ON THE MULTIPLICATION OF FOLLICLE CELLS IN THE OOCYTE OF THE GOLDFISH, *CARASSIUS AURATUS**

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In all animals having follicular eggs, the cells of the follicular epithelium show the same function and play important role in the nutrition or yolk formation of the eggs.

On the other hand, it is well known that the follicles in mammals enlarge through mitotic proliferation of the follicle cells and accumulation of liquor folliculi secreted by granulosa cells. In other vertebrates such as lizards or hens, the multiplication of the follicle cells is also accomplished by their mitotic division occurring throughout the whole period of oocyte growth (Brambell 1956). In fishes, Mendoza (1943) and Takano (1962), using the viviparous fish *Neotoca bilineata* and *Lebistes reticulatus* respectively, observed dividing cells in the follicular epithelium. Chaudhry (1956) suggested in other teleosts that the follicle cells may increase in number by means of their division, but Schmidt (1898) failed to find any dividing follicle cells in the elasmobranches.

In fishes therefore, no decisive conclusion has yet been obtained whether the multiplication of the follicle cells results from their mitotic division or is accomplished by other means. The present study was carried out in an attempt to ascertain the mechanism of the multiplication of follicle cells in the oocyte of the goldfish, *Carassius auratus*.

Before going further, the writer wishes to express his hearty thanks to Professor Kiichiro Yamamoto of the Faculty of Fisheries, Hokkaido University, for his kind encouragement in the prosecution of the work and his kind criticism during the preparation of this manuscript. Thanks are likewise offered to Mr. Kazunori Takano for his advice in the course of the present study.

Material and Methods

The goldfish used in the present study were cultured in an aquarium located on the campus of the Fisheries School of Hokkaido University. The female goldfish were sacrificed at various time of the year. The ovaries were fixed with Bouin's solution. Serial sections of 10 micra from pieces of ovaries were prepared

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by the usual paraffin method and stained with iron-haematoxylin-light green or Delafield's haematoxylin-eosin. In addition to them, in order to count exactly the number of the dividing follicle cells, the author made serial sections of 7 micra thickness from three females kept in an aquarium of 30×45×30 cm at 17–19°C for two weeks in February.

Results

No follicle cell was observed around the dividing or resting oogonia (Fig. 1). But the cells made appearance in the oocytes of the early stage of development and they developed into follicular epithelium surrounding completely the oocytes by the time of the early peri-nucleolus stage. At this stage, the cells of the follicular epithelium were flat and very few in number. When the oocytes have grown up to the early yolk vesicle stage at which they measured about 150 micra in diameter, follicle cells began to increase rapidly in number.

On the other hand, the division of a follicle cell was found first in the oocyte of early peri-nucleolus stage. At that stage the diameter of the oocyte was about 80 micra (Fig. 3). As the oocyte grew above 150 micra, many cases of mitosis of follicle cells were observed in the follicular epithelium. These dividing cells measured about 4–8 micra in diameter; they were about the same in size as resting follicle cells, but much smaller than the dividing oogonia which showed a diameter of about 14 micra (Figs. 1, 2). The axis of the division was usually tangential but sometimes perpendicular or oblique to the surface of the oocytes.

