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Ultrastructural Observations of Spermatogenesis Following Transplantation of Rat Testis Cells Into Mouse Seminiferous Tubules

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ABSTRACT: The testes of busulfan-treated immunodeficient mice receiving seminiferous tubule injections of testis cells from rats were examined by light and electron microscopy. The presence of active rat spermatogenesis was verified by criteria that are known to characterize spermatogenic cells of this species. In addition, spermatogenesis from the mouse was identified as taking place in some seminiferous tubules as the result of reinitiation of spermatogenesis after busulfan treatment. Rat spermatogenesis in mouse seminiferous tubules showed the generally recognized associations of cells known to characterize stages of spermatogenesis of the rat. The Sertoli cells associated with rat spermatogenesis were identified ultrastructurally as being of mouse origin. Thus, rat spermatogenesis, which has a cycle length that is 50% longer than mouse spermatogenesis, can proceed among mouse Sertoli cells, which supposedly exert much shorter cyclic influences in concert with mouse germ cell development. Studies are needed to determine if the timing of rat spermatogenesis is controlled by the germ cells or the Sertoli cells. These observations are considered preliminary since a thorough study of somatic-germ cell relationships was not undertaken. It is concluded that a mouse Sertoli cell in the environment provided by the mouse testis can produce both mouse and rat gametes.

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

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The recent report of xenogeneic transfers of spermatogonia (Clouthier et al, 1996) suggests that immunological difference between transplanted cells and host cells can be overcome using immunodeficient mice. The outcome of transplantation of rat spermatogonia to the mouse testis is that the donor cells are capable of differentiating to form sperm morphologically characteristic of the rat. While the efficiency of donor spermatogenesis in the mouse is not great, the model system developed (Clouthier et al, 1996) shows that either the germ cell has, to a large extent, the innate property of determining the characteristic shape of the sperm of the species and/or there is considerable flexibility in the somatic cells of the testis to allow the development of donor sperm. For example, the cycle length of the rat is about 50% longer than that of the mouse. Our current concept of Sertoli cell function is that it, too, has a cycle that corresponds with the cycle of germ cell development (Parvinen, 1993).

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The initial report of xenogeneic transplants verified donor spermatogenesis using an enzyme marker present in donor cells of the rat. The donor cells contained the *Lac Z* gene, which encodes the enzyme β-galactosidase, which can be visualized histochemically and used as a marker for transplanted cells. This histochemical demonstration, along with demonstration of typical-appearing rat sperm in the epididymis, have been, to date, the only structural observations used to assess spermatogenesis in this transplant model. The present report describes the light and electron microscopic appearance of mouse testes that have received transplanted rat germ cells.

Methods

Animals and Transplantation Procedure

Donor rat testis cells were collected by procedures previously described (Brinster and Avarbock, 1994; Clouthier et al, 1996). Basically, the testes of Sprague-Dawley rats 12- to 17-days old were subjected to a two-step enzyme digestion procedure, and the resulting cells, enriched for germ cells, were concentrated by centrifugation and resuspension in an injection medium (Brinster and Avarbock, 1994). The concentration of cells in the suspension used for injection ranged from 40 to 80 × 10^6 cells/ml, and

Table 1. *Spermatogenesis following rat testis cell transplantation to mouse seminiferous tubules*

Recipient mouse number	Donor rat cell strain	Mouse recipient genotype	Days after transplant to sacrifice	Spermatogenesis in seminiferous tubules (%)‖					
				Testis I*			Testis II		
				Full†	Intermed.‡	Empty§	Full	Intermed.	Empty
1117-1095	Sprague-Dawley**	Nude††	59	0	14.95 (13)	85.1 (74)	0	20.0 (15)	80.0 (60)
762-1084B	Sprague-Dawley	SCID	194	5.75¶ (5)	3.4 (3)	90.9 (80)	13.4 (25)	20.9 (39)	65.7 (123)
768-1083A	Sprague-Dawley	SCID	177	30.3 (71)	38.0 (89)	31.7 (74)	54.7 (70)	35.9 (46)	9.4 (12)
765-1083B_2	Sprague-Dawley	Nude	183	63.5 (33)	30.8 (16)	5.7 (3)	48.1 (37)	51.9 (40)	0
764-1084A	Sprague-Dawley	Nude	188	83.3 (125)	14.0 (21)	2.7 (4)	32.6 (61)	11.8 (22)	55.6 (104)

* At sacrifice, testes were not designated left or right.
† Full—all cells of a cell association present.
‡ Intermediate—spermatogonia primary spermatocytes or few spermatids at the most are present.
§ Empty—only Sertoli cells.
¶ There was no determination of whether mouse or rat spermatogenesis was taking place in these tubules.
‖ In parenthesis, number of tubules examined.
** Sprague-Dawley donor rat testes cells were from 12–17-day-old animals.
†† Nude and SCID mice were treated with 32 mg/kg busulfan to destroy endogenous spermatogenesis. This treatment regimen allows the partial return of endogenous spermatogenesis.

0.5 ml of cell suspension was used to inject the seminiferous tubules of the two testes of each recipient mouse. The recipient mice were either NCr Swiss nude (nu/nu), which lack B-cells, or severe combined immunodeficient (SCID), which lack B-cells and T-cells (Taconic, Germantown, New York). Endogenous spermatogenesis in the recipient males was destroyed by an intraperitoneal injection of busulfan (32 mg/ml) at least 4 weeks prior to donor cell transplantation (Brinster and Avarbock, 1994). This level of busulfan allows the partial return of endogenous spermatogenesis. The period necessary for complete spermatogenesis to occur from stem cell to spermatozoa is approximately 35 days in the mouse and 52 days in the rat. Recipient mice were sacrificed between 177 and 194 days following injection of donor testis cells, which represents approximately 5 and 3.5 times the period necessary for complete mouse and rat spermatogenesis, respectively.

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), dyhydrated 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

Light microscopy showed that spermatogenesis was taking place in mice in some tubules and not in other tubules (Fig. 1). There was considerable animal-to-animal variability in the number of tubules with active spermatogenesis. As a rough indication of the degree of spermatogenesis, this process was classified as "full" spermatogenesis if all the cell types of a cell association were present (qualitatively), as "intermediate" spermatogenesis if germ cells other than spermatogonia were present but elongate spermatids were lacking, and as "Sertoli cell-only" if Sertoli cells, or perhaps Sertoli cells and occasional spermatogonium, were present (Table 1). The mean percentage of tubules in recipient nude mice (six testes) and SCID mice (four testes) combined containing full spermatogenesis was 31.9 ± 11.3 (Table 1). Intermediate spermatogenesis occupied 25.2 ± 6.3%, and 27.2% of the tubules in both nude and SCID mice presented only Sertoli cells. Thus, slightly less than half of the tubular profiles lacked germ cells more mature than spermatogonia and most of these contained Sertoli cells only.

Because busulfan treatment does not completely prevent spermatogenesis from reinitiating from the stem cell population, it was always found that some endogenous

