SPERMATOGENESIS IN THE ALBINO RAT AS REVEALED BY ELECTRON MICROSCOPY*

A PRELIMINARY REPORT

by

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Methods are presented here for the preparation of tissue sections of rat testis for the electron microscope which show a resolution of better than 100 A for certain cytoplasmic cell components. These methods include the usual fixation in osmic acid, the omission of washing of fixed tissue, the necessity for alcohol dehydration, and removal of embedding material by a sublimation process.

A preliminary description of certain features of spermatogenesis revealed by these techniques is included. New observations of the cell structure are the peripheral location of young spermatid mitochondria, the filamentous structure of the caudal sheath of the developing spermatid nucleus, and a description of structural details of the acroblast.

MATERIALS AND METHODS

This tissue preparation may conveniently be divided into categories of fixation, dehydration, embedding, cutting, and removal of embedding material.

The general procedure followed is tabulated below:

1. Fixation in 2% osmic acid for 15 minutes to 1 hour. No washing.
2. Dehydration in 60%, 80%, 90%, 95%, and 100% ethyl alcohol for 1 hour in each.
3. Embedding in butyl methacrylate monomer with 2% catalyst** in equal portions with absolute alcohol for 1 hour; then in the monomer with catalyst for 12 to 15 hours at about 5 degrees Centigrade.
4. Polymerization in #1 gelatin capsules at 45 degrees Centigrade, about 8 hours.

The methacrylate embedding procedures follow, with minor changes, those outlined by NEWMAN *et al.*[1] Cutting is done with a Spencer Model 820 microtome modified with a glass wedge as described by PEASE AND BAKER[2]. A steel knife is used, sharpened on glass as described by HILLIER[3]. The sections are floated from the knife onto 35% dioxane in water containing a small amount of antioxidant*** suggested by HILLIER in a private communication. They are picked up on 100 mesh silica-coated collodion-covered screens and after drying are transferred to the evaporator. Here they are placed 1 inch from a tungsten coil with a sheet of mica about 0.001 inch thick interposed between the coil and the screens. When a vacuum of 0.2 μ is reached, the coil is heated by a current equal

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** Di-chlorobenzoyl peroxide in equal proportions with dibutyl phthalate.

*** A sufficient quantity of sodium chrom glucosate is dissolved in water to produce a definite yellow color and the filtered solution is used to dilute the dioxane. The material may be obtained from D. W. Haering and Company, Inc., P. O. Box 6039, Harlandale Station, San Antonio, Texas.

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to about one-half the current required to melt the tungsten, and that temperature is held for one minute. The filament is closewound from 1–1/2 inch of 0.020 inch diameter wire into a cone with 1/8 inch base diameter. This last step removes nearly all the embedding material from the sections by sublimation. The mica sheet prevents tungsten from being deposited on the sections. The screens are now ready to be examined.

The best preparations obtained have been of testis and lung. In testis from which the tunic has been removed the tubules will separate from one another when placed in the osmic acid fixative. Thus a tissue with a large surface-to-volume ratio is easily obtained without manipulation. Lung, owing to its structure, also has a large surface-to-volume ratio. Less easily penetrable tissues such as liver have not been so successfully prepared.

Although good preparations of testis and lung have been made where washing of the tissue after fixation was part of the procedure, in general the quality is good more consistently when the washing is omitted.

Dehydration in alcohol is necessary for good results with osmic fixation. Methyl, ethyl, and *iso*-propyl alcohols have been tried with essentially the same results. These alcohols reduce osmic acid and it is felt that this characteristic is significant in the fixation process. Dioxane does not reduce osmic acid and gives poor results when used as a dehydrating agent.

The sublimation method outlined for removing the embedding material from sections avoids the distortion introduced by the use of solvents and may be carried to completion more successfully than sublimation in the electron beam of the microscope. The temperature must be controlled rather carefully to avoid removal of portions of tissue and at the same time to permit complete clearing of the section. Silica coating of the collodion supporting film is essential to provide the strength required for the sublimation procedure.

RESULTS AND DISCUSSION

The pictures which follow, Fig. 1 through 3, are of cells in the germinal epithelium of normal adult rat testis. These pictures are representative of the results obtainable with the techniques described in the sense that a reasonable portion of tissue-bearing fields is acceptable. Letters on the figures simplify identification of structures referred to in the text.

