VII. OBSERVATIONS OF THE DEFINITIVE SEX-CELLS OF THE CHICK*

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The Theory of Germinal Continuity as elaborated and strongly defended by Weisman undoubtedly furnishes the basis of our modern conceptions of Heredity. If an unbroken chain of germ cells through sucessive generations cannot be demonstrated histologically then the Weisman hypothesis has not proven entirely satisfactory.

Two rival theories, founded upon direct observation, have been advanced and defended by many investigators. Strangely enough the history of the sex-cells in the chick has been cited by two very competent investigators as proof of each of the two theories.

Considering, then, only the chick we may designate Swift as leader in the defense of direct germinal continuity and Firket as his opponent. Swift (15) after carefully following the germ cells from an extra-embryonic origin until about ten days after hatching definitely states that the primordial sex-cells in both the male and female, which migrate into the sex-gland, become the definitive sex-cells; while the peritoneal cells of the germinal epithelium present in the sex-cords develop the follicular epithelium. An unbroken chain of cells, at least from the primordial germ cells to the definitive sex-cells, was thought by him to be established.

However, Firket working about the same time and also basing his conclusions upon observations on the chick, states that the process of segregation and migration does take place but that the primordial germ cells after migration disintegrate in the germ gland and that the definitive sex-cells arise from the surrounding

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peritoneal cells, although admitting that definite cells might develop from the primordial germ cells.

These two theories have many defenders among workers on other forms but because of lack of time will not be discussed here.

Considering the differences in opinion it was decided to reinvestigate the germinal history of the chick.

It has been established, even by those holding opposing views, that primordial sex-cells do appear and are not changed before the fourth or fifth day. Consequently a series of embryos ranging from three days incubation to two days after hatching was secured at intervals of one-half day periods. The tissue was fixed either in Bensley's acetic-osmic bichromate mixture, or Boum's picroformal acetic fixing sclution and stained either by Bensley's aniline fuchsin with Wright's blood stain as a counter stain, or Heidenhain's iron-haematoxylin, counterstained with Orange G. These are practically the same methods used by Swift in his work on the chick.

Before taking up the present work let us review briefly the history of the germ cells.

The period of the indifferent gonad has caused very little controversy. Swift and Firket, holding conflicting views concerning the origin of the definitive germ cells, agree fairly well upon this period. The primordial germ cells first appear arranged in the form of a crescent at the anterior and antero-lateral border of the area pellucida. The primordial sex-cells are carried from here by the blood to all parts of the body. When the embryo has reached the twentieth to twenty-second somite stage the germ cells leave the blood vessels and migrate into the mesodermal tissues. Some of them migrate out of the vessels in different parts of the body and there disintegrate but the majority find their way to the mesodermal tissue of the splanchnic mesoderm near the angle of the coelom, where they are incorporated into the thickening germinal epthelium. Here they are readily recognized by; heir immense size, correspondingly large nuclei, clear cyotplasm containing especially in the younger cells an abundance of vitellus, and a little later an attraction sphere. These characteristics remain fairly constant throughout the period studied.

The sex of each embryo was carefully distinguished and recorded in order to detect any difference in development. The development of the gonads are arbitrarily divided into periods for ease in description.

Six to Eleven Days Incubation

This period might be called the period of cord formation in both sexes. The cords first appear as bulges on the under surface of the epithelium giving it a way appearance. These cords carry with them the primordial germ cells which assume a quiescent state and take no active part in the formation of the cords. This is the only cord formation in the male.

In the female the cords of the first proliferation are formed in much the same manner but never become large, always separating in a short time from the epithelium and carrying very few sex-cells with them. The epithelium becomes thickened, while in the male it becomes thinned almost to a single layer of cells. In the male the cords are separated from the epithelium by the formation of the tunica albuginea. The seminiferous cords increase in mass as well as length and make up the greater part of the testis. The thin film of mesenchymal tissue between the cords is beginning to thicken toward the latter end of this period. The germ cells, as in the earlier part of the period, remain comparatively quiescent, with a few scattered mitoses.

