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Ultrastructural Observations of Spermatogenesis in Mice Resulting From Transplantation of Mouse Spermatogonia

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ABSTRACT: The objective of the present study was to provide a morphological characterization of spermatogenesis following germ cell transplantation into the seminiferous tubular lumen of another mouse. The recipient mice (W-locus) were sterile because of a defect in spermatogenesis resulting from the failure of virtually all germ cell precursors to migrate to the genital ridge during embryonic development. Recipient mice containing intratubular injections of testis cell suspensions from C57 mice were allowed to develop for over 1 year, whereupon animals were sacrificed and testis tissue examined by light and electron microscopy. Donor mouse cells formed normal cell associations (stages) as viewed in cross-sectioned tubules. Spermatogonia were found exclusively in the basal compartment, indicating that they were translocated from the tubule lumen through the Sertoli cell junctions, eventually to reside on the basal lamina. Some tubules looked entirely normal from both a quantitative and qualitative standpoint. Others showed qualitative and quantitative impairment. In some tubules a generation of cells was missing from a cell association. A variety of degenerating cells and structural abnormalities were responsible for this impairment, however, the most common abnormalities were seen during the elongation phase of spermatogenesis. Elongation abnormalities and the subsequent degeneration of these cells led to the presence of fewer-than-expected elongate spermatids. There were regions of the testis where no spermatogenesis was noted and only Sertoli cells were present. These regions were generally typical of the testis histology seen in animals not exposed to injected germ cells. However, Sertoli cells in these regions phagocytosed sperm produced in spermatogenically active regions of the tubules. Because transplantation of germ cells, either from fresh or from frozen cells, had wide-ranging implications in biology and medicine, characterization of spermatogonial transplants is an important step in improving this procedure.

Key words: Testis, mouse, spermatogenesis, electron microscopy.

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Compartmentalization of the seminiferous tubules into two (Dym and Fawcett, 1970) or three (Russell, 1977) distinct regions has given rise to the idea that there are distinct cell types within the seminiferous epithelium in each compartment and that as germ cell development proceeds there is translocation of cells from one compartment to another. Spermatogonia are exclusively resident in the basal compartment and have relatively free access to blood-borne substances. Young spermatocytes resting on the basal lamina and positioned in the basal compartment are transferred toward the lumen to a transient compartment, termed the intermediate compartment, formed by the Sertoli cells (Russell, 1977). Shortly thereafter, these cells enter the adluminal compartment, which is considered to be an immunopriviledged environment. It is generally believed that many nutritive materials must pass through Sertoli cells to reach germ cells or to be modified by Sertoli cells prior to consumption by germ cells (Sylvester and Griswold, 1994). The basally positioned Sertoli cell barrier formed by a continuous ring of Sertoli cell tight junctions around the tubule is responsible for the segregation of spermatogonia and the adluminal isolation of germ cells.

Because the stem cell spermatogonia are located in a very small, inaccessible, and confined region of the seminiferous epithelium, little thought has been given to transplantation of cells to this compartment to determine their viability and growth potential. The recent reported development of techniques to transplant spermatogonia from one mouse to another (Brinster and Avarbock, 1994;

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Table 1. *Spermatogenesis following transplantation of C57BL/6 testes donor cells into the seminiferous tubules of W-mutant mice*

Recipient mouse number	Recipient genotype	Days after germ cell transfer	Spermatogenesis in seminiferous tubules (%)					
			Testis I*			Testis II		
			Full†	Intermed.‡	Sertoli-only§	Full	Intermed.	Sertoli-only
724-1078D	W^v/W	349	30.7 (50)¶	3.1 (5)	66.2 (108)	—‖	—	—
702-1078B	W^{44}/W^{44}	363	54.0 (41)	9.2 (7)	36.8 (28)	6.9 (4)	3.5 (2)	89.6 (52)
703-1078C	W^v/W^v	363	17.3 (9)	5.8 (3)	76.9 (40)	20.5 (16)	0	79.5 (62)
632-1078A	W^v/W^v	408	0	0	100.0 (79)	0	0	100.0 (55)
704-1082C	W^v/W	411	4.0 (2)	0	69.0 (48)	78.3 (47)	6.7 (4)	15.0 (9)
678-1082B	W^{44}/W^{44}	425	33.3 (139)	7.2 (30)	59.5 (248)	—	—	—
655-1082A	W^{44}/W^{54}	440	0.7 (2)	0.7 (2)	98.6 (286)	44.5 (153)	9.9 (34)	45.6 (157)

Mutant mice were maintained on the C57BL/6 genetic background or the closely related WB/ReJ background (The Jackson Laboratories). Donor testes cells were collected from animals 5–15 days of age and transplanted into recipient animals 4–6 weeks of age.

* At sacrifice, testes were not designated left or right.

† Full—all cell association present but no quantitative aspects are implied.

‡ Intermediate—spermatogonia, spermatocytes, and perhaps round spermatids present.

§ Empty—Sertoli cells or Sertoli cells and an occasional spermatogonium present.

¶ Number in parenthesis is number of tubules analyzed.

‖ Testis was primarily fibrous tissue.

Brinster and Zimmerman, 1994) has shown that germ cell transplantation and growth may be accomplished even if stem cells are injected into regions not considered to be their normal environment. In these reports Brinster and colleagues have successfully transplanted spermatogonia isolated from mice at prenatal and postnatal periods into recipient mice that have no endogenous spermatogonia. Injections of spermatogonia into the lumen of seminiferous tubules of these animals resulted in quantitatively complete spermatogenesis in regions of the testis. Furthermore, animals that were once sterile could produce gametes that would sire young. The proof that transplanted cells were growing in the recipients was the use of a genetic marker that could have been transmitted only by donor cells. A more recent report by Jiang and Short (1995) suggests that spermatogonial precursor cells from rats will initiate intralumenal spermatogenesis in recipient rats. The intralumenal cell associations formed in that experiment were coordinated with that of the epithelium of the same tubule.

Spermatogenesis in recipient mice has been roughly characterized by light microscopy of paraffin-embedded tissues with the main emphasis being the histochemical demonstration, using the *Lac Z* gene as a donor marker, that donor cells were successfully transplanted and undergoing spermatogenesis in the recipient testis. The present study expands on the initial findings by providing additional light microscopic and ultrastructural evidence of the status of spermatogenesis in recipient males. We show here that spermatogonia reside in their normal position in the basal compartment and that subsequent germ cell development takes place from these seeded stem cells in cellular associations that are typical of the normal mouse testis.

Methods

Animals and Transplantation Procedure

Donor testis cells were collected from C57BL/6 mice by procedures previously described (Bellvè et al, 1977; Brinster and Avarbock, 1994). Following removal of the testes from the mouse, the tunic was removed and the tubules exposed to collagenase and then trypsin. The cell suspension was centrifuged and the pellet resuspended in injection medium (Brinster and Avarbock, 1994). The germ cell component of the cell suspension was enriched by this isolation procedure. The concentration of cells in the injection medium suspension ranged from 15 to 32×10^6 cells/ml, and 0.4 ml of cell suspension was used to inject the testes of each mouse. For the injection of some recipient mice, additional Sertoli cells (0.5 to 3.5×10^6 cells/ml) were added to the testis cells (recipient males 632, 655, 678) or additional TM4 cells (10×10^6 cells/ml) were added (recipient males 702, 703, 704). The TM4 cells were from an immortalized Sertoli cell line (Mather, 1980). In other experiments now analyzed, the addition of Sertoli cells to injected testis cells did not appear to influence colonization of recipient seminiferous tubules by transplanted donor cells (Brinster and Avarbock, 1994). Recipient males were mutant W mice, which are immunologically compatible with the C57BL/6 donor mice. In these experiments recipient males carried the W, W^v, or W^{44} mutant alleles in the homozygous or compound heterozygous combination, and spermatogenesis does not occur in the testes of these mice (Brinster and Avarbock, 1994). Recipient animals were sacrificed for analysis approximately 12 to 15 months following donor testis cell transplantation.

