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# THE PHYSIOLOGY OF REPRODUCTION OF SOME TELEOST FISHES

## II. Study on the Hypophysis of Silver Carp (*Hypophthalmichthys molitrix* C. & V.)[1]

FAH-HSUEN LIU[2] AND TIEN-HSI TAN[3]

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ABSTRACT

The hypophyses of 3 carps (silver carp, *Hypophthalmichthys molitrix* C. & V., common carp, *Cyprinus carpio* L. and golden carp, *Carassius auratus* L.) were dissected out seasonally for comparison. Paraffin sections were prepared and stained with PAS, Mallory's triple stain and Heidenhein's modification of Mallory's triple stain. The activity of gonadotrophs was found to possess seasonal periodicity corresponding to the stage of gonad maturity. The various stages of the gonadotrophic activity indicate clearly the sequences of the development of gonadotropin. The structure of the hypophysis and the cell types were described.

Fish culture is a very important part of the fishery industry in Taiwan. The shortage of fry supply has limited the development of this industry. This shortage is due to the fact that fishes popular to fishculturists, such as milk fish (*Chanos chanos*), grey mullet (*Mugil cephalus*), the straw and silver carps (*Ctenopharyngodon idellus*, *Hypophthalmichthys molitrix*) do not spawn in local culture ponds. It will be a tremendous offer to fishery industry if these fishes can be made to spawn in local ponds. The adoption of hormones to induce spawning of fishes is practiced in large scales in various countries especially Brazil, USSR, and U. S. A., but the methods are found not applicable to the straw and silver carps. These fishes must have some physiological problems relating to their reproduction. The morphology and physiology of the hypophysis of these fishes may be one of the most important problems to investigate.

Voluminous literature regarding fish hypophysis can be found. Among which, the physiology of the pituitary gland of fishes by Pickford & Atz (1) is the most important one. It has reviewed the works on fish hypophysis in all directions. Besides, Kerr (2), Mathews (3, 4) and Scruggs (5) have also given valuable informations to the hypophysis of various teleost in histological structure, in cell types and their variations, in activity of gonadotrophs and in relationship between pituitary and gonad. No work, however, can be found about the hypophysis of the silver carp. The present investigation, therefore, attempts to find out the general structure of the hypophysis of this fish and special attention will be made regarding the activity of the gonadotrophs and their relation to the development of gonads of this fish.

## MATERIALS AND METHODS

Most of the silver carps used in this study were caught in the culture ponds and some were bought from local fish market. For comparison of the gonadotrophic activity, quite a number of *Cyprinus carpio* and *Carassius auratus* were used simultaneously with silver carp. The fishes were sacrificed in the four different seasons of a year, and their number is indicated in the table.

Different methods of dissection of the hypophysis were tried according to previous workers.

1 Supported in part by the National Council on Science Development.

2 Professor, Department of Zoology, National Taiwan University and research fellow, Institute of Zoology, Academia Sinica, Taipei, Taiwan.

3 Research assistant, Institute of Fishery Biology, National Taiwan University, Taipei, Taiwan.

It was found difficult to pull the hypophysis out of the brain from dorsal side of the skull because it is embeded in a socket of sphenoid bone. All the specimens were therefore dissected from the ventral side of the skull. This was done firstly by removing the whole lower jaw and then by removing the covering of the connective tissue from the palate. A white spot was located under the transparent membranous vomer. This bony membrane was cut and the whole hypophysial body was exposed. It was then taken out from the skull with the brain after the surrounding bony structures were cleared out.

The hypophyses were fixed in Zenker's or Formal-sublimate. Paraffin serial sections were made 4–5 $\mu$ thick and stained in Mallory's triple stain, Heidenhein's azan modification of Mallory's triple stain and PAS. Some of the PAS-stained sections were counterstained with Heidenhein's iron hematoxylin.

## TERMINOLOGY

There is a great confusion in the terminology of the parts of fish hypophysis because the terms bear little resemblance to those of tetrapod vertebrates and vary greatly. However, a conclusive comparison of the names of the different regions used by various authors was listed in the table and a new set of names was given by Pickford & Atz (1). These new names are proadenohypophysis (tuberalis of tetrapods), mesoadenohypophysis (pars anterior), metaadenohypophysis (pars intermedia) and neurophypophysis (pars posterior). This terminology is adopted in the present investigation.

## OBSERVATION

The hypophysis of silver carp, very much like that of *Cyprinus carpio*, is a dorso-ventrally compressed, heart-shaped structure located on the ventral side of the brain to which it is connected by a short stalk at the infundibulum. Its anterior end is usually broader than the posterior end. Three parts can be distinguished externally. They are the proadenohypophysis, the small anterior-dorsal portion, the mesoadenohypophysis, the large anterior-ventral portion and the metaadenohypophysis, the posterior portion which is separated from the mesoadenohypophysis by a narrow groove.

