BIOLOGY OF REPRODUCTION 44, 656–662 (1991)

Cyclic Localization Change of Golgi Apparatus in Sertoli Cells Induced by Mature Spermatids in Rats

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ABSTRACT

Previously we reported that the intracellular localization of the Golgi apparatus of rat Sertoli cells changes during the seminiferous epithelial cycle, and that the cyclic changes seem to be correlated to specific generations of germ cells. To ascertain which generations of germ cells are responsible for the cyclic changes, we determined the relative volume of the Golgi apparatus within the basal, mid, and apical cytoplasm of Sertoli cells in testes with and without mature spermatids. In normal adult rats, the Golgi apparatus was usually localized exclusively in the basal cytoplasm, whereas at stages VII–IX it increased remarkably in mid and apical cytoplasm, with a concomitant decrease in the basal cytoplasm. In young adult testes without spermatids at steps 15–19 of spermiogenesis (2nd layer spermatids), the Golgi apparatus was localized in the basal cytoplasm throughout the seminiferous epithelial cycle. Orchiopexy maintained for 35 days following 60 days of cryptorchidism allowed germ cells to regenerate to spermatids at steps 1–14 of sperminogenesis (1st layer spermatids), but failed to change the intracellular localization of the Golgi apparatus in Sertoli cells. At 50 days after orchiopexy, when all generations of germ cells appeared in the tubules, the cyclic changes in localization of the Golgi apparatus were restored similar to those in normal adult testes. These findings indicate that the cyclic change in localization of the Golgi apparatus in Sertoli cells is evoked by the presence of 2nd layer spermatids.

INTRODUCTION

Sertoli cells constitute a part of the seminiferous epithelium along with germ cells, which mature from the basal to the apical part of the seminiferous epithelium with a definite cellular association. Sertoli cells provide germ cells with an optimal microenvironment. A reduced production of round spermatids has been reported in Sertoli cell-depleted testes of rats treated neonatally with an antimitotic drug [1]. The viability of germ cells is improved by coculture with Sertoli cells in a serum-free medium [2]. On the other hand, Sertoli cells have been known to change their functions during the seminiferous epithelial cycle. Incorporation of ^{3}H-proline and ^{3}H-cystine into Sertoli cells has been reported to reach a maximum at stages VII and VIII [3]. An increase in secretion of androgen binding protein, plasminogen activator, and a meiosis-inducing substance at stages VII and VIII has been shown in a review article [4]. Morales et al. [5] revealed the cyclic changes of mRNA levels of transferrin and sulfated glycoprotein-2 in Sertoli cells using an in situ hybridization technique combined with quantitative radioautography. Ultrastructural alterations of Sertoli cells have also been described qualitatively [6–8] and quantitatively [9–11].

These changes in the ultrastructure and function of Sertoli cells appear to reflect an intimate relationship between Sertoli cells and germ cells. Previously we demonstrated quantitatively the cyclic changes of rat Sertoli cells during the seminiferous epithelial cycle [12]. The most interesting finding was the cyclic change in intracellular localization of the Golgi apparatus. This organelle is usually localized exclusively in the basal cytoplasm, but at stages VII–IX, it increases in relative volume within the mid and apical cytoplasm with a concomitant decrease in the basal cytoplasm, probably representing a shift from the basal to mid and apical cytoplasm. The functional significance of this phenomenon might be suggested by events occurring in the germ cells at around stage VIII. A question arises as to which generations of germ cells are responsible for the cyclic changes of Sertoli cells. It is known that germ cells at certain stages of maturation affect Sertoli cell function. It has been reported that secretion of plasminogen activator is reduced when preleptotene spermatocytes decrease in number [13], and that secretion of androgen binding protein increases when Sertoli cells are cocultured with pachytene spermatocytes [14].

There are several methods for producing testes in which germ cells at specific maturation steps are present; of these, orchiopexy following cryptorchidism is especially useful. Since germ cells are known to regenerate at a certain rate, one can obtain tubules containing germ cells at various maturation steps in accordance with the time after orchiopexy. By comparing the seminiferous tubules with and without germ cells at a specific stage of maturation, it may be possible to determine which generation of germ cells evokes the cyclic change in localization of Sertoli cells. However, the surgical stresses of cryptorchidism and orchiopexy may impair the physiological relationship between germ cells and Sertoli cells. In addition, Sertoli cells in seminiferous tubules that have once undergone spermiation may have

Accepted November 20, 1990.
Received August 3, 1990.
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acquired a chronological rhythm that would impose cyclical changes on the intracellular localization of Golgi apparatus. If such a rhythm would persist even after the disappearance of spermatids by cryptorchidism, orchiopexy would be ineffective as a method for resolving the question. To rule out these possibilities, seminiferous tubules in young adult testes that had never undergone spermiation were also examined in the present study.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats in three different conditions were used. The first group consisted of 5 normal adult rats 70 days old. The second group consisted of 20 rats that underwent orchiopexy following cryptorchidism. The third group consisted of 16 normal young adults 33–42 days old. In the second and third groups, one can expect to examine the Sertoli cells that come in contact with spermatids at a given step among steps 1–19 for the first time after the disappearance of mature spermatids or after birth. The differences between these two groups is that the seminiferous tubules in the second group will have undergone the seminiferous epithelial cycle one time before cryptorchidism.