Fig. 1 shows a cross-section of a late phase 8 tubule. The phase designation follows that of ROOSEN-RUNGE AND GIESEL[4] described in their excellent statistical study of spermatogenesis in the rat. A few sperm heads (A) may be seen in the lumen at the very bottom of the figure near the right corner. A number of vacuolated bodies (B) in the same general region which contain clusters of heavily staining fat globules are the spermatid cast-offs. Most of the section is dominated by young spermatids (C) which occupy that half of the seminiferous epithelium towards the lumen. The nuclei have become eccentric and are in contact with the cell wall. The nuclear cap is heavily stained and occupies about one-third of the periphery of the nuclear cross section. In several cells the acrosome in its early, uncondensed state may be seen in the center of the nuclear cap. The spermatid mitochondria characteristically adhere to the cell membrane.

The cells immediately above the spermatids are primary spermatocytes (D) which at this stage are fairly large. Their outline is irregular and shows indentation by surrounding cells. The large idiosome (D_1) has a diameter about one-third that of the nucleus. Two small cells tentatively identified as Type B spermatogonia (E) may be seen at the basement membrane (H). A large oval SERTOLI nucleus (F) with a deep indentation also lies at the basement membrane. The SERTOLI cytoplasm (G) streams down among the other cells and shows no obvious cell membrane.

In good preparations for the electron microscope it has been our experience that membranes between cells in general which are in contact are usually quite distinct. However, observations by the author have never disclosed a membrane separating two SERTOLI cells whose cytoplasm is in contact. This may throw some light on the question

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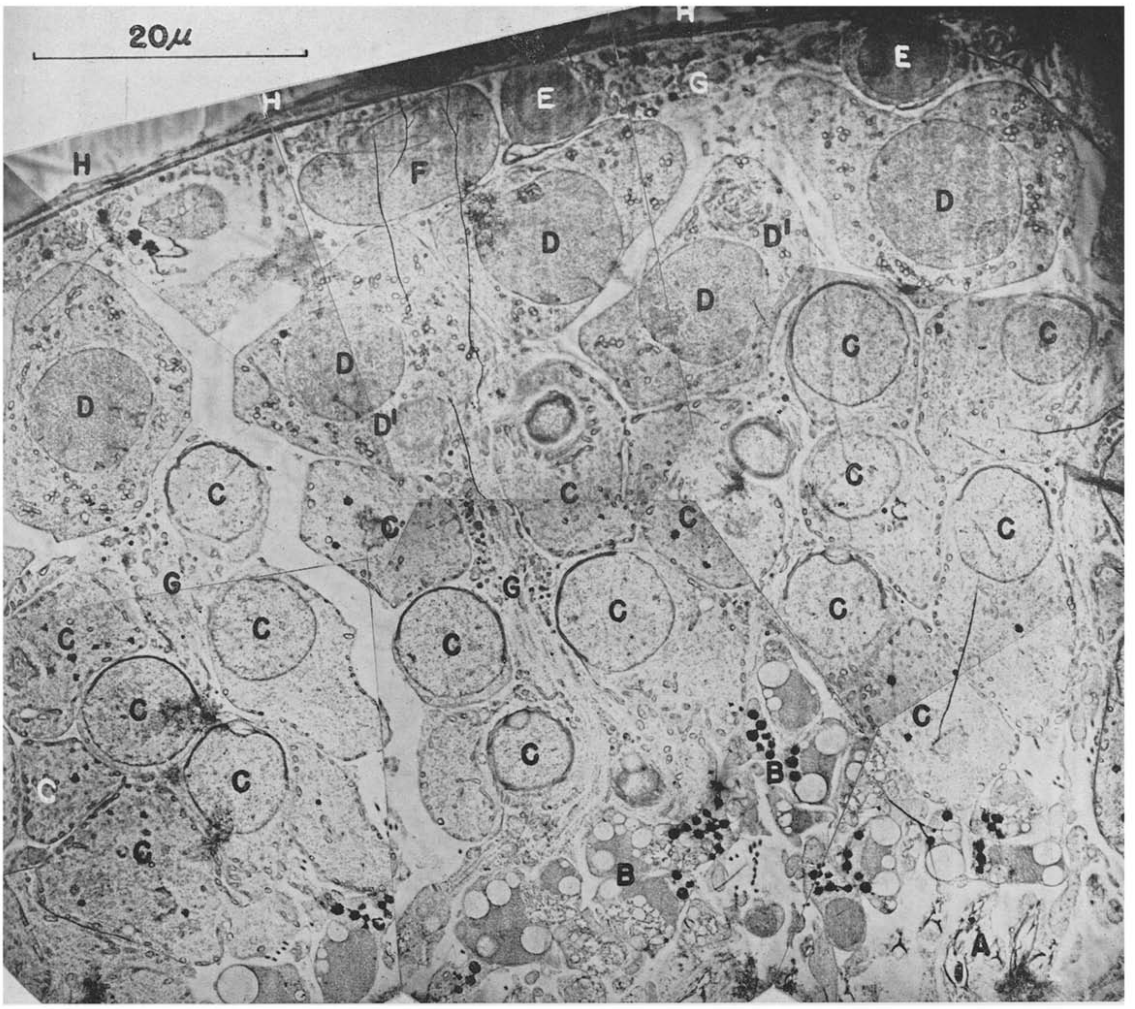

