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ELECTRON MICROSCOPY OF THE HUMAN SEMINIFEROUS TUBULES

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The germinal epithelium of the testis is composed of different cell types which constantly undergo changes through a complicated combination of proliferation, differentiation and renewal. Clermont has postulated (1-3) that human germ cells are systematically arranged into six basic stages, and has proposed models for the development and renewal of spermatogonia in man. On the other hand other authors have stated that the cellular division and combinations in the human testis lack the synchronism and steadiness which is seen in other mammals (4-6).

With the development of the electron microscope, certain ultrastructural characteristics of the human testis have been investigated. Data have been accumulated with respect to the testicular connective tissue (7,8), the Sertoli cells (9-13) and the spermatids (14-16).

The present paper is directed towards obtaining information which would provide a basis for more detailed investigation into the histophysiology and histopathology of the germinal epithelium. In recent years research in cellular pathology has emphasized the importance of cytoplasmic(17) rather than nuclear changes, as these appear to be the earliest cellular modifications following diverse types of injury. Therefore, identification and description of the normal germinal cell ultrastructural characteristics are essential to the understanding of morphological events following injury or disease processes.

MATERIAL AND METHODS

For these studies biopsy specimens were obtained from 10 healthy

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men who had no record of former testicular disease. Biopsy was performed by a modification of Charny's technique (18). The material was prepared for electron microscopy following the method already reported (19).

RESULTS

Tubular Wall

Electron microscopical studies of the wall of the human seminiferous tubules clearly showed two main components: a) The internal one, attached to the germinal epithelium is the basal membrane presenting a lamellar structure, the lamellae are separated by spaces of low electron-density(18). This structure presented infoldings which appeared simple or rather labyrinthic and included some collagen fibers or cytoplasmic processes of the wall cells. The depth of indentation reached one or two microns. b) The external layer showed an alternate arrangement of collagen fibers oriented at different angles together with cells. The cells are extremely elongated, their endoplasmic reticulin was poorly developed and was mainly in the form of lamellae. Fine filaments aggregated in bundles are seen in the cytoplasm of the cells which were found to be somewhat similar to smooth muscle fibers (20).

Germinal Epithelium

The germinal epithelium is composed of spermatogenic or germinal cells and Sertoli cells. It is now a widely accepted belief that there are two independent cell lines in the mammalian testis.

The Sertoli cells are most conspicuous in this epithelium. The germinal cells, with the exception of the earlier spermatogonia are dispersed amongst the Sertoli cells and are separated from the basement membrane by the cytoplasmic processes of the Sertoli cells, which in many cases are not wider than 200Å.

Sertoli Cells. These showed a well developed endoplasmic reticulum which appeared as flat and parallel aggregated cisternae, with associated ribosomes arranged on the membranes in single strands. This type predominated in the cytoplasmatic processes surrounding the germinal cells. In the basal portion of the cell, which contains its characteristically lobulated nucleus with a prominent nucleolus, ribosomes were arranged in membrane-free clusters, and the endoplasmic reticulum appeared in Caufield's solution-fixed preparations as numerous empty smooth-surfaced vesicles of about 600 to 1800Å in diameter. Fixation with glutaraldehyde changes this appearance into a rather tubular form: short, tortuous, interconnected tubules averaging 240-300Å in diameter with localized dilatations were seen in the central portions of these cells, while in the periphery they formed arrays of long, flat membranous profiles. The ground sub-

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stance of the cytoplasm appeared as a dense material made of granules smaller than ribosomes. As the density of the ground substance is higher than that of the germinal cells, the body of the Sertoli cells and their processes clearly stood out over the surrounding elements. Regarding the density of the ground substance, two types of Sertoli cells can be distinguished; dark and light. At present we are not able to relate these two types to any functional stage or ascertain if they correspond to the two different types of cells demonstrated by Johnsen at light microscopical level (21). Ground substance also included bundles of microtubules of about 300Å. Microfibrils with diameter averaging 35Å were particularly numerous around the nuclear membrane. They were prominent near the head of the spermatids where they appear in bundles parallel to the axis of the spermatid (12). Sertoli cells show wide attachment to the lamellar basement membrane, whose characteristic "pegs" protrude into these cells (7,8).

