Nuclear blebs in oocytes of the fish *Clarias batrachus*

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Summary. Large nuclear blebs have been observed in oocytes of the fish, *Clarias batrachus.* The bleb, which contains nucleoplasm, is finally extruded from the nucleus. The extruded body is DNase-sensitive, and it implies that some amount of DNA is extruded from the nucleus of these oocytes.

In many oocytes, at the onset of vitellogenesis, blebs have been seen to arise from the nuclear envelope[2]. These blebs are responsible for the production of annulate lamelli[3]. Inside the annulate lamelli are heavy bodies which may be the repository of ribonucleoprotein or mRNA[4-6]. Blebs, pockets, and projections on the nuclear surface of immature cells of the myeloid series have been described in certain cases of leukemia and lymphoma[7]. Belsare[8] has observed cytoplasmic bodies in oocytes of *Clarias batrachus.* In the present communication, we describe the formation of nuclear blebs in oocytes of the fresh-water, air-breathing fish, *Clarias batrachus.*

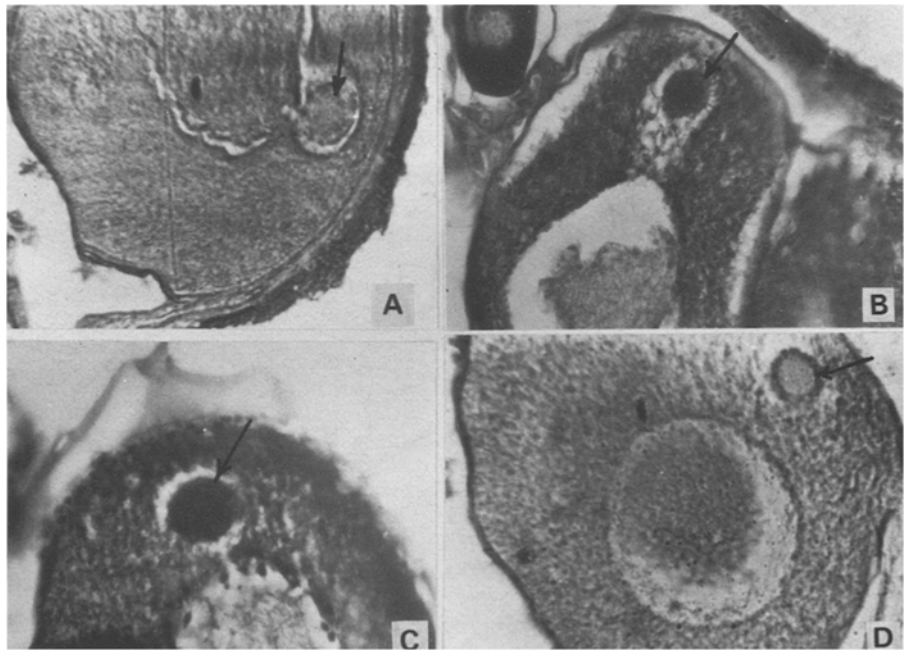
Material and method. The ovary of *C. batrachus,* belonging to zero-year class, was dissected out and fixed in Davidson's fixative for 20 h[9]. Sections (6 µm) were stained with biebrich scarlet-fast green, iron alum haematoxylin, crystal violet, methyl green-pyronin, and Feulgen's leuco-basic fuchsin. Some sections were treated with DNase and RNase[10] (1 mg/ml) for 2 h at 37 °C.

Results and discussion. Nuclear blebs were commonly observed in oocytes of about 150-250 µm in diameter. These blebs were not observed in oocytes of smaller diameter. Figure A shows the formation of nuclear bleb. Some amount of nuclear material is being sequestered to be finally extruded from the nucleus. In figure B, the nuclear material is almost completely extruded from the nucleus and is placed in cytoplasm of the oocyte. There is a translucent cytoplasm around the extruded material and the latter appears to be in the process of condensation. In figure C, the condensation of extruded material appears to be almost complete. In some cases, the extruded material appears to be differentiated into an inner, less dense core and an outer denser rim (figure D). The extruded material has also been observed to impinge on the cell membrane of oocytes.

When sections are stained with iron alum haematoxylin, crystal violet, and methyl green-pyronin, and Feulgen's leuco-basic fuchsin, the extruded body takes up the stain characteristic of chromatin. In order to ascertain whether this extruded body is the reservoir of ribonucleoprotein or RNA, some sections were incubated with RNase. These bodies appear to be RNase-resistant. On the other hand, incubation with DNase leads to the breakdown of the structure leaving a gap in the cytoplasm. These results demonstrate that the substance extruded from the nucleus of oocytes contains DNA. It is difficult to explain why these DNA-containing bodies are extruded by the nucleus.

Blebbing phenomena have been demonstrated for both nuclear membranes[11]. Increased bleb formation in cells may be a sequel to poison treatment, nutrient deprivation, irradiation and thermal treatment[12]. Such blebs, containing some nucleoplasm, have been seen for a variety of cellular situations, besides their frequent occurrence in meiotic prophase[11]. We believe that nuclear bleb formation in oocytes of *C. batrachus* can be explained in one of the following ways:

1. It may be presumed that the formation of blebs in the fish oocyte, which is in meiotic prophase, is a normal cytological process rather than a reflection of its response to stress or cellular injury. 2. It is possible, but not proved, that bleb formation is a means to selectively eliminate extra DNA. Selective elimination is a phenomenon of widespread occurrence in animals[13]. 3. Formation of blebs in the fish oocyte offers presumptive evidence of a deranged DNA metabolism.



