PERMEABILITY OF SERTOLI CELL TIGHT JUNCTIONS TO LANTHANUM AFTER LIGATION OF DUCTUS DEFERENS AND DUCTULI EFFERENTES

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ABSTRACT

The permeability of Sertoli cell tight junctions to lanthanum administered during fixation has been compared in rats after ligation of the ductus deferens and after ligation of the ductuli efferentes. In both control and vasoligated testes, lanthanum penetrated only short distances into the Sertoli cell tight junctions before stopping abruptly. The tight junction, consisting of numerous pentalaminar fusions of contiguous Sertoli cell membranes, prevented diffusion of lanthanum into the adluminal compartment of the seminiferous epithelium. In rats with ligated ductuli efferentes, lanthanum completely permeated many Sertoli cell tight junctions and occupied intercellular spaces of the adluminal compartment. In spite of their newly acquired permeability to lanthanum, tight junctions retained characteristic ultrastructural features, including numerous membrane fusions. When lanthanum-filled tight junctions were sectioned en face, membrane fusions appeared as pale lines in lakes of electron-opaque tracer. These linearly extensive fasciae occludentes occasionally ended blindly, suggesting that lanthanum may have traversed the junction by diffusing around such incomplete barriers. The increased permeability of Sertoli cell tight junctions after efferent ductule ligation, which caused rapid testicular weight gain followed by atrophy, indicates that tight junctions are sensitive to enforced retention of testicular secretions inside the seminiferous tubules. The apparent normalcy of Sertoli cell tight junctions after vasoligation, which had no effect on testis weight, supports the view that blockage of testicular secretions distal to the epididymis is relatively innocuous.

INTRODUCTION

Tight junctions composed of linear fusions between contiguous cell membranes are the site of an effective barrier to extracellular diffusion in many epithelia (1, 2). These junctions have been implicated in the impermeability of seminiferous tubules to various substances from blood plasma. In the rat, an incomplete system of tight junctions between myoid cells forms a partial permeability barrier to such tracers as ferritin, peroxidase, and lanthanum, while an apparently complete network of Sertoli cell tight junctions blocks all extracellular routes to the lumen of the seminiferous tubule (3, 4). Hence, the ultimate component of the blood-testis barrier is an extensive tight junction that occurs between Sertoli cells and divides the seminiferous epithelium into a basal compartment, containing spermatogonia, and an adluminal compartment, containing spermatocytes and spermatids (4).

The blood-testis barrier preserves a special fluid environment in the adluminal compartment that is uniquely favorable to spermatogenesis (5). A more specific role of the barrier involves isolation of spermatozoal antigen inside the seminiferous tubule and prevention of autoallergic orchitis (6). The resistance of the blood-testis barrier to induced damage is an important factor in shielding the testis from autoimmune disease, yet very little is known of the permeability of the barrier after experimental manipulation of the male reproductive tract.

Previous studies indicate that ligation of the efferent ductules results in rapid elevation of fluid pressure within the seminiferous tubules (7, 5), whereas ligation of the ductus deferens fails to produce such dramatic change (7). These two operations offer an opportunity to determine if enforced retention of secretions inside the seminiferous tubules can affect the blood-testis barrier. The status of the blood-testis barrier after blockage of the ductus deferens is particularly worthy of investigation due to the possibility that autoimmunization with spermatozoa may occur following vasectomy (8–10).

In the present study, the blood-testis barrier of rats was examined following ligation of the testicular excurrent ducts at two points, the ductuli efferentes and the ductus deferens. At intervals after the operation, permeability of Sertoli cell tight junctions to various lanthanum solutions administered during fixation was assessed by electron microscope observation. The distribution of lanthanum observed in operated testes suggested that Sertoli cell junctions become leaky after efferent ductule ligation but remain tight after vasoligation.

MATERIALS AND METHODS

Preliminary Observations

The effects of efferent ductule ligation were initially assessed in 3-mo old Holtzman rats (Holtzman Co., Madison, Wis.) at 1, 2, 3, and 7 days after operation. The efferent ductules of the left testis were ligated in ten rats, two rats were sham operated on the left side, and two remained intact. The testes of these rats were fixed in a solution containing lanthanum hydroxide and were studied in the electron microscope. Later, a lanthanum silicate solution was used in the fixation of testes from eight more Holtzman rats 1 day after unilateral ligation of the efferent ductules and from eight normal Holtzman rats. Observation

of lanthanum distribution in these testes facilitated planning of the experiment described under the following heading.

The permeability of Sertoli cell junctions after ligation of the ductus deferens was initially examined in eight Fisher/DA rats 4 mo after unilateral operation. Lanthanum hydroxide was used in the fixation of these testes as well as testes from four normal Fisher/DA rats, the external controls. Later, testes of ten Holtzman rats were studied 3 mo after unilateral vasoligation; these testes were fixed in a solution containing lanthanum silicate.

