in distilled water for 24 h and then the roots were fixed and squashed as above.

Mitotic arrest was the most salient feature of treated cells (figure 1). Deformed nuclei and nuclear lesions were also common in such preparations. Deeply pycnotic nuclei presumably formed by arrest in metaphase were also common (figure 2). Arrest in prophase and elongation of nuclei were also frequently encountered (figure 3). Early splitting of chromosomes was noticed and this in association with nondisjunction of daughter chromosomes could possibly result in polyploidy.

Figure 4 shows a triploid cell in which early splitting of chromosomes is very evident. Sister-strand exchanges were also observed. The spindle mechanism was found to be disturbed in the treated cells. The display of metaphase chromosomes in 3 different groups could also be noticed in some preparations (figure 5). Metaphase clumping (figure 6) and neocentric activity of telomeres (figure 7) were also encountered. In materials treated with the drug for 24 h and then allowed to grow in distilled water for another 24 h, there was a drastic decrease in the mitotic index. The cytological effects of different treatments with nimbidin are presented in table 1. (A minimum of 500 cells was observed for each timing and treatment.)

The Allium test was undertaken for rapid screening of drugs for antimitotic activity, since it is very convenient, inexpensive and provides hundreds of cells in mitosis in a single preparation. It is proposed to carry out further tests with sarcoma 180, adenocarcinoma 755 and leukemia 1210, according to the international scheme[7] for establishing the anticancer activity of this drug.

The results clearly indicate that nimbidin is a strong antimitotic agent. At higher concentrations, the drug is able to arrest cell division immediately upon treatment. The effect seems to be primarily in the kinetochore of chromosomes, as shown by the acute clumping of chromosomes and neocentric activity of telomeres. Disturbances in chromosome movement may be a secondary effect due to kinetochore inactivation. Such effects have been induced by UV-microbeam irradiation of kinetochores[8]. However, this does not interfere with the molecular and effective replications of chromosome strands. Thus this drug behaves similarly to colchicine and vinca alkaloids. From a comparison of these results with those obtained by us with adriamycin[9], it appears that nimbidin is a more powerful antimitotic agent than adriamycin, currently used in cancer chemotherapy.

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Vitellogenesis in the air breathing fish, *Channa punctatus* (Bloch)

G.P. Verma, K.C. Sahu and U.R. Acharya[1]

Post-graduate Department of Zoology, Berhampur University, Berhampur-760007 (Orissa, India), 17 March 1980

Summary. Two types of yolk develop in the oocyte of *Channa punctatus*. The carbohydrate yolk, which develops from the material present in the ooplasm, breaks up for the use of the growing oocyte before ovulation takes place. The proteid yolk, developing from the extraoocytic material, finally crams the fully mature oocyte, perhaps to participate in the process of embryogenesis.

Nath and Nangia[2], Chopra[3], Belsare[4], and Shahi et al.[5] have studied yolk formation in the oocyte of *Channa punctatus*, and Guraya[6] has studied it in *Channa maruleus*. An account of vitellogenesis in *Channa punctatus* is given here.

Material and methods. Specimens were collected from local ponds, and ovaries were processed[7] for the detection of proteins, carbohydrates, lipids and nucleic acids (table).

Results. 4 vitellogenic stages can be distinguished in developing oocytes: 1. in the young oocytes (160 µm in diameter), which appear as pre-vitellogenic ones in the preparations for histological studies and for detection of protein and lipid content, carbohydrate yolk develops (figure 1) as globules scattered randomly in the ooplasm; 2. when the oocyte measures 260 µm in diameter the carbohydrate yolk becomes vacuolated and starts to disintegrate, pouring its contents into the ooplasm (figure 2). This dissolution starts in the central zone and proceeds towards the peripheral ooplasm; 3. during the dissolution of the carbohydrate yolk, the accumulation of protein-containing precursor granules for the synthesis of another type of yolk, the proteid yolk, may be observed in the peripheral ooplasm (figure 3). These precursor granules eventually coalesce together, enlarge in size and change into proteid yolk, which gradually invades the whole ooplasm. Ultimately the carbohydrate yolk disappears and the mature oocyte is packed only with the proteid yolk.

Discussion. Yolks of varying structure and chemical nature develop in fish oocytes[8]. Thus, whereas 2 types of yolk, namely yolk globules and yolk granules, occur in *Liopsetta obscura*[9], *Carassius auratus*[10], and *Tilapia mossambica*[11], the mature oocyte of *Hypomesus japonicus*[12] contains 3 types of yolk: yolk vesicles, yolk globules and lipid globules. When the chemical nature is considered, it has been shown that two types of yolk - carbohydrate and proteid - develop in *Mystus tengara*[13], but Guraya[6] reports the elaboration of fatty yolk in addition to carbohydrate and proteid yolk in the oocyte of *Channa maruleus*. *Channa punctatus* develops 2 types of yolk; carbohydrate yolk developing de novo disintegrates in the growing oocytes and only proteid yolk, which is synthesized from the infiltrating protein-positive precursor granules, persists in the mature oocyte. It seems possible to visualize that the carbohydrate yolk is utilized by the growing oocyte before ovulation and the proteid yolk is used after fertilization during embryogenesis.