Section through oocytes of *C. batrachus* showing the formation of nuclear blebs: *A* the bleb is arising from the nucleus; *B* the bleb containing nuclear material is cut off from the nucleus and is surrounded by translucent cytoplasm; *C* the material of the extruded body is in the condensed form; *D* the extruded body is differentiated into an outer denser rim and an inner less dense core. All stained with iron-alum haematoxalin-eosin. × 320.

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Fluorophores as visualization aides in agar growth media

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Summary. A survey of 26 fluorophores revealed 5 which were non-inhibitory to *Staphylococcus aureus* and *Escherichia coli* and produced low background and high colony fluorescence.

Use of optical brighteners for staining living microorganisms has been reviewed by Paton and Jones[1]. They reported that some microorganisms could be stained by growth on agar which contained diamino-stilbenedisulfonate or oxycyanine compounds. Weaver and Zibilske[2] examined growth of bacterial suspensions in the presence of similar compounds and concluded that protein adsorption of the brighteners was the primary staining mechanism.

Other types of fluorescent compounds, those whose fluorescence is highly dependent on the immediate environment, have been widely used to study the conformation of membranes and proteins[3]. 2 factors which are important in determining the quantum yield of fluorophores are the viscosity and polarity (hydrophobicity) of the microenvironment. The bacterial envelope is a region of hydrophobicity and increased viscosity due to the presence of lipids, proteins, polysaccharides, and other closely related compounds. In designing a system for fluorescent detection of bacteria, one must take these properties into account. Compounds which have a high quantum yield when associated with membranes and proteins and a low quantum yield in an aqueous environment, such as an agar medium, are desirable. One fluorophore, 8-anilino-1-naphthalenesulfonate (ANS), was used by Cramer and Phillips[4] to study colicin-induced changes in *Escherichia coli* membrane conformation. We have observed that ANS could be incorporated into agars resulting in growth of highly fluorescent but otherwise normal bacterial colonies. This facilitated detection of transparent colonies and of colonies on opaque media, such as chocolate agar[5]. Media incorporating ANS and specific biochemicals were found to be useful in distinguishing between gram-positive and gram-negative colonies and in identifying bacterial species[6].

This report is a survey of fluorophores as additives to growth media with the objective of defining the characteristics of useful additives.

The compounds listed in the table were incorporated at 80 µg/ml in Nutrient Agar (Difco, Detroit, MI). The media were autoclave sterilized and plates poured. Separate plates were streaked for isolated colonies with *Escherichia coli* or *Staphylococcus aureus* and incubated overnight at 37°C. Plates were examined using long-wavelength UV-light. Each fluorophore was evaluated in terms of background fluorescence and overall visibility (fluorescence) of the colonies versus the agar, as described in the table. The

Effectiveness of fluorophores in nutrient agar for fluorescent labeling of *Escherichia coli* or *Staphylococcus aureus* colonies

Fluorophore	Agar surface[i]	Colony versus background[j]
A) Effective fluorophores		
1 8-Anilino-1-naphthalenesulfonic acid, Na or Mg salt[a]	−	+ +
2 2-p-Toluidino-6-naphthalenesulfonic acid[a]	−	+ +
3 1-p-Toluidino-8-naphthalenesulfonic acid[a,h]	−	+ +
4 Acridine orange[a]	−	+ +
5 1-Pyrenebutyric acid[a]	+	+ +
B) Less effective fluorophores		
1 1-Naphthalenesulfonic acid, Na salt[a]	−	− −
2 2-Naphthalenesulfonic acid, Na salt[a]	−	−
3 1-Naphthol-2-sulfonic acid, K salt[a]	−	− −
4 1-Naphthol-4-sulfonic acid, Na salt[a]	+ +	− −
5 2-Naphthol-6-sulfonic acid, Na salt[a]	+	− −
6 1-Naphthol-8-sulfonic acid, Na salt[a]	−	−
7 2-Naphthol-8-sulfonic acid, K salt[a]	+ +	−
8 7-Anilino-1-naphthol-3-sulfonic acid[b]	+	−
9 N-ethyl-naphthyl-2-amino-6-sulfonic acid[e]	+ +	− −
10 N-octadecylnaphthyl-2-amino-6-sulfonic acid[e]	− −	−
11 1-Naphthalenesulfonyl chloride[a]	−	−
12 2-Naphthalenesulfonyl chloride[a,h]	−	− −
13 5-Dimethylamino-1-naphthalenesulfonyl chloride[a]	+ +	−
14 2-p-Toluidino-6-naphthalenesulfonyl chloride[a]	−	− −
15 Lissamine rhodamine B sulfonyl chloride[a]	−	−
16 4-Amino-1-naphthol hydrochloride[a,h]	−	− −
17 Cycloheptamylose-dansyl chloride[d]	+ +	− −
18 Dansyl ethylamine[e]	− −	−
19 Rose bengal[a,g]	−	− −
20 Auramine O[f]	−	−
21 Fluorescamine[c]	+	− −

[a] Eastman Kodak Co., Rochester, N.Y. [b] Aldrich Chemical Co., Milwaukee, Wisc. [c] Hoffmann-La Roche, Inc., Nutley, N.J. [d] Pierce Chemical Co., Rockford, Ill. [e] ICN Pharmaceuticals, Cleveland, Ohio. [f] Fisher Scientific Co., Pittsburg, Pa. [g] Growth of *Staphylococcus aureus* inhibited. [h] Fluorogenic agent incompletely soluble at 90°C. [i] Fluorescence of colony-free agar surface: − low, + high, + + very high, − − poor. [j] Fluorescence of colony versus agar surface: − − poor, − mediocre, + good, + + excellent.