BIOLOGICAL STUDIES ON ASCARIS EGGS

V. ON THE STAINING REACTIONS OF ASCARIS EGGS TO PHOSPHOR, CALCIUM AND SOME OTHER SUBSTANCES IN THE PROCESS OF THEIR OÖGENESIS

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INTRODUCTION

In the former report, the author made a research on the distribution of nucleic acid, fat and glycogen in process of the ascaris eggs. In this report, the observation on phosphor and calcium was achieved, because they are considered to have an important role in the metabolism of the tissue. In addition, the observation on mucus and hyalin substance etc were also made.

MATERIALS AND METHODS

As materials genital organs of Ascaris suilla were used. They were taken from a fresh female collected at the slaughter house. The initial, middle and posterior portions of the ovary, and several parts of the uterus and vagina, were cut off and fixed by 30% neutral formalin or pure alcohol. The duration of fixing was 3 hours in each cases. After fixing, materials were transferred into 70% or pure alcohol in a usual manner, then removed into n-buthanol, xylol, fluid paraffin successively and finally embedded in hard paraffin (melting point 52°C) at 54°C about 1 hour. Then, the section was made on each material 10 micron thick. Grandis & Mainini’s method, Kossa’s method and the calcium sulphate proving method for calcium, Angeli’s method for phosphor, thionin staining for mucus were then performed on each cut material1) 2). Van Gieson’s staining was also tried.

RESULTS OBTAINED

1. Findings by Calcium Staining

The purplin staining by Grandis and Mainini was applied to the sections after deparaffinization. In general, alcohol is used for the fixation of calcium proving, but in this study 30% formalin was also used for fixing. After the staining by purplin, the tissues were washed with distilled water 2 minutes, differentiated with 70% alcohol, then enclosed by glycelin. For a control, the specimen treated with 1/10N-chloric acid 2 minutes was used to compare. By these observations, calcium was not proved in eggs and surrounding tissues throughout every stage of ascaris oögenesis. In order to prove the calcium
sulphate crystal, the specimens were deparaffinized and added with one drop of 3% sulfuric acid. During the observation specimens were covered with cover glass, and closed its surrounding with waselin. In this case also no crystal was found. Applying Kossa’s silver nitrate method no calcium was proved, too.

2. Results of Observation of Phosphor

The phosphorus compounds should take, if they exist, a form of salt or organic compound also in ascaris eggs. Here the author tried to prove chiefly the organic compound, applying the Angeli’s method to the material fixed with 30% formalin. At that time 0.5 g of ammonium molybdate was used for 20 cc of 30% chloric acid according to Serra & Queiroz Lopes modification. As a preliminary experiment, the solution of stanno-chlorid was dropped into the former solution and a little quantity of coloured precipitate appeared, but its disturbing effect against experimental result remained almost none, when specimens were washed by water sufficiently. To prove inorganic phosphorus compound, a group of specimens without decomposition were treated with the former solution without hydrochloric acid, but the reaction was found negative. For organic phosphorus compound, decomposition was carried 20 minutes by the former solution, but with such a duration of time a specific reaction was not found. When the time of decomposition was prolonged to 40 minutes, a positive colour reaction was recognized. This time in the ovary the external stratum took a tone of most intensive blue colour and the nucleus or the stem substances in the initial part revealed to take more intensive colour than cytoplasm of the germ cells. But an uneven distribution of colour, as such was the case found by pyronin staining within the cytoplasm of egg cell was not found. In the oviduct, the external stratum of wall was coloured blue most strongly, and epithelial cytoplasm took only rarely slight blue tinge, while in egg cells the nucleus and granular substance in the external cytoplasm were proved to be slightly positive. In the seminale receptacle, the most external stratum took a most intensive colour, while the internal side, the nucleus of epithel and the sperm cell in egg were proved to be slightly positive. In the cytoplasm of epithelial or egg cell, no recognizable reaction was found. The sperm cells outside of eggs were positive in reaction and in the nucleus of epithelial cells the nucleolus and nucleous membrane reacted conspicuously positive. In the uterus, the most external stratum as such was the case in the seminal receptacle, took an intensive blue tone, and also the reaction of the epithelial cells were about the same as in the case of the latter, except that sub-epithelial stratum showed marked positive reaction. In eggs, the protein coat took the same strong blue tone as the external stratum of the uterus tissue, but the shell showed no positive reaction. The cytoplasm of egg showed a slightly positive reaction, but not so remarkable as the epithelial cytoplasm. It should be noted that generally throughout all tissues slight destruction of the tissue was found by the procedure of the phosphorus proving technic.
3. Findings by Mucus Staining and Van Gieson's Picro-fuchsin Method

The thionin staining for mucus was performed on the materials fixed by formalin or alcohol. At that time 0.5–0.01% thionin solution was applied for staining about 10–30 minutes. As a result of this procedure the internal side of the germ cell in the initial portion of ovary, the epithelial cytoplasm of the seminale receptacle or uterus, the nucleolus of the epithelial cells and the outline of the sperm within the eggs were intensively coloured, but a specific metachromasia for mucus was not found. The other tissue was found unstained. By applying the toluidinblue, the same results as by thionin was obtained. Van Gieson's staining method was then tried. The recept of the stains was: 9 parts of water solution saturated with picric acid and 1 part of acid-fuchsin water solution. The nucleous staining by Mayer's hemalaun was carried on the materials fixed with formalin or alcohol, then the staining by picro-fuchsin and dehydration were done as rapidly as possible. When observed the stained preparations, in the ovary the external stratum was coloured red and the cytoplasm of the germ cell stained yellow. In the latter there was found a different grade of staining by picric acid according to the locality. In the oviduct, the external part of the wall stained red and the other parts had a tinge of yellow of yellowish brown. In the seminale receptacle, the most external stratum, the sub-epithelial tissue and the external part of the egg shell stained reddish, while the other portions took a colour of yellow or yellowish brown. In the uterus, the findings on the wall was almost the same as the seminale receptacle, but the protein coat of the egg was coloured red, while the egg shell not stained and the cytoplasm of the egg cell had generally a tinge of yellow. In the hematoxylin-eosin staining made as a cotrol, the protein coat of the egg was found stained especially by eosin.

SUMMARY

The author examined several substances in the stages of ascaris oogenesis by histochemical technics and the result is as follows:

1. No calcium was found by means of Grandis & Mainini's, Kossa's method and the gyps proving method.

2. The phosphorus compound was examined by Angeli's method. The external stratum of ovary, oviduct, seminale receptacle and uterus showed a positive reaction for phosphor. The protein coat of the egg reacted also positive. But no difference of staining intensities was found in the cytoplasm of egg cells.

3. On mucus staining by thionin or by toluidinblue, no specific metachromasia was found in every tissue.

4. By Van Gieson's staining method, the external part of the wall and the protein coat of the egg from ovary to uterus took a remarkably red colour and by hematoxylin-eosin staining the protein coat was also stained intensively with eosin.
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REFERENCES