Recipient Animals

Seven recipient mice were examined either by light or electron microscopy (for details see Table 1). Recipient mice were sacrificed 12 to 15 months following cell transplantation (Table 1).

Tissue Preparation

Animals were perfused according to the method provided by Sprando (1990). Briefly, after a saline wash to clear the testes, they were perfused with 5% buffered glutaraldehyde for 30 minutes and then postfixed in the same solution overnight. After three washes in buffer, small tissue cubes were postfixed in a mixture of 1% osmium and 1.5% potassium ferrocyanide (final concentration), dehydrated in ethanols, infiltrated in propylene oxide, and embedded in Araldite (CY 212). Tissue sections showing silver and silver-gold interference colors were examined with a Hitachi (H-500) electron microscope.

Results

Table 1 shows the basic data relating to the status of spermatogenesis in seven animals receiving cell suspension transplants. Twelve testes were examined from seven animals. Two testes were used for other purposes. By light microscopy, the mean percentage of tubules with "full" spermatogenesis in 12 testes was 26.6 ± 5.3 (SE) (range 0–78%). The term "full" as applied to spermatogenesis is used to denote the presence of all cell types of a cell association. The term is not used to imply quantitative changes that made them different from normal animals. An "intermediate" level of spermatogenesis was characterized by the presence of spermatogonia and spermatocytes and, at the most, the presence of young spermatids. The mean percentage of tubules showing intermediate spermatogenesis was 4.1 ± 1 (range 0–9.9%). Overall, approximately 69.3% of tubular profiles were devoid of germ cells. Although testes were sectioned transversely, the few seminiferous tubules showing full spermatogenesis that were sectioned longitudinally showed full spermatogenesis along the entire length of the sectioned tubule. Figure 1 shows the variations of tubular epithelium height in a typical transplanted testis.

The same types of tubules characterized by light microscopy (above) were noted during electron microscopic observation of tubules. Full spermatogenesis commonly was noted (Figs. 1B and 2) and, in some instances, little difference between these tubules and spermatogenesis in historical controls examined by this investigator (Russell et al, 1990) over many years was discerned. The common difference noted in these tubules between historical controls and transplanted animals was that the epithelium of many tubules was not as thick as in historical control testes.

The position of germ cells was examined in each tubule with active spermatogenesis. Spermatogonia appeared normal from an ultrastructural standpoint and were situated on the basal membrane of the seminiferous tubule and resided between adjoining Sertoli cells. A Sertoli–Sertoli junctional complex overlay spermatogonia. At these sites, the plasma membrane of Sertoli cells demonstrated areas of fusion characteristic of junctional sites of normal mice (Figs. 2 and 3).

Attention was paid to stages of spermatogenesis near the time of sperm release because these spermatids represented the final product of spermatogenesis. Figure 4 shows a section of a stage VII tubule and the association of spermatids at step 16 with Sertoli cells. Most step 16 spermatids appear typical of mouse spermatids, as has been previously published (Russell et al, 1990). The relationship of the Sertoli cells to the heads of these spermatids appeared normal.

Completely normal spermatogenesis was noted in

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FIG. 1. **(A)**, Low power light micrograph of several seminiferous tubules in a testis transplanted with mouse germ cells slightly over 1 year previously. The epithelium is of variable height and some tubules show only Sertoli cells displaying a vacuolar appearance. **(B)**, A typical tubule as seen in recipient mice contains all cell types of a cell association and would be described as "full," but some cells (elongated spermatids in particular in this micrograph) may be reduced in number. **(C)**, Low magnification electron micrograph showing an area of the tubule with no pachytene spermatocytes, a reasonably normal complement of step 7 spermatids (s-7), and few step 16 (s-16) spermatids. Those germ cells present display normal morphology. A, ×350; B, ×700; C, ×4,100.

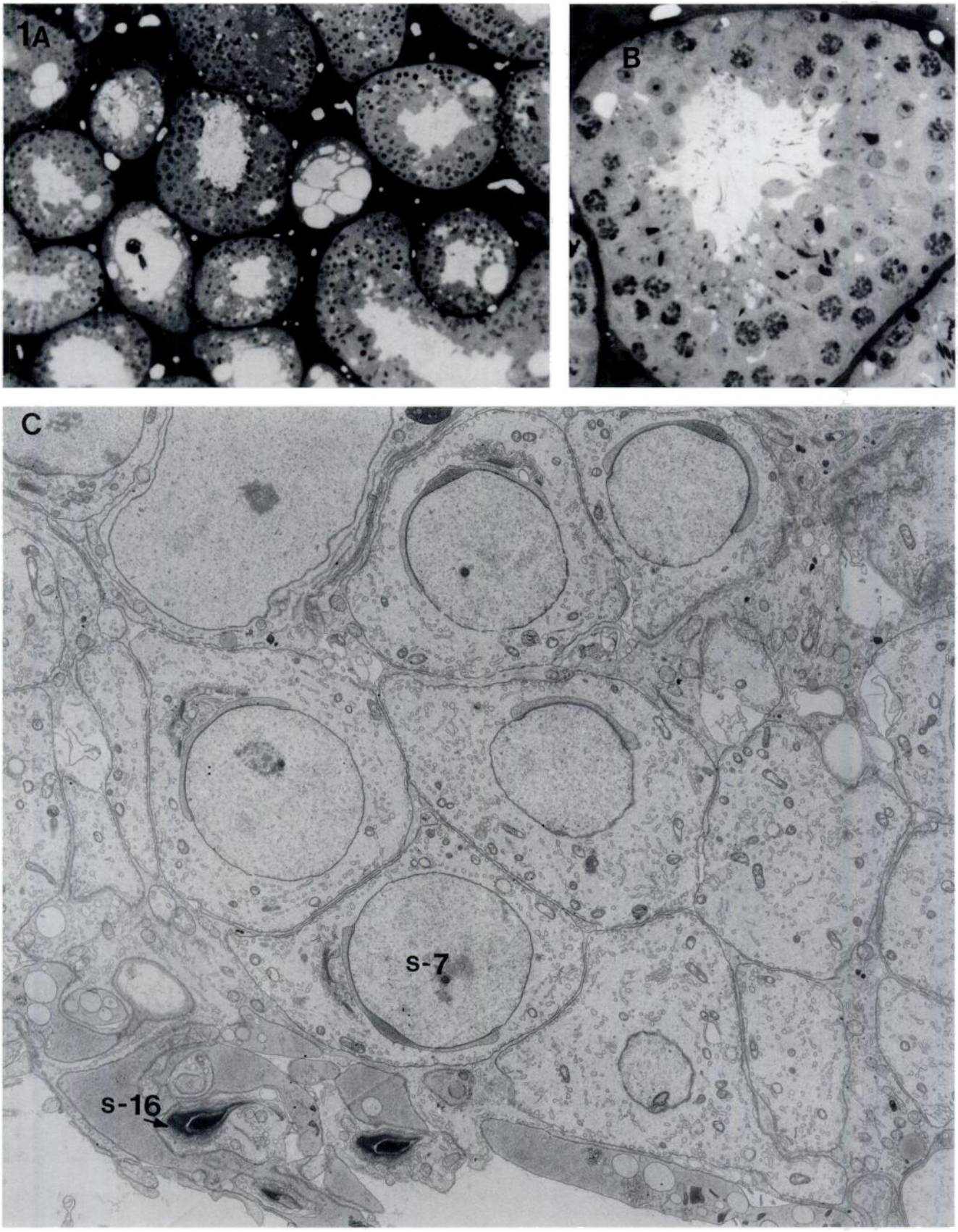
FIG. 2. Micrographs depicting the basal **(A)** and the apical **(B)** aspect of a recipient mouse seminiferous tubule showing "full" spermatogenesis. The cell types indicated in this stage II tubule are type A spermatogonia (gonia), pachytene spermatocytes (cytes), step 2 spermatids (2 tids), and step 14 spermatids (14 tids). The Sertoli cells (S) show a typical nucleus and nucleolus (N), a junctional complex (jc), and normal-appearing configurational relationships with germ cells. There is slight infolding of the boundary tissue of the tubule (isolated arrow). A and B, ×4,800.