Spermatogonia. Different types of spermatogonia can be demonstrated with the electron microscope. In the following description we are going to avoid identification by using the alphabet. There are two reasons for this: first, there is more than one paper dealing with spermatogonia where similar letters refer to different cells (1,22). Secondly, when following alphabetical order it could be assumed that this order is related to the sequence of the cells in the spermatogenic line.

The flat type spermatogonium appeared as a rather small, oval-shaped cell. Its larger diameter was parallel to the basement membrane to which it was attached. The nucleus contained one or two conspicuous nucleoli composed of coarse, dense strands which branched and anastomosed in an irregular network. The ground substance of the cytoplasm contained disseminated microtubules and very abundant, uniformly scattered fine granules, 120Å in diameter, which made these cells appear darker than other spermatogonia and spermatocytes, although lighter than Sertoli cells. The endoplasmic reticulum was poorly developed and consisted of a few flat membranous profiles, while occasional scattered clusters of four or five larger and denser ribosomal granules were seen. The round or ovoid mitochondria were relatively large and contained a dense matrix with clearly marked parallel cristae; they tended to aggregate close to the poles of the nucleus. The unremarkable Golgi apparatus also appeared in the vicinity of the nucleus.

Following glutaraldehyde fixation some of the flat spermatogonia showed a circular rarefaction in the center of the nucleoplasm, which appeared as a "vacuole" of less density, but without any limiting membrane or special structure within it. This characteristic was not exclusive to flat spermatogonia as similar "vacuolizations" could also occasionally be seen in round spermatogonia, preleptotene spermatocytes and in spermatids.

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The round type spermatogonium, a larger, circular cell, was also attached to the basement membrane. Its round, homogeneous nucleus was lighter and contained a small nucleolus which frequently did not show up in the ultra-thin sections. The ground substance of the cytoplasm was less dense and contained no microtubules, although the Golgi apparatus was more conspicuous. While the mitochondria were smaller, they showed well-developed cristae in a dense matrix. In contrast to the flat type spermatogonia, they were scattered throughout the cytoplasm. Small crystal like structures were seen. They presented an irregular shape and were formed of bundles of fibrils or tubules aligned in the long axis. The nucleus of human spermatogonia has been studied recently by Tres and Solari (23). Five different types of nuclear bodies were observed in type A (flat) spermatogonia. Type I bodies are typical nucleoli. Types II, III and V are considered to be atypical nucleoli. Type IV bodies are small chromatin condensations. These authors stated that they could not establish a valid criterion for distinguishing the different kinds of A gonia at the ultrastructural level although they suggested the existence at least 2 types of A gonia. In these authors' criteria the different nuclear bodies probably reflect different metabolic activities.

A third type of cell was noted which seemed to correspond to the usual descriptions for preleptotene primary spermatocyte. The cytoplasmic subcellular characteristics of this cell were very similar to those of round spermatogonia and showed little resemblance to those of primary spermatocytes. They differed from the round type spermatogonia in that they were separated from the basement membrane, as well as from neighboring cells, by the previously described ramifications of the Sertoli cell cytoplasm. It was frequently noted that intercellular bridges existed between these preleptotene cells and round spermatogonia.

Although in the course of meiosis primary spermatocytes underwent remarkable changes in size as well as in the characteristics of the nucleus, the subcellular features of the cytoplasm remained stable. These changes in the nucleus will be presented later on by Dr. Solari. The numbers of polyribosomes dispersed throughout the ground substance were greater than in any other germ cell type. But since the ground substance contained little else, the primary spermatocytes were the lightest cells in the germinal epithelium. Short and very flat membranous profiles were scattered throughout the cytoplasm, and flat saccules were located throughout the nucleus. The prominent Golgi apparatus, round and rather compact in form, was situated at one of the poles of the nucleus. The cristae mitochondriales did not show the straight parallel pattern which was seen in the spermatogonia; instead, they followed an irregular course and frequently coalesced with the outer membrane, giving the mitochondria a "vacuolated" appearance (24). Microtubules were scattered throughout the cytoplasm, and although their appearance was undis-

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tinctive, they seemed to be unrelated to the microtubules of the spindle during the first meiotic division.

The earliest modification of primary spermatocytes, which enabled those cells to be distinguished from spermatogonia, was the appearance of very fine single leptotene threads. These were always present even in the youngest spermatocytes. We were, therefore, unable to find a "resting" stage that could be interposed between the spermatogonial mitosis and the meiotic prophase.

