oocyte ratio. The striking reduction in the relative volume of nucleus merits attention. It probably reflects on the nucleo-cytoplasmic intimacy and the controlling of synthetic activities going on in the cytoplasm, by nuclear contents in some way.

Yamamoto (1956) reported in *Liopsetta obscura* the lampbrush bivalents to appear in the early yolk vesicle stage oocytes and to become indistinct after this stage, while Arndt (1960) in his studies on some fresh water cyprinoids emphasized that the lampbrush bivalents dissolve as soon as the vitelline bodies appear in the ooplasm. In contrast, Bara (1960) observed the lampbrush bivalents of *Scomber scomber* to appear in the stage 6 (late perinucleolus stage) oocytes retaining their individuality and always staining with certain dyes in later stages of oogenesis also, which corroborates with the findings of Marechal (1905) for *Gasterosteus*. The lampbrush bivalents of *A. cuchia* appear in the late perinucleolus stage oocytes and are com-

paratively far more distinctly and neatly organized. Evidence presented supports the view of Arndt (1960) on the disappearance of chromosomes in the yolky oocytes though differs in the manner of their disappearance. According to Arndt (1960) the chromosomes undergo dissolution. In the present fish the lampbrush bivalents show signs of decondensation during the yolk vesicle stage and with the deposition of yolk globules disappear from view by loss of stainability. They reappear in the migrating nucleus in a shrunken and indistinct form.

There is quite a plethora of observed facts and views regarding the multinucleolate condition of the oocytes of fishes and other animals. Formation of the extra nucleoli by budding or fragmentation of the large original nucleolus has been reported by Cunningham (1898), Nussbaum (1913), Eggert (1929), Chaudhry (1951) and Mas (1952), while the appearance in the nuclear cavity at random has been described by Cunningham (1898), Marechal (1905), Stephen (1902), Jorgensen (1913), Subramaniam and Aiyar (1935), Ito (1938), Yamamoto (1956) and Bara (1960). Yamamoto (1956) reported the extra nucleoli associated always with heterochromatic Feulgen-positive granules, while Arndt (1960) observed the earliest extra nucleoli arising in close contact with the heterochromatic Feulgen-positive segments of several chromosomes lying near the nuclear membrane. Chouinard (1963) recorded a heterochromatic Feulgen-positive body lying attached to one side of the original nucleolus, which contains nucleoli organizing centres of nucleolar chromosomes and gives off extra nucleoli on the other side of the original nucleolus. This author further reported that non-heterochromatic chromosomal regions distinct from the nucleoli organizing regions of the usual nucleolar chromosomes are instrumental in the formation of extra nucleoli. In *A. cuchia*, the budding of nucleoli was evident in many instances, and the sites of contact of many chromosomal bivalents with the original Feulgen-positive nucleolus, were also recognized as the nucleoli organizing centres. It is of interest to note that the extra nucleoli are formed at the heterochromatic Feulgen-positive loci on many chromosomes even after the organization of lampbrush bivalents. The process of extra nucleoli formation in the species investigated begins at about the post-synaptic stage and continues till the late yolk vesicle stage is reached. The stop of extra nucleoli formation correlates to some extent with the disappearance of chromosomes. The present observations contradict the reports of Yamamoto (1956), Arndt (1960) and Chouinard (1963) in that the heterochromatic Feulgen-positive nucleoli organizing centres on the chromosomes are functional till the latter's optical disappearance. On the other hand, above authors reported that the nucleoli forming activity of the heterochromatic Feulgen-positive centres is in later stages, transferred to the non-heterochromatic nucleoli organizing centres located on the chromosomes. The present finding supports the view of Arndt (1960) that there is an intrinsic connection between the disappearance or dissolution of lampbrush bivalents and simultaneous appearance of minute nucleoli scattered in the centre of the nucleus.

Whether the lampbrush chromosomes play any role in the formation of extra peripheral nucleoli or all the peripheral nucleoli in an oocyte are formed before the organization of lampbrush bivalents, are the questions of intrinsic importance in both fish and amphibian oocytes (Duryee 1950, Gall 1954, Callan and Lloyd 1960, Chouinard 1963, Macgregor 1965). In amphibian oocytes two mechanism have been defined to be operating concerning the multinucleolate condition of the oocyte nuclei. The first is that no part of the lampbrush bivalents is involved in the formation of extra nucleoli, and that the latter are formed before the chromosomes assume lampbrush form and the second is that the nucleoli organizing centres on the lampbrush chromosomes are involved in the process. Judging from the data presented by previous authors it appears that no attention has been paid to this aspect of the subject, where the fish are concerned. In the fish in question here, as is evident in the foregoing pages, the lampbrush chromosomes are also involved actively in the formation of extra nucleoli. The thick lateral projections (Feulgen-positive sites of the shedding of extra nucleoli) and the fusing knots on the lampbrush bivalents of fish in question show resemblances to the giant fusing loops of the lampbrush chromosomes of amphibian oocytes within the command of light microscopy. Formation of extra nucleoli at the heterochromatic Feulgen-positive nucleoli organizing centres on lampbrush bivalents seems to be in a way parallel to the shedding of extra nucleoli at the sites of giant fusing loops in the amphibian oocytes. Similar cytochemical reactions shown at such sites are the strongly recommendatory evidences in this connection.

