STUDIES OF THE INTERSTITIAL CELLS OF LEYDIG.

No. 2.—Their Postembryonic Development in the Pig.

BY

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With 5 Text Figures.

In a recent article[1] I presented the results of a study of the embryonic development of the interstitial cells of Leydig in the pig. In the present article I wish to give a brief account of the findings in a study of their postembryonic development in the same animal. The methods employed were the same as those described in the first article, to which I may refer also for the more important facts in the literature of the subject.

The youngest pig in my series was one month old. In sections of the testis at this age, compared with one from the embryo pig near term, the cross-sections of seminal tubules are somewhat more numerous and closer together, and the masses of interstitial cells are proportionally smaller. Beneath the albuginea the cells are much reduced in size, and are arranged in a few more or less parallel rows, separated by small bundles of connective-tissue fibres. In the deeper portions of the gland, however, the cells are still of about the same size. Three main types of cells can be observed, as follows (Fig. 1).

1. Cells with cytoplasm condensed around an eccentric nucleus, while the periphery is extensively vacuolated. The vacuoles have more or less uneven, ragged margins. Some of them hold inclusions which vary in size; the largest of these have the granular appearance and staining reactions of the cytoplasm, while others are more hyaline in appearance. Occasionally structures entirely similar to these inclusions are found between the cells.

2. Cells whose cytoplasm is condensed around an eccentric nucleus, while their periphery is much clearer, containing only a few scattered cytoplasmic threads.

[1] Amer. Jour. Anat., Vol. 3, No. 2, 1904.

3. This variety is similar to the preceding, but is characterized by the presence of large acidophile granules, which have about the same size as those noted in the germinal epithelium of the early embryo. They are more granular in appearance, however, than the latter, and in preparations stained by Mallory's method they take the acid fuchsin, whereas the granules of the epithelium are stained by the aniline blue. With Mann's mixture of methyl blue and eosin they are stained by eosin. Although acidophile, they do not stain with as much intensity as the granules of eosinophile leucocytes. The granules are situated, for the most part, in the peripheral portion of the cytoplasm; occasionally a cell is found which seems loaded with them throughout, but, as a general rule, they are largest and most numerous near the periphery. No such granules were seen within the seminal tubules; in the spaces between the Leydig's cells, however, small collections of them were rather frequently encountered, which in most cases were undoubtedly small por-

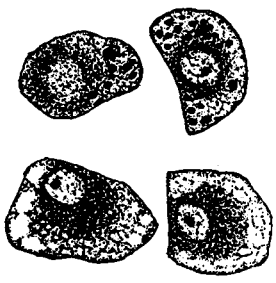

FIG. 1.

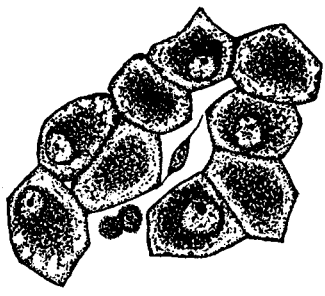

FIG. 2.

FIG. 1. Types of Leydig's cells in pig one month old. × 800.
FIG. 2. Pig three months old. A small group of Leydig's cells. × 800.

tions sectioned from the periphery of granule-bearing cells, but in some instances seemed to be free. All the cells of these three varieties have rather coarse, well defined cell-boundaries, especially marked in the case of the vacuolated cells. These boundaries frequently stain differently from the cytoplasm proper; in preparations stained by Mann's solution of methyl blue and eosin the cell-boundaries quite commonly are blue, while the cytoplasm takes the eosin. Many of the cells have two nuclei, but no mitotic figures were observed. A review of my preparations of the testis from the embryo just before term shows that all these varieties of cells are present there; indeed the principal difference between the two glands, so far as Leydig's cells are concerned, is the atrophy of the subalbugineal layer in the pig one month old. The granules, however,

in the granule-bearing cells of the embryonic gland are smaller and not so limited to the periphery of the cells.

In preparations stained with Sudan III or osmic acid numerous globules of fat, oftentimes very large, are found constantly in the seminal tubules; but the interstitial cells contain at the most only a few fine droplets—many of them contain no fat whatever.

The collections of cells have a rich blood-supply through a network of thin-walled capillaries. A rather striking feature in the testis at this age is the large number of eosinophile leucocytes, both in capillaries and free among the interstitial cells.