The PAS-stained sagittal sections of the hypophysis show clearly the four parts: proadenohypophysis, metaadenohypophysis and neurohypophysis as indicated by A, B, C, and D respectively in *Fig. 1.*

The proadenohypophysis is the smallest portion of the gland. The cells are polygonal in shape and stained orange in Mallory's sections, but pink in PAS sections. Some of the cells were looked like squamous epithelial cells. Clefts or sinusus and fine capillaries are often observed.

The mesoadenohypophysis is the largest portion of the gland in which four main cell types can be clearly identified. The basophils are stained red by PAS and blue by aniline blue, and can be distinguished into two types morphologically as indicated by DB and BB in the figures. They are the larger ones, stained more intensely by PAS and the smaller ones, comparatively few in number, occuring in groups at certain region and stained less intensely by PAS. The latter also show distinct nuclear structure in PAS sections, counterstained by iron-hematoxylin but not the former which, however, often possess a strongly stained sphere at the center of the cell in place of the nucleus as shown in *Fig. 3.* The latter cells are also shown in PAS sections when the former cells are inactive. It is supposed that the former are identical with the delta-basophils, the gonadotrophs and the latter identical with the beta-basophils, the thyrotrophs of the mammalian pituitary. Only one type of acidophils can be definitely found as indicated by AA in *Fig. 2.* They are the large acid fuchsin and azocarmine-stained cells scattered all over the mesoadenohypophysis and mingled among the basophils. These large acidophils are supposed to be identical with the alpha-cells, the somatotrophs. Very seldom, small orange cells are found at the boundary between proadenohypophysis and mesoadenohypophysis. These cells may be the epsilon-cells, the luteotrophs of mammalian pituitary as they were reported to be present in mesoadenohypophysis of fish by previous workers (1). They may also be cells of the proadenohypophysis invading into the mesoadenohypophysis. The last type of cells are the chromophobes as shown by letter CH in *Fig. 3.* They are usually large and oval and do not take much stain. Blood capillaries are scattered between cells in this part of the gland.

The metaadenohypophysis is composed of cuboidal cells usually arranged in follicles. The cells are stained orange in Mallory's and azocarmine's sections and do not show granulation. Some of the cells do not arrange in follicles but in groups and sometimes invade into the adjoining mesoadenohypophysis.

The neurohypophysis is simply the extension of the stalk. When it enters into the gland from the mesoadenohypophysis it branches like the