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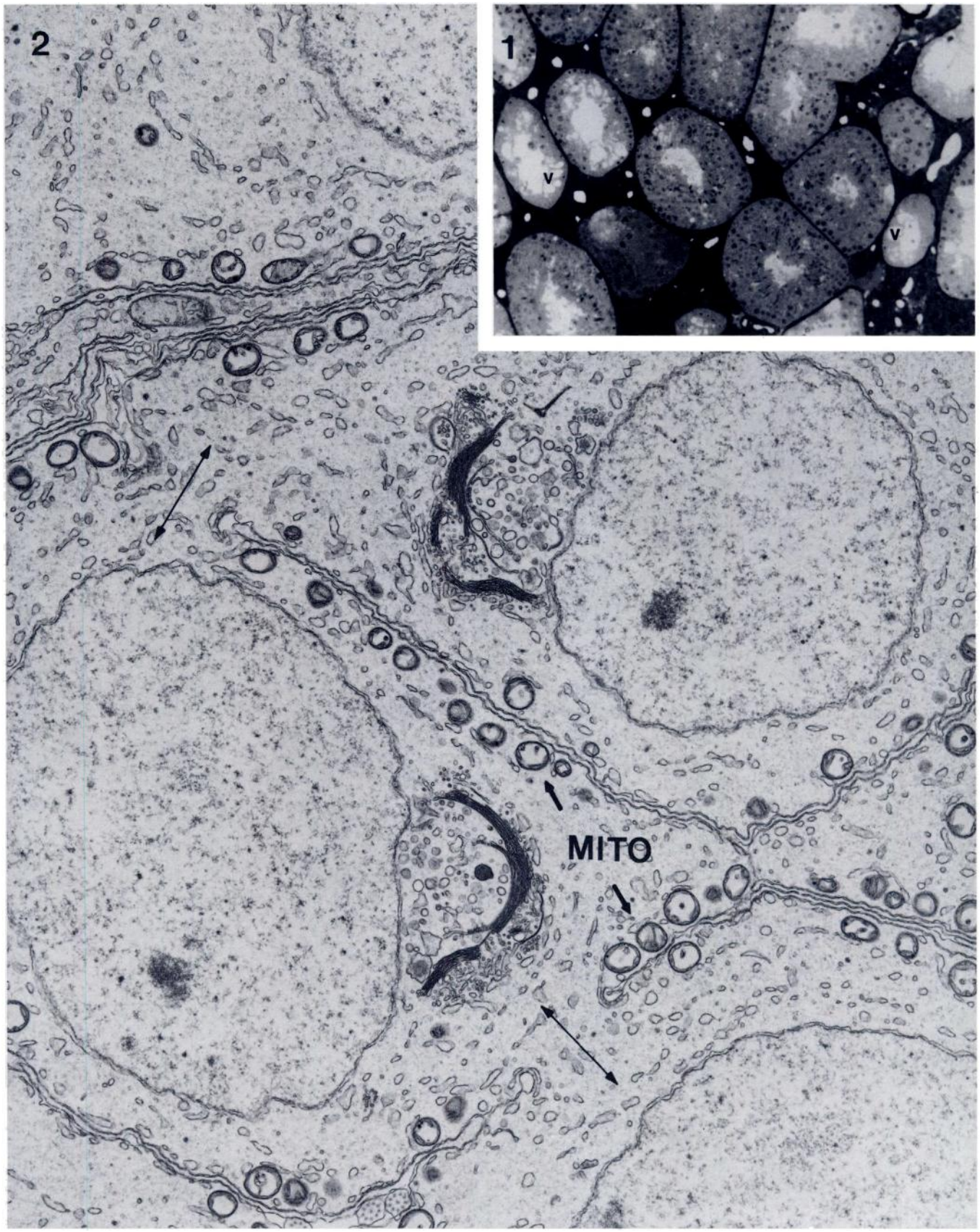
FIG. 1. Light micrograph of a busulfan-treated mouse testis transplanted with a suspension of cells from the rat testis approximately 6 months previously. The tubular morphology is variable with some tubular profiles showing active spermatogenesis and others with reduced numbers of germ cells. Vacuolation of Sertoli cells (v) is common in Sertoli cell-only mammals. ×330.

FIG. 2. Identification of rat spermatids in the tubular epithelium of the mouse by their mitochondrial position. Spermatids are of rat origin when all mitochondria (MITO) lie along the plasma membrane. Also indicated are normal-appearing intercellular bridges (double-headed arrow) and developing acrosomes (single-headed arrow). ×14,000.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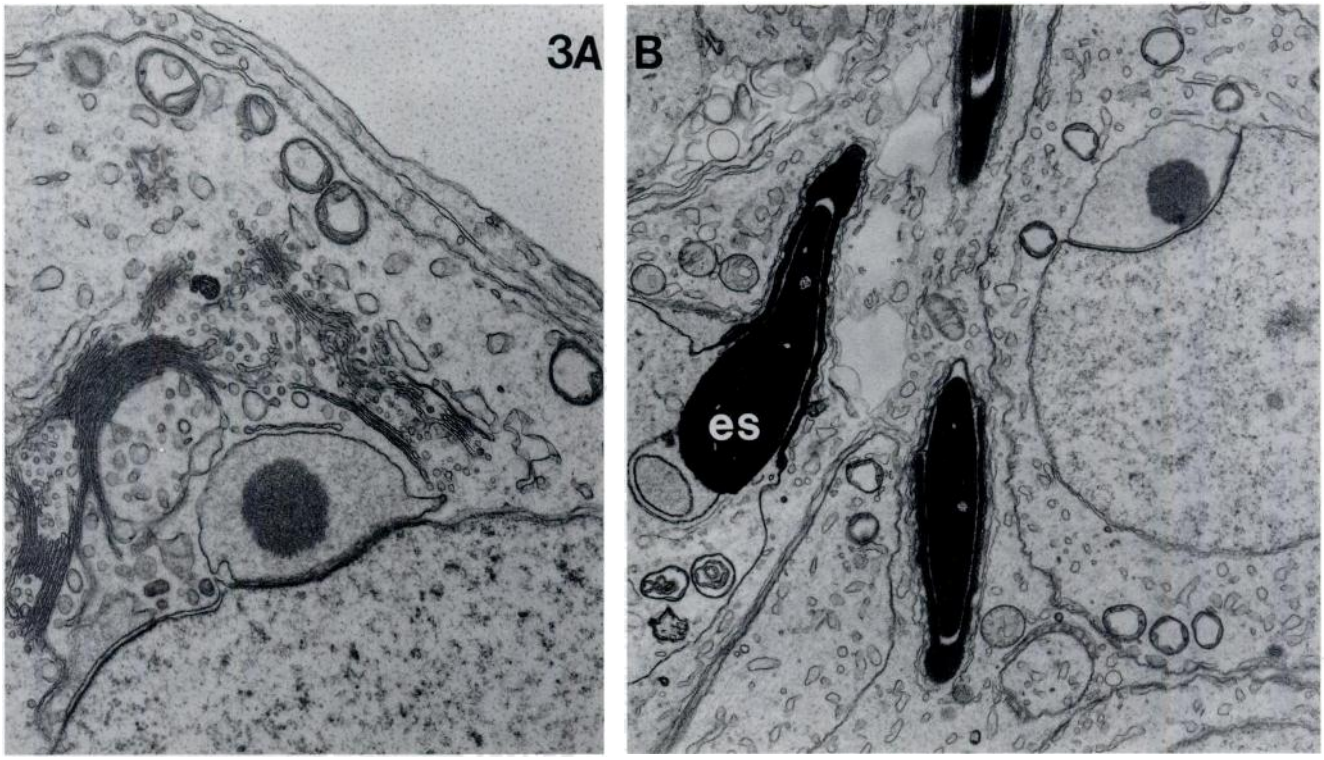

FIG. 3. Differences in the shape of the acrosome of early spermatids in rat **(A)** and mouse **(B)** are one of the characteristics that can be used to differentiate mouse and rat spermatids. In **A** the acrosomal vesicle lies on the rounded nucleus and indents it very little, whereas in **B** the acrosomal vesicle has greatly indented the nucleus. Note also that the position of mitochondria in the mouse and rat round spermatids is characteristic of the species, lying peripherally along the plasma membrane in the rat and randomly scattered in the cytoplasm in the mouse. Relatively normal-appearing elongated spermatids (es) of the mouse are also seen in **B**. A, ×19,500; B, ×;9,600.

mouse spermatogenesis was present in the recipient mice sacrificed after transplantation. Light microscopy was not usually sufficient to determine if donor (rat) spermatogenesis or endogenous (mouse) spermatogenesis was taking place in a given seminiferous tubule. There were some indications by the shape of the elongate spermatids and by the degree of condensation of the spermatid heads at particular stages that a particular tubule was either rat or mouse spermatogenesis. But given that electron microscopy did not sample a large number of tubules, the precise percentages of tubular profiles with either rat or mouse spermatogenesis was not determined. It was our impression that slightly less than half of the tubules with active spermatogenesis contained rat spermatogenesis.

