IV. THE GERM CELLS IN THE DEVELOPING GONAD OF THE TROUT

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The history of the germ cells has long been a subject involving much controversy. The question of the origin of the genital ridge and the germ cells later found there has been argued extensively. Some investigators (Dustin, Spehl and Polus, Bouin, etc.) believe that the germ cells are of mesodermal origin; while others (Allen, King, Swift, Bounoure, etc.) firmly adhere to the belief that they are of endodermal origin. Among the above named workers and many others there seem to be divided opinions as to the origin of the germ cells and their activities after reaching the gonads. Most investigators agree in at least one essential point. They all believe that in some way or other the germ cells migrate into the region of the gonad, but some contend that they degerenate and new or secondary germ cells are derived from the germinal epithelium.

The material used in this work consisted of trout embryos and fishes from the time of fertilization until the time that the mature sperm and eggs make their appearance. While material is at hand and to a certain extent has been studied, it is not the purpose of this paper to discuss the entire activities of the germ cells, but to discuss only the early stages and up to the time when one can easily say that the sex cells under observation are the ones that actually gives rise to the functional germ cells. In addition an attempt was made to follow the sex cells in their migration.

The Life History of the Trout

The material was obtained by the writer from Mr. Webster Carney, proprietor of the Ozark Trout Farm located near Neosho, Missouri. To Mr. Carney the writer is very grateful for his help in providing material for this study. Without his generous assistance the study would have been impossible. At this hatchery the eggs are fertilized by the dry method; that is, in a pan without brook water. The males and females are stripped, and their spawn mixed in a pan with a feather. As soon as the eggs cease to stick to the sides of the pan, the milt is washed off and the eggs are placed in wire trays that are submerged in troughs of running water. Before hatching takes place, the trays of eggs are placed over other larger and deeper trays, perforated with holes which are just too small to allow the fish to pass through. When the fish are hatched, they fall through the wire mesh of the tray into the perforated tray. Thus, they may be kept clean very easily because all the debris falls through into the trough.

All age computations are from the time of fertilization. Two hours after the eggs and milt have been mixed the pronuclei are formed and are lying side by side. On the fifth day the germ ring stage is reached; on the ninth day the blastopore closes. The fish hatch approximately on the twenty-first day. The male trout mature at the age of 12 months; the female do not lay eggs until they are 22 months old.

The temperature of the water varies in winter and summer between the two extremes of 56 and 58 degrees. At the Petkin, Colorado Hatchery where the writer also observed the method of dealing with trout eggs, the temparature is kept at about 52 degrees, and the trout hatch is about 30 days

The History of the Problem

Since time does not permit a thorough treatment of the history of this problem, it is deemed wise to simply insert here a brief outline of the works of others in this field.

Name	Year	Form Studied	Activities of Germ Cells
Waldeyer	1870	Chick	Sex cells come from primordial cells which arise from germinative epithelium.
R. Hertwig	1880	Sagitta	Early segregation. (gastrula.)
Boveri	1887	Ascaris	Early segregation. (first cleavage.)
Hoffman and Hollander	1892	Birds	Early segregated cells degenerate.
B. M. Allen	1904	Pig and rabbit	Definitive sex cells of peritoneal origin.
Lams	1906	Amphibians	Small degenerating sex cells.
Dustin	1907	Rana and Bufo	Early sex cells degenerate. New cells develop from peritoneal cells.
King	1907	Bufo	No degeneration.
Elpatiewsky	1909	Ascaris	Germ cells come from the single original egg.
Winiwarter	1909	Cat and Rabbit	Showed two epochs of degeneration.
Kingsbury and Hirsch	1912	Desmognathus	Whole cysts and lobules degenerate. (See King, 1907.)
Spehl and Polus	1912	Axolotl	Germ cells degenerate. New ones come from epithelium.
Firket	1914	Chick	Primary genital cells migrate into the germ gland, then degenerate. New ones arise from epithelium.
Swift	1914-15-16	Chick	Enter mesoderm by amoeboid movement, then enter blood stream. Go to gonad through blood.
Hargitt	1924	Amphibia	Most of the primordial germ cells degenerate. The definitive germ cells may come from peritoneal epithelium.
Gatenby	1924	Chick	Definitive germ cells come from peritoneal cells.

Name	Year	Form Studied	Activities of Germ Cells
Simpkins	1925	Trionyx	Germ cells arise from stroma or germinative epithelium. No evidence of extra-regional origin of sex cells is found.
Swingle	1926	Rana	Germ track is continuous. (See Hargitt, 1924.)
Bounoure	1927	Rana	Germ track is continuous. He uses mitochondria as criterion of distinction.
Goldsmith	1928	Chick	Germ cells do not degenerate. Early segregation.
Simpkins	1928	Man	Early germ cells degenerate; definitive sex cells differentiate from somatic cells.
Swezy and Evans	1930	Man	Proliferation from germinal epithelium.
		FISH	
Nussbaum	1880	Trout	Blastomeric origin and migration into sex gland. Continuous germ track.
Eigenman	1891	Micrometrus	Early segregation.
Woods	1902	Acanthias	Early segregation and migration. Continuous germ track.
Bohi	1904	Trout and Salmon	Early segregation.
Dodds	1910	Lophius	Early segregation and migration.
Allen	1911	Lepidosteus and Amia	Early segregation. No degeneration.
Okelberg	1921	Brook lamprey	Early segregation. Degeneration may take place.
Richards and Thompson	1921	Fundulus	Early extra-embryonic origin and migration into region of gonad.
Foley	1926	Umbra	In successive crops of cells the stroma gives rise to definitive sex cells.
Butcher	1929	Lake lamprey	Transformation of sex cells from peritoneal cells. Degeneration may take place.