Cryptorchidism and Orchiopexy

At the age of 56 days, rats were made bilaterally cryptorchid. Sixty days after surgery, a few testes were examined preliminarily by light microscopy; they contained seminiferous tubules consisting of Sertoli cells and a few spermatogonia. Orchiopexy was then performed on the testes of remaining rats. At 35 days and at 50 days after orchiopexy, testes from 5 rats on each day were fixed to obtain seminiferous tubules without and with spermatids at steps 15–19 of spermiogenesis (2nd layer spermatids), respectively.

Preparation of Samples

Testes were perfused through the abdominal aorta, as described previously [12]. About 40 tissue blocks chosen randomly from each testis were immersed overnight in the same fixative, postfixed with 1% osmium tetroxide for 2 h, dehydrated, and embedded in epoxy resin. Sections 0.5 μm thick were stained with toluidine blue to determine the stages of the seminiferous epithelial cycle. Each section contained 5–30 profiles of the tubules at various stages of the cycle. Two to three tubules were chosen for each stage from among the 40 tissue blocks in each testis, on the basis of criteria that the tubules were preserved well and cut transversely. Ultrathin sections stained with uranyl acetate and lead citrate were observed with a JEOL 100 CX electron microscope.

Morphometric Procedures

Seminiferous tubules examined in the present study were those at stages II, IV, VI, VII, VIII, IX, XII, and XIV of the cycle in normal adult rats [15]. Stages VII, VIII, and IX are those before, during, and after spermiation, respectively. Stages XII, XIV, and II correspond to those before, during, and after the meiotic divisions of spermatocytes, respectively. Since normal adult rats demonstrate a remarkable change of intracellular localization of the Golgi apparatus around the stage of spermiation, as shown in our previous report [12], seminiferous tubules in the testes with orchiopexy following cryptorchidism were analyzed at specific stages before and after spermiation. In the young adult rats, the tubules were examined at stages identical to those in normal adult rats.

Sertoli cell cytoplasm was divided into three parts: basal, mid, and apical portions. At stages VII, VIII, and IX, Sertoli cell cytoplasm of approximately 75 μm in height was divided equally into three portions of about 25 μm in height. At remaining stages, the cytoplasm 90–100 μm in height was divided equally into three portions of approximately 30–33 μm in height. Twenty, five, and five electron micrographs were taken in basal, mid, and apical portions, respectively, from different Sertoli cells at each stage examined per animal and were enlarged to a final magnification of 11 000 ×. The Golgi apparatus in the basal portion of Sertoli cells appeared to be distributed evenly in the basal cytoplasm, except for the absence in the narrow peripheral cytoplasm just beneath the plasma membrane. Since the micrographs were taken to include as wide a cytoplasmic area as possible, the presence or absence of nuclear profiles in the basal portion seemed not to affect the detectability of the Golgi apparatus. Total area of Sertoli cell cytoplasm examined was 3 180–4 230, 570–860, and 630–900 μm^2 per stage per animal for basal, mid, and apical portions, respectively.

In the present study, an abundance of the Golgi apparatus was expressed as relative volume of Golgi area within the cytoplasm, because the change in volume of the Golgi area paralleled that of the volume of the Golgi apparatus itself in our previous examination in normal adult testes [12]. The relative volume of Golgi area in each cytoplasmic portion was determined with a "Digigrammer" (Model G, Mutoh Kogyo, Tokyo, Japan) by tracing a boundary of the Golgi area (cytoplasmic area in which only the Golgi apparatus was contained; see areas encircled by a dotted line in Fig. 1) and the plasma membrane of Sertoli cells.

Statistical Analysis

The morphometric data were analyzed statistically with Student's *t*-test. Numerous differences were detected among stages of the cycle, cytoplasmic portions, and experimental conditions. However, the significance is indicated in the text figures only within the same stages in different experimen-

tal conditions, because the present study examined the changes produced by different experimental conditions.

RESULTS

Normal Adult Testes

The Golgi apparatus of Sertoli cells in normal adult testes showed a prominent change in intracellular localization during the seminiferous epithelial cycle. It was localized exclusively in basal cytoplasm at stages other than VII, VIII, and IX, whereas it was located predominantly in mid and apical cytoplasm at stages VII–IX (Fig. 1). This was clearly shown by morphometric analysis (dotted thin line in Figs. 2 and 3). The peak values in mid and apical cytoplasm were remarkably higher than that in basal cytoplasm.