By electron microscopy, the criteria to differentiate rat from mouse spermatogenesis were those provided in published micrographs of mouse and rat spermiogenesis (Russell et al, 1990). The key to the ready identification of the species origin of a particular germ cell type was found to be primarily in the positioning of the mitochondria (Fig. 2), as well as in the appearance of the acrosomal system of the spermatids. Mitochondria within rat spermatids become aligned along the plasma membrane in mid-stage I of the spermatogenic cycle (Dym, 1977). In the mouse these organelles remain randomly scattered within the cytoplasm of spermatids. Figures 2 and 3A show round spermatids from the donor (rat) cells containing peripherally located mitochondria. This is contrasted with Figures 3B and 4A, which show endogenous mouse spermatogenesis as evidenced by the presence of round spermatids possessing mitochondria randomly scattered throughout the cytoplasm.

Subtle differences in the positioning and form of the acrosomal system of the mouse and the rat were noted and could be used as markers (Fig. 3). In the mouse the acrosomal vesicle markedly indented the nucleus after it

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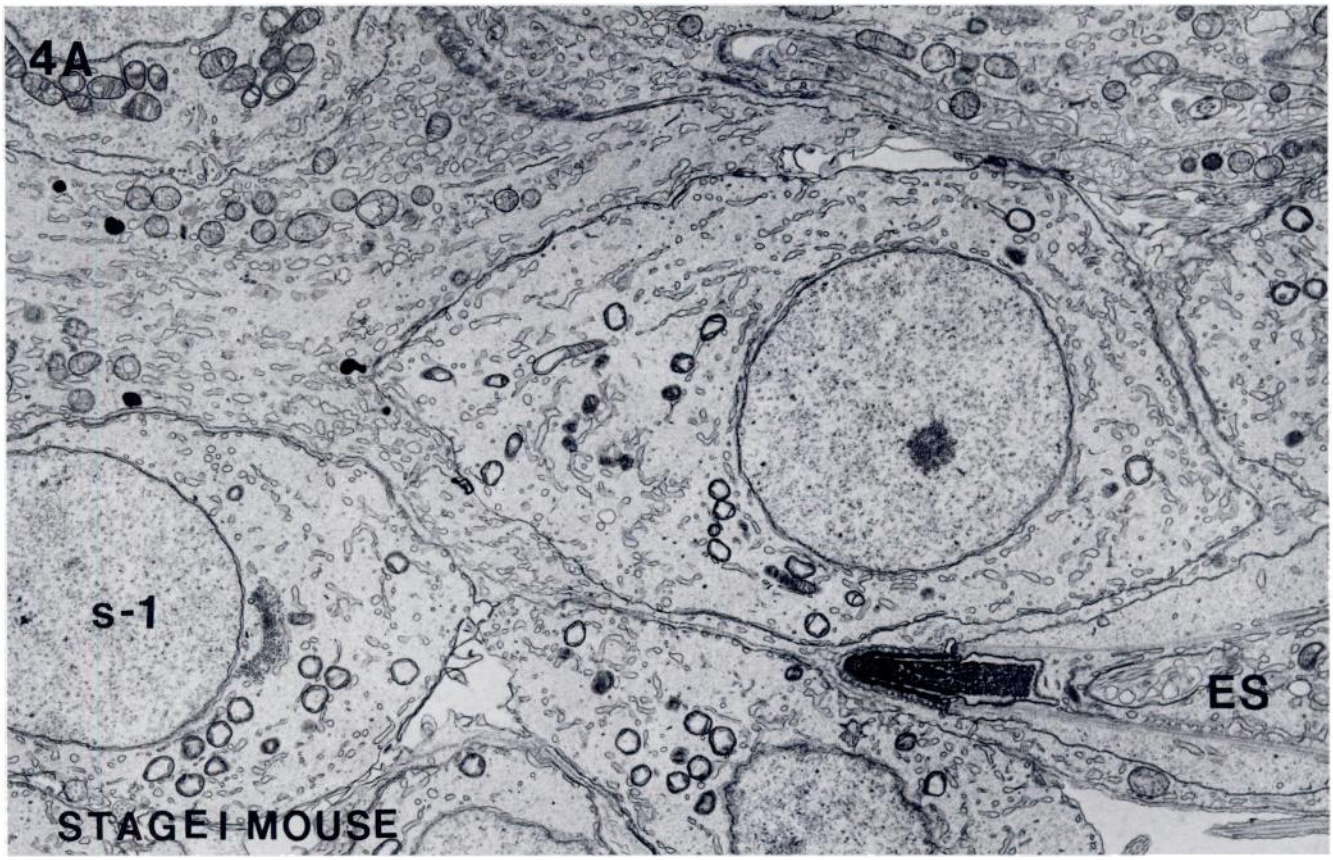
FIG. 4. Micrographs illustrating the species-related synchronization of cell types to form cell association in mouse **(A)** and rat **(B)** spermatogenesis. In both **A** and **B**, nuclear condensation of elongating spermatids (ES) is taking place. In **A**, which is mouse spermatogenesis, chromatin condensation of the elongate spermatids is taking place among step 1 spermatids (s-1). Thus, the tubule is characterized as stage I. In **B**, which is rat spermatogenesis, chromatin condensation is taking place among large pachytene spermatocytes (PS) and would be typical of what might be designated in the rat as stage XI. Note that the position of mitochondria in the mouse spermatids is scattered throughout the cytoplasm. A, ×6,600; B, ×7,200.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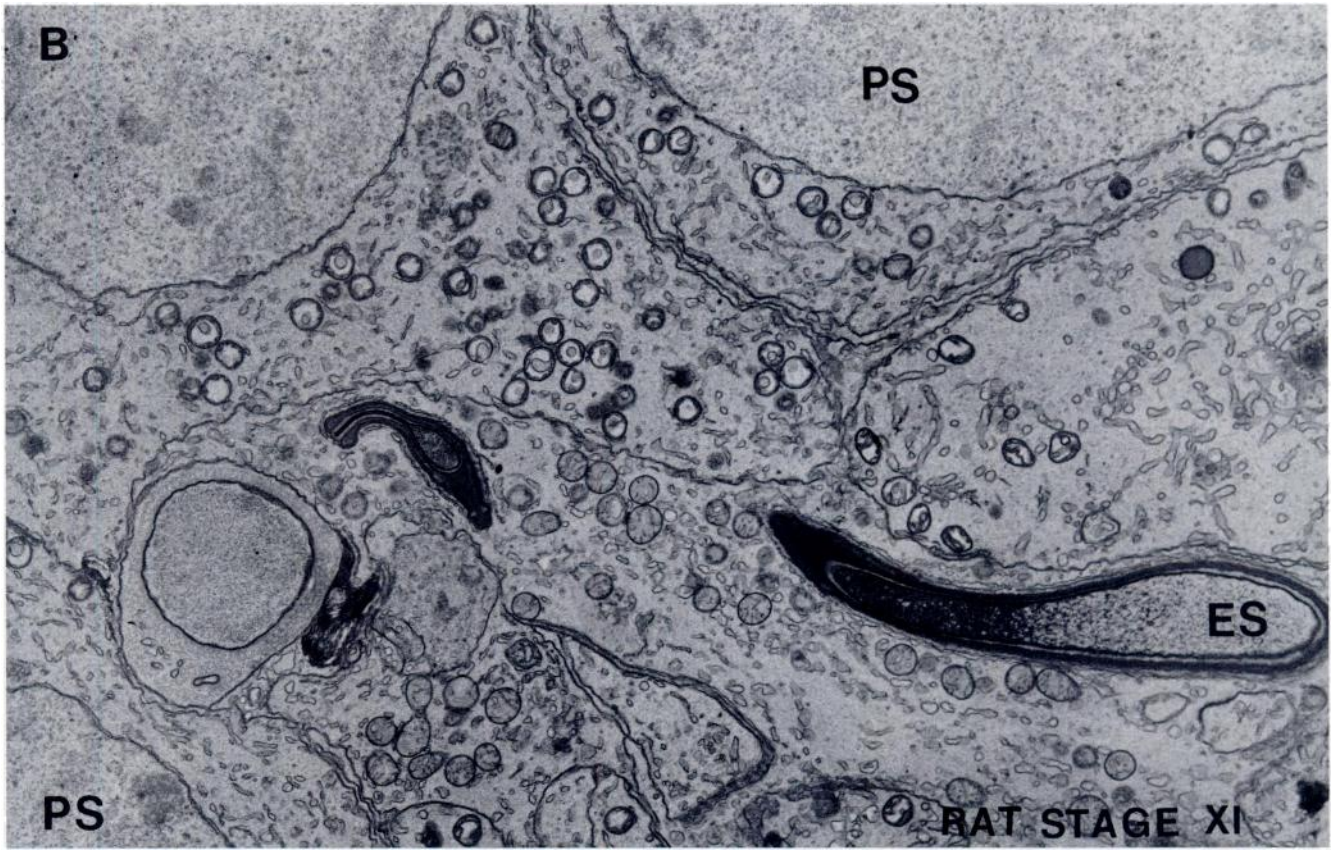
STAGE I MOUSE


B
PS
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RAT STAGE XI

made contact with the nucleus (Fig. 3B). In contrast, in the rat the acrosomal vesicle lay on the rounded spermatid and indented the nucleus only slightly (Fig. 3A). These differences have been described previously (Russell et al, 1990).