Many investigators (Eggert 1929, Subramaniam and Aiyar 1935, Narain 1937, Ito 1938, Chaudhry 1951, Dodson 1953, Yamamoto 1956, Bara 1960) have described the occurrence of nucleolar extrusions in the course of oogenesis in a number of teleosts. Most of the authors have variously postulated the time and the role of nucleolar extrusions and treated them of compelling significance in the process of oogenesis, but since this aspect does not come within the compass of this paper, it will be discussed elsewhere. However, it will be worthwhile to record here that one of the major roles of nucleoli is the intervention in the chromosomal control of synthetic activities going on in the ooplasm. The present work records nucleolar extrusions during the late phase of previtellogenesis and up to the late phase of vitellogenesis. In conformity with the reports of Narain (1937) and Stolk (1959) in this fish also the nucleoli break bodily into the ooplasm along with which some amount of nucleoplasm is also released. It is of interest to report here that a large number of peripheral nucleoli are lost by extrusion and new ones are produced at nucleoli organizing centres due to which there is a turnover in the population of extra nucleoli during the process of oogenesis. However, there exists no relationship between the number of nucleoli organizing centres and number of extra peripheral nucleoli; the latter are produced at a indefinite rate.

Marza *et al.* (1937) reported the chromosomes of *Fundulus* oocytes to be Feulgen-negative until the diakinetik stage; Yamamoto (1956) observed the presynaptic, synaptic and lampbrush chromosomes of *Liopsetta* oocytes to be Feulgen-positive and Hisaoka and Firlit (1962) reported the chromosomes of oogonia and yolk free oocytes of *Brachydanio* to be Feulgen-positive. In *A. cuchia* the chromosomes of the oogonia and oocytes up to late perinucleolus stage stain with methyl green, being Feulgen-positive. This positivity decreases

gradually to become almost negative in the later stages, except at certain loci, where localized synthesis of DNA takes place and which bear strong Feulgen-positivity in later stages also. It may follow the suggestion of Hisaoka and Firlit (1962) that the DNA of chromosomes (except at the nucleoli organizing centers) is in a depolymerized state during the later stages of oogenesis. The nucleoplasm also never gives a positive reaction with Feulgen's stain, probably due to the depolymerized state or too much diluted form of DNA to be stainable even slightly. It may not be out of line to mention here that it has been established that there is a definite amount of DNA in each nucleus of a species and the same amount of DNA is possessed by somatic nuclei, follicular cell and ovarian stroma cell nuclei, which stain intensely purplish-violet by Feulgen-stain. The same amount of DNA is diffused in a large area of the oocyte nucleus due to which the concentration becomes very low and hence not easily detectable. Brachet (1960) observed such a feature in amphibian oocytes. It is of significance that the lateral projections of lampbrush bivalents, showing Feulgen-positivity, detach either as a whole or in parts reaching the periphery of nucleus to act as extra nucleoli, which are usually RNA-containing bodies. It indicates that locally synthesized DNA on the chromosomes is transferred to the extra nucleoli produced at such sites or the DNA-containing outgrowths detach as such. These DNA-containing outgrowths on the chromosomes have RNA-positive areas, which may be compared with the RNA-puffs and RNA-clumps. Occurrence of such RNA-puffs has been considered due to the raised synthetic activity of the cell. This aspect indispensably warrants further investigation.

Arndt (1960) reported the absence of detectable RNA in the nucleoli during previtellogenesis; RNA appears in them only with the beginning of vitellogenesis. In *Lebistes*, Vakaet (1955) reported a decrease in RNA of nucleoli towards the end of vitellogenesis to finally become totally negative. Chouinard (1963) observed no detectable amount of DNA in the nucleoli of *Salvelinus*. Presence of small amounts of detectable DNA, in addition to RNA is shown in the nucleoli of lampbrush and yolk vesicle stage oocytes of *A. cuchia*. It is possible that DNA substance present at the nucleoli organizing centres of the chromosomes penetrates into the RNA-containing nucleoli at the time of their shedding and does not disappear or separate till late. It is worth mentioning here that the presence of DNA has been noted in the liver nucleoli and amphibian oocyte nucleoli by Brachet (1950) and Panijel (1951) suggesting that this DNA is born on the chromosomes. Positive protein reaction in the nucleoli by ninhydrin/Schiff's technique, as recorded by Vakaet (1955), Yamamoto (1956) and Arndt (1960) is shown in the nucleoli of this fish.

Presence of DNA-like material in the ooplasm of amphibian and sea-urchin eggs has been defined. In the present fish, since the peripheral nucleoli possess a small amount of DNA along with a large amount of RNA and are extruded into the ooplasm as such, possibility of the presence of DNA in the ooplasm is worth consideration.

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