Plan of the Experiment

After preliminary observations indicated a change in the permeability of Sertoli cell junctions following efferent ductule ligation but not vasoligation, an experiment was designed to compare simultaneously the effects of the two operations at three postoperative intervals. 30 male Holtzman rats, all born on the same date 3 mo earlier, were obtained from the breeders 1 wk before beginning the experiment. Five of these rats were randomly assigned to each of six groups. Two groups were operated at the beginning of the experiment, one undergoing unilateral vasoligation and one, unilateral ligation of the efferent ductules. 3 wk later, two more groups were operated similarly. The last two groups were operated 24 h before termination of the experiment. The contralateral testis of every rat in the experiment was subjected to the same surgical manipulations as its partner, with the exception of actual ligation, at the time of operation. During the experiment, all rats were housed together in our animal colony. At termination, testes were weighed to the nearest 0.005 g and immersed in aldehyde fixatives containing lanthanum (solutions a or c described below). Testis weight and permeability of Sertoli cell junctions to lanthanum were compared between operated and control testes in each animal and between operated testes of the two groups at each time interval.

Vasoligation

After anesthetization with intraperitoneal injection of chloral hydrate (36 mg/100 g body weight), the vasoligation procedure was performed on the left ductus deferens via scrotal incision. In the experimental groups, a control operation involving all steps except actual ligation was performed on the right ductus deferens. Using sterile technique, the tunica vaginalis was opened and the testis exposed. The ductus deferens was separated from its accompanying artery at a point approximately 5 mm from the cauda epididymis. A 5-0 silk ligature (Ethicon, Inc., Somerville, N. J.) was passed around the ductus deferens and tightly knotted. The incision in the

tunica vaginalis was closed with 3 interrupted sutures of 5-0 chromic gut, and the scrotal incision was closed with 9 mm autoclips. Rats were examined regularly after the operation to ensure that the testes remained in the scrotal position.

Efferent Ductule Ligation

Using chloral hydrate anesthesia and sterile technique, efferent ductule ligation was performed on the left side. In the experimental groups, a control operation involving all steps except actual ligation was performed on the right side. The testis was exposed through incisions in the scrotum and tunica vaginalis, and the caput epididymis was gently retracted from the surface of the testis to expose its connections with that organ. Proceeding from the cranial pole of the testis, these connections are a thickened fold of visceral tunica vaginalis, the efferent ductules, and blood-vascular connections of the testis. The testicular blood vessels were separated from the efferent ductules near their origin at the oval avascular area of the testicular surface, and two ligatures of 5-0 cotton (Ethicon) were passed around the ductules and tightly knotted. In the control operation, the ligatures were removed from the ductules without knotting. Otherwise, manipulation of the blood vessels and ductules were identical in both operations. The testis was returned to its normal position, and the incision in the tunica vaginalis was closed with three interrupted 5-0 chromic sutures. The scrotal wound was closed with 9-mm autoclips. Rats were examined after the operation to ensure that surgically induced cryptorchism did not complicate the results.

Lanthanum Solutions

Initial exposure of the rat testis to lanthanum occurred during fixation. The solutions used were (a) lanthanum hydroxide prepared at pH 7.8 and administered in cacodylate-buffered glutaraldehyde at the same pH, (b) the same solution as (a) but administered at pH 7.3, and (c) lanthanum silicate prepared according to the following description and administered in cacodylate-buffered glutaraldehyde at pH 7.3.

Lanthanum hydroxide (11) was prepared by slowly adding drops of 0.01 N NaOH to 2% $La(NO_3)_3$ (the hexahydrate; Matheson, Coleman and Bell, Cincinnati, Ohio) until the solution reached pH 7.8. This opalescent solution was mixed with an equal volume of cacodylate-buffered glutaraldehyde to make a fixative containing 1% lanthanum and 2% glutaraldehyde (70%; Ladd Industries, Ltd., Burlington, Vt.) in 0.1 M cacodylate at pH 7.8 or 7.3. Lanthanum silicate (J. P. Revel, personal communication) was prepared by mixing equal volumes of 0.1% sodium silicate (the pentahydrate; Matheson,

Coleman, and Bell) and 4% $La(NO_3)_3$. This translucent milky solution was mixed with an equal volume of cacodylate-buffered glutaraldehyde to make the standard fixative at pH 7.3.

Tissue Preparation

Two methods of administration of the lanthanum solutions gave good results as judged by the uniformity of lanthanum distribution in the extracellular spaces of the basal compartment of the seminiferous epithelium. These were perfusion fixation of the intact testis using buffered solutions of glutaraldehyde and lanthanum (4) and immersion fixation of testicular fragments in similar solutions. Since superior results were not noted in the perfused tissue, the simpler technique of fixation by immersion was employed in our experiment.