Differences in the head shape, head size, and acrosomal shape and degree of chromatin condensation of elongating and elongate spermatids were also used as criteria to differentiate rat from mouse cells. For example, condensation of mouse elongate spermatid nuclei (steps 12–13) begins in stage XII and/or I, which is the period during meiotic cell division (metaphase, anaphase, and telophase) and after cell division (Fig. 4A). In the rat nuclear condensation begins in stage XI before meiotic divisions occur (Fig. 4B). In every instance where complete mouse and rat spermatogenesis were found, germ cells were always in cell associations that were appropriate for the species. There was never an instance where mouse and rat spermatogenesis were found to be intermixed.

Spermatogonia were always found in the basal compartment of the testis (not shown). Intercellular bridges between like cell types were formed (Fig. 2). Thus, a stage VII tubule would contain type A spermatogonia (not always seen due to low frequency), preleptotene spermatocytes, pachytene spermatocytes, step 7 spermatids, and step 16 (mouse) or step 19 (rat) spermatids. In some instances one or two generations of cells would be missing or greatly reduced in number (Fig. 5). Missing germ cell layers were more commonly found in tubules where rat spermatogenesis was present than where mouse spermatogenesis was noted, although spermatogenesis of both species displayed this pathology within recipient testes. Nuclear, acrosomal, and flagellar defects were common in some tubules showing rat or mouse spermatogenesis (Fig. 6).

Not only were qualitative differences evident, but quantitative differences were noted as well. Usually, less than the expected numbers of cells would be present in any tubule. Degeneration of germ cells was often seen (Fig. 5). Generally, when quantitative differences were noted it was the elongate spermatids that were relatively deficient (Fig. 5). Phagocytosis of abnormally appearing cells, whether they be mouse or rat, was common in stage VIII, at or after the expected time of sperm release. Since elongation of spermatids was a block to completion of spermatogenesis in both rat and mouse (see below), fewer than normal about-to-be-released spermatids were present. Figure 6A,B shows a comparison of stage VII (late) tubules containing rat and mouse spermatogenesis, respectively. Elongated spermatids were few in number and could either be relatively normal appearing or abnormal appearing. Both species showed the reduction in elongated spermatids.

In each tubule where rat spermatogenesis was noted, the adjacent Sertoli cells were examined to determine if Sertoli cells from the rat, possibly injected as part of the cell suspension, had also colonized the tubules. The difference between rat and mouse Sertoli cells has been described (Russell and Griswold, 1993), and such cells are readily determined by their shape and the internal configuration of their mitochondria. Mouse Sertoli cell mitochondria are rounded and, more importantly, show a char-

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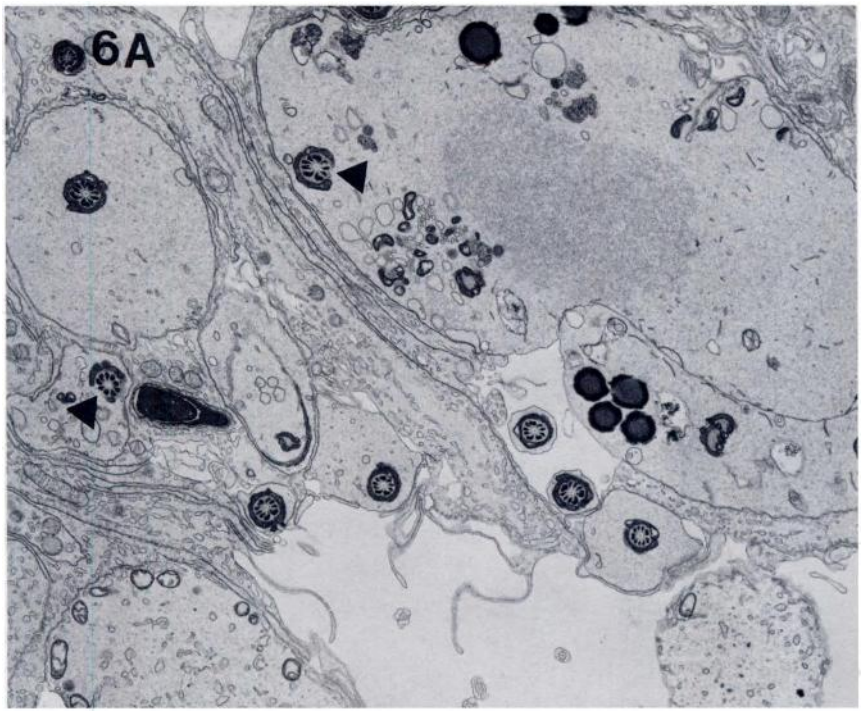
FIG. 5. Light micrograph showing three tubules in which elongate spermatids are lacking as a component of the cell association. At least one of these (bottom left tubule) appears to be rat spermatogenesis, given that the cell borders in step 7 (s-7) spermatids can be visualized. The ability to visualize cell borders is due to the vague presence of the peripherally positioned mitochondria (arrowhead). A degenerating cell is indicated (open arrow). ×300.

FIG. 6. Rat **(A)** and mouse **(B)** spermatogenesis at stage VII depicting normal-appearing and abnormal-appearing elongated spermatids that characterize step 19 and step 16 cells of each species, respectively. **(A)**, A sectioned profile of a nucleus and numerous flagella show that most cells appear morphologically normal. Some rat flagella contain more than the normal amount of cytoplasm surrounding them, and some appear to lack a full mitochondrial sheath (arrowheads). **(B)**, Some mouse elongated spermatid nuclei are decondensed (d) and acrosomal separation from the nucleus is common (arrows are insinuated between acrosome and nucleus in abnormal spermatids). Generally the predecessor step 7 of both mouse and rat cells are normal appearing. A, ×4,500; B, ×3,600.

FIG. 7. Figure illustrating the mitochondrial characteristic of mouse Sertoli cells. Mouse Sertoli cell mitochondria are generally rounded and oval with expanded cristae (star within cristae). The adjacent spermatids show mitochondria (MITO) lying along the plasma membrane and thus are of the rat type. ×18,000.

FIG. 8. Step 6 spermatids and step 18 spermatids of rat lineage in a stage VI tubule. Numerous step 6 spermatids are present and show a typical configurational relationship to the Sertoli cell. The Sertoli cell is of mouse origin since it contains the unique mitochondrial cristae typical of this species. Elongate spermatids can be seen within crypts of this Sertoli cell. Nine such spermatids are enumerated, a number slightly less than the mean (10.3) found in the normal rat (Russell and Peterson, 1984). Some abnormalities of the acrosomes of the elongate spermatids are seen, such as separations of the acrosome from the nucleus, although most look normal. ×9,000.