In the female the primordial sex-cells pass through a period of rapid multiplication beginning about seven or eight days of incubation. These cells push into the stroma of the gonad forming the cords of second proliferation or the cortical cords. The sex-cells at this time are quite conspicuous with their clear cytoplasm.

Twelfth to the Eighteenth Day of Incubation

The seminiferous cords do not increase much in mass from the tenth to the thirteenth day but, by fusing with each other in all directions, form a continuous network. The primordial germ cells at about eleven and a half to thirteen days enter into a period of active cell diversion which lasts through this period. After about fourteen days a change takes place in part of the germ cells, which change is thought by Swift to be the formation of spermatogonia. At the end of this period the spermatognia are quite numerous and show a slight decrease in size.

As the cortical cords increase in size the cords of the first proliferation are pushed farther into the stroma of the gonad. The cords of the first proliferation later form the medullary cords of the ovary. The further development of these medullary cords was not followed, but has been fully described by Firket for the chick and by Allen in mammals. The fate of the sex-cells carried by them was not therefore discovered but it is thought that they

may distintegrate and thus offer some of the evidence upon which so much difference of opinion has been founded. They remain large and rounded and their nucleus is in a typical resting state, which is in marked contrast to the condition of rapid multiplication occuring in the germ cells of the cortex. Certain it is, however, that the sex-cells of the cortical cords do not disintegrate.

During this period there is, as in the male, a change in the appearance of the germ cells. A darkened area is seen in the cytoplasm on one side of the nucleus. It corresponds to the "Mitochondral Crescent" of Swift and to the "couche vitellogene" and "nucleus of Balbiani," as described by D'Hollander. If this darkened area is the same as the Mitochondral Crescent of Swift, then we are in full agreement with him when he asserts that the mitochondrial crescent appears in the cells which are the result of primordial germ-ecll division; in other words, the oogonia and spermatogonia.

Nineteen Days Incubation to Two Days After Hatching

In great contrast to their former scattered condition the spermatogonia have become arranged at the periphery, with their large excentrically placed nucleus directed toward the center of the cord. The nuclei of the peritoneal cells on the other hand migrated into close juxtaposition with the basement membrane of the cords. The individuality of the cells in the center of the cords gradually became lost and a syncytium was formed. This process is in reality the first indication of a lumen in the seminiferous cords. The cords from this time on, all show the developing lumen and were beginning to orient themselves in reference to the adult structure of the testis.

About the only change noticable in the female was an increase in the size of the cortical cords due to increase in numbers of the germ cells and, to a certain extent, to the follicular cells. Many authors have described disintegrating germ cells as having their nucleus highly vacuolated and the chromatin arranged in large irregular blotches. Our preparations showed a few cells with these characteristics. However when such cells were found the tissue around them showed similar signs of disintegration. Upon careful study it was learned that these were cases of poor fixation.

Two sources of disintegrating cells might have furnished this evidence; they being the sex-cells that never reach the germ analage and those carried by the cords of the first proliferation in the female. No evidences of disintegration were found in the germ cells of the gonads.

No transforma.ion of the peritoneal cells into germ cells was observed.. The nuc.eus of the peritonial cells in the majority of cases was elongated and very regu ar. The chromatin was granular and from one to three nucleoxi were present suspended by a granu.ar network of chromatin. The nuclei were surrounded by varying amounts of cy.oplasm with no, or at most very indistinct, cell walls. No cases were found where this cell wall was nearly as complete or deeply staining as were the walls of the germ cells. Occasiona ly these nuclei do round up and thus appear larger but never are they surrounded by a complete cell wall filled with a lighter staining cytoplasm as in the case of the primordial germ cells.

Our studies indicate that the primordial sex-cells found in the germinal epithelium of the developing gonad give rise to the germ cells found in the testis of the chick three or four days after hatching. From this we concluded that the primordial germ cells do give rise to the definite sex cells.

Our findings then are in support of the work of Swift, with whom we agree in all the major points and in most of the details.