TABLE I

*Reaction of hypophysis to PAS stain in various fishes*

| Kind of fish | Sex | Weight, g | Length, cm | Age, year | Date of killing | Reaction |
|---|---|---|---|---|---|---|
| Hn 1 | M | 1,110 | 49.0 | 4 | Oct. 1961 | ++ |
| Hn 2 | M | 1,180 | 48.5 | 4 | 〃 | ++ |
| Hn 3 | F | 1,060 | 48.6 | 4 | 〃 | + |
| Ca 1 | F | 67 | 11.9 | 1 | 〃 | + |
| Ca 2 | M | 69 | 12.2 | 1 | 〃 | ++ |
| Ca 3 | F | 67 | 12.6 | 2 | 〃 | ++ |
| Ca 4 | M | 45 | 10.0 | 1 | 〃 | + |
| Cc 1 | F | 156 | 16.7 | 2 | 〃 | ++ |
| Cc 2 | M | 114 | 14.7 | 2 | 〃 | ++ |
| Hn 11 | M | 1,120 | 47.8 | 4 | Jan. 1962 | ++ |
| Hn 12 | M | 1,470 | 49.8 | 5 | 〃 | ++ |
| Hn 13 | M | 1,200 | 48.0 | 4 | 〃 | ++ |
| Hn 14 | F | 1,160 | 49.3 | 4 | 〃 | + |
| Hn 16 | F | 1,150 | 49.0 | 4 | 〃 | + |
| Cc 11 | M | 190 | 25.5 | 3 | 〃 | − |
| Cc 13 | M | 205 | 29.0 | 4 | 〃 | − |
| Cc 14 | F | 188 | 24.0 | 3 | 〃 | − |
| Cc 15 | F | 176 | 24.0 | 3 | 〃 | + |
| Cc 16 | M | 149 | 22.8 | 3 | 〃 | − |
| Ca 12 | M | 67 | 13.4 | 2 | 〃 | − |
| Ca 13 | F | 43 | 11.3 | 1 | 〃 | − |
| Ca 15 | F | 105 | 15.6 | 3 | 〃 | − |
| Ca 16 | M | 45 | 11.0 | 2 | 〃 | − |
| Ca 17 | M | 47 | 11.1 | 2 | 〃 | − |
| Ca 18 | F | 52 | 13.0 | 2 | 〃 | − |
| Hn 22 | M | 1,850 | 48.3 | 5 | Apr. 1962 | − |
| Hn 23 | M | 1,860 | 48.9 | 5 | 〃 | + |
| Hn 26 | F | 1,750 | 43.3 | 5 | 〃 | + |
| Hn 27 | F | 1,830 | 44.9 | 5 | 〃 | − |
| Ca 21 | F | 74 | 13.2 | 2 | 〃 | + |
| Ca 22 | F | 71 | 13.1 | 2 | 〃 | − |
| Ca 23 | M | 72 | 13.4 | 2 | 〃 | − |
| Ca 24 | M | 73 | 14.1 | 2 | 〃 | − |
| Ca 25 | M | 68 | 14.0 | 2 | 〃 | − |
| Cc 21 | M | 196 | 25.2 | 3 | 〃 | − |
| Cc 22 | F | 178 | 24.2 | 3 | 〃 | + |
| Cc 23 | M | 200 | 26.5 | 3 | 〃 | − |
| Cc 25 | F | 191 | 25.3 | 3 | 〃 | + |
| Hn 41 | M | 1,450 | 48.5 | 5 | July 1962 | + |
| Hn 42 | F | 1,780 | 50.8 | 5 | 〃 | + |
| Hn 43 | M | 1,950 | 52.2 | 5 | 〃 | ++ |
| Hn 44 | M | 1,750 | 51.1 | 5 | 〃 | + |
| Hn 45 | F | 1,400 | 50.0 | 5 | 〃 | ++ |
| Hn 46 | F | 1,050 | 46.0 | 4 | 〃 | ++ |
| Cc 41 | M | 280 | 27.2 | 3 | 〃 | + |
| Cc 42 | F | 270 | 27.1 | 3 | 〃 | + |
| Cc 43 | M | 240 | 26.2 | 3 | 〃 | + |
| Cc 44 | M | 290 | 27.3 | 3 | 〃 | ++ |
| Cc 45 | F | 285 | 27.1 | 3 | 〃 | + |
| Ca 41 | F | 74 | 12.2 | 2 | 〃 | + |
| Ca 43 | F | 71 | 12.6 | 2 | 〃 | + |
| Ca 44 | M | 68 | 12.8 | 2 | 〃 | ++ |
| Ca 45 | F | 77 | 13.1 | 2 | 〃 | ++ |
| Ca 46 | M | 73 | 13.4 | 2 | 〃 | ++ |

Hn: *Hypophthalmichthys molitrix*
Cc: *Cyprinus carpio*
Ca: *Carassius auratus*
− No reaction to PAS stain.
+ Positive reaction to PAS stain.
++ Strong reaction to PAS stain.

root of a plant and penetrates into all the other three parts of the hypophysis.

*Activity of gonadotroph*

The activity of gonadotrophs of silver carp can be detected by the staining intensity of PAS and by the number of cells stained as shown in the figures. The mesoadenohypophysis of some hypophysis is heavily stained by PAS (*Figs. 1* and *4*) and that of others is lightly stained (*Figs. 3, 5* and *6*) or not at all. *Fig. 6* shows a few of the gonadotrophs with only the central portion stained while *Fig. 3* indicate a large number of the gonadotrophs taking the stain. In *Fig. 5* and *Fig. 4*, many gonadotrophs are heavily stained and mostly the whole cell. Therefore the gonadotrophs are shown to have different degree of activity.

Table I shows the reaction of hypophysis of the silver carp, *Cyprinus carpio* and *Carassius auratus* to PAS. It can be found that the hypophyses of all fishes examined in October reacted positively with PAS and most of them reacted very strongly. The hypophyses of all *Cyprinus carpio* and *Carassius auratus* showed no reaction but those of silver carp showed strongly positive reaction when examined in January. In April, only two out of the four of silver carps (Hn 27 possesses well developed ovary), two out of the four *Cyprinus carpio* and one out of the five *Carassius auratus* showed slightly positive reaction, while all the others showed no reaction. In July, all the fishes showed positive reaction and most reacted strongly.