FIG. 9. Shown are examples of cell degeneration and abnormal spermatids in tubules with rat spermatogenesis. **(A)**, Light micrograph; the tubule contains numerous spermatids that failed to elongate (arrowheads), whereas one spermatid (arrow) has begun elongation and appears relatively normal. **(B)**, Pachytene spermatocytes in the early phase of degeneration. **(C)**, An early degenerating step 9 spermatid. Acrosomal discontinuities are evident. The adjoining Sertoli cells contain mitochondria of the mouse type. **(D)**, A spermatid at about step 9 of spermiogenesis, showing an acrosomal break (arrow) and abnormal manchette (M) development. The manchette has greatly deformed the nucleus and has formed finger-like appendages extending from the nucleus. A, ×300; B, ×7,200; C, ×10,800; D, ×15,000.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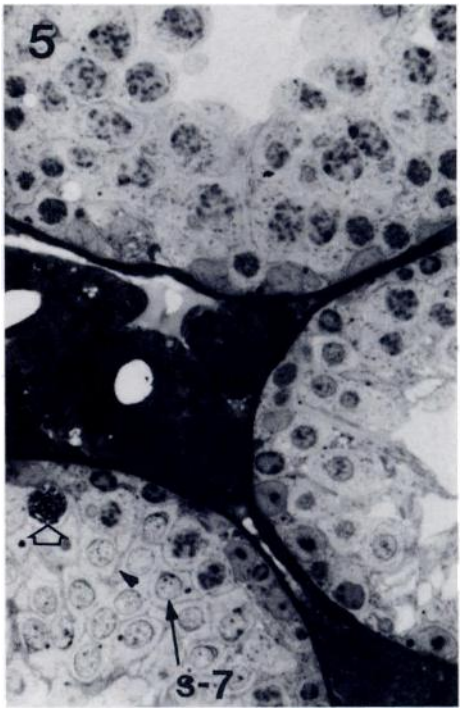
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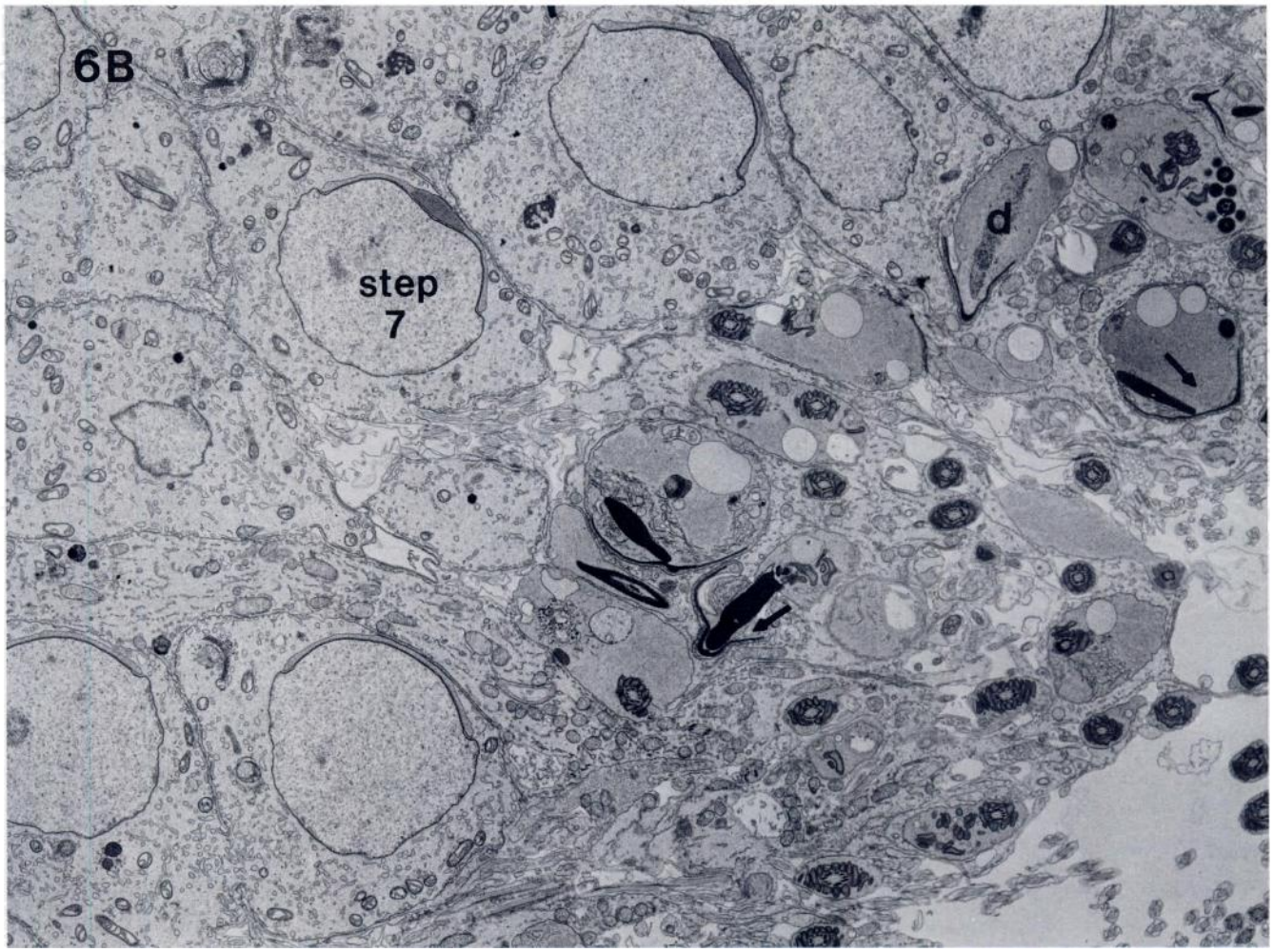
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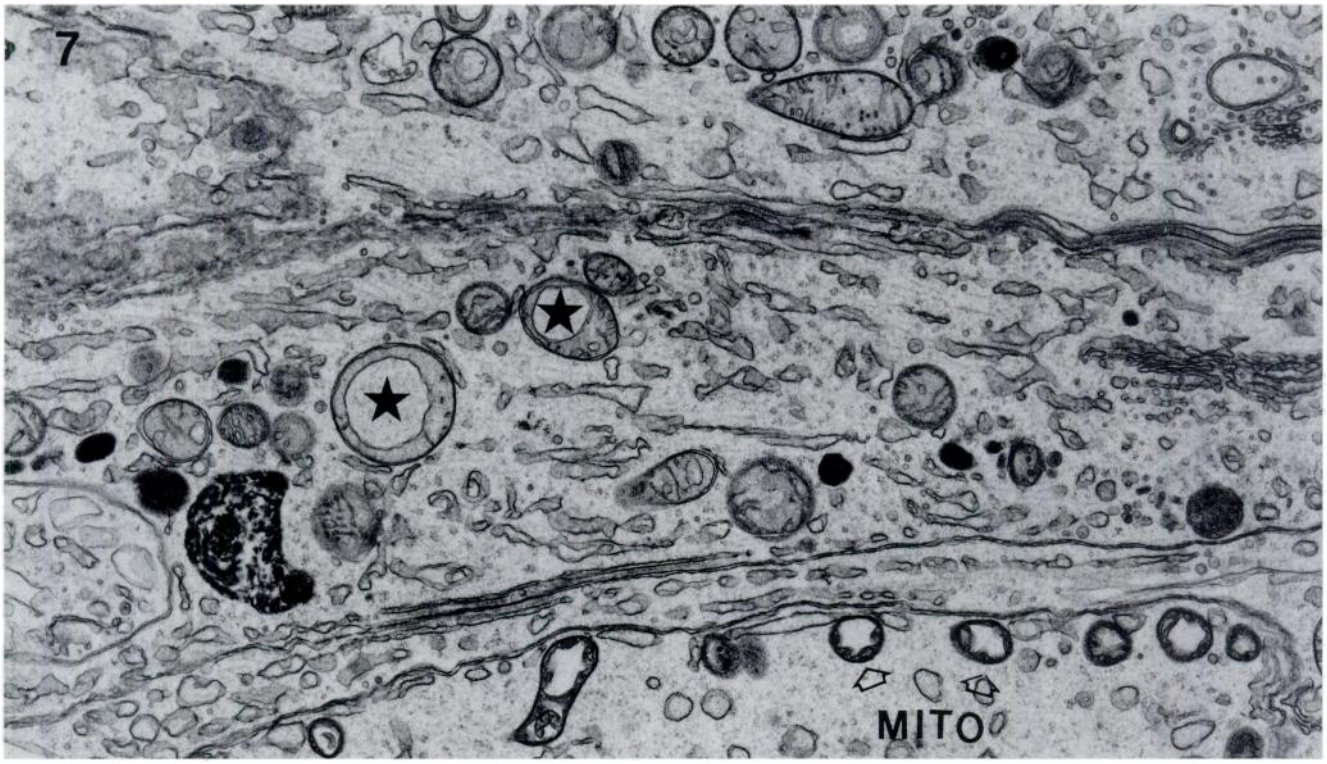
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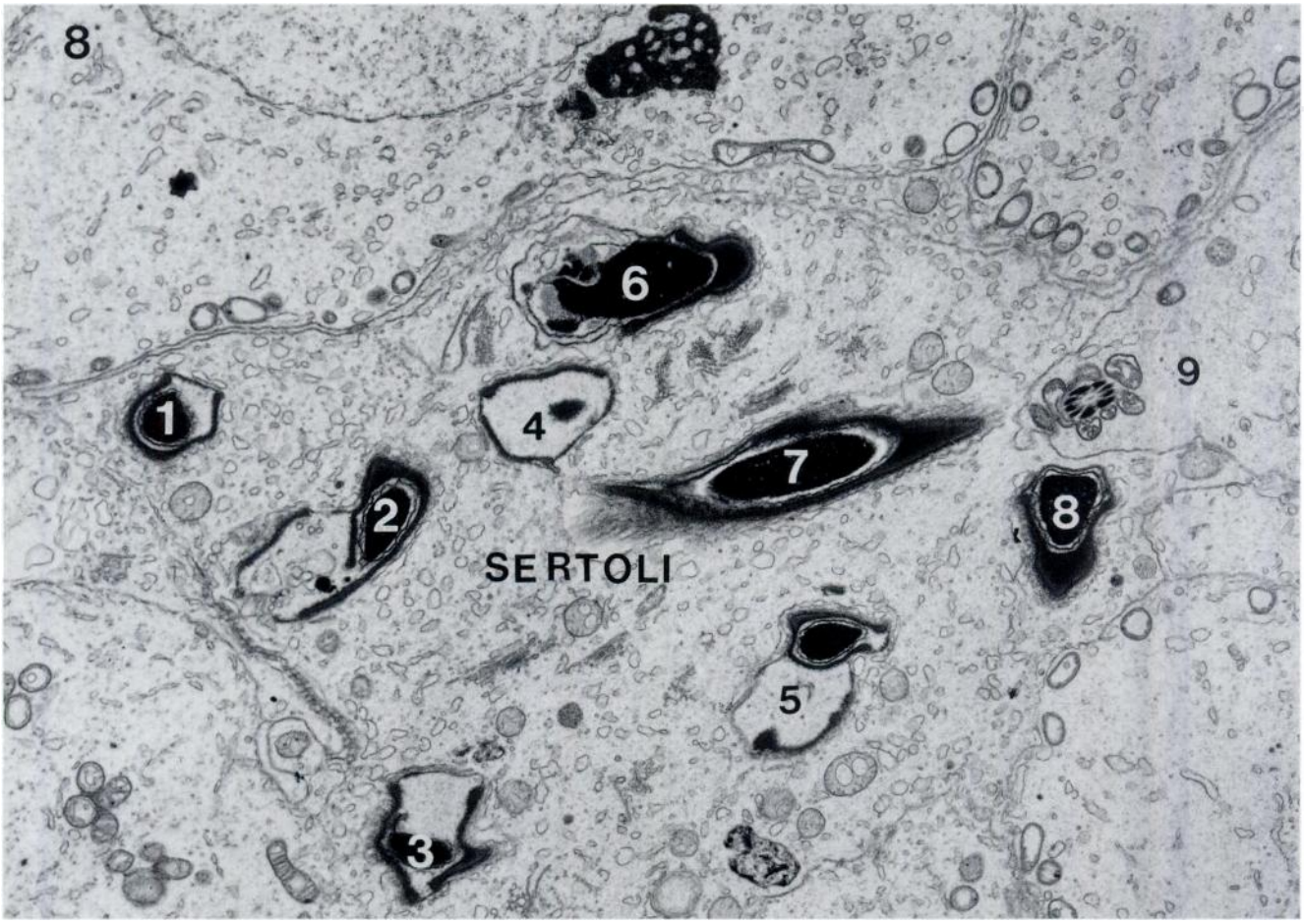
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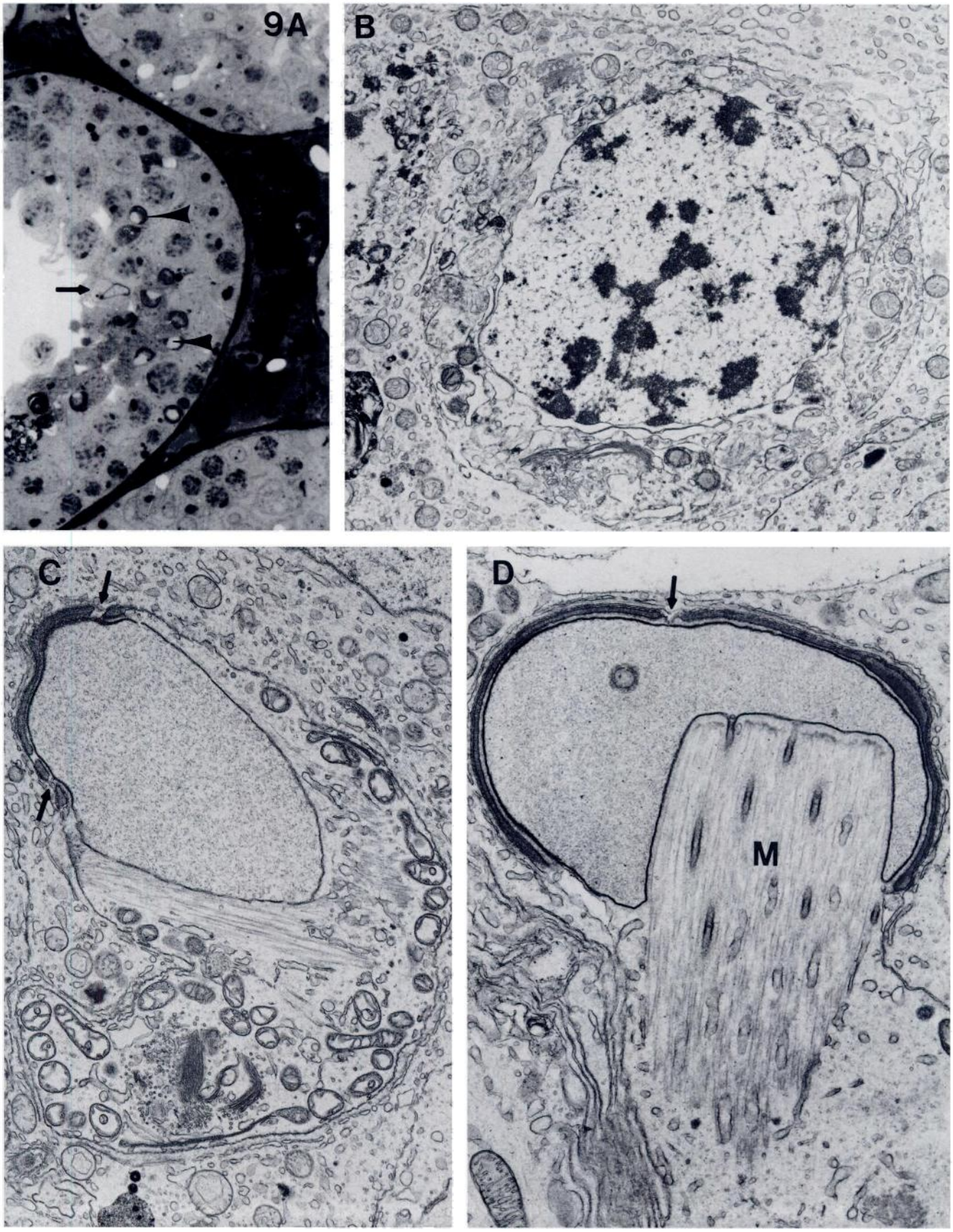
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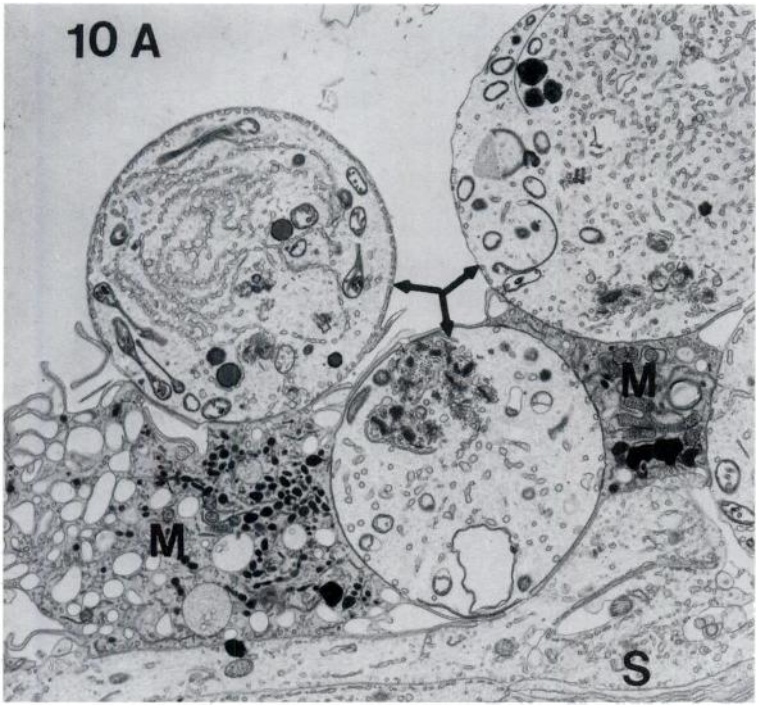