FIG. 3. Sertoli–Sertoli junctional complexes appear normal as evidenced by the presence of ectoplasmic specialization (es) and the periodic membrane translucencies (arrowheads) indicating the presence of occluding junctions. ×108,000.

FIG. 4. Lumenal aspect of a seminiferous tubule showing full spermatogenesis; numerous step 16 spermatids (16) line the epithelium. The relationship of the spermatid heads (h) and their cytoplasmic lobes (CL) to the Sertoli cell (S) appears normal. A portion of a tubulobulbar complex is shown (open arrows). ×16,800.

FIG. 5. Lumenal aspect of a seminiferous tubule showing step 16 spermatids, 2 of which demonstrate abnormally shaped heads (arrows). ×9,600.

FIG. 6. Typical view of a Sertoli-only epithelium shown by light **(A)** and electron **(B)** microscopy. **(A)**, A tubule with qualitatively complete spermatogenesis is adjacent to a Sertoli cell-only tubule. The Sertoli cell-only tubules show numerous step 16 spermatids outside and within the cytoplasm of Sertoli cells (arrowheads). **(B)**, Sertoli cells have engulfed and are in the process of phagocytosing the heads (h) and flagella (f) of step 16 spermatids. The junctional region between Sertoli cells appears normal (broad, open arrows). A, ×700; B, 6,600.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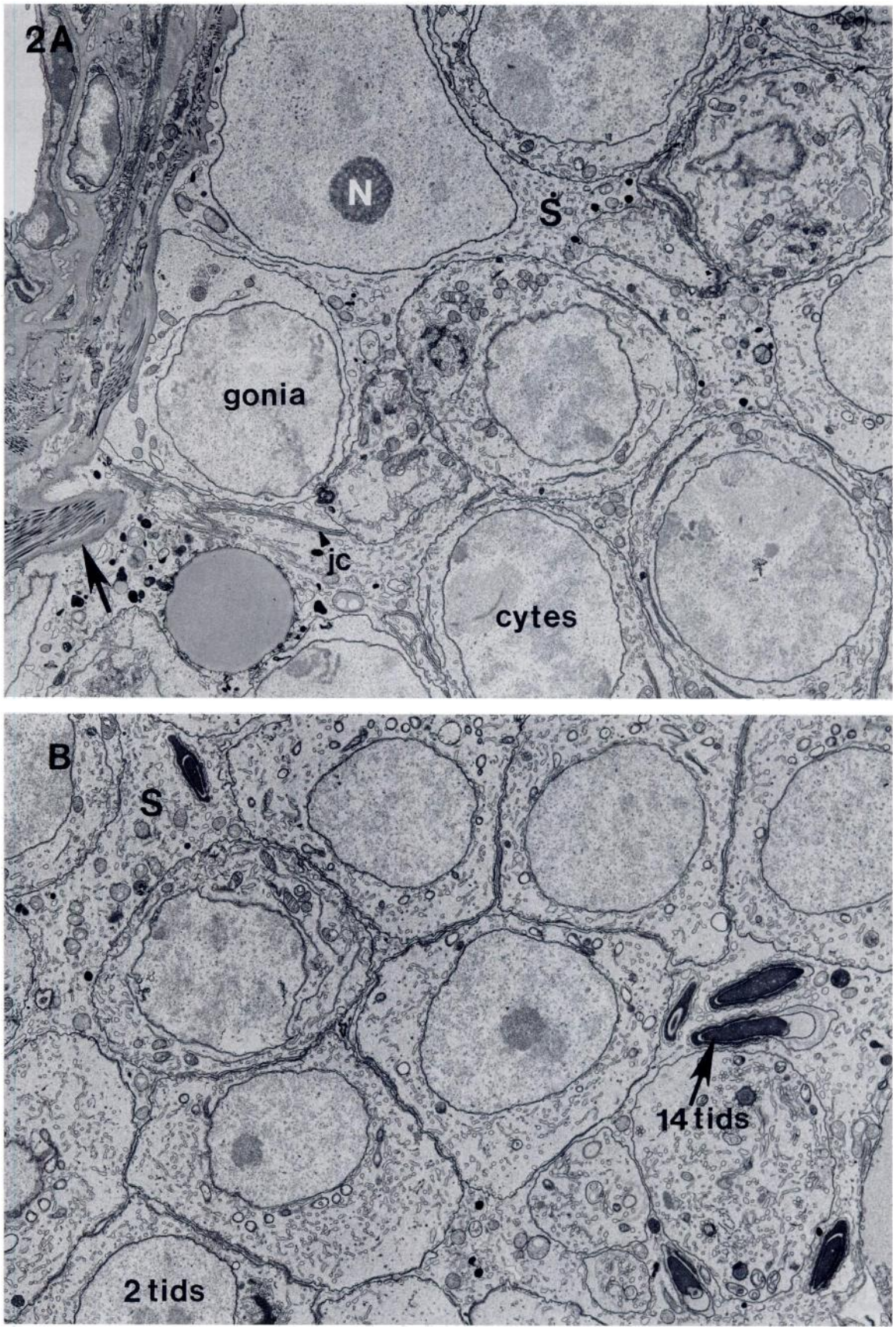