Testes with Orchiopexy Following Cryptorchidism

At 60 days after cryptorchidism, seminiferous tubules consisted of Sertoli cells and a few spermatogonia (Fig. 4A). Fifteen days after orchiopexy, primary spermatocytes had regenerated in about one fourth of the seminiferous tubules (Fig. 4B). At 35 days after orchiopexy, germ cells had matured up to spermatids at steps 1–14 of spermiogenesis (1st layer spermatids) in about one fourth of the seminiferous tubules, thus enabling us to determine the stages of the cycle (Fig. 4C). The numerical ratio of 1st layer spermatids to Sertoli cells was almost equal to that in normal adult testes. The Golgi apparatus in these tubules was located almost exclusively in the basal cytoplasm throughout the seminiferous epithelial cycle (Fig. 5, A and B). Morphometric analysis revealed a localization of Golgi area only

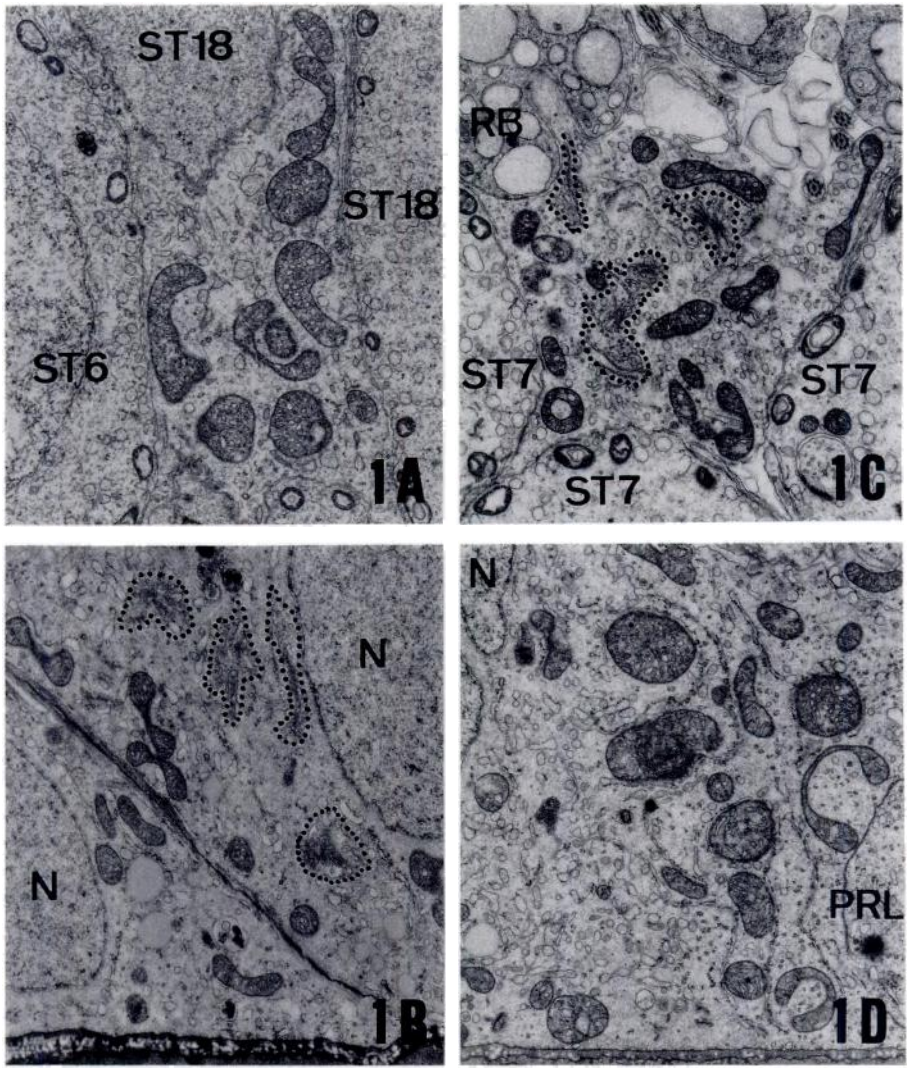

FIG. 1. Representative electron micrographs of Sertoli cells in normal adult testes. The Golgi apparatus was localized not in the apical (A) but in the basal cytoplasm (B) at stage VI; it increased in the apical (C) and decreased in the basal cytoplasm (D) at stage VII. Golgi area is encircled by a dotted line. N: Sertoli cell nucleus; PRL: preleptotene primary spermatocytes; ST6, ST7, and ST18: steps 6, 7, and 18 spermatids, respectively; RB: residual body of mature spermatid. × 8 000.