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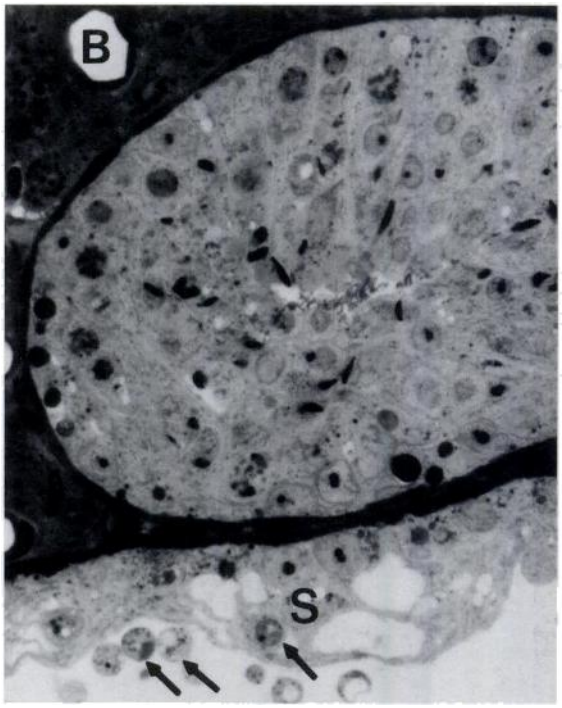
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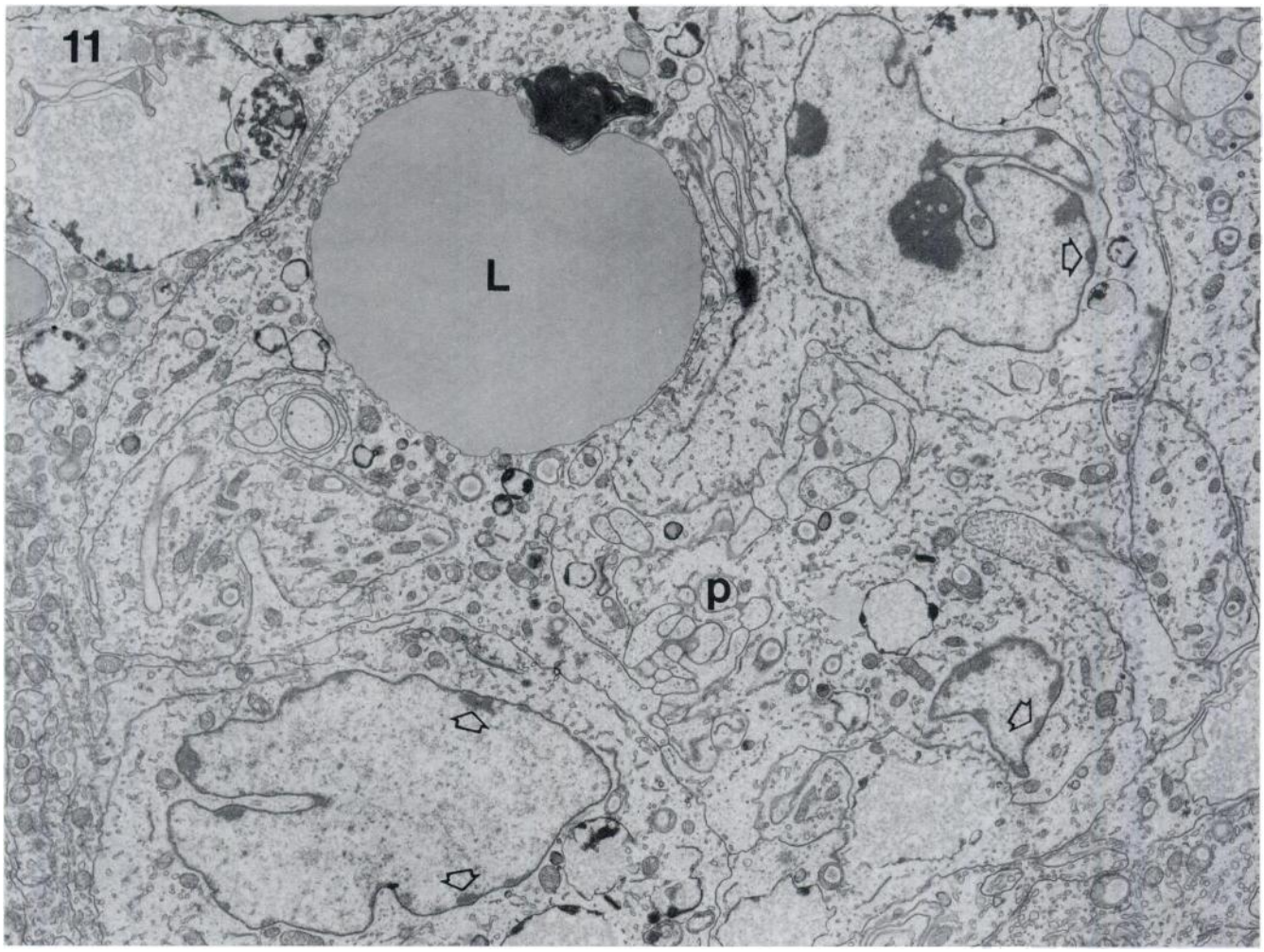
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acteristic expansion of the intercristal space. Other features, such as the appearance of the nucleolus, can be used to distinguish cells of these two species. In every instance where rat spermatogenesis had colonized the mouse tubules, the adjoining Sertoli cells were of the mouse type (Fig. 7). No evidence of rat Sertoli cells was seen in any region of the mouse testis.