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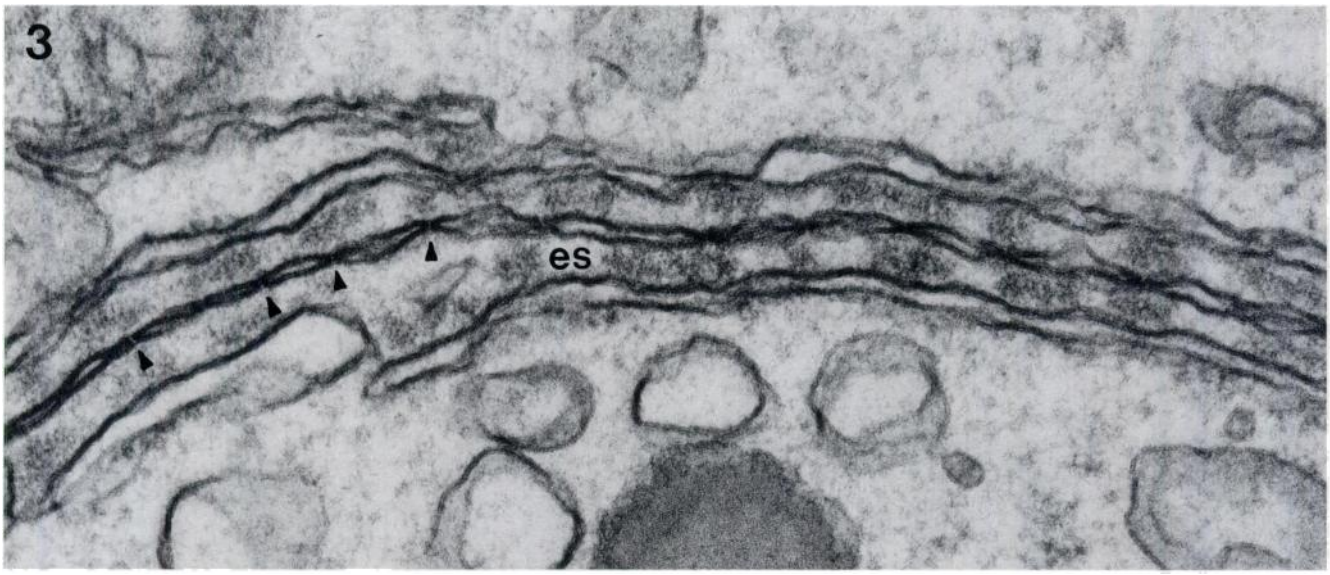
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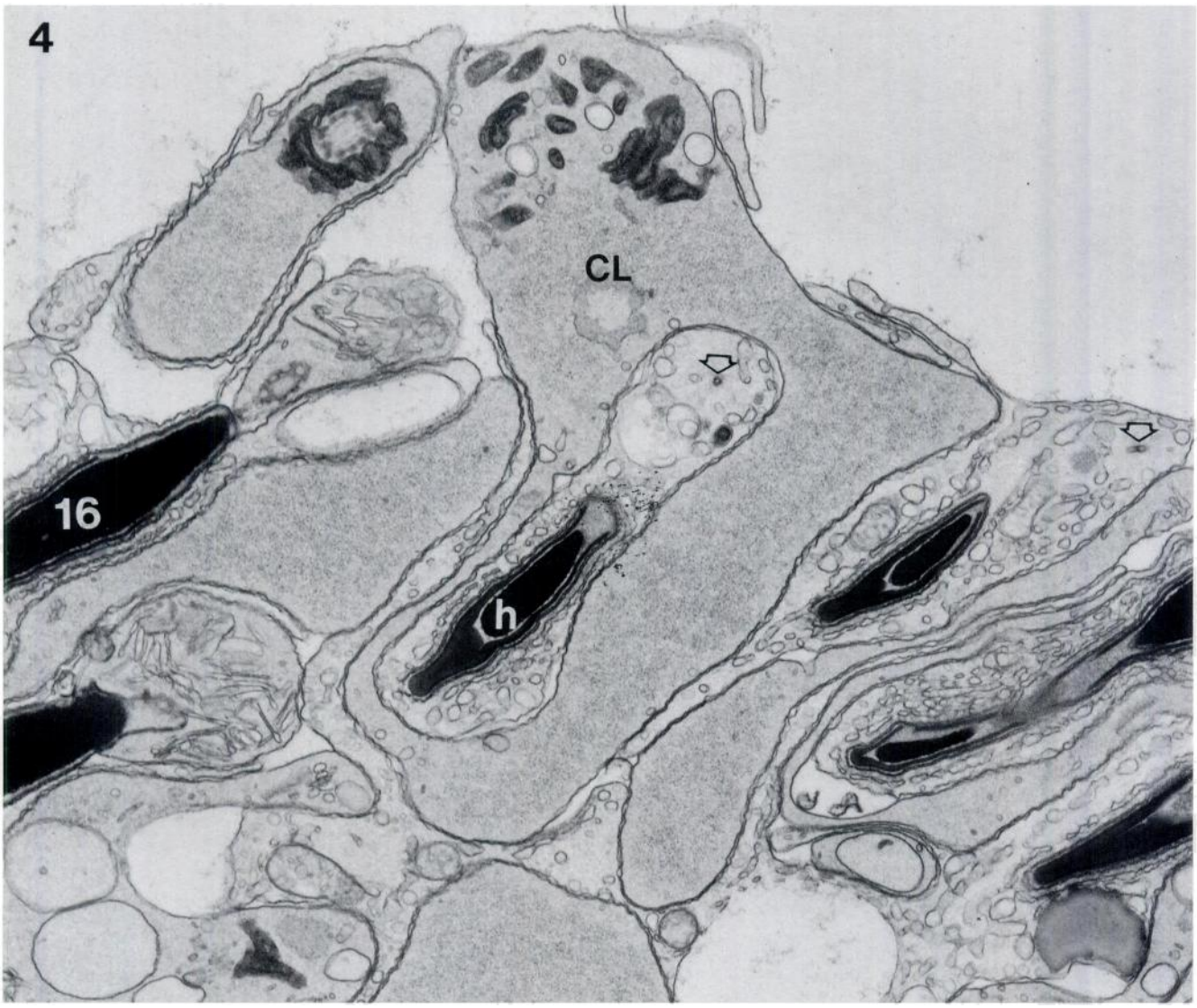
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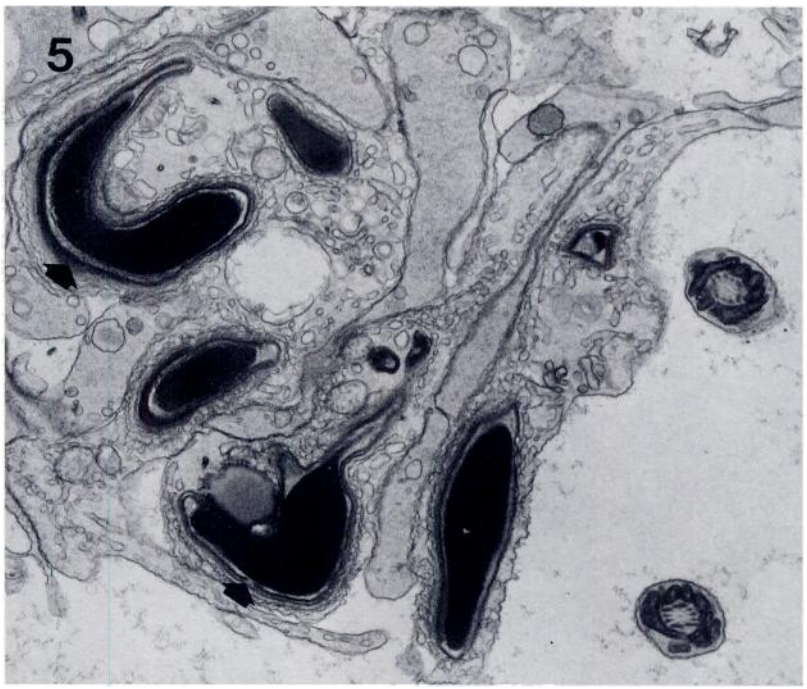
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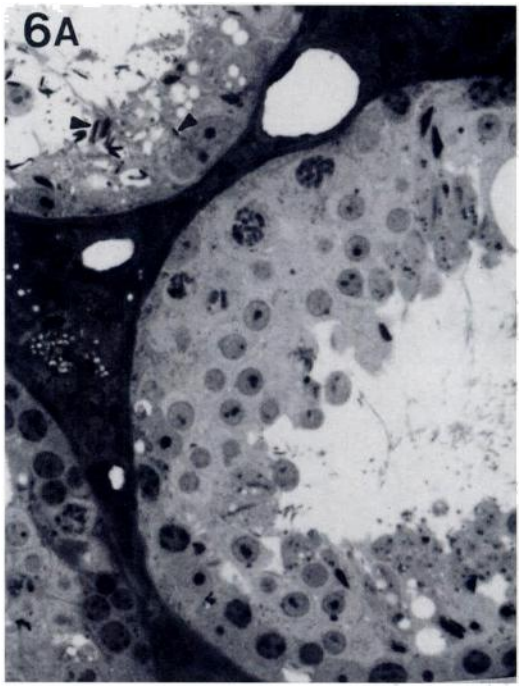
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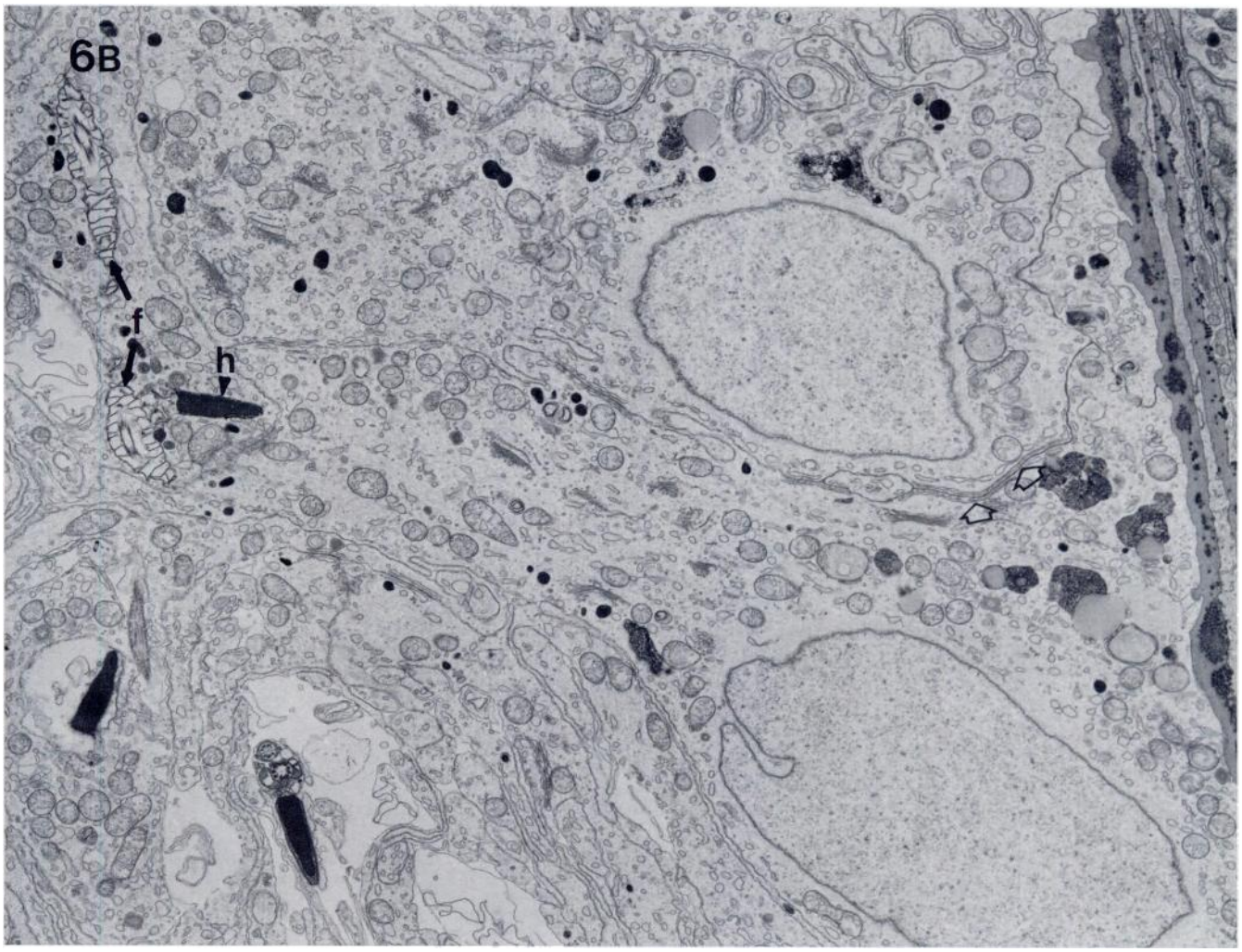
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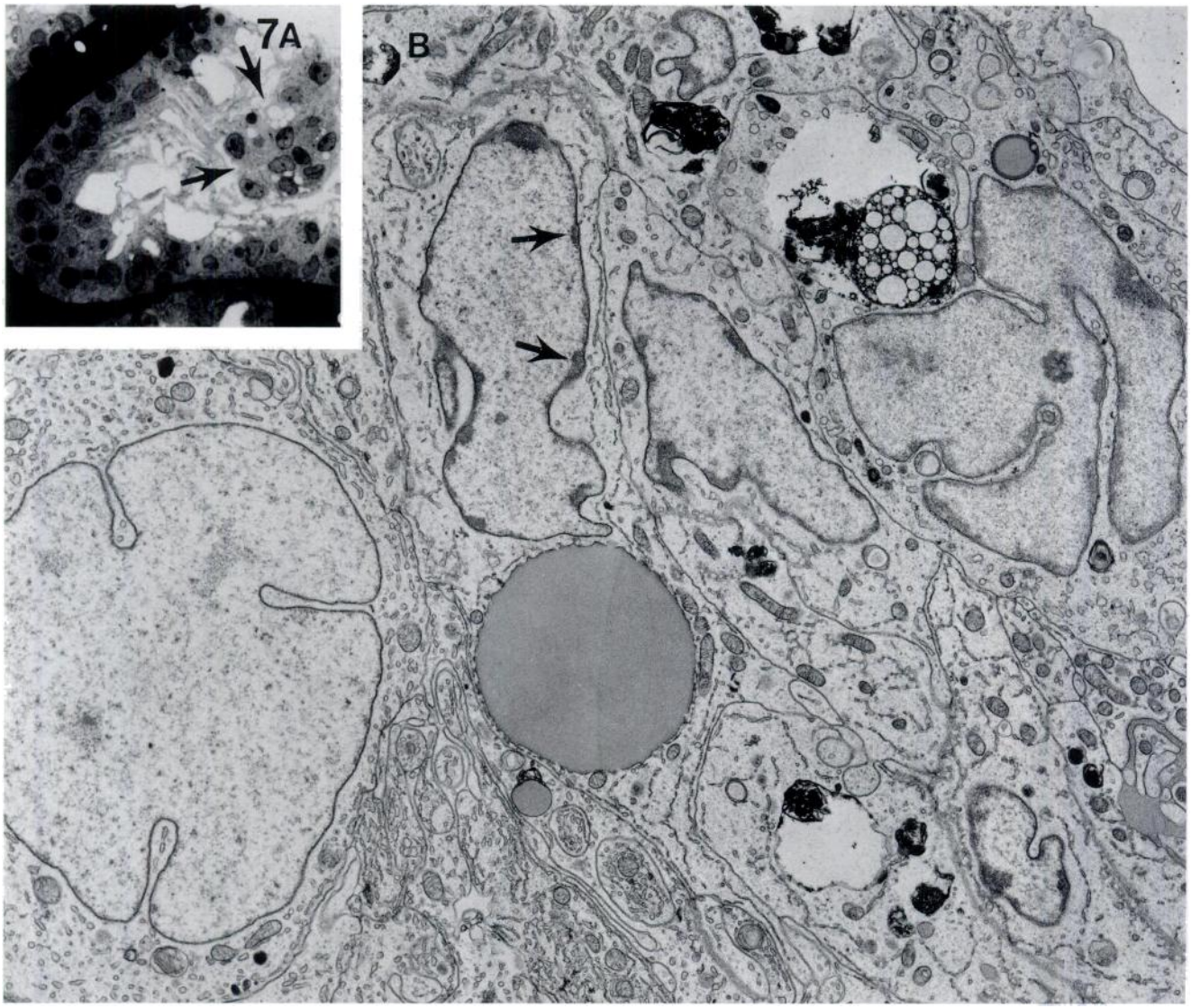