Fig. 1. Low power view of a portion of a late phase 8 tubule. A few sperm heads (A) are still present at the lumen. Cast-offs (B), remnants of the spermatid cytoplasm abandoned by the developed sperm, line the lumen at this stage. Developing spermatids (C) are present in large numbers. The older primary spermatocytes (D) have grown to large size and contain a prominent idiosome (D_1). The next generation of primary spermatocytes (E) are small and nearly spherical and adhere to the basement membrane (H). The SERTOLI nucleus (F) is close to the basement membrane in all phases of spermatogenesis. Its cytoplasm (G) surrounds the spermatogenic cells and appears to be continuous with that of all the other SERTOLI nuclei in the tubule.

of the existence of the "SERTOLI syncytium" which has received much attention in the years since the first description of these cells by SERTOLI[5].

Fig. 2 shows a young spermatid in a somewhat earlier phase than those in Fig. 1. The mitochondria (A) are clearly seen generally in contact with the cell membrane. They are somewhat variable in form but tend to be spherical. A number of pale staining bodies (B) are scattered throughout the cytoplasm. These bodies have a variable form corresponding to the stage development of the spermatid. In the cell shown they are elongated, lightly-staining structures with a maximum dimension of about 2000 A. At

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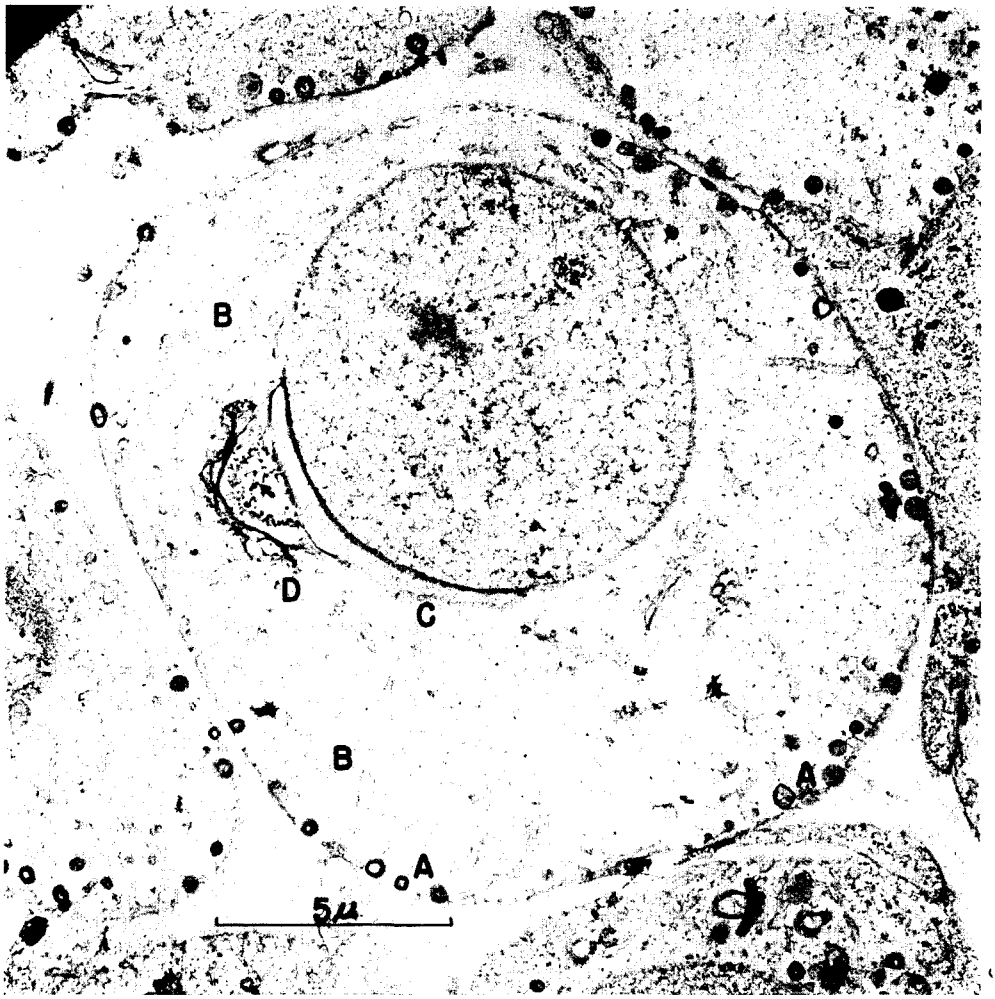