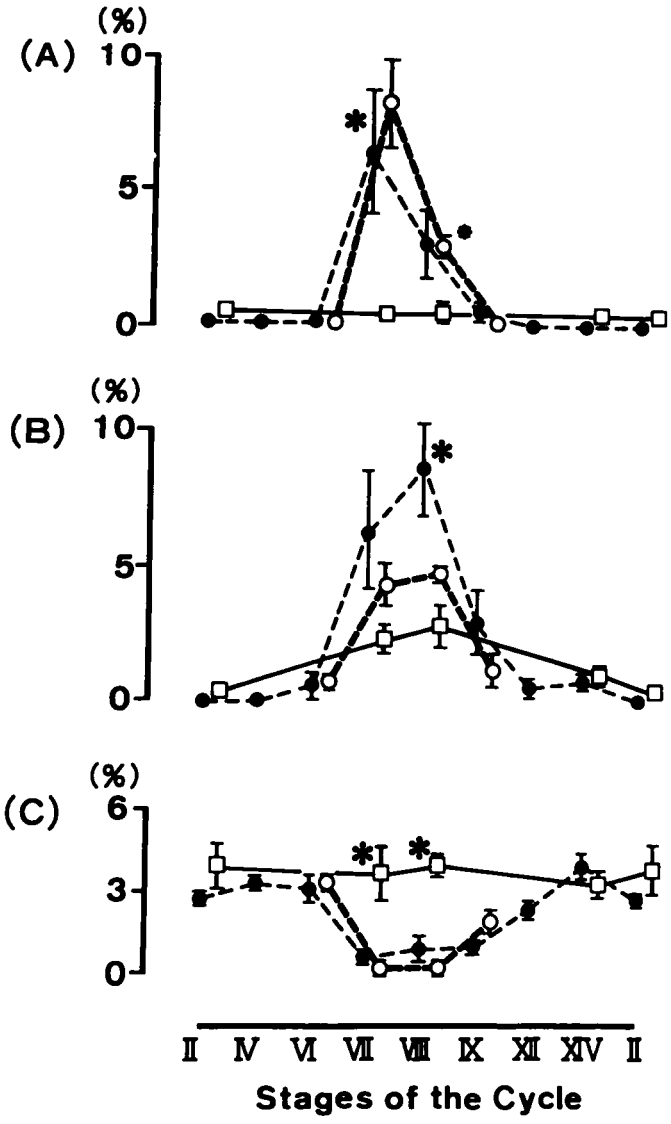

FIG. 2. Morphometric data showing a relative volume of Golgi area within the Sertoli cell cytoplasm during the cycle in normal adult testes (●-----●), testes at 35 days after orchiopexy (□——□), and at 50 days after orchiopexy (○——○). In the presence of 2nd layer spermatids, values in apical and mid cytoplasm were high and low in basal cytoplasm at stages VII and VIII, similar to normal adults. In the absence of 2nd layer spermatids, the Golgi area was located in the basal cytoplasm throughout the cycle, with a slight abundance in the mid cytoplasm at stages VII and VIII. A, B, and C: apical, mid, and basal cytoplasm, respectively. Large asterisks indicate a significant difference ($p < 0.05$) between the tubules with 2nd layer spermatids (normal adult testes and testes at 50 days after orchiopexy) and the tubules without 2nd layer spermatids (testes at 35 days after orchiopexy) at each stage. A small asterisk at stage VIII in (A) indicates a significant difference ($p < 0.05$) between tubules at 35 and 50 days after orchiopexy.