FIG. 7. Light **(A)** and electron **(B)** micrographs showing a portion of an aggregation or "ball" of Sertoli cells at the lumen of the tubular epithelium. **(A)**, A ball of Sertoli cells is seen within the tubular lumen (arrows). **(B)**, Two morphological types of Sertoli cells are seen in a Sertoli cell-only (empty) tubule. On the bottom left a typical Sertoli cell nucleus is seen in cells that make contact with the basal lamina (not shown). These nuclei appear typical of those of normal Sertoli cells. In a lumenal position (to the right), several other Sertoli cells of the kind that were never found attached to the basal lamina are seen. Their nuclei appear smaller, more irregular, and contain numerous heterochromatic masses (arrows) along the nuclear envelope. A, ×500; B, ×9,600.

slightly less than 50% of tubules where germ cells more advanced than spermatogonia were present. In more than half of the tubules, in which cells more advanced than spermatogonia were present, quantitative and qualitative defects in spermatogenesis were apparent. Areas of tubules lacking a cell type or showing a paucity of specific cell types within a cell association were noted (Fig. 1C). Most commonly, the most advanced generation of cells was lacking (Fig. 1C). For example, spermatocytes or elongate spermatids would be sparse, while in the same tubule the other cell types of the cell association would be quantitatively normal (Fig. 1C). Testis pathology was noted in transplanted testes and included germ cell degeneration (not shown), abnormal shaping of the sper-

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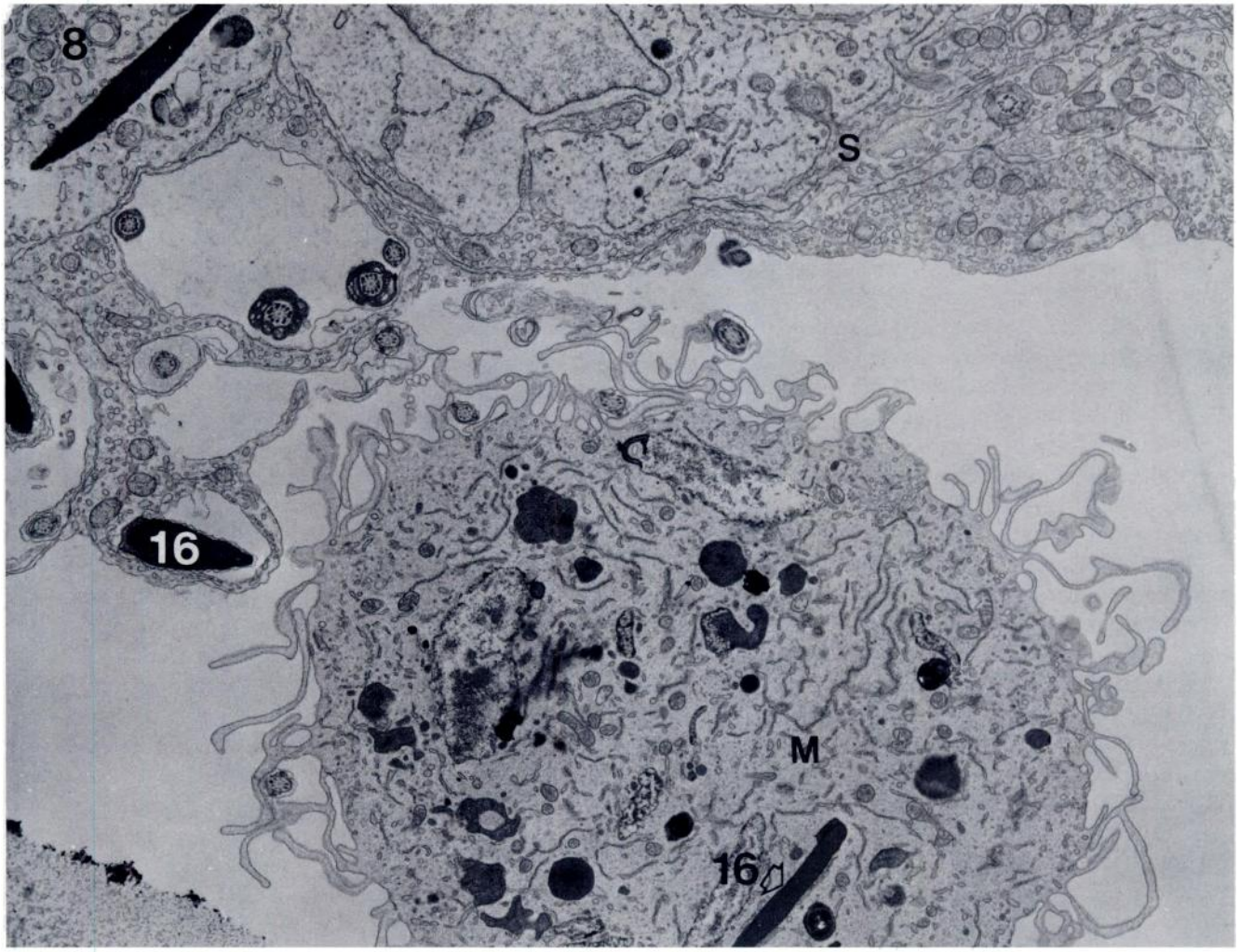
FIG. 8. A macrophage (M) is seen in the lumen of a tubule containing only Sertoli cells (S). Step 16 (16) spermatids have been phagocytosed by the macrophage as well as by the Sertoli cell epithelium. ×6,000.