Fig. 2. Early resting spermatid. Mitochondria (A) adhere to the cell membrane. Microsomes (B) of variable form are found throughout the cytoplasm. The nuclear cap (C) has not yet condensed as much as the nuclear caps of spermatids in Fig. 1. The acroblast (D) is still in contact with the nuclear cap. The acrosome is not shown in this section.

later development they become spherical and much smaller. These bodies appear to be similar to structures noted by PORTER AND THOMPSON[6] (Fig. 1 of their paper) in a normal tissue cultured chicken macrophage and referred to by them as microsomes or "components of the ground substance".

The nuclear cap (C) is at an early stage of development and later becomes much condensed. The acroblast (D), a term used by KING[7] and later by BOWEN[8] to denote the "idiosomal" structure from which the acrosome is formed and which contains the centrioles, is in contact with the nucleus near the edge of the cap. The acroblast is a bell-shaped structure with the osmiophillic cap lying distal to the nucleus. Numerous small spherical objects are inside the acroblast. These have diameters of the order of 200 to 300 A.

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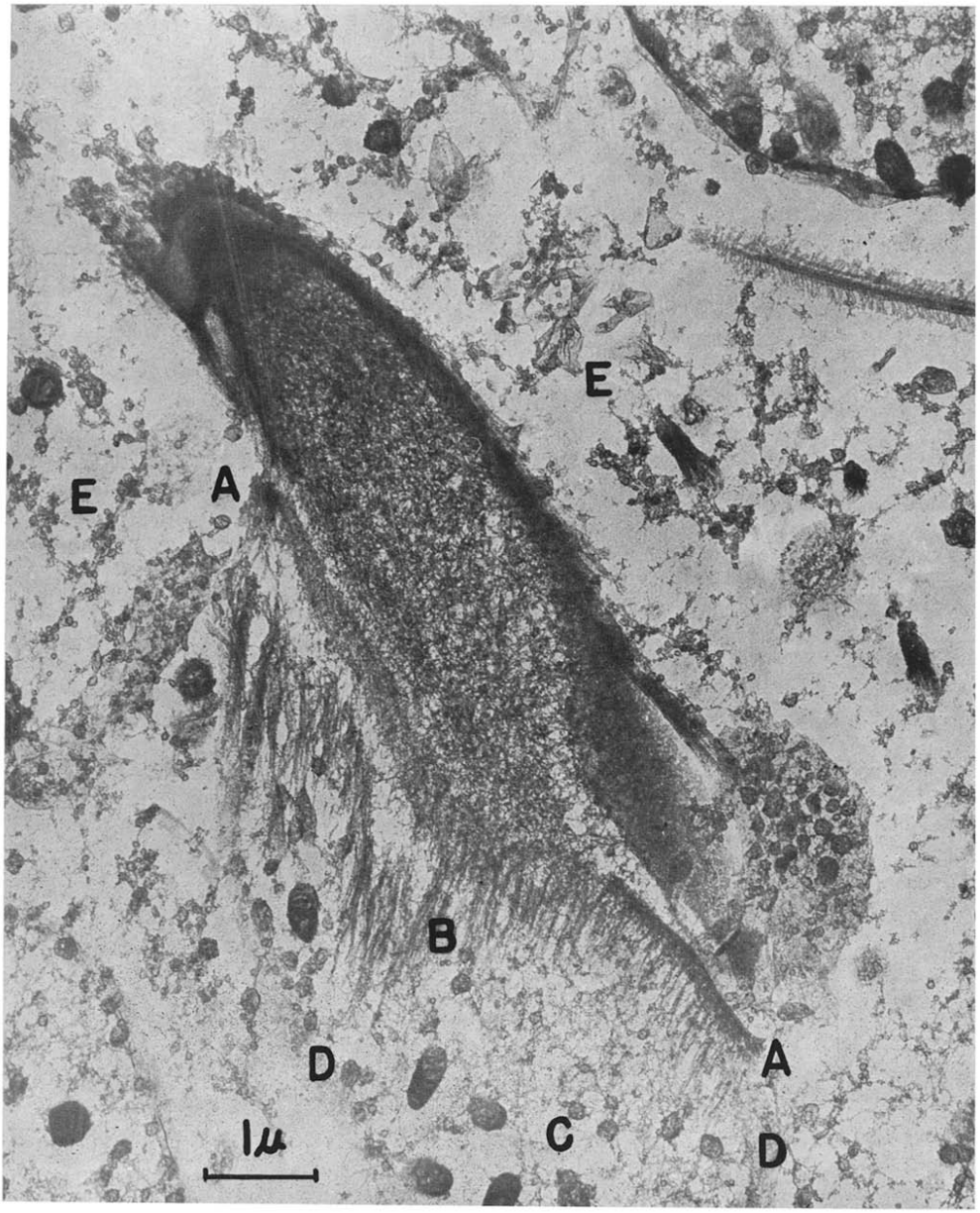