The above observation shows that the activity of gonadotrophs of *Cyprinus carpio* and *Carassius auratus* possesses a seasonal cycle which bears relation to the maturity of the gonad but not that of silver carp. The former fishes spawn in late February and early March and so in January, the gonads are full of mature eggs or active sperms. The inactiveness of the gonadotrophs when examined in April may be due to the inhibitory force of the mature germ cells and or to the needlessness of the gonadotropin. It may be more likely that the unused gonadotropin in the blood has the ability to inhibit the further secretion of gonadotrophs. This explanation of the seasonal cyclic activity of gonadotrophs is further strengthened by that exhibited by the silver carp. As shown in the table, the gonadotrophs of silver carp are active in all seasons of the year except one male and one female which possess comparatively well developed gonads. Only two of all the examined silver carps possess comparatively well developed ovary and all the males are immature. The spawning time lasts from May to July. The non-cyclic activity of the gonadotrophs of the silver carp does not mean that they are potentially dislike that of *Cyprinus carpio* and *Carassius auratus* but it is due to the immaturity of this fish and thus the development of the germ cells needs the stimulation of the gonadotropin.

The activity of the gonadotrophs of silver carp is found as normal as that of *Cyprinus caripo* and *Carassius auratus* but the relation of gonadotropin to the development of germ cells is not understandable because the development of the female gonad of this fish at the same age varies greatly even observed at the same time. A six-year-old female does not even show fully developed ovary. The stage of maturity of the male is much slower than the female.

*Development of gonadotropin*

The different degree of the activity of gonadotrophs as shown by staining intensity indicates a clear sequence of the development of gonadotropin. In many instances the gonadotrophs possess only a centrally located sphere stained by PAS (*Fig. 6*). The spheres indicate the earliest stage of the development of gonadotropin. Later these spheres seem to give their material away gradually to other part of the cytoplasm and then they disappear as shown in *Figs. 3, 4* and *5*. In *Fig. 3* the cytoplasm of the gonadotrophs are stained in various degrees and many of the central spheres still exist. *Fig. 5* shows a still later stage of development in which some gonadotrophs still possess a central sphere but most of the central spheres show expansion. When the expansion reaches to the periphery of the cell there seems to be a process of disintegration and the whole mass is broken into fine granules (*Fig. 4*) which then through liquification diffuse into the cytoplasm. This diffused stage is supposed to be the stage ready for secretion.

## CONCLUSION

The general structure of the hypophysis of the silver carp is very much like that of *Cyprinus carpio* and *Carassius auratus* possessing four general regions; the proadenohypophysis, the mesoadenohypophysis, the metaadenohypophysis and the neurohypophysis. Four cell types can be identified in the mesoadenohypophysis: the beta-basophils, delta-basophils, alpha-acidophils, and chromophobes while the epsilon-acidophils can not be identified with certainty.

The activity of the gonadotrophs of silver carp is different from that of the *Cyprinus carpio* and *Carassius auratus* because they are active throughout the year and do not show seasonal cyclic variation. However, this does not mean that the gonadotrophic activity of this fish is potentially different from the other two species or does not vary cyclically with the season. It is because the gonads of the examined silver carp are all immature while in *Cyprinus carpio* and *Carassius auratus* the activity of the gonadotrophs is closely related to the gonadal maturity. The gonadotrophs become inactive when gonads become fully mature. The inactiveness of the gonadotrophs is supposed to be due to the inhibitory effect of the unused gonadotropin remaining in the body fluid.

Various degree of the activity of gonadotrophs show a clear sequence of the development of gonadotropin. It appears firstly as a central sphere. Then it expands and disintegrates into fine granules which finally liquify and diffuse into the cytoplasm ready for secretion.

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*Fig. 1.* Sagittal section of silver carp showing general topography of the hypophysis stained by PAS, 40×.

A...proadenohypophysis.
B...mesoadenohypophysis.
C...metaadenohypophsis.
D...neurohypophysis.

*Fig. 2.* Portions of mesoadenohypophysis stained in Mallory's triple stain, 97×.

DB...delta-basophil (gonadotroph).
BB...beta-basophil (thyrotroph).
AA...alpha-acidophil (somatotroph).

*Fig. 3.* Portion of mesoadenohypophysis stained in PAS counterstained by iron-hematoxylin, 97×.

CH...chromophobe.
N....nucleus of acidophil.
DB...gonadotrophs with nucleus shadowed by PAS stained sphere.