In the pig two months old Leydig's cells, in general, are smaller than in the preceding specimen. The varieties of cells described above are still present, but with some differences.

FIG. 3.

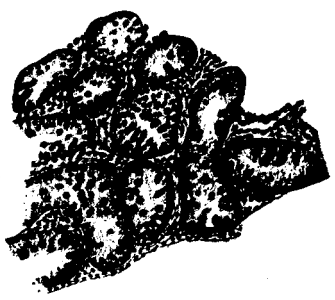

FIG. 4.

FIG. 3. A small area of testis in pig one month old. × 50.

FIG. 4. Small area of testis in pig five months old. × 50.

The granule-bearing cells are scanty, while the cells with pale periphery are quite abundant, as are also the vacuolated cells, the two together forming the great majority of the interstitial cells. In the case of the vacuolated cells the number of inclusions is noticeable. In many of these cells the septa between the vacuoles are breaking down.

At three months the convoluting of the seminal tubules has increased considerably, so that many more cross-sections of them are seen, and the interstitial cells are divided up into smaller collections. The breaking down of the septa between vacuoles and the concentration of cytoplasm around the nucleus have also progressed, so that now the individual cells are much smaller than in the preceding stages, and most of them present the pale periphery (Fig. 2). A very few containing acidophile granules may be seen.

These two processes, growth of the tubules and atrophy of the interstitial cells, continue at such a rapid rate that in the five-months pig the tubules greatly predominate over the interstitial cells (compare Figs. 3 and 4). The latter are now so reduced in size as to almost be identical in appearance with the subalbugineal cells of the pig at one month. Individual cells are shown in Fig. 5. Many of them are like the central one in the figure, others entirely lack the distinct cell-boundaries and are little more than naked nuclei, and others show distinct cell-boundaries only at intervals, especially at the margin of a vacuole.

Of the three adult testes at my disposal two were evidently pathological, as the tubules in one case contained no sexual cells, and in the other only a few spermatogonia; probably they were ectopic testes, and need not be considered here. The third one, however, was normal, and spermatogenesis was quite active. Sections show that the growth and convoluting of the seminal tubules have progressed still further, with the result that there are very few Leydig's cells between the albuginea and

FIG. 5. Types of Leydig's cells in pig five months old. × 800.

the bases of the tubules, but they have been crowded against the lines of attachment of the septa to the albuginea. In the deeper portions of the sections the general appearance of the cells is quite similar to that found in the five-months pig, with the exception that they are somewhat larger. They do not contain any acidophile granules, nor could Reinke's crystalloids be demonstrated in them. The subdivision of the groups of Leydig's cells has increased, and in many situations there are none between adjacent tubules.

While, of course, this study does not warrant conclusions as to the function of Leydig's cells in the adult, we may at least inquire if it furnishes any data in support of any of the hypotheses which have been advanced as the result of histological investigation of adult conditions. In favor of the theory of v. Bardeleben, that Leydig's cells replace Sertoli cells as the latter are worn out in the performance of their function, I can find no evidence in any of the preparations. After the basement membrane of the tubules is laid down it forms a barrier which completely prevents the passage of interstitial cells into the tubules. So also as to the view

of Plato, that the function of Leydig's cells is to store up fat and pass it on through the walls of the tubules to be used as pabulum in spermatogenesis, the evidence is negative. The Leydig's cells of the pig's testis contain little or no fat, while the tubules show large quantities of that substance; nor could I detect the minute canals described by him in the walls of the tubules. Moreover, if recent investigations upon fat metabolism are to be accepted, fat entering the tubules from the outside would probably pass through their walls, not as such, but rather as its two liquid components. Some support, however, might be derived for an extension of Plato's theory as suggested by v. Lenhossek, according to which the function of the interstitial cells is to store up, not merely fat, but other material as well, to be used as pabulum by the tubules. The most important facts in the development of Leydig's cells, it seems to me, are the alternating periods of hypertrophy and atrophy, and the structural characters of these cells during the stage of hypertrophy. The periods of hypertrophy precede, while those of atrophy are synchronous with, periods of rapid growth by the seminal tubules. Moreover the changes in the interstitial cells, though occupying much more time, are comparable, to some extent, with those which occur in secreting cells. So that the appearances described might be interpreted as possibly indicating that the Leydig's cells elaborate a specific pabulum for the tubules during the development of the testis.

I wish here to thank Professor F. P. Mall for the courtesy of a seat in his laboratory while this article was in preparation.