Fig. 3. Developing spermatid nucleus. The filamentous caudal sheath (B) emerges from the uncapped portion of the nucleus (A–A) into the spermatid cytoplasm (C). The spermatid cell membrane is shown at (D). The capped portion of the nucleus projects into the SERTOLI cytoplasm (E). Microsomes of various sizes are scattered throughout both the spermatid and SERTOLI cytoplasm. Mitochondria are seen in the spermatid cytoplasm and have commenced to leave the cell membrane and migrate to the tail filaments which are not shown in this section.

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Fig. 3 shows the nucleus of a phase 3 developing spermatid. The neckpiece is not included in the section. The anterior part of the head lies toward the top of the illustration. That portion (A–A) of the head from which numerous fine filaments (B) project downwards consists mostly of the uncapped part of the nucleus. To this part of the nucleus the cytoplasm (C) of the spermatid clings. The faint outlines of the spermatid cell membrane (D) may be discerned starting at either end of the filamented region and extending downwards. Between the cell boundaries lie a number of heavily-stained mitochondria and a number of the lightly-stained bodies described in Fig. 2. The capped part of the head projects into the SERTOLI cytoplasm.

The filaments mentioned above constitute the caudal sheath first described by MEVES[9]. The caudal sheath forms about the posterior part of the head enclosing the neckpiece in a funnel-like structure. In the rat the caudal sheath does not appear to be the precursor of a future structure, but shortens as development proceeds and finally disappears.

SUMMARY

Omission of washing of osmic-fixed tissue and use of alcohol dehydration permit consistently better preparations of tissue for sectioning for the electron microscope. A sublimation method removes almost all embedding material from sections with little distortion.

Applying these techniques to the seminiferous epithelium of adult rat it has been found that the young spermatid mitochondria adhere to the cell wall. The spermatid acroblast has a complicated internal structure of globular bodies and the caudal sheath is filamentous.

RÉSUMÉ

Lorsque l'on omet le lavage et que l'on déshydrate à l'alcool les tissus fixés à l'acide osmique, l'on obtient des préparations meilleures pour coupes destinées au microscope électronique. Une méthode de sublimation permet d enlever des coupes presque toute la matière environnante en ne causant qu'une légère difformation.

En appliquant cette technique à l'épithélium séminifère de rats adultes nous avons trouvé que les mitochondries des jeunes spermatides adhèrent à la paroi cellulaire. L'acroblaste des spermatides adhèrent à la paroi cellulaire. L'acroblaste des spermatides a une structure interne compliquée de corps globulaires et la gaine caudale est filamenteuse.

ZUSAMMENFASSUNG

Bei der Herstellung von Schnitten für das Elektronenmikroskop empfiehlt es sich, die mit Osmiumtetroxyd fixierten Gewebe nicht zu waschen und mit Alkohol zu entwässern. Mit Hilfe einer Sublimationsmethode kann man beinahe das gesamte umgebende Material mit geringer Deformation aus den Schnitten entfernen.

Durch Anwendung dieser Arbeitstechnik auf das samentragende Epithel erwachsener Raten, wurde gefunden dass die Mitochondrien der jungen Spermatide der Zellwand anhaften. Der Acroblast der Spermatide hat eine komplizierte innere Strukturund enthält kugelförmige Körper; die caudale Scheide ist faserig.

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