The secondary spermatocytes were relatively small cells, slightly larger than young spermatids, and obviously smaller than the neighboring spermatocytes. They were seen infrequently and appeared close to or partially in the lumen of the seminiferous tubules. The nucleus was round with a rather homogeneous aspect; the nucleolus was very inconspicuous and was absent much of the time. The endoplasmic reticulum was made of rosaries of small flat saccular profiles which were arranged concentrically in relation to the nucleus. The limiting membranes of these saccules were agranular and tortuous. Scattered among the endoplasmic reticulum,round mitochondria were found,which present the same vesiculization seen in the primary spermatocytes and spermatids. Resting and dividing secondary spermatocytes were generally seen co-existing in the same portion of the tubule.

During the course of spermiogenesis, the spermatids showed changes in the configuration of the acrosome which did not differ from those classically known (14,15). Five different stages of progressive change in the morphology of the spermatid were easily characterized.

In Stage A, corresponding to the beginning of spermiogenesis, the spermatids were round, with a round homogeneous nucleus. Toward the end of this phase the acrosome granule, as well as the acrosome vesicle, could be seen.

The cap finally became established during Stage B, and the nucleus could be seen starting its elongation, although the appearance of the nucleoplasm remained unchanged. During this stage the microtubule of the manchette first appeared, while within the cytoplasmic branches of the Sertoli cell similar microtubules became arranged parallel to the axis of the spermatid.

Elongation proceeded during Stage C, but the most remarkable change occurred in the nucleoplasm where chromatin-like material became condensed in flocs or large irregular granules, which gave the nucleus a "tigroid" appearance.

During Stage D, progressive reduction in nuclear size could be observed, the chromatin flocs formed wider clusters, and the nucleus

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became more and more compact until all the interstices among the flocs were obliterated and the nucleus again presented a homogeneous appearance.

In the final phase, Stage D2, most of the cytoplasm of the spermatid became eliminated, and residual bodies could be seen; either partially free in the lumen of the tubule, or in the process of being "phagocytozed" by the Sertoli cells.

DISCUSSION

Each cell in the human germinal epithelium shows, in both the cytoplasm and the nucleus, particular subcellular characteristics which allow its identification in electron micrographs. For the cytologic study of the testis, electron microscopy offers undoubted advantages over light microscopy. The distinctive features of the germinal cell types that can be established with the light microscope, either in normal or pathological conditions, represent primarily morphological configuration of the chromatin and the nucleolus, since the cytoplasmic details are poor and the cellular limits are difficult, or impossible to determine. Furthermore, different fixatives give diverse chromatin patterns, and it is sometimes difficult to classify spermatogonia or correlate the different classifications which appear in the literature (3,22).

Clermont (1) recognized two types of A spermatogonia (A dark and A pale), the first one having a large vacuole-like cavity in the nucleus. He gave a ratio of Ad:Ap of 1:1. We were not able to establish with the electron microscope the same discrimination among spermatogonia. Following fixation with glutaraldehyde, we observed in some nuclei a rarefaction of the nucleoplasm which might appear as a vacuole-like structure, but since procedures in the preparation of the specimen are different, it is difficult to affirm that this "vacuole" corresponds to that described by light microscopists. On the other hand, our ratio between the "vacuolated" and "non-vacuolated" flat spermatogonia is not 1:1, but closer to that of Roosen-Runge and Barlow (5) who found vacuoles in 96 of 1000 nuclei. We observed in the fragments fixed in glutaraldehyde, that similar "vacuoles" could be found in other types of germinal cells (gonovytes, spermatogonia, round spermatids, etc.). Thus it appears that this "vacuole" is not a distinctive characteristic of any type of cell, or of any specific stage, but a kind of modification of the nucleus of the germinal cells due to the aldehyde fixation. Its significance we are not yet able to evaluate.

We did not find an identifiable resting stage of primary spermatocyte that would be interposed between the last spermatogonial mitosis and the meiotic prophase. The youngest spermatocyte seemed to be already in meiosis. Sotelo and Trujillo-Cenoz (25) reported the same finding in other species, and stated that "the appearance

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of very fine chromosome threads (leptotene threads) seems to be the better mark to recognize the transformation of spermatogonia into spermatocytes. The preleptotene cell, here described, presented subcellular characteristics, which corresponded to those of B spermatogonia rather than spermatocytes, except for the fact that they were not attached to the basement membrane but were surrounded by Sertoli cells and so separated from the other cells and the basement membrane. The existence of cellular bridges between these cells and B spermatogonia unquestionably indicates their origin. The preleptotene stage probably represents a more differentiated type of B spermatogonia moving into the core of the germinal epithelium.