The reader will note that workers on cyclostomes, elasmobranchs, and teleosts are agreed that the sex cells are early segregated. But there is some disagreement as to their fate after reaching the gonad. However,

very few (Okelberg, Butcher) have found any evidence of degenaration. It is quite odd that investigators working with the same or very closely related forms obtained contradictory results; thus Lams, Dustin, Spehl, and Polus found that the early germ cells degenerate and new ones arise from peritoneal epithelium in the amphibians. On the other hand, Swingle and Bounoure found that the sex cells do not degenerate and the germ track is continuous.

Most of the material used in this investigation was fixed in Bouin's fluid, embedded and sectioned in paraffin, and stained by Heidenhain's iron haemotoxylin method. Last year, 1929, Doctor Lynch, then of the Zoology Department of the Oklahoma Agricultural and Mechanical College, suggested the use of Smith's fluid as a fixative. This was tried and found to give excellent results. Since the yolk is rendered rather soft, and though they were rather thick, sections could be cut in paraffin.

Observations

The primordial germ cells of the trout can be easily recognized by their large size, clear nuclei, and lightly staining cytoplasm. The chromatin is frequently condensed in large granules resembling nucleoli. Because of the brief time available only a summary of the observations can be given here.

Positive identification of the sex cells was first made in the 21-day fish; then from that point their history was followed back to the 9-day embryo, and forward to the 104-day stage in which sex differentiation is obvious.

The germ cells, so far as observed, first make their appearance in the mesentery between the gut and the Wolffian duct. Very soon the numbers increase considerably, though not until the 27-day stage is reached is there any good evidence of mitotic divisions; neither is there any evidence of proliferation from epithelial cells. The explanation for such an increase is obscure, but it is possible that these cells are potential germ cells of blastomeric origin, and are just at this time taking on the distinctive characters of primordial germ cells.

In early stages the germ cells are situated rather far back, and as the animal grows they migrate forward, those on the right side preceding those on the left, until they reach a location in the anterior of the coelomic cavity just below the Wolffian duct where the gonad forms.

From the 31-day stage on, the mitotic figures are common, and as the sex cells increase in numbers, there is an increase in the epithelial cells of the gonad. At first the germ cells hang in the thin layer of peritoneal cells; then they are later surrounded by epithelial cells, so that a thickened ridge is produced that extends almost the entire length of the coelomic cavity.

Summary and Conclusions

1. The germ cells of the trout are segregated early in the formation of the embryo, and are first found in the splanchnopleure of the 9-day fish.
2. There is a migration through the mesentery of the gut to the position of the future gonad just below the Wolffian duct.
3. The peritoneal epithelium surrounds the germ cells after they have reached their destination.
4. The sex cells first appear in the posterior region of the embryo and then migrate anteriorly.
5. The primordial germ cells give rise to the definitive sex cells in the trout.
6. No degeneration or subsequent transformation from epithelial cells occur in the trout.

7. Sex differentiation occurs between the 95-day stage and the 104-day stage.
8. Although the male trout are not ready to spawn, mature sperm cells occur in the testis at least as early as 22 weeks after fertilization.

Bibliography

Bachmann, Freda M., The Migration of the Germ Cells in Amiurus nebulosus. Biol. Bul. Vol. 26:351-366, 1914.

Bohi, U., Beitrage zur Entwicklungsgeschichte der Leibeshohle und der Ersten Genitalanlage bei den Salmoniden. Morph. Jahrb. Bd. 32, 1904.

Dodds, G. S., Segregation of the Germ Cells of the Teleost, Lophius. Jour. Morph. Vol. 21, 1910.

Eigenmann, C. H., On the Precocious Segregation of the Sex Cells in Micrometrus aggregatus. Jour. Morph. Vol. 5, 1891.

Hann, H. W., The History of the Germ Cells of Cottus bairdii. Girard. Jour. Morph. and Physiol. Vol. 43. No. 2. March 5, 1927.

Macleod, J., Recherches sur la structure et la development de l' appareil reproducteur femelle des teleosteens. Arch. de Biol. T. 2, 1881.

Morgan, T. H., The Formation of the Fish Embryo. Jour. Morph. Vol. 10, 1895.

Nussbaum, M., Zur Differenzierung des Geschlechts im Tierreich. Arch. f. mikr. Anat. Bd. 18, 1880.

Okkelberg, Peter, The Early History of the Germ Cells in the Brook Lamprey [Entosphenus Wilderi (Gage)]. Up to and including the period of sex differentiation. Jour. Morph. Vol. 35:1-151,1921.

Richards, A. and Thompson, J. T., The Migration of the Primary Sex Cells of Fundulus Heteroclitus. Biol. Bul. Vol. 40, 1921.

Wilson, H. V., Embryology of the Sea Bass. Bul. U. S. Fish. Comm. Vol. 9, 1889.

Wilson, E. B., The Cell in Development and Heredity, 1925.

Woods, F. A., Origin and Migration of the Germ Cells in Acanthias. Amer. Jour. Anat. Vol. 1:307-320, 1902.