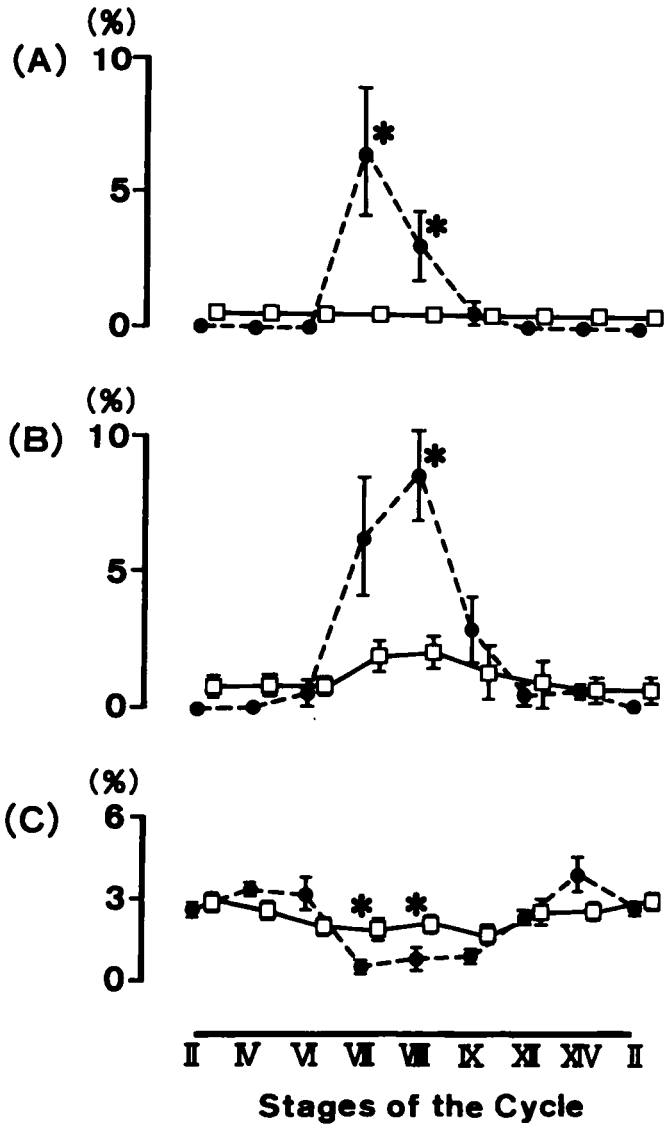

FIG. 3. Morphometric analysis on the relative volume of Golgi area in young adult testes (□——□) in comparison with normal adult testes (●-----●). The localization pattern in young adult testes was similar to that in the testes at 35 days after orchiopexy, as shown in Figure 2. A, B, and C: apical, mid, and basal cytoplasm, respectively. Asterisks indicate a significant difference ($p < 0.05$) at each stage.

in the basal cytoplasm, except for a slight abundance in mid cytoplasm at stages VII and VIII (solid line in Fig. 2). Fifty days after orchiopexy, seminiferous tubules contained a full population of germ cells (Fig. 4D), similar to normal adult testes. A survey of the fine structure and morphometric analysis revealed that the Golgi apparatus in these tubules changed in intracellular localization during the cycle, i.e., at stages VII–IX, there was predominant localization in mid and apical cytoplasm with a decrease in the basal cytoplasm, similar to the localization pattern in normal adult rats (dotted thick line in Fig. 2, and Fig. 5, C and D).

Young Adult Testes

Only seminiferous tubules containing numerous 1st layer spermatids and lacking 2nd layer spermatids were examined. The most prominent difference from tubules containing 2nd layer spermatids in normal adult testes was a lack of alteration of intracellular localization of the Golgi apparatus during the cycle (Fig. 3). Many other organelles showed a distribution pattern similar to that in normal adult

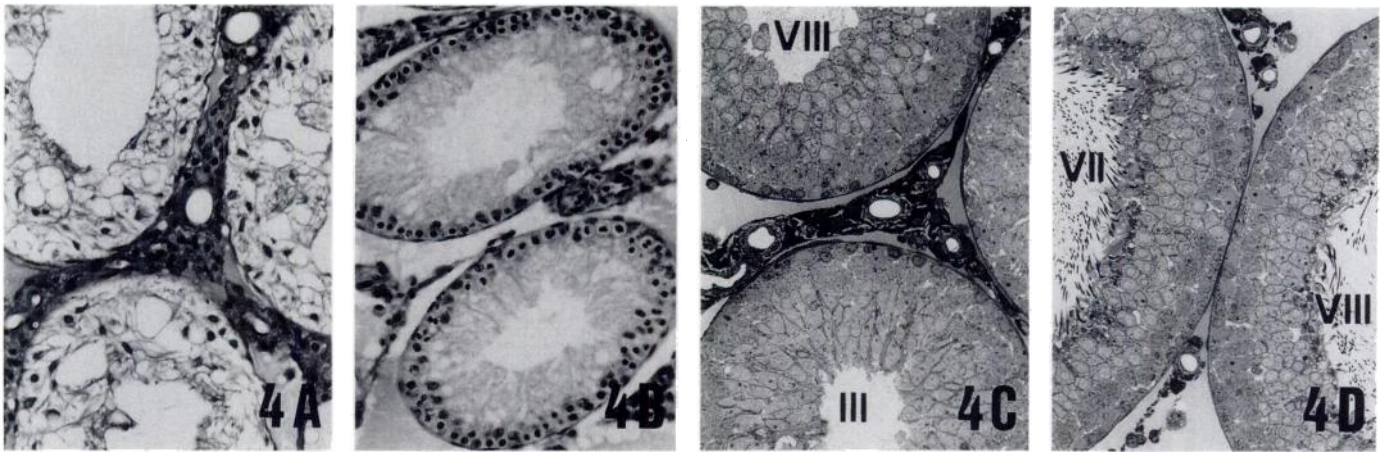

FIG. 4. Light micrographs showing regeneration of germ cells after orchiopexy following cryptorchidism. A: At 60 days of cryptorchidism, seminiferous tubules consisted of Sertoli cells and only a few spermatogonia. B: At 15 days after orchiopexy, tubules contained Sertoli cells, spermatogonia, and primary spermatocytes. C: At 35 days after orchiopexy, germ cells regenerated up to 1st layer spermatids. D: At 50 days after orchiopexy, tubules showed a full recovery of spermatogenesis. The stages of the seminiferous epithelial cycle are labeled. × 180.

testes (data not shown). Morphometric analysis revealed the Golgi area to be localized in the basal cytoplasm throughout the seminiferous epithelial cycle, with a slight abundance in the mid cytoplasm at stages VII and VIII (solid line in Fig. 3). This localization pattern coincided with that in testes 35 days after orchiopexy, when 2nd layer spermatids had not yet regenerated.