Fragments of testis containing 2-3 mm segments of seminiferous tubules were immersed in one of the lanthanum solutions and left at room temperature. 5 h later, tissue samples were rinsed in the same buffered lanthanum solution but without glutaraldehyde, where they remained overnight at 4°C. The tissues were postfixed 8 h in 1% OsO_4 in 0.1 M collidine buffer containing 1% of the same lanthanum at the same pH as in previous steps. After overnight staining in 0.5% aqueous uranyl acetate, the tissues were dehydrated in alcohol and propylene oxide and embedded in Epon. Embedded tissue was trimmed to expose cross sections of intact seminiferous tubules approximately midway between their cut ends. This precaution avoided the possibility of encountering luminal deposits of lanthanum that had diffused a short distance into the tubules through their cut ends. Silver to pale gold sections of these tubules were stained lightly with lead citrate and examined in a Zeiss EM9S electron microscope.

RESULTS

Blood-Testis Barrier in Normal Testes

Normal testes included the sham-operated testis in unilaterally vasoligated rats and in rats with unilateral ligation of the efferent ductules as well as testes of unoperated rats. When these testes were fixed in solutions containing lanthanum hydroxide or lanthanum silicate, extracellular distribution of lanthanum confirmed the existence of a partial barrier in the myoid cell layer surrounding the seminiferous tubules (3, 4). Tight junctions between myoid cell processes excluded lanthanum from the seminiferous epithelium in approximately 60% of the tubular cross sections examined in this study. Whenever lanthanum traversed the myoid layer and occupied inter-
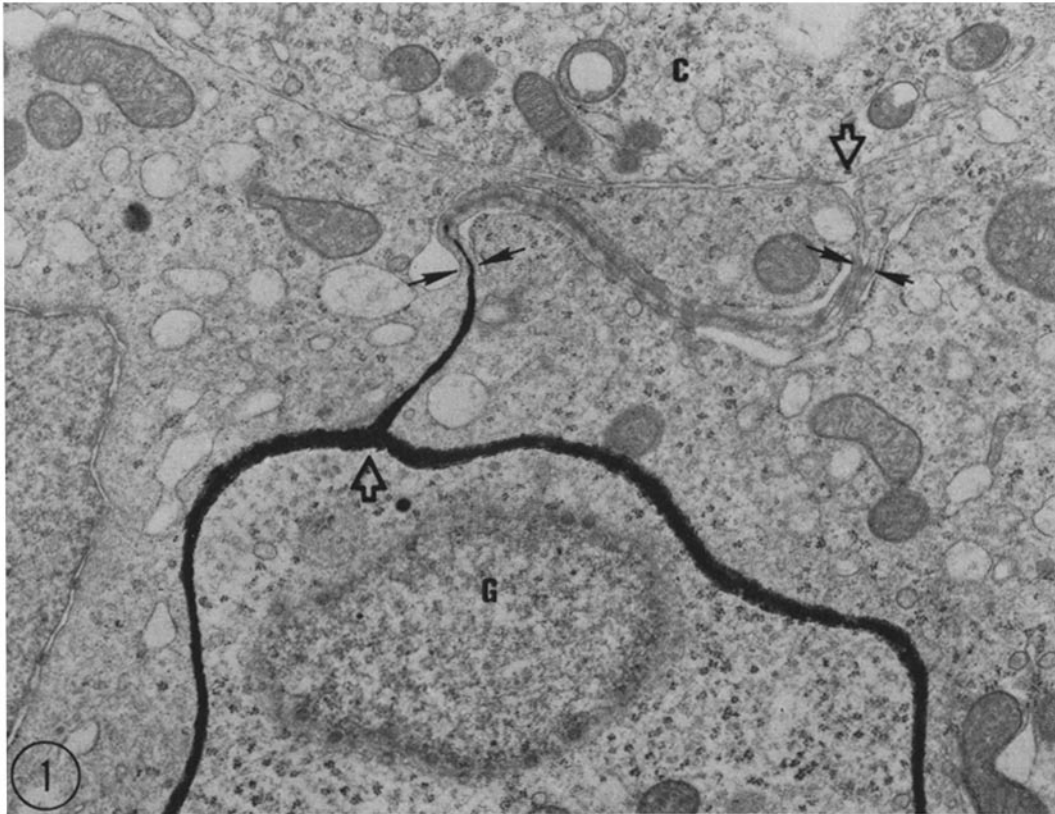
FIGURE 1 A portion of the blood-testis barrier is formed by tight junctions joining adjacent Sertoli cells, seen here in a junctional complex extending between pairs of small arrows. These junctions normally block diffusion through extracellular clefts (between large arrows) that would otherwise connect spaces around spermatogonia and spermatocytes. Here lanthanum has filled extracellular spaces around the spermatogonium (G) and between Sertoli cell processes below the junctional complex. Lanthanum stops abruptly a short distance into the junction, which blocks access to extracellular space around the spermatocyte (C). From the sham-operated testis of a rat with efferent ducules unilaterally ligated 1 day earlier. × 16,500.

cellular spaces at the base of the seminiferous epithelium, its distribution was checked by a more effective barrier formed by tight junctions between Sertoli cells (Fig. 1). Hence, the blood-testis barrier in control testes comprised a partial barrier in the myoid layer and a complete barrier between Sertoli cells (4). Regardless of which lanthanum solution was used, Sertoli cell tight junctions in normal testes showed no indication of permeability to the tracer.