It could be speculated that the establishment of the above mentioned relationship between the Sertoli and the preleptotene cell is an unavoidable determining factor that induces, after the last spermatogonial division, the transformation of the spermatogonium into a primary spermatocyte, which in time will be able to accomplish meiotic division. The function of the Sertoli cells is not yet known, but it was suggested long ago that the products of the secretion of these cells could supply nutrition to the germinal cells (26). Also, it has been shown that they can act as an active transporting system as labelled substances injected into adult rats leave the circulation in the intertubular spaces and rapidly move into the seminiferous tubules, diffusing through the cytoplasm of the Sertoli cells (10). Christensen and Fawcett(27) have advanced the idea that the site of hormone production in steroid producing cells is in the agranular endoplasmic reticulum. The agranular endoplasmic reticulum of the Sertoli cell could be considered a morphological base to support the claim that these cells produce steroid hormones, but no consistent proof has been found at the present time. As was also speculated by Christensen (8) the parallel microtubules in the Sertoli cytoplasm could be related to the mechanism of the release and holding of the spermatids.

The supporting cells reach maturity at pubertal ages, simultaneously with the beginning of spermatogenesis, and an obvious regulation by the hypophysis occurs. We can only conjecture as to whether gonadotropins act upon both germinal and Sertoli cells or whether the primary target is the supporting cells (Sertoli) which then could influence the germinal ones. Courot (28) injected gonadotropic preparations into lambs whose testes were not yet showing spermatogenic activity and observed that while no important evolution of the gonocytes was seen, a fairly constant stimulation of precursors of Sertoli cells was observed.

Mancini et al. (22) have described additional types of spermatogonia in the adult human testis; binucleated, hypertrophic and pyknotic. While binucleated spermatogonia, as well as other binucleated types, are seen frequently and merely indicate a delay of

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the cytokinesis as Fawcett and Burgos (14) have reported, hypertrophic and pyknotic cells are seen rather seldom, and in our opinion have little significance. Large spermatogonia have been interpreted as polyploid cells (5), and pyknosis probably indicates failure of isolated cells to pursue the complete evolution toward spermatozoa.

Fig. 1. Human Sertoli cell. Note the well developed nucleolus. × 17,000

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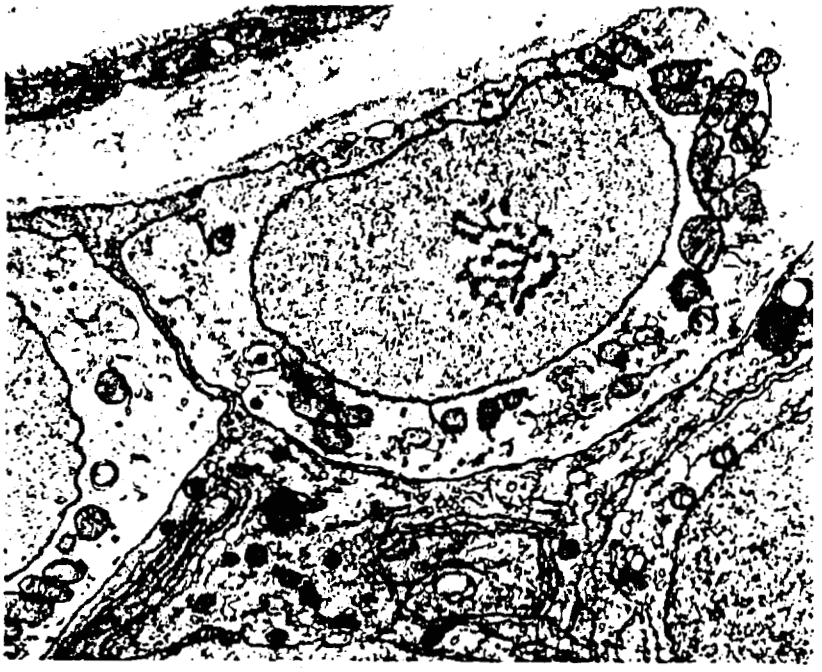

Fig. 2. Flat spermatogonium in contact with the basal lamina. x 30,000.