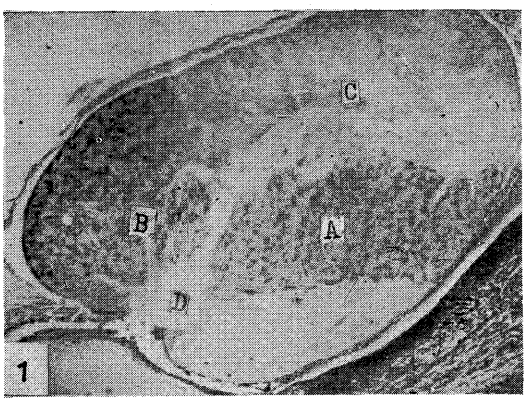
*Fig. 4.* Portion of mesoadenohypophysis stained by PAS, 97×.

DB...gonadotrophs with the central PAS-stained sphere expanded to the whole body of the cell, upper right.
DB...gonadotrophs with diffused PAS-stained substance, lower left.

*Fig. 5.* Portion of mesoadenohypophysis stained by PAS, 97×.

DB...gonadotroph with distinct central PAS-stained sphere and some diffused PAS-stained material at periphery.

*Fig. 6.* Portion of mesoadenohypophysis stained by PAS showing central PAS-stained spheres of gonadotrophs, 97×.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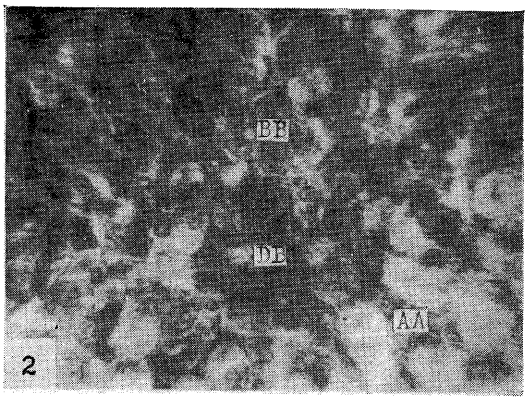
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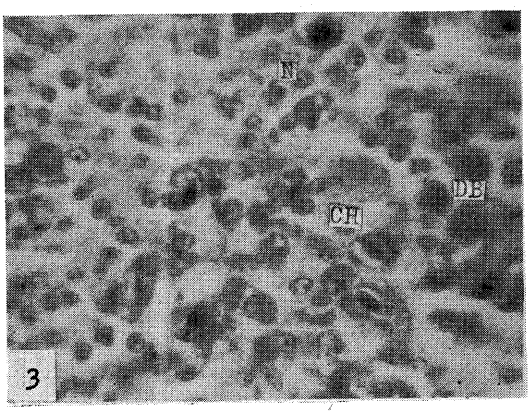
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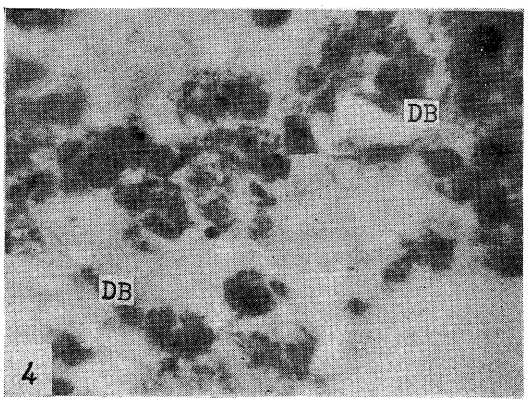
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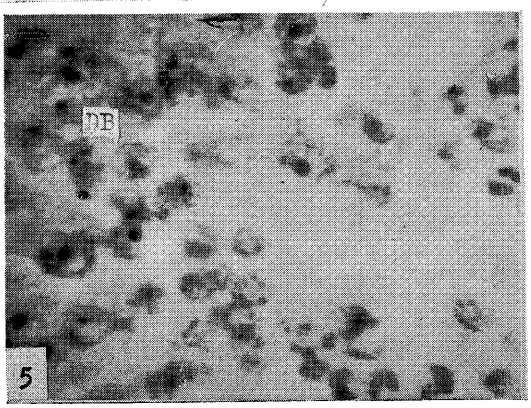
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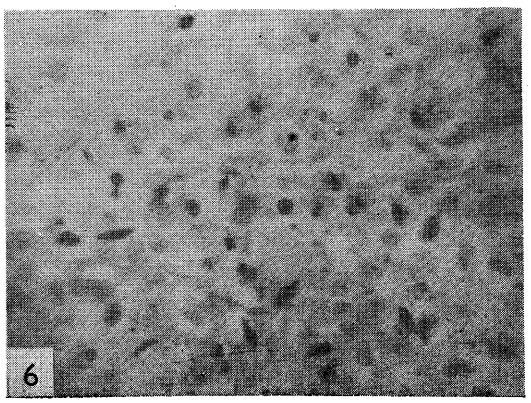
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