## III. THE RELATION OF THE DEFINITE OVA TO THE PRIMODIAL GERM CELLS OF THE DOMESTIC FOWL*

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From the time of the publication by Waldeyer in 1870 of his observations on the origin of germ cells in the embryo, there have been many contributions to the knowledge of this subject. Waldeyer's view that the germ cells arise from a germinal epithelium in localized area of the peritoneum on the dorsal side of the coelom in vertebrates has been supported by many subsequent observations. Nussbaum, however, in 1880, reached the conclusion that the germ cells are not so derived, but come from cells which are segregated at a much earlier period in the formation of the embryo and indeed he contends that their origin is traceable to the preceeding germ cells. Nussbaum's view has been very stimulating to research on this problem. It has received corroboration from so many investigators in all the vertebrate classes with the exception of the mammals that it may be said to be the prevailing view at the present time. However, there are now two distinct schools of investigators, both of whom use the chick embryo as material for investigation and have reached conflicting results from their studies. These two schools hold views which correspond to those of Waldeyer and Nussbaum, and so far as the fowl is concerned may be represented by the work of Firket on one hand and by that of Swift on the other.

Both schools agree on the earlier stages; that the primordial germ cells arise anterior and antero-lateral to the embryo in a specilized region of the germ wall endoderm, just at the margin of the area pelucida, during the primitive streak stage and up to the three somite stage, and are found in the space between the ectoderm and endoderm. Later the mesoderm arises and the primodial germ cells, by amoebid movement, pass into this mesoderm and into the blood vessels being formed there. They are carried at first by their own locomotive power and later by the flow of the blood streams into all parts of the embryo and the vascular system. They remain distributed in this manner until the embryo reaches the twenty-two somite stage, at which time they become relatively more numerous in the splanchnic mesoderm.

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From this point the one school, represented by Swift on the chick, holds that the primordial germ cells increase by division in the gonads until about the seventeenth day of incubation, at which time they go into the quiescent stage of the normal resting germ cell. The primordial germ cell of the female enter, at this time, into the growth period. The cells begin to fill with deutoplasm and this process continues until the time of ovulation. They show no signs of degeneration of the primordial germ cells, nor do they show any corresponding proliferation of the splanchnic epithelium to develop later into ova.

The other school as characterized by the work of Firket, also on the chick, holds to the view that the primordial germ cells, which arise in the eextra-embryonic regions and migrate to the gonads, degenerate after they reach the gonads, and that the definitive germ cells arise from the proliferation of the splanchnic epithelium.

Because of the divergent views of these two schools it has seemed desirable to reinvestigate the germ cell history in the fowl, and accordingly the project has been under way in the zoological laboratory of the University of Oklahoma since 1924. Hulpieu, 1925, while working in this laboratory carried this experiment from the indifferent stage of the three day embryo to the time of hatching. His work, in the main agrees with that of Swift. He found no widespread degeneration of the primordial germ cells. Hulpieu's material shows that immediately following the period of increased proliferation (eight to thirteen days incubation) the primordial germ cell of the female enter into the growth period characterized by the laying down of the deutoplasm or yolk material around the nucleus of these primordial germ cells, and by the rearrangement of the small cells derived from the splanchnic epithelium into the follicle cells.

The material used in the present study was pure bred Rhode Island and Leghorn fowls. Samples were taken from eight to fifteen days intervals from the time of hatching to the adult stage. The technic procedures do not differ materially from that of Swift and Firket. The material includes samples taken at the following ages: 1, 10, 15, 20, 25, 30, 37, 46, 52, 65, 69, 77, 88, 99, 115, 126, 140, 150, 161, 170, and 180 days. Since this is a continuous process, one condition merging into the next, a detailed description is given for the 1, 10, 39, and 69 day old stages only, taking these as the representative stages of the ovarian development.

In the one day chick the definitive ova have attained a size considerably larger than that of the original primordial germ

cells, due to the accumulation of the deutoplasm. They stain very lightly and are quite numerous throughout the ovary and are surrounded by the follicles cells. They do not as yet show any differentation into the white and yellow yolk such as can be seen in the later stages. Nor are any large or small yolk granules to be seen. It is a homogenous mass of yolk material composed of very fine granules. The nucleus is in the center of the yolk mass and is in a reticulated condition showing no signs of activity. Not all of the primordial germ cells enter into this growth period but only relatively a few. It may be that those cells which are destined to be extruded during the first ovulation period are the ones that exhibit this growth. The follicle cells have begun to form the ring around the definite ova, being forced into their position by the increase in size of the ova; but they are not yet compact nor arranged into the condensed ring of the later stages.

In the thirty-seven day old chick the definitive ova have increased in size in a marked manner. The amount of deutoplasm is increased to such an extent that the follicle cells are forced out against the tubular walls and are very dense in staining reaction. They are still cube shaped but instead of the longitudinal axis of the cells being parallel to the egg nucleus they now lie at right angles to it. The nucleus has not yet started its migration to the periphery of the ovum, being still at or near the center of the deutoplasm. The deutoplasm remains in the fine granular condition as previously described.

In the sixty-nine day old chick the nucleus has become definitely activated, perhaps temporarily, and is passing through the prochromosome stages. The number of these chromatin bodies correspond to the number of the chromosomes as counted by Guyer. These prochromosome stages persist up to the late spireme stage. Up to the time of writing no later stages have been seen. Since it is reported that the ova do not enter into the maturation divisions until after the breaking of the follicles, these prochromosome stages may represent a pseudoactivation. It would seem that the oogonial nuclues returns to the typical resting condition following this temporary activity, and the growth of the ova continues uninterrupted until the time of ovulation.

This study has corrobated the work of Swift in that no widespread degeneration of the primordial germ cells in the domestic fowl has been found. Following the period of migration, the primordial germ cells come to rest in the gonads and, as seen in the embryo of eight to thirteen days of age, pass through a period of rapid increase in number by division,

during which time the gonads increase in size in proportion to the amount of cell division that takes place inside. Following this period of increased mitotc activity the definitive ova which are the primordal germ cells begin to fill with deutoplasm. This process of increase in amount of yolk material continues until the egg is mature and ovulation takes place.

This study has not demonstrated that no degeneration of the definitive germ cells of the female of this form takes place. Such cells simply have not yet been seen. However, it has certainly been demonstrated that degeneration is not the usual process and that if it does occur in some cases enough primordial germ cells remain behind to account for the definitive germ cells without calling in the germinal epithelium.

A considerable part of the interest in the problem of the origin of the germ cells is traceable to the germplasm theory of Weismann that the multicellular organism consists of two distinct portions, the soma and the germ cells, both of which are derived from the preceding germ cell. As the germ plasm doctrine was formerly interpreted, it is not in accord with the view of Waldeyer, and that of the school to which he belongs, that already differentiated somatic cells could give rise to germ plasm. Perhaps this fact has been largely responsible for the great amount of interest in this problem. Recent studies have brought about a reinterpretation of Weismann's hypothesis and it is now held that the chromatin represents the germ plasm, while the cytoplasm is the soma. According to this view all cells contain both soma and germ plasm. Those in which the cytoplasm is especially differentiated becoming the somatic, while those which retain their general embryonic characters provide the germ cells. It is evident that upon this interpretation the problem of the origin of the germ cells become of interest chiefly from an embryological rather than a genetic point of view. It may be said, however, that the work here reported offers no evidence that the germ cells are not segregated at an early period in the development of the body of the embryo chick nor that there may not be a quite definitely maintained continuity of the germ plasm in the earlier sense of this form.

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