Fig. 3. Flat spermatogonium. Note the "vacuolization of the nucleus" x 27,300.

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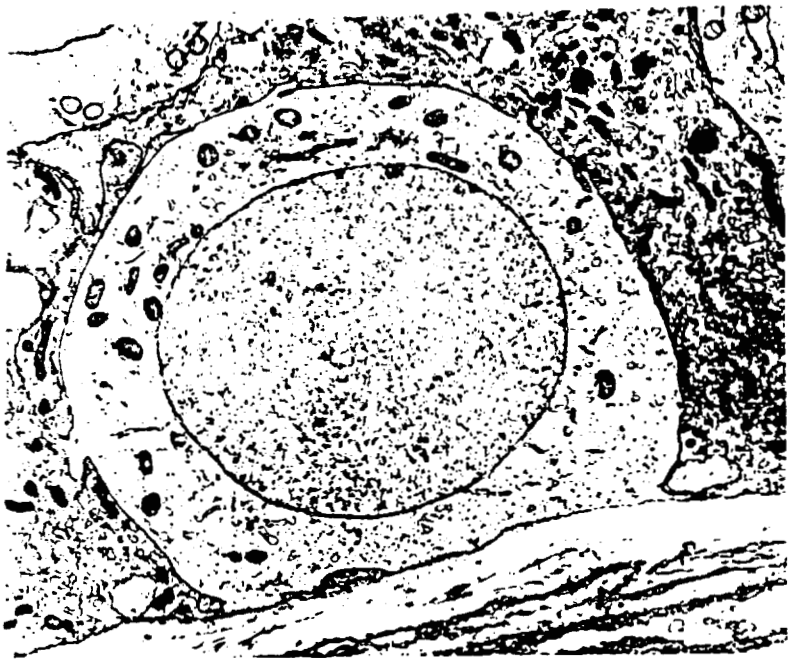

Fig. 4. Round spermatogonium. Note that the basal lamina forms "pegs" which project into the Sertoli cells. x 18,000.

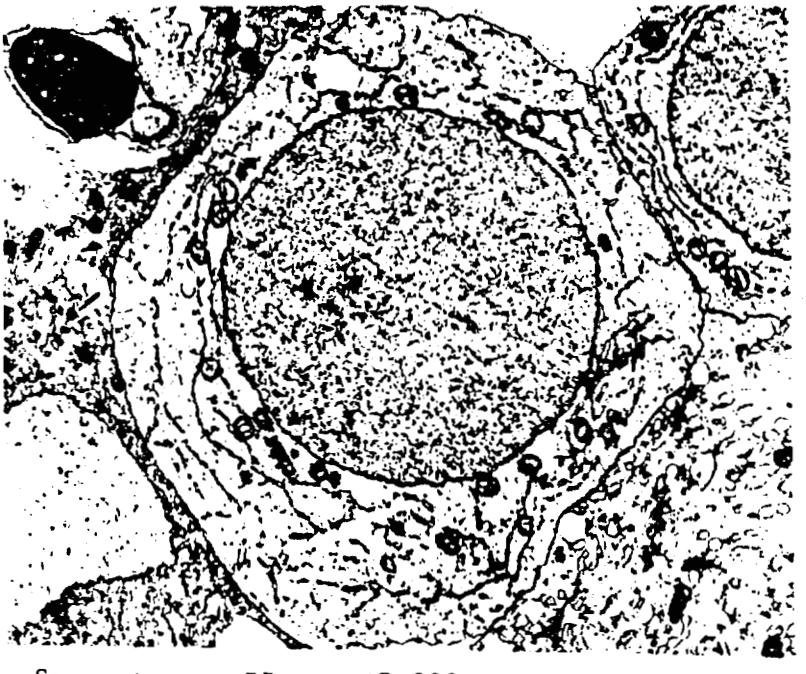

Fig. 5. Spermatocyte II. x 17,000.

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Fig. 6. Spermatid B. Note the tubules of the "manchette" as well as those in the cytoplasm of Sertoli cell. x 40,000.

Fig. 7. Spermatid D. Note condensed nuclear material and the connecting piece. x 27,300.

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