The structure of the barrier between Sertoli cells corresponded to that described earlier in the rat (4). Although the specialized zone of contact between Sertoli cells contains several different junctional types, this study was concerned with the

tight junction. The tight junction, consisting of numerous pentalaminar fusions of continguous cell membranes and intervening intercellular spaces, effectively prevented the transepithelial passage of lathanum in all normal testes.

Blood-Testis Barrier after Vasoligation

Ligation of the ductus deferens had no discernable effect on the distribution of lanthanum in the testis. As in control testes, lanthanum traversed the myoid cell layer in less than half of the tubular cross sections studied. Once inside the myoid layer, lanthanum filled intercellular clefts at the base of the seminiferous epithelium, but its diffusion to-
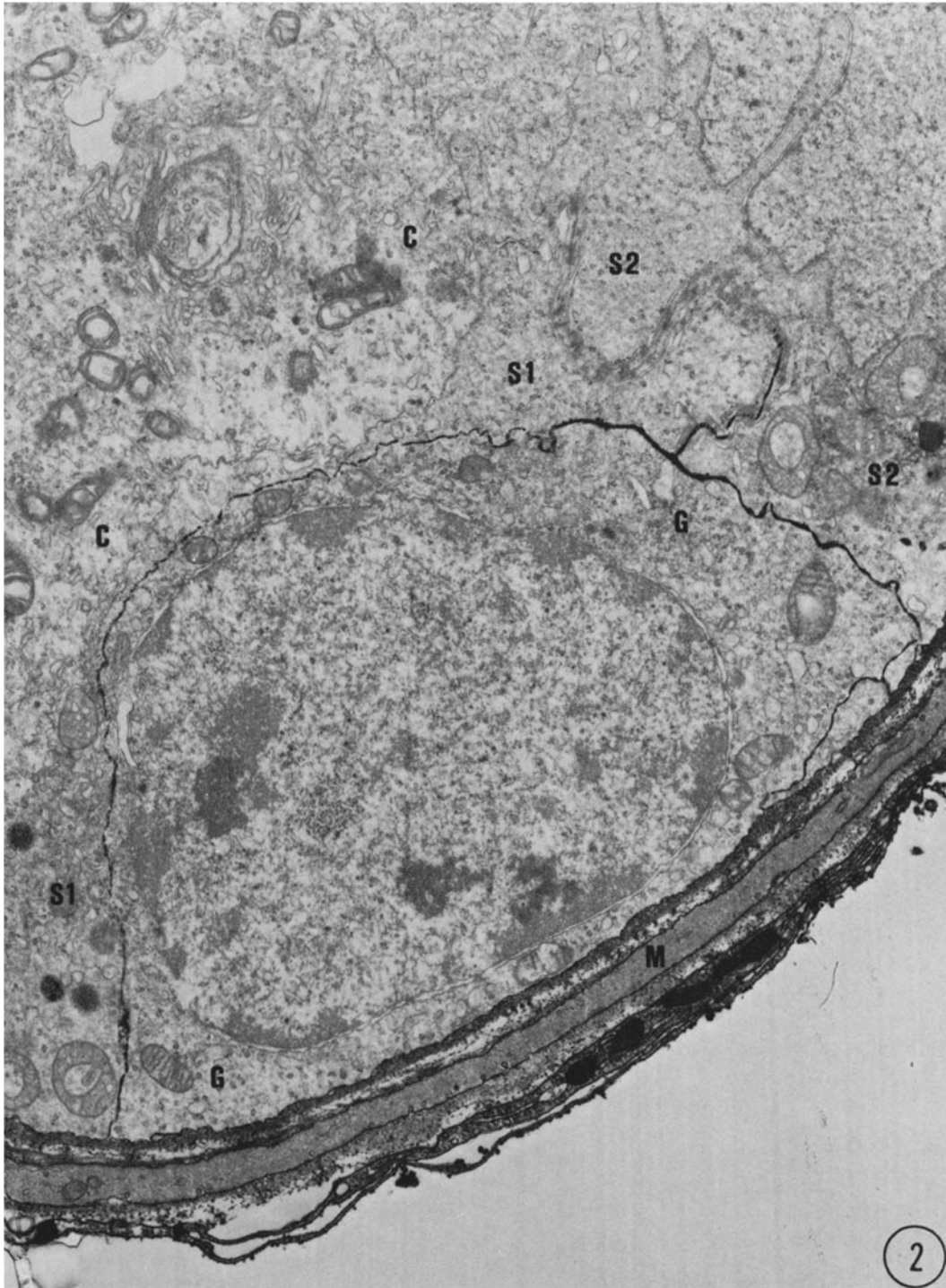
FIGURE 2 Following vasoligation, lanthanum continues to be excluded from the adluminal compartment of the seminiferous epithelium. The ultimate component of the blood-testis barrier occurs in a zone of specialized contact between two Sertoli cells (S1 and S2). Electron-opaque lanthanum, which has filled the intercellular cleft around the spermatogonium (G), extends only a short distance into the contact zone, where a tight junction stops the tracer before it reaches clefts around the spermatocyte (C). An incomplete component of the blood-testis barrier occurs between myoid cells (M) and is also unaffected by vasoligation. From the testis of a rat vasoligated 4 mo earlier. × 12,000.