DISCUSSION

Sertoli cells, as nourishing and sustentacular cells, facilitate maturation of germ cells. On the other hand, their function, and possibly their morphology, is affected by specific generations of germ cells, as cited in the introduction [13, 14]. The present study demonstrated that the intracellular localization of Golgi apparatus changed during the seminiferous epithelial cycle, and that the change in localization seemed to be regulated by 2nd layer spermatids. We found previously that in normal adult testes the Golgi apparatus of Sertoli cells is localized exclusively in the basal cytoplasm at stages other than VII–IX; at stages VII–IX, the Golgi apparatus increases remarkably in relative volume within the mid and apical cytoplasm, with a concomitant decrease in the basal cytoplasm [12]. The present study revealed that the Golgi apparatus was localized in the basal cytoplasm throughout the cycle in young adult testes without 2nd layer spermatids. In the absence of step 19 spermatids at 35 days after orchiopexy following cryptorchidism in adult rats, the Golgi apparatus of Sertoli cells at stages VII–IX was mostly localized in the basal cytoplasm, similar to other stages. However, step 19 spermatids reappeared at 50 days after orchiopexy and the same cyclic changes in localization occurred as in normal adult rats.

The above findings indicate that cyclic changes in the intracellular localization of the Golgi apparatus in Sertoli cells are closely correlated with changes in adjoining germ cells. There are two interpretations that support a functional significance of this phenomenon. First, it seems that the Golgi apparatus appears in mid and apical cytoplasm at stages VII–IX to synthesize glycoproteins in response to needs of plasma membrane and lysosomes. Formation of the tubulobulbar complex between Sertoli cells and mature spermatids just before spermiation [16] and phagocytosis of large residual bodies of mature spermatids appear to stimulate the Sertoli cells to increase the plasma membrane covering apical cytoplasm. Primary lysosomes increase in mid and apical cytoplasm to dispose of the phagocytosed residual bodies, as shown in our previous report [12]. Because the Golgi apparatus is a critical organelle in membrane biogenesis [17], and incorporation of ^{3}H-fucose into Golgi apparatus is followed by that into lysosomes in Sertoli cells [18], the Golgi apparatus may be compelled to synthesize glycoproteins for plasma membrane and lysosomes in these regions. It appears likely that the Golgi apparatus moves upwards at stages VII–IX to meet these needs. This hypothesis proceeds from the following observations. The Golgi apparatus in basal cells of the chick corneal epithelium shift twice from the apical to basal cytoplasm, concurrently with the appearance of an acellular collagenous matrix beneath the epithelium—probably to play a role in the production and discharge of matrical substances [19]. Moreover, when cultured fibroblasts move to fill the empty area produced by an experimental wound, the Golgi apparatus and microtubule-organizing center are located predominantly forward of the nucleus of fibroblasts facing the wound [20].

A second interpretation is that Sertoli cells may nourish germ cells prone to be injured. Russell and Clermont [21] observed an increased degeneration rate of mid-pachytene spermatocytes and spermatids of steps 7 and 19 in hy-