Rat spermatogenesis, when found in the mouse, was species-representative, meaning that the cell types were characteristic of that described for the species. In addition, the appearance of all sectioned cell profiles and the cell association was typical of that described in the literature (Russell et al, 1990). Additionally, in some regions of seminiferous tubules, spermatogenesis appeared quantitatively normal, yielding the approximate expected number of elongate spermatids per Sertoli cell (Fig. 8). Sporadic germ cell degeneration was noted in tubules with both rat and mouse spermatogenesis (Fig. 9).

Occasionally, there was evidence in tubular profiles containing both rat and mouse spermatogenesis, indicating that the elongation of spermatid heads and acrosomal shaping were a major stumbling block to completion of spermatogenesis. Such abnormalities were variable in nature and not quantified, but it was our impression that they were slightly more common in tubules containing rat than mouse spermatogenesis. Figure 9 shows some examples of abnormalities noted in elongating spermatids.

In regions of the testis where neither mouse nor rat spermatogenesis were present, only Sertoli cells or occasionally Sertoli cells and a few spermatogonia were noted. These sometimes contained phagocytosed sperm, as has been described in the accompanying manuscript (Russell et al, 1996). Occasional macrophages showing evidence of phagocytic activity were seen in Sertoli cell-only tubules. Macrophages appeared to phagocytose cytoplasmic remnants (species unknown) of elongated spermatids, as did nearby Sertoli cells (Fig. 10).

Clusters or "balls" of Sertoli cells, identified as being of mouse origin by their characteristic mitochondria, were often seen (Fig. 11). They appeared to have no contact with the basal lamina. Their nuclei were smaller and more heterochromatic and irregularly shaped than those attached to the basal lamina.

Discussion

Xenogeneic transplantations of organs and tissues are largely unsuccessful because of immunologic intolerance phenomena. However, the testis is often viewed as an immunoprivileged site (literature cited in Bellgrau et al, 1995) because autoantigens present on germ cells do not normally elicit an immune response even though some may reside on the surface of cells of the basal compartment of the testis (Yule et al, 1988). Several factors contribute to immunotolerance. First, the testis contains high levels of steroids, molecules that are immunosuppressive by nature. Second, Sertoli cell secretes immunosuppressive substances (Bellgrau et al, 1995). However, the most commonly recognized mechanism for immune tolerance of autoantigenic molecules is the physical isolation of cell surface antigens by the Sertoli cell barrier. The tight junctions between Sertoli cells sequester postmeiotic germinal cells into an adluminal compartment where it is generally thought that they are not accessible to the immune system. It might be hypothesized that cells with foreign antigenic properties, such as cells from another species, should be tolerated in the testis. Thus far, attempts to transplant rat cells into mice with normal immune systems have proved unsuccessful. Therefore, the present study employed mice with defective immune systems to achieve successful spermatogenesis from rat testes donor cells (Clouthier et al, 1996).

Because recipient mice regenerated some endogenous spermatogenesis after busulfan treatment, it was necessary to differentiate rat and mouse spermatogenic cells. This was not a difficult task with electron microscopy. Light microscopy could identify only certain stages of the spermatogenic cycle that were unique to mouse or rat. Clearly, there is a need to develop a model in which only donor cells have the capability to produce sperm.

The busulfan-treatment model is also somewhat inadequate because endogenous spermatogenesis does not regenerate to a state characteristic of the normal mouse. Commonly, elongate spermatids of both species are abnormally shaped and some degenerate; furthermore, there is evidence that many are phagocytosed at the time of expected sperm release. Because both rat and mouse spermatids show the same features, it seems possible that

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FIG. 10. Phagocytosis of germ cell cytoplasm. Rounded masses (arrows) of germ cell cytoplasm were often detected lining the lumens of tubules. These were taken up both by macrophages (M; **A**) or by the Sertoli cell-only epithelium (S; **B**). Occasionally macrophages are seen near the lumen, especially near the tubule lumen of Sertoli cell-only tubules. In **A**, the thin cytoplasmic processes of a macrophage surround several cytoplasmic fragments of germ cells. A, ×4,800; B, ×1,450.

FIG. 11. The general shape of Sertoli cells within a "ball" or aggregate is rounded, although numerous small cell processes (p) are noted. The nuclei of Sertoli cells are highly irregular and abnormally demonstrate speckled heterochromatin along the nuclear envelope (broad arrow). Typical tripartite nucleoli are not seen. Large lipid droplets (L) are common. ×6,000.

there is some permanent damage of Sertoli cells due to busulfan treatment. It is also possible that prolonged inactivity of Sertoli cells, such as seen in the W-locus mutation (Russell et al, 1996), makes them poor nurturers of germ cells.

In some tubular profiles it was noted that a specific cell type comprising a cell association was missing. Since massive degeneration of postspermatogonial cells was not seen, the absence of cell layers suggests that there is either degeneration of relatively immature spermatogonia or there is not regular recruitment of differentiated spermatogonia as one might observe in a normal animal. If a stem spermatogonium did not divide to form cells committed to undergo spermatogenesis, a "window" of missing germ cells would result. As cells repopulate a seminiferous tubule after damage, they must first build up the numbers of stem cells sufficiently before committed cells can differentiate to produce active spermatogenesis. The same situation might apply to the regions of seminiferous tubules undergoing repopulation by transplanted donor cells. At the edge of the growth zone, spermatogenesis might be expected to be less efficient than within a central region of the zone. A study of longitudinal sections of seminiferous tubules might provide an explanation for the missing cell layers.

Occasional macrophages were noted in the lumen of seminiferous tubules. They showed the ability to engage primarily elongated spermatids with their cellular processes, to internalize them, and to degrade them. The manner by which macrophages entered the seminiferous tubules was not clear. Presumably, they were not remnants of the cell suspension injected because the life span of a macrophage ranges from 2 to 4 months (Parslow, 1994).

Little attention has been given in the literature to the significance of positioning of mitochondria in spermatids. Fortunately, the rat spermatid is atypical with respect to the alignment of mitochondria along its cell surface (Dym, 1977), and the difference in mitochondria position allowed ready identification of mouse and rat cells in recipient animals showing both types of spermatogenesis. It has been inferred that the movement of mitochondria to the cell surface is due to the influence of the surrounding Sertoli cell (Dym, 1977). This now seems unlikely, because mitochondria moved to the cell surface of the rat germ cells transplanted into the mouse, whereas mouse Sertoli cells do not induce the mitochondria of mouse spermatids to move peripherally. The location of spermatid mitochondria appears to be controlled by the spermatids themselves.

The present study shows that the germ cell alone possesses the programming to develop morphological characteristics that are typical of the species. Thus, the somatic cells of a mouse do not induce the rat germ cell to form a sperm in the likeness of a mouse sperm. Although the mouse and rat sperm head shapes are generally sickle-shaped, there remain considerable differences in the dimensions and shapes of their sperm heads and, furthermore, they show differing morphologies during development. Some have discussed a role for the Sertoli cell in spermatid head shaping (Fawcett et al, 1971), a role, if any, that is surely not species specific given that a rat spermatid will achieve the shape of a rat sperm while surrounded and supported by mouse Sertoli cells. The length of the rat sperm tail also remains characteristic of the rat and not the mouse (Clouthier et al, 1996).

The present study has shown that rat germ cells develop in the presence of mouse Sertoli cells. Although Sertoli cells from the donor rat probably were injected into recipient mouse testes, there is no remaining evidence of these mouse Sertoli cells, either in the lumen or established on the basement membrane of the seminiferous tubule. Sertoli cells at the ultrastructural level make their identity clear (Russell, 1993a), particularly by the unique morphology of the mitochondria and to a lesser extent the appearance of the nucleolus. The structural appearance of the mitochondria provide unequivocal evidence that rat germ cells establish themselves among mouse somatic cells.

The findings of this study indicate that Sertoli cells, which normally function to produce mouse spermatozoa, are capable of supporting the differentiation of germ cells from the rat. Therefore, germ cells of the rat must influence recipient Sertoli cells to provide appropriate support. While there has been apparently 10 to 11 million years for genetic diversity to occur (Catzeflis et al, 1993), the signals necessary for germ cell–Sertoli cell interaction must have been conserved. Given this reasonably close species relationship, one cannot make too many assumptions about the nature of the factors emanating from the Sertoli cell in these two species that would suggest that the Sertoli cell of the mouse cannot support rat spermatogenesis. The general morphological relationships between the mouse Sertoli cell and the rat germ cell appear normal (Russell, 1993b). However, the junctional connections have not yet been studied in detail.

During germ cell development, the cycle of the seminiferous epithelium is of relatively constant duration for any species and is usually not susceptible to major change. When germ cells are present, there is also a corresponding cycle in Sertoli cells. A temporal relationship exists that relates the configurational, nutritive, and secretory functions of the Sertoli cell to specific germ cell associations during the spermatogenic cycle (Parvinen, 1993). The results of this ultrastructural analysis of rat spermatogenesis in mouse seminiferous tubules indicate considerable plasticity in the Sertoli cell, the germ cells, or both. Clearly, one or both of these cell types must alter their biology in order to generate rat sperm in a mouse

Sertoli cell environment. The timing and spatial configuration of this interspecies process can tell us much about the regulation of spermatogenesis in all species.

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