TABLE I

Comparison of Body, Testis, and Epididymis Weights in Rats at Three Intervals after either Ductus Deferens Ligation (DDL) or Efferent Ductule Ligation (EDL)

Experimental group*	Body weight	Testis weight		P‖	Epididymis weight		P
		Right‡	Left§		Right	Left	
	g	g			g		
DDL One Day	473±34¶	1.95±0.12	1.92±0.11	>0.70	0.64±0.03	0.75±0.03	<0.001
EDL One Day	457±15	1.92±0.11	2.42±0.09	<0.001	0.64±0.03	0.70±0.07	>0.10
DDL One Week	470±19	1.82±0.11	1.94±0.13	>0.10	0.61±0.05	0.87±0.32	>0.10
EDL One Week	476±17	2.01±0.11	1.49±0.06	<0.001	0.68±0.05	0.56±0.02	<0.001
DDL One Month	483±32	1.80±0.13	1.86±0.09	>0.30	0.60±0.07	0.61±0.06	>0.80
EDL One Month	491±26	2.09±0.18	1.15±0.12	<0.001	0.64±0.05	0.42±0.04	<0.001

* Five rats per group.
‡ Sham-operated side.
§ Ligated side.
‖ Probability that the sample means are not different by Gosset's t test.
¶ Mean ± standard deviation.

ward the lumen was checked by Sertoli cell tight junctions (Fig. 2). Frequently, lanthanum penetrated a short distance into the tight junction, filling intercellular spaces between sites of membrane fusion before stopping abruptly. Discontinuities in the terminal distribution of lanthanum, caused by exclusion of the tracer at sites of membrane fusion, were particularly visible in areas where the plane of section passed obliquely through the membranes of the junction. Limited penetration of the basal aspect of Sertoli cell tight junctions by lanthanum was commonly observed in control testes as well. With respect to structural integrity and lanthanum permeability, neither the partial barrier in the myoid layer nor the complete barrier betweeen Sertoli cells was altered by vasoligation.

Gravimetric comparison of vasoligated and control testes was consistent with the observed normalcy of the blood-testis barrier after blockage of the ductus deferens (Table I and Fig. 3). Vasoligated testis weight was not significantly different from that of sham-operated controls at any interval after operation. However, epididymis weight was elevated by 24 h after vasoligation and rose even higher in some rats operated 1 wk earlier. Increased variance in epididymis weight 1 wk after vasoligation reflected a tendency for some rats to have a near-normal epididymis, while in others, it was about twice its usual size. Rats with large epididymides had not yet formed a sperm cyst at the site of ligation, while the others showed small cysts averaging 0.15 g each. By 1 mo after vasoligation, all rats had normal epididymides,
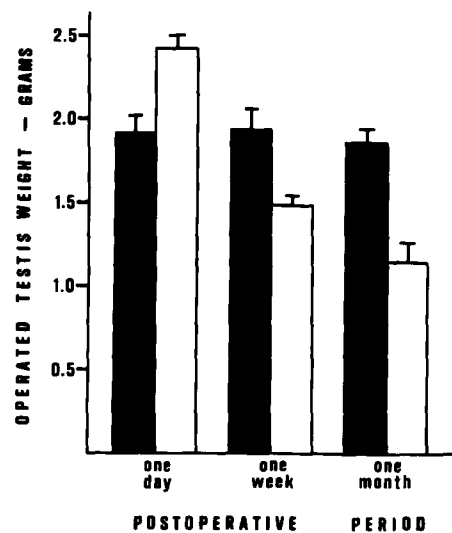

FIGURE 3 Mean testis weight on the operated side is displayed for each experimental group at three intervals after surgery. Solid bars represent vasoligated groups while open bars represent groups with ligated efferent ductules. Brackets indicate one standard deviation above the mean. See Table I for details.