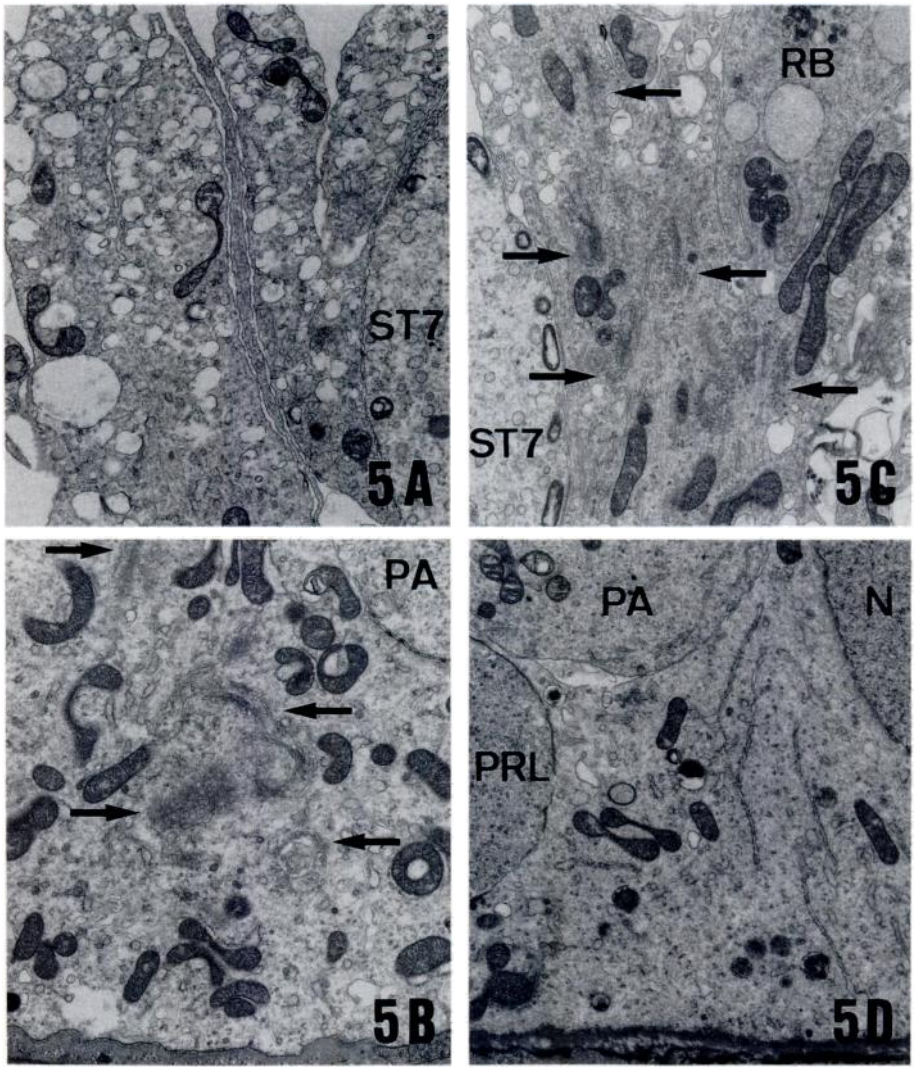

FIG. 5. Electron micrographs of Sertoli cells at stage VII after orchiopexy following cryptorchidism. At 35 days after orchiopexy, Golgi apparatus (arrows) was localized not in the apical (A) but in the basal cytoplasm (B), whereas at 50 days after, it was located in the apical (C) and mid cytoplasm and was scarce in the basal cytoplasm (D). N: Sertoli cell nucleus; PA and PRL: pachytene and preleptotene primary spermatocytes, respectively; ST7: step 7 spermatid; RB: residual body of mature spermatid. × 8 000.

pophysectomized rats. Step 7 spermatids adjoin the mid and apical portions of tall columnar Sertoli cells at stage VII, and step 19 spermatids adjoin the apical portion at stages VII and VIII, although pachytene spermatocytes are in contact with the basal part of the Sertoli cells. In the present study, seminiferous tubules containing spermatids of steps 7 and 19 showed a remarkable increase in the volume of the Golgi area in mid and apical cytoplasm of Sertoli cells. Tubules containing step 7 spermatids but not step 19 spermatids in testes of young adults or in testes at 35 days after orchiopexy in mature rats revealed a slight increase in the Golgi area in mid but not apical cytoplasm of Sertoli cells. The proximity of the Golgi apparatus of Sertoli cells to these sensitive germ cells may provide the latter cells with a favorable environment, e.g. by secreting some glycoproteins.

At first, we wondered if Sertoli cells in tubules that had once undergone spermiation might have acquired a chronological rhythm that would impose cyclic changes in the intracellular localization of the Golgi apparatus. Results indicate that the Sertoli cells did not acquire such a rhythm. The duration of one cycle from stage I to stage XIV is 13 days. The change in localization of the Golgi apparatus was not observed even at 35 days after orchiopexy.

The recovery of impaired function by Sertoli cells after orchiopexy following cryptorchidism has been reported to be poor. Levels of androgen binding protein in efferent ducts are low even at 100 days after orchiopexy following 10 days of cryptorchidism in mature rats [22], although no significant decrease has been observed in immature rats treated with a similar procedure [23, 24]. In the present study, or-

chiopexy following cryptorchidism resulted in poor recovery of spermatogenesis, as occurs in rats [22], mice [25], and lambs [26]. Spermatogenesis was restored in only one fourth of the seminiferous tubules, with 1st layer spermatids reappearing 35 days after orchiopexy and 2nd layer spermatids 50 days after orchiopexy following 60 days of cryptorchidism. In Sertoli cells, the cyclic changes in localization of the Golgi apparatus were restored—albeit in a limited number of seminiferous tubules—50 days after orchiopexy.

