Phase Contrast Microscopy of Liver Cells of a Fish (Oryzias latipes)

II. Changes of Intracellular Structures of the Fresh Crush Preparations during the Application of Fixing Solutions.

By

Morio Ihnuma

(Department of Anatomy, School of Medicine, Keio-Gijuku University, Tokyo)

In the ordinary histological procedures, the treatments like fixing, dehydrating, heating or staining may exert certain influence upon the cells and some changes may be caused. Applying various fixatives to the fresh crush preparations of liver cells of Oryzias latipes, the author examined the changes of the intracellular structures of cells. In this paper the author reports the resulted effects upon the fine structure of these cells.

Material and Methods

Crush preparations of fresh liver cells of Oryzias latipes were made by the same method as described in the foregoing paper (Ihnuma, 1952). Exchanging the physiological saline solution for a series of various fixatives, sealing the cover glass with paraffin, the observation under a phase contrast was started in approximately thirty minutes.

Eleven kinds of fixatives were tried in the experiment. The phase contrast microscope, made by Tiyoda Optical Co. Ltd., was used. Oil immersion lenses of dark contrast medium and low were used (abbreviated as D. M. and D. L. respectively).

Observations

1) 70% and absolute alcohols:
   With the D. M.:
   Both alcohols gave almost the same results. In fresh preparation no network could be seen within the nucleus before applying alcohols,
but in alcohols the network gradually appeared. In the cytoplasm the filamentous bodies were visible, which might be the deformed mitochondria. The "Special Granules" (Tsu k u d a, 1952) were also recognized (Fig. 1 and 2).

2) 10% Formalin solution:
   With the D. M.:
   No network was seen in the nucleus, but the nuclear membrane could be demonstrated. In the cytoplasm, the "Special Granules" stood out black, and fat droplets appeared light. The shape of mitochondria was similar to that of the fresh preparations, but somewhat shorter and sometimes nodular (Fig. 3).

3) Zenker-formalin solution:
   With the D. M.:
   Fine intracellular structures were difficult to recognize. The nuclear membrane was indistinct. In the cytoplasm light filamentous elements were visible around the nucleus, but it is difficult to decide what they are (Fig. 4).

4) Bouin's solution:
   With the D. M.:
   A network was clearly seen in the nucleus, and within the nucleolus a network could be observed. The filaments in the cytoplasm are considered as the deformed mitochondria (Fig. 5).

5) Carnoy's solution:
   With the D. M.:
   The nucleolus was swollen, and the network in it was visible. The filaments, which are considered as the deformed mitochondria, were seen but few in number (Fig. 6).

6) Absolute aceton:
   With the D. M.:
   The cells shrank considerably. The nucleus showed specklike structure, but the network could not be seen. In the cytoplasm, short and nodular filaments, probably the deformed mitochondria, were recognized (Fig. 7).

7) Regaud's solution:
   With the D. M.:
   The nucleus had no structure. In the cytoplasm, the mitochondria were seen comparatively undeformed, but they were few in their number. The "Special Granules" and fat droplets were also visible (Fig. 8).

8) Levi's solution:
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The shrinkage of cells was rather slight.

With the D. M.: The mitochondria were not deformed, and their shape was almost similar to that of the fresh preparations (Fig. 9).

With the D. L.: The mitochondria was more faintly visible than with the D. M. Near the nucleus black and thick filaments were seen mixed among the mitochondria. These filaments are considered to be Golgi apparatus (Fig. 10).

9) Champy's solution:

With the D. M.: In the nucleus no structure was found. The mitochondria were faintly visible.

With the D. L.: Around the nucleus there was a network composed of filaments which were thicker and darker than the mitochondria. They are readily distinguishable from the mitochondria, and are considered probably to be the Golgi apparatus (Fig. 11). The picture of this apparatus was less distinct than that of the fixed-stained preparation by Saka (1951).

10) Flemming's stronger solution:

With the D. M.: The network of the nucleus was distinctly seen, and the mitochondria were also clearly visible, the structure, which is considered as the Golgi apparatus, was not found with the D. M., and the D. L. gave also the same result (Fig. 12).

Summary

1. Fresh liver cells of Oryzias latipes, treated by eleven fixatives, were examined by means of the phase contrast microscopy.

2. The materials treated with Levi's, Champy's, 10% formalin and Regaud's solution gave more similar pictures than the other to those of the fresh material, but not quite the same.

3. Acetic acid containing fixatives, i.e. Bouin's, Carnoy's and Flemming's stronger solutions, were able to make the network of nucleus clearly visible, which was invisible in the fresh material.

4. The best preserving fixatives for Golgi apparatus are Levi's and Champy's solutions.

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References


Explanation of Figures

Fig. 1 70% alcohol, D. M.
Fig. 2 absolute alcohol, D. M.
Fig. 3 10% Formalin solution, D. M.
Fig. 4 Zenker-formalin solution, D. M.
Fig. 5 Bouin’s solution, D. M.
Fig. 6 Carnoy’s solution, D. M.
Fig. 7 Absolute aceton, D. M.
Fig. 8 Regaud’s solution, D. M.
Fig. 9 Levi’s solution, D. M.
Fig. 10 Levi’s solution, the same cell of Fig. 9, D. L.
Fig. 11 Champy’s solution, D. L.
Fig. 12 Flemming’s stronger solution. D. M.
PHASE CONTRAST MICROSCOPY OF LIVES CELLS OF A FISH

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 12

Morio Ihnuma