and the sperm cysts averaged 0.46 g each. The epididymis remained normal in rats operated for longer periods, and the sperm cysts did not become larger, although their contents became less watery and more compact. Enlargement of the epididymis soon after operation followed by formation of a sperm cyst at the ligature verified effective blockage of the ductus deferens in these studies.
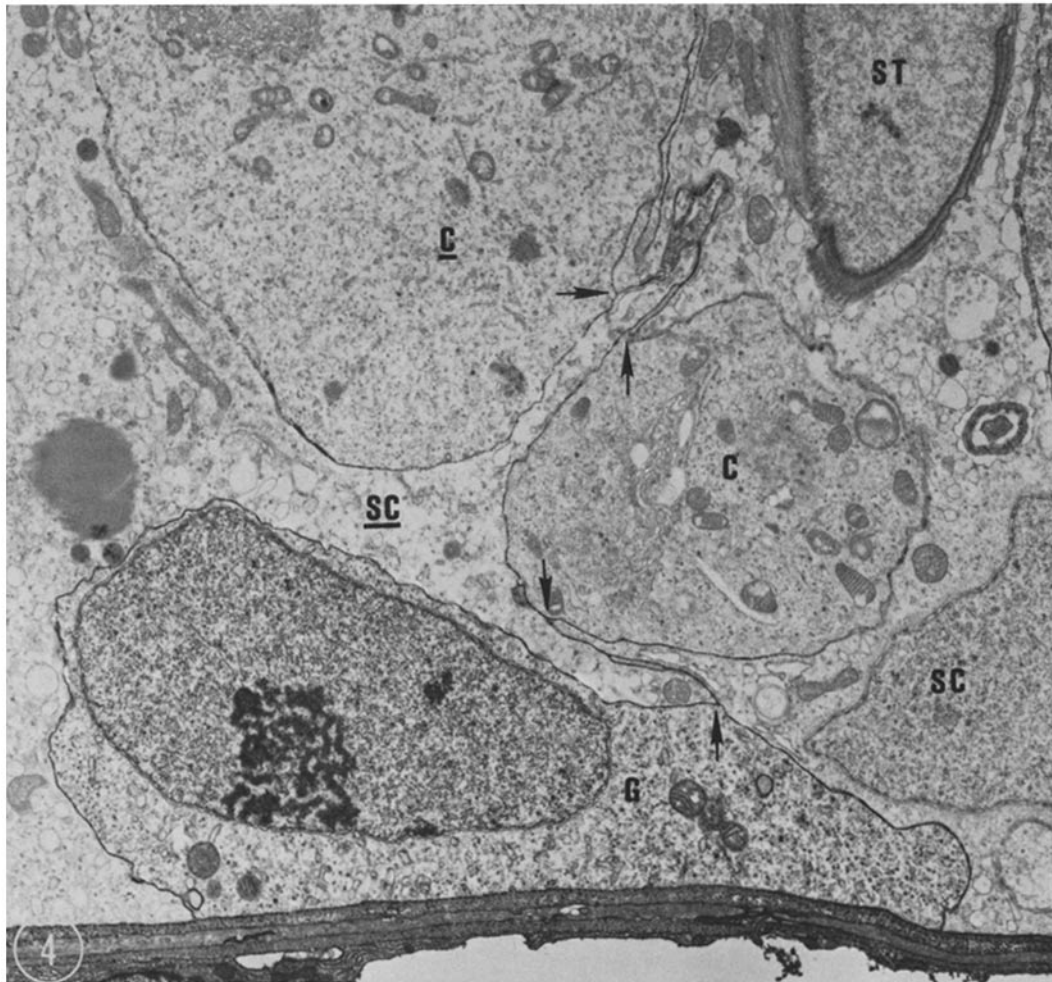
FIGURE 4 Within a day of efferent ductule ligation, the blood-testis barrier becomes leaky to lanthanum at many points along the seminiferous tubules. In addition to its usual presence around a spermatogonium (*G*), lanthanum penetrates junctional complexes (between pairs of arrows) of adjacent Sertoli cells (*SC* and *SC*) and fills extracellular space around spermatocytes (*C* and *C*). Some distortion of the seminiferous epithelium, presumably due to intralumenal pressure, is evident in the tubule. Note the spermatid (*ST*) intruding in the lower level of the epithelium. From a rat testis 1 day after efferent ductule ligation. × 6,300.

Blood-Testis Barrier after Ligation of Efferent Ductules

In the postoperative periods studied, lanthanum crossed the myoid cell layer of most seminiferous tubules in testes with ligated efferent ductules. In many tubular profiles examined at each time interval, lanthanum also permeated the Sertoli cell tight junctions and entered the adluminal compartment of the seminiferous epithelium (Figs. 4–6). Leaky tight junctions were observed after efferent ductule ligation regardless of which lanthanum solution was used during fixation.