FIG. 9. A macrophage (M) in the intratubular area shows evidence of internalization and degradation of material. Nearby Leydig cells (L) show normal ultrastructural features. ×10,560.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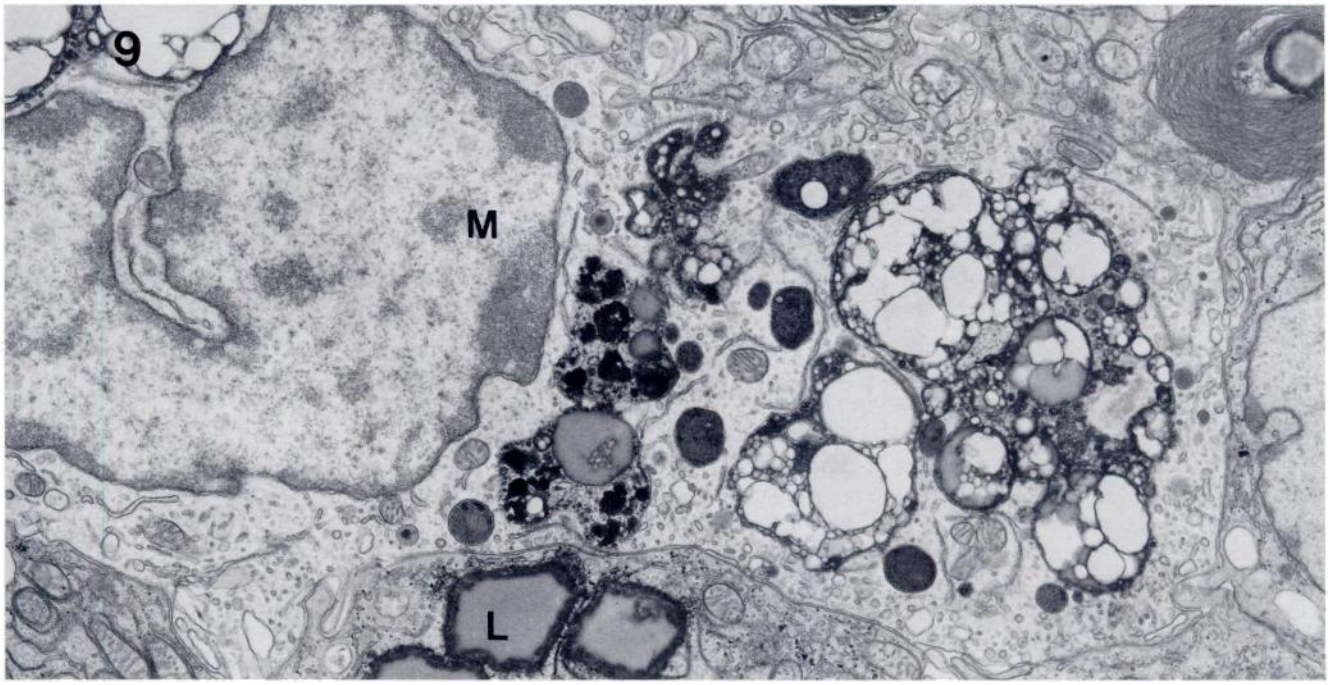
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matid head (Fig. 5), and retention of step 16 spermatids and their cytoplasm (Fig. 6).

In all animals, tubules were noted that were devoid of active spermatogenesis and could be described as Sertoli cell-only from the standpoint that no active spermatogenesis was taking place. Although these tubules lacked spermatogenesis, they were not always devoid of germ cells. Step 16 spermatids could be commonly seen in tubules without any other germ cell type. Given that these spermatids were always at approximately step 16 of development, it appeared that tubules devoid of spermatogenesis were actively phagocytosing sperm released in other portions of the same tubule. Moreover, because almost every tubule profile containing inactive spermatogenesis showed these spermatids, it suggested that portions of every tubule were releasing sperm and that these were picked up and phagocytosed by the Sertoli cell-only epithelium (Fig. 6).

The lumenal edge of seminiferous tubules displayed two features that were not reported in animals of the W-locus mutation (Kurohmaru et al, 1992; França et al, 1994). First, there were aggregations of Sertoli cells or "Sertoli balls" totally composed of Sertoli cells with no apparent cytoplasmic attachment to the basal lamina (Fig. 7). Their nuclei were more convoluted and contained more heterochromatic bodies along the nuclear envelope than Sertoli cells attached to the basal lamina. We initially suspected these cells to be part of the original cell suspension from the donor mouse, but mouse Sertoli cell "balls" were also seen after injection of rat germ cells into sterile mice (see Russell and Brinster, 1996, for criteria for identifying species characteristics of Sertoli cells). Second, occasional macrophages lined the lumen of the tubule. These frequently contained phagocytosed sperm (Fig. 8).

The seminiferous tubules of one recipient animal were extremely small, containing only Sertoli cells without a tubular lumen (animal 632-1078A). Numerous mast cells were seen among Leydig cells, especially near the capsule of the testis. Because the response of this animal was not typical of most recipient mice, this effect was not illustrated.

Leydig cells were typical of those described for the mouse (Russell, 1995). However, macrophages which intermingled with Leydig cells, contained evidence of phagocytosed debris (Fig. 9).

Discussion

Transplantation experiments with germ cells have wide-ranging consequences in medicine and show great potential in basic research for understanding spermatogenesis (see, e.g., Dym, 1994; Lemonick, 1996; Lovell-Badge, 1996). The reports from Brinster's laboratory that germ cells can be frozen and subsequently transplanted or transplanted directly from one mouse to another represent a technological breakthrough (Brinster and Avarbock, 1994; Brinster and Zimmerman, 1994; Avarbock et al, 1996). Characterization of the transplanted cell population has thus far been only by light microscopy and by using a transgenic strain bearing the *Lac Z* gene that serves as a histochemical reporter of cell origin. Further proof that spermatogenesis is from the donor animals has been provided by mating experiments of recipient animals in which the *Lac Z* gene is inherited and expressed in the F_1 generation. The present report describes features of spermatogenesis in recipient mice. It demonstrates that relatively normal spermatogenesis has been established in some seminiferous tubules, and yet other tubules show both a quantitative diminution and qualitative features associated with abnormal spermatogenesis. A description of spermatogenesis in recipient mice serves as a foundation from which improvement of the transplantation technique can occur.

In previous reports (Brinster and Avarbock, 1994; Brinster and Zimmerman, 1994) and in an accompanying commentary (Dym, 1994), it has been assumed that spermatogonia are the cells giving rise to spermatogenesis in recipient animals. Certainly it is possible that more advanced cell types could seed and produce sperm in some recipient animals if the donor cells are more mature than spermatogonia. However, according to our current concepts of stem cell renewal (de Rooij et al, 1989), they would not be capable of acting as stem cells to begin this process anew. The animals examined in the present study had transplants over a year earlier, sufficient time for cells more mature than spermatogonia to have completed spermatogenesis. In all recipient animals we have seen numerous typical spermatogonia, which indicate that the transplanted cells giving rise to spermatogenesis are from the stem cell population.