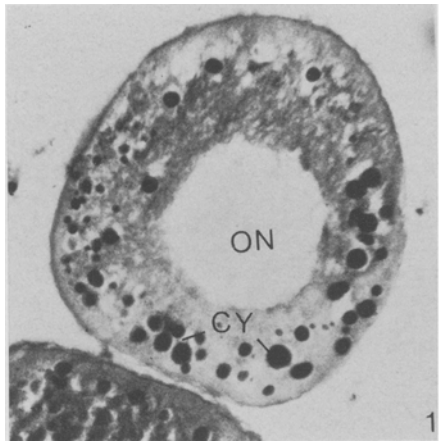

Fig. 1. Development of carbohydrate yolk (CY) in a young oocyte around the oocyte nucleus (ON) (PAS, 400:1).

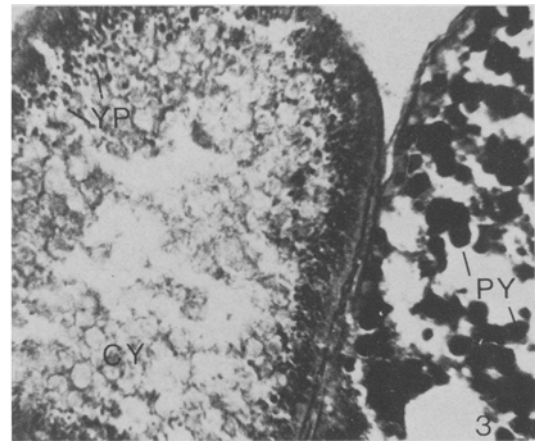

Fig. 3. Protein-positive precursor granules (YP) accumulated in the peripheral ooplasm. Negative reaction in CY and strong reaction in proteid yolk (PY) in the adjacent mature oocyte (HgBPB, 400:1).

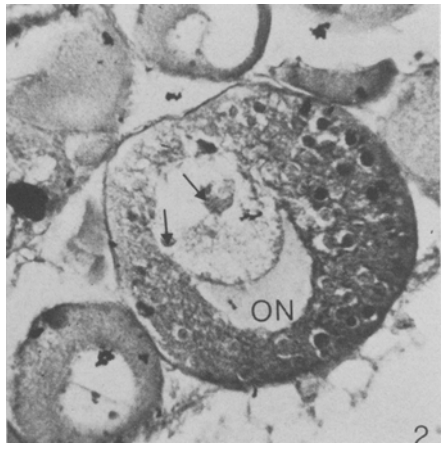

Fig. 2. Disintegration (arrows) of CY (PAS, 400:1).

The histochemical nature of yolk

Test	Carbohydrate yolk	Proteid yolk
PAS (Period acid-Schiff)	3	0
PAS after acetylation	0	0
PAS after deacetylation	3	0
PAS after trypsin	3	0
PAS after malt diastase	0	0
HgBPB (Mercuric bromphenol blue)	1	3
Hg BPB after malt diastase	0	3
SBB (Sudan black B)	0	1
SBB after pyridine	0	1
SBB after chloroform and methanol	0	1
Acid haematein	0	1
Acid haematein after pyridine	0	1
MG/PY (methyl green/pyronin Y)	0	Red
MG/PY after TCA	0	Red

3, strong reaction; 2, moderate reaction; 1, weak reaction; 0, no reaction.

Chopra[3] and Shahi et al.[5] also described the development of both carbohydrate and proteid yolk in this species of fish but failed to note the disintegration of the former in the growing oocyte. These yolk bodies correspond to the 'fatty' and 'albuminous' yolks described by Nath and Nangia[2] respectively. Chopra[3] did not find lipids in the carbohydrate yolk and this is in agreement with the present findings. It therefore seems untenable to describe it as fatty yolk. The results obtained (table) show that the carbohydrate yolk contains 1:2 glycol groups and glycogen and no protein (Chopra[3] and Shahi et al.[5]), lipid or nucleic acid (Shahi et al.[5]), while the proteid yolk contains only protein and no carbohydrate, lipid (Chopra[3] and Shahi et al.[5]) or nucleic acid. Though the present authors support the findings of Shahi et al.[5] as regards the de novo origin of carbohydrate yolk, they do not agree with their view with respect to the participation of nucleolar extrusions in the synthesis of proteid yolk, because although the nucleoli are arranged along the nuclear membrane, none of them migrate to the ooplasm. The arrangement of nucleoli along the nuclear membrane may be attributed to their participation in the supply of ribosomal or other species of RNA to the ooplasm. The role of nucleolar extrusions in vitellogenesis has also been denied in *Channa maruleus*[6] and *Mystus tengara*[13]. The appearance of minute protein-positive precursor granules in the peripheral ooplasm during stage 3 suggests their infiltration from outside through the surrounding follicular epithelium. The infiltration of extraoocytic material (vitellogenin) for the synthesis of proteid yolk has already been established in a number of animals, including fish, with the help of autoradiography and electron microscopy[14].

1 Acknowledgments. The authors are grateful to Professor C.C. Das, for his encouragement and the laboratory facilities. The award of a U.G.C. junior fellowship to one of us (U.R.A.) by the Berhampur University is thankfully acknowledged.
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