Although permeable to lanthanum, Sertoli cell tight junctions retained their characteristic ultrastructural features. Cytoplasmic specializations that normally parallel the junction, including cisternae of endoplasmic reticulum and cytoplasmic filaments, persisted after the operation (Figs. 6 and 7). Numerous fusions of contiguous cell mem-
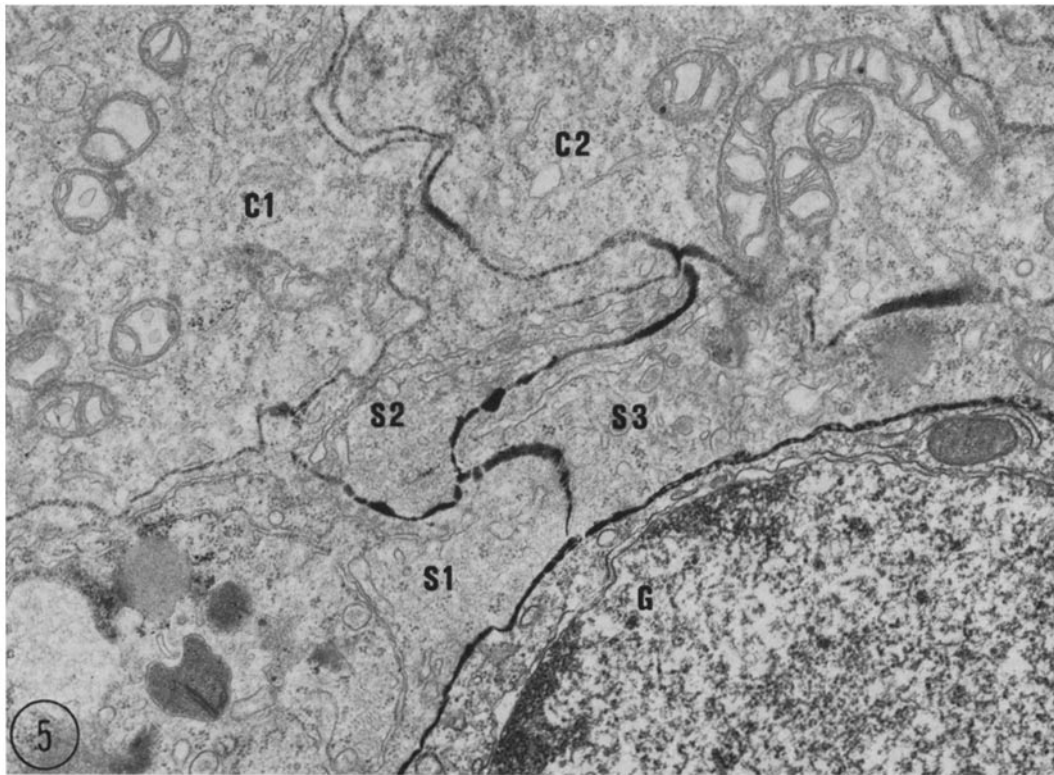
FIGURE 5 Following efferent ductule ligation, lanthanum is no longer confined to the basal compartment of the seminiferous epithelium. In addition to its usual presence between Sertoli cells (*S1* and *S3*) and spermatogonia (*G*), lanthanum extends beyond the blood-testis barrier into spaces between Sertoli cells (*S1*, *S2*, and *S3*) and spermatocytes (*C1* and *C2*). From a rat testis 1 day after efferent ductule ligation. × 18,000.

branes continued to appear in the junction (Fig. 7). The only apparent difference in the tight junction after efferent ductule ligation was its complete permeation by lanthanum, which filled all extracellular spaces in the junction. Although lanthanum traversed the tight junction, it continued to be excluded from the actual sites of membrane fusion. In areas of the tight junction that were sectioned obliquely, the dark strip of lanthanum in the intercellular cleft was repeatedly divided by light crossbands caused by exclusion of lanthanum at fusions of contiguous Sertoli cell membranes (Figs. 6 and 7). When tight junctions permeated with lanthanum were sectioned *en face*, it became evident that membrane fusions extended linearly over the contiguous cell surfaces (Figs. 8–10). Lanthanum in extracellular spaces within the junction demarcated membrane fusions, which appeared as pale lines in lakes of electron-opaque tracer. These pale

lines were considered to correspond to the fasciae occludentes of freeze-fractured tight junctions (2). In parts of the junction, linear membrane fusions appeared in parallel array and were aligned with bundles of filaments in Sertoli cell cytoplasm (Fig. 8).

The extent of linear membrane fusions was difficult to assess in this material, but it was apparent that some branching or joining occurred (Fig. 8), while other fusions appeared to end blindly (Figs. 9 and 10). If linear membrane fusions ultimately ended blindly in testes with ligated efferent ductules, lanthanum may have traversed the tight junction by diffusing around the ends of these fasciae occludentes. Unfortunately, it was impossible to compare the incidence of blind-ending fusions in control and vasoligated testes due to the very restricted extent of lanthanum penetration into these junctions.

The consequences of efferent ductule ligation were reflected in testicular weight changes as well. Preliminary observations indicated a 20–30% rise in testis weight within 24 h of operation. Testis weight remained elevated through the third postoperative day but dropped 20–30% by the end of the first week. The experimental groups confirmed the initial rise and subsequent fall of testis weight (Table 1 and Fig. 3). By 1 mo after operation, testis weight had declined almost 50%. During the period of elevated weight, operated testes were obviously swollen. In spite of their turgidity, these testes appeared well vascularized. Small testes at longer intervals were distinctly flaccid. Epididymis weight was unchanged 24 h after the operation, slightly depressed at 1 wk, and significantly reduced at 1 mo.

DISCUSSION

Ligation of the efferent ductules causes rapid changes in the testis, with increased testis weight occurring within 12 h of operation (7). For more than 24 h after efferent ductule ligation, testis weight increases at about the rate that fluid is normally secreted by the seminiferous tubules (5). Swelling of the tubules is proportional to testicular weight gain (7), suggesting that the increase in testis weight is caused by accumulation of testicular fluid secretion inside the seminiferous tubules. Failure of the blood-testis barrier to block lanthanum diffusion after efferent ductule ligation may also be related to intratubular accumulation of testicular fluid. Leakiness develops in the barrier as soon after operation as the occurrence of previously reported changes in testis weight and tubule diameter. Other rapidly acting but nonspecific influences of the operation on the blood-testis barrire include such possibilities as local inflammation following surgery and temporary interference with blood flow. These alternative mechanisms of change are unattractive due to the normalcy of the blood-testis barrier after sham operation and to the absence in all cases of widespread structural deterioration that might be attributed to vascular insufficiency.

While efferent ductule ligation is very effective in eliciting change in the testis, vasoligation appears to be relatively innocuous. Up to 90 days after unilateral vasoligation, Smith (7) found no difference in weight, in seminiferous tubule diameter, or in spermatogenesis between operated and contralateral testes. Other workers have reported no change in the rat testis after various vasectomy procedures (12–15), while some have described conflicting results (16–20). At intervals between 1 day and 4 mo after vasoligation, the rats in the present study showed no change in testis weight or in permeability of the blood-testis barrier. The absence of any indication of elevated intratubular pressure in vasoligated testes is probably due to resorption of testicular fluid secretions by the intact efferent ductules and epididymis (5).

If increased intratubular fluid pressure following efferent ductule ligation is causally related to leakiness of the blood-testis barrier, it may appear paradoxical that the barrier became permeable to lanthanum by 24 h while fluid pressure, as indicated by increased testis weight, remained elevated at 72 h after operation. Resolution of this dilemma requires quantitative assessment of experimentally induced permeability, which lanthanum distribution cannot provide. However, it is conceivable that the barrier acquires sufficient permeability to dissipate additional testicular fluid secretions when a certain intratubular pressure is attained.

Considering the reported weakness of the blood-testis barrier in the rete testis (6), it is surprising that efferent ducutle ligation can raise fluid pressure beyond the rete into the seminiferous tubules. Nevertheless, efferent ductule ligation does elevate intratubular pressure, as shown by increased testicular weight and seminiferous tubule diameter (7), and also affects the relatively strong blood-testis barrier in the tubules, as indicated by lanthanum permeability in this study.

In using lanthanum to monitor the permeability of the blood-testis barrier, one must consider that the manner in which the tracer is administered to the tissue may determine whether it penetrates tight junctions. For example, Machen et al. (21) observed that administration of ionic lanthanum to gallbladder and intestine in vitro resulted in penetration of the so-called "tight" junctions by the tracer. Noting that colloidal lanthanum added during fixation does not penetrate tight junctions of liver (22) whereas it does pass through these junctions when administered to living animals (23, 24), Machen et al (21) concluded that ionic and colloidal lanthanum may give similar results if the tissues are incubated with the tracer before fixation. Hence, if lanthanum is used to assess the tight junction's role as a barrier to extracellular diffusion across an epithelium, it is important that both control and experimental tissues be identically
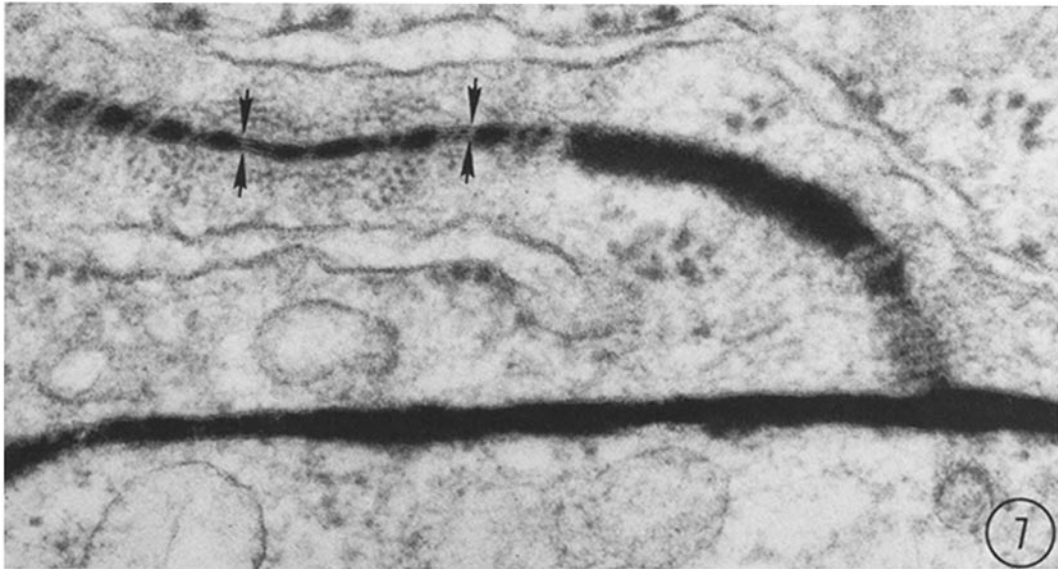