Identification and characterization of the stem cell spermatogonia in rodents has not been fully achieved, but most workers believe the stem cell is the spermatogonium without intercellular bridges or A-isolated cell (A_S), so designated by Huckins (1978). Successful transplants can be achieved using donor animals that are perinatal, indicating that the precursor cells, or gonocytes, are capable of maturing to stem cells that give rise to spermatogenesis. The most successful transplants are from cell preparations in older animals exhibiting partial spermatogenesis. The reason for the higher success rate reported in older animals is not known, and this higher success rate is not expected, given that stem cell spermatogonia represent an increasingly smaller percentage of total testes cells as animals achieve puberty. It is known that type A spermatogonia increase their absolute numbers until ap-

proximately 28 days of age (Vergouwen et al, 1993). There is no need to characterize the population of donor cells used for transplantation to determine what percentage of cells injected are spermatogonia.

The most interesting aspect of testis transplantation is the organization of the seminiferous epithelium that must occur after introduction of isolated donor cells into the lumen of recipient mouse seminiferous tubules. Spermatogonia must be translocated from the tubular lumen to the basal compartment of the testis. In our examination of recipient tubules, spermatogonia were seen only in this compartment and none remained in the lumen. The microinjected spermatogonia do not induce the formation of a basal compartment since the W/W strain of animals have already been shown to have a well-developed system of Sertoli–Sertoli junctions (Kurohmaru et al, 1992; França et al, 1994). Finding that spermatogonia have been translocated to the basal compartments implies that the Sertoli cells are able to recognize these cells and to coordinate their movement basally. Movement across the barrier, from basal compartment toward the lumen, is thought to be coordinated by the Sertoli cell because this cell possesses the cytoskeletal elements and cell configuration changes that are normally associated with such movements (Russell, 1977). Thus, perhaps one function of Sertoli cells, in addition to those already known (Russell and Griswold, 1993), is to sort germ cells according to type and position them within the epithelium. Sorting transplanted cells would appear to be an unnatural function for Sertoli cells because this process does not occur in normal animals. However, sorting of cells within an epithelium that has been damaged by some other means may be the way spermatogenesis can be rejuvenated. In the present study, spermatogonial cells were not seen in the process of translocation from one compartment to another, but the mechanism involved in this translocation would best be studied in recipient animals shortly after transplantation of cells.

From examination of longitudinal seminiferous tubules during our random sectioning procedure, it was noted that stretches of seminiferous tubules showed active spermatogenesis. This question arises: "Are the longitudinally sectioned tubules with active spermatogenesis resulting from the seeding of one stem cell or from the seeding of multiple stem cells?" At injection, dye included with cellular suspension would often occupy a large percentage of the surface tubules. Tubules other than those that were injected would also fill via the interconnections of seminiferous tubules with the rete testis (Brinster, personal observations). Often, nearly 100% of tubules would fill with blue dye. If numerous stem cell seedings occurred in these animals, one would expect recipient spermatogenesis to occur over a wide area and to be present in many tubules of the testis. We felt that nearly all tubules of the testis contained active spermatogenesis because there were indications from the presence of phagocytosed sperm that spermatogenesis was in virtually every tubule. However, most of the tubular profiles were without spermatogenesis, leaving open the question of how seeding occurs.

Do the appropriate cell associations form from injected spermatogonia? Our examination of numerous cross sections of tubules have shown that they do form. The identifying criterion for a particular stage is its spermatids, whose acrosomes and nucleus are characteristic for specific developmental steps in the history of these cells. There was always an appropriate cell association according to previously published cycle maps (Dym and Clermont, 1970; Russell et al, 1990). The question then arises: "Do the cell associations form a *wave* of spermatogenesis, as has been previously described for the rat?" (Perey et al, 1961). To obtain these data, serially sectioned testes of both normal and transplanted testes will be needed, a project currently underway in our laboratories.

Spermatogenesis in recipient animals was not always quantitatively and/or qualitatively normal. Not only were fewer than normal germ cells observed, but degenerating cells were present, and elongating and elongate spermatids were sometimes abnormally shaped. Abnormalities were common in spermatids as they began to elongate in stages VIII–XI. It is not clear if the defects seen were due to the model itself (W-locus mouse), an animal model whose testes have never seen germ cells, or were due to some features associated with recipient cells or the transplantation procedure. In an accompanying paper (Russell and Brinster, 1996) we show that endogenous mouse spermatogenesis after busulfan treatment shows similar quantitative and qualitative alterations (pathologies), suggesting that the testis virtually without germ cells is somewhat impaired in its capability to fully regenerate spermatogenesis. Thus, the qualitative and quantitative inadequacies in spermatogenesis may be due to the somatic cells of the W-locus mouse rather than the inability of donor cells to seed and proliferate.

Sperm reaching the epididymis from recipient mice have been shown capable of fertilizing eggs in natural mating trials (Brinster and Avarbock, 1994). It is clear from the present study that released sperm do not all reach the epididymis, probably accounting for the low fertility rates noted. Spermatogenesis is regional in the recipient testis, i.e., extending along a tubule to a limited extent but probably not filling the entire tubule. Examination of areas without spermatogenesis (Sertoli cell-only) frequently showed elongate spermatids that had been taken up by Sertoli cells and phagocytosed. From these observations it is suggested that Sertoli cells not supporting spermatogenesis develop specific surface features that can recognize elongate spermatids, attach to them, and subsequently degrade them.

The presence of intralumenal "balls" of Sertoli cells protruding from the epithelium and extending into the lumen suggested to us initially that Sertoli cells were injected in the Sertoli cell suspension with the germ cells. The clumps morphologically appear typical of Sertoli cells that have lost their attachment with the basal lamina (Russell et al, 1990, Fig. B). There is no colonization of these cell aggregations by germ cells. In the rat-to-rat transplant study of Jiang and Short (1995) colonization was intraluminal and presumably involved both donor germ cells and donor Sertoli cells. Injection of multiple cell types, such as peritubular cells, Sertoli cells, and germ cells, may lead to intralumenal colonization of the Sertoli cells within the tubule in a situation in which the epithelium of the recipient animal is intact at the period of injection.

The observation that some cell types of a cell association are lacking or greatly deficient suggests that either germ cell degeneration is great or that cells entering spermatogenesis do so as to skip a generation of cells. Only occasionally did we see evidence of degeneration of cells. It appears that spermatogonia sometimes divide to yield cells committed to spermatogenesis, but sometimes they do not divide or they may degenerate.

In summary, the major finding of the present studies were as follows: (1) normal cell associations or stages were formed from transplanted spermatogonia, (2) spermatogenesis was sometimes similar to that of control animals but sometimes showed quantitative and qualitative abnormalities, (3) areas of the seminiferous tubules in which colonization did not take place showed only Sertoli cells that actively phagocytosed released sperm, and (4) elongation and chromatin condensation of spermatids was a major barrier to completion of spermatogenesis, a feature that may be attributed to the somatic cells of the genetic model used rather than the failure of germ cells to develop. Characterization of spermatogenesis in the recipient testis represents an important step in improving the spermatogonia transplantation technique.

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