REFERENCES

1. Orth JM, Gunsalus GL, Lamperti AA. Evidence from Sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. Endocrinology 1988; 122:787–794.
2. Tres LL, Kierszenbaum AL. Viability of rat spermatogenic cells in vitro is facilitated by their coculture with Seroli cells in serum-free hormone-supplemented medium. Proc Natl Acad Sci USA 1983; 80:3377–3381.
3. Irons MJ. Formation of the flagellum in the rat spermatid. Montreal, PQ: McGill University, 1980. Ph.D. thesis.
4. Parvinen M. Regulation of the seminiferous epithelium. Endocr Rev 1982; 3:404–417.
5. Morales C, Hugly S, Gliswold MD, 1987. Stage-dependent levels of specific mRNA transcripts in Sertoli cells. Biol Reprod 36:1035–1046.
6. Posalaki Z, Szabó D, Bácsi E, Ökrös I. Hydrolytic enzymes during spermatogenesis in rat: An electron microscopic and histochemical study. J Histochem Cytochem 1968; 16:249–262.
7. Chen I-L, Yates RD, 1975. The fine structure and phosphatase cytochemistry of the Golgi complex and associated structures in the Sertoli cells of Syrian hamsters. Cell Tiss Res 157:227–238.
8. Fawcett WD. Ultrastructure and function of the Sertoli cell. In: Astwood EB, Greep RO, Hamilton D (eds.), Handbook of Physiology. Endocrinology V. Washington, DC: American Physiology Society; 1975:21–55.
9. Assaf AA. Cyclic changes of cytoplasmic components in rat Sertoli cells. Montreal, PQ: McGill University; 1980. MSc thesis.
10. Chemes H. The phagocytic function of Sertoli cells: a morphological, biochemical, and endocrinological study of lysosomes and acid phosphatase localization in the rat testis. Endocrinology 1986; 119:1673–1681.
11. Kerr JB. An ultrastructural and morphometric analysis of the Sertoli cell during the spermatogenic cycle of the rat. Anat Embryol 1988; 179:191–203.
12. Ueno H, Mori H. Morphometrical analysis of Sertoli cell ultrastructure during the seminiferous epithelial cycle in rats. Biol Reprod 1990; 43:769–776.
13. Vihko KK, Suominen JJO, Parvinen M. Cellular regulation of plasminogen activator secretion during spermatogenesis. Biol Reprod 1984; 31:383–389.
14. Galdieri M, Monaco L, Stefanini M. Secretion of androgen binding protein by Sertoli cells is influenced by contact with germ cells. J Androl 1984; 5:409–415.
15. Leblond CP, Clèrmont Y, 1952. Definition of the stages of the cycle of the seminiferous epithelium in the rat. Ann NY Acad Sci 1952; 55:548–573.
16. Russel LD. Spermatid-Sertoli tubulobulbar complexes as devices for elimination of cytoplasm from the head region of late spermatids of the rat. Anat Rec 1979; 194:233–246.
17. Farquhar MG, Palade GE. The Golgi apparatus (complex) (1954–1981)—from artifact to center stage. J Cell Biol 1981; 91:77s–103s.
18. Lalli MF, Tang X-M, Clermont Y. Glycoprotein synthesis in Sertoli cells during the cycle of the seminiferous epithelium of adult rats: a radioautographic study. Biol Reprod 1984; 30:493–505.
19. Trelstad RL. The Golgi apparatus in chick corneal epithelium: changes in intracellular position during development. J Cell Biol 1970; 45:34–42.
20. Kupfer A, Louvard D, Singer SJ. Polarization of the Golgi apparatus and microtubule-organizing center in cultured fibroblasts at the edge of an experimental wound. Proc Natl Acad Sci USA 1982; 79:2603–2607.
21. Russell LD, Clermont Y. Degeneration of germ cells in normal, hypophysectomized and hormone treated hypophysectomized rats. Anat Rec 1977; 187:347–366.
22. Jégou B, Laws AO, de Kretser DM. The effect of cryptorchidism and subsequent orchidopexy on testicular function in adult rats. J Reprod Fertil 1983; 69:137–145.
23. Karpe B, Plöen L, Hagenäs L, Ritzén EM. Recovery of testicular functions after surgical treatment of experimental cryptorchidism in the rat. Int J Androl 1981; 4:145–160.
24. Jégou B, Peake RA, Irby DC, de Kretser DM. Effects of the induction of experimental cryptorchidism and subsequent orchidopexy on testicular function in immature rats. Biol Reprod 1984; 30:179–187.
25. Nishimune Y, Aizawa S, Komatsu T. Testicular germ cell differentiation in vivo. Fertil Steril 1978; 29:95–102.
26. Monet-Kuntz C, Barenton B, Locatelli A, Fontaine I, Perreau C, Hochereau-de-Reviers MT. Effects of experimental cryptorchidism and subsequent orchidopexy on seminiferous tubule functions in the lamb. J Androl 1987; 8:148–154.