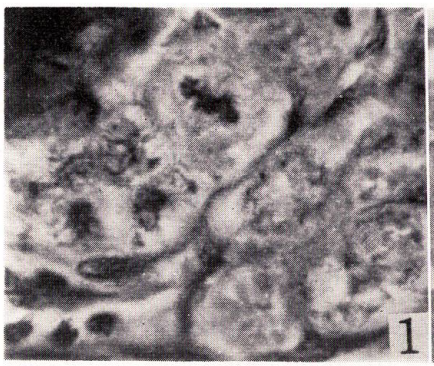


Fig. 1. Dividing oogonium ×1130

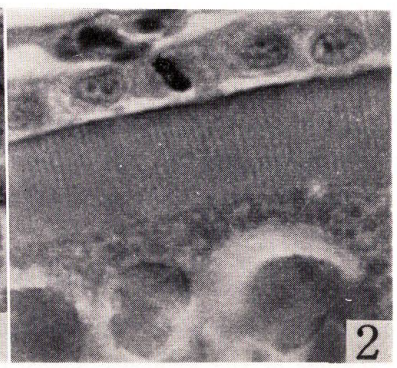
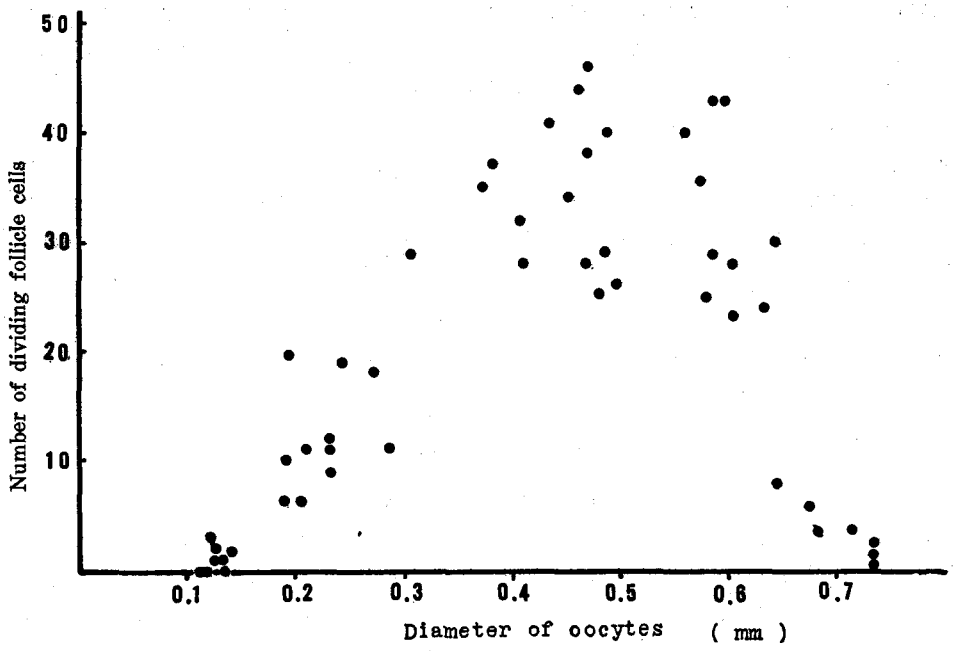
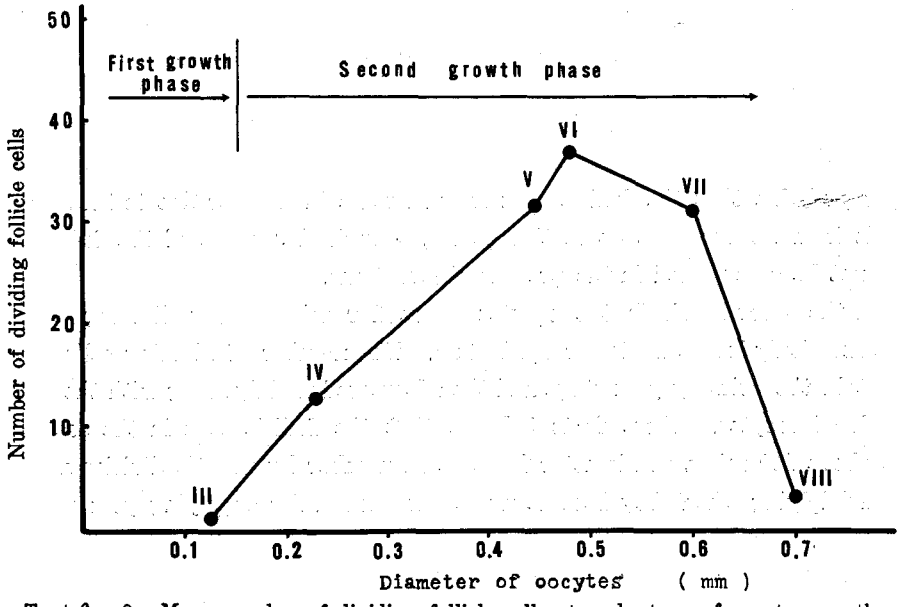


Fig. 2. Dividing follicle cell ×1130

The total number of cells in course of division (Figs. 4, 5, 6, 7, 8) was counted in the oocytes of various size. The results are shown in Text-fig. 1. The mean number of dividing cells at each stage of oogenesis is shown in Text-fig. 2. From



Text-fig. 1. Number of dividing follicle cells in oocytes of various size



Text-fig. 2. Mean number of dividing follicle cells at each stage of oocyte growth. III: late peri-nucleolus stage, IV: early yolk vesicle stage, V: late yolk vesicle stage, VI: primary yolk stage, VII: secondary yolk stage, VIII: tertiary yolk stage.

these results it is clear that the division of follicle cells occurs very frequently in the oocytes measuring from 0.4 to 0.6 mm in diameter. The oocytes in these dimensions spread over the stages from the late yolk vesicle stage to the secondary yolk stage. The largest occurrence of division of follicle cells was found in the oocytes of the primary yolk stage the count being 37 in average per one oocyte (Text-fig. 2). In these stages the cells of the follicular epithelium were large and sometimes cubical in form. As the oocytes grew further, the dividing cells rapidly decreased in number, and they were rarely detected in the tertiary yolk stage. No dividing cell of the follicular epithelium was observed in the oocytes of 0.75–1.0 mm just before ovulation. The follicle cells in these oocytes had become flat again.

Discussion

Up to date, numerous morphological observations have been reported on the ovaries of various kinds of teleost fishes. However, the follicular epithelium of the oocytes of fishes has received even less attention than those of other vertebrates such as mammals or birds. The follicle cells are accepted as the same in function in all animals having follicular eggs (Raven 1961), moreover they may be multiplied by the same means, namely by their mitotic devision (Brambell 1956, Raven 1961). However no detailed study has been made on the multiplication of the follicle cells in fishes, except that of Mendoza (1943) who reported a mitotic figure in *Neotoca bilineata*.

In the present study, no dividing cell was observed in the follicular epithelium until the oocytes arrived at the dimension of 80 micra. From this fact it seems highly probable that the follicle cells may increase in number due to the accretion of cells from other sources up to this period, though the source of the follicle cells could not be ascertained in the present observations.

On the other hand, in the oocytes above 150 micra many dividing cells appeared suddenly in the follicular epithelium. The number of dividing cells became largest in the primary yolk stage and then decreased rapidly along with the further growth of oocytes. These facts indicate that the follicle cells in the oocytes at least above 150 micra in diameter increase in number by mitotic division and they stop multiplying when they become numerous enough for supplying yolk materials to oocytes.

The growth of the oocyte may be divided into two phases, the first and the second, the boundary dimension of the two phases being 150 micra. The growth of the first phase occurs irrespective of the existence of the pituitary gland, while the second growth phase is intimately concerned with pituitary gonadotrophin

(Yamazaki 1961). As mentioned above the dividing cells of the follicular epithelium were demonstrated in the second growth phase. Therefore the follicle cells seem to be activated directly or indirectly into division by the pituitary gonadotrophin. Chester Jones & Ball (1962) expressed the same opinion in mammals that the secretion of liquor folliculi, the mitotic proliferation of follicle cells and so on are dependent on the follicle stimulating hormone of the pituitary gland.

Summary

1. The follicular epithelium consists of a single layer throughout the period of growth of oocytes, but it changes in thickness.

2. Dividing cells in the follicular epithelium were frequently observed in the oocytes above 150 micra in size but not in those below 80 micra.

3. The middle sized oocytes had the most numerous dividing cells. As the oocyte grew further, the dividing cells decreased rapidly in number and disappeared in the oocyte just before maturation.

4. The division of follicle cells is considered to be dependent upon the pituitary gonadotrophin.

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Explanation of Plate

PLATE I

dc dividing follicle cell

Fig. 3. The smallest oocyte having a dividing follicle cell ×350

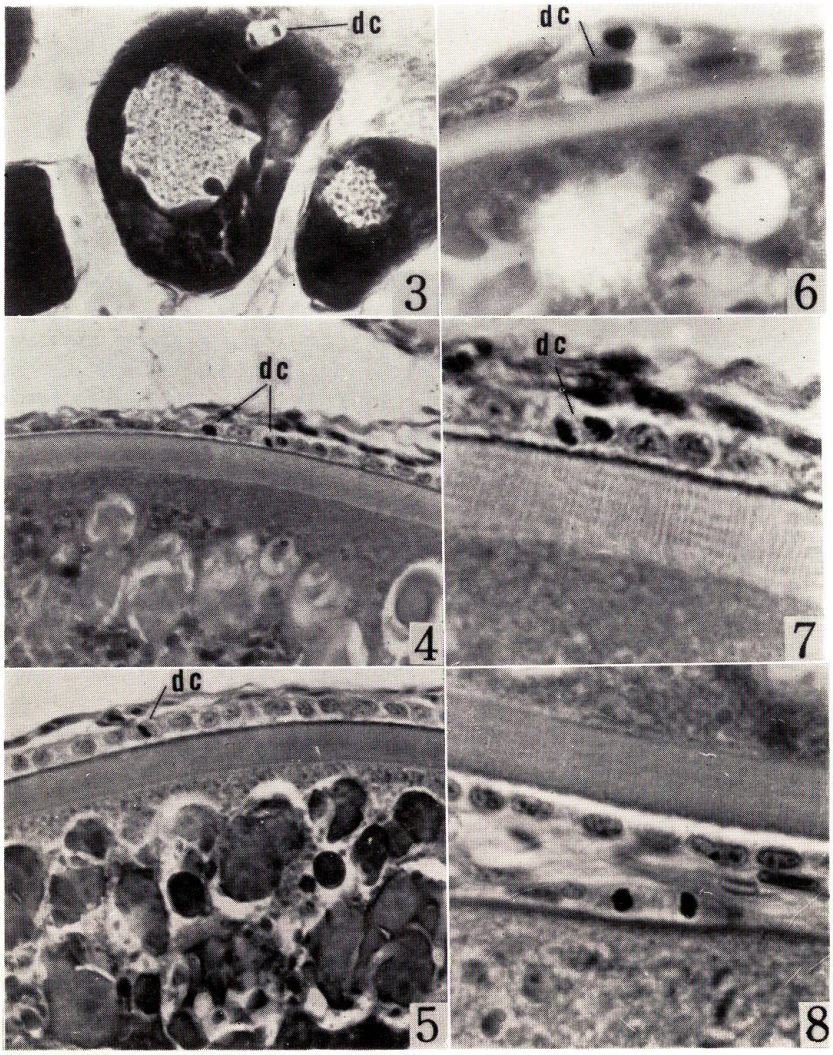
Fig. 4. Dividing cells in the follicular epithelium ×445

Fig. 5. Metaphase ×445

Fig. 6. Early anaphase ×1130

Fig. 7. Early telophase ×1130

Fig. 8. Telophase-interphase following separation of daughter cells ×1130



F. Yamazaki: Multiplication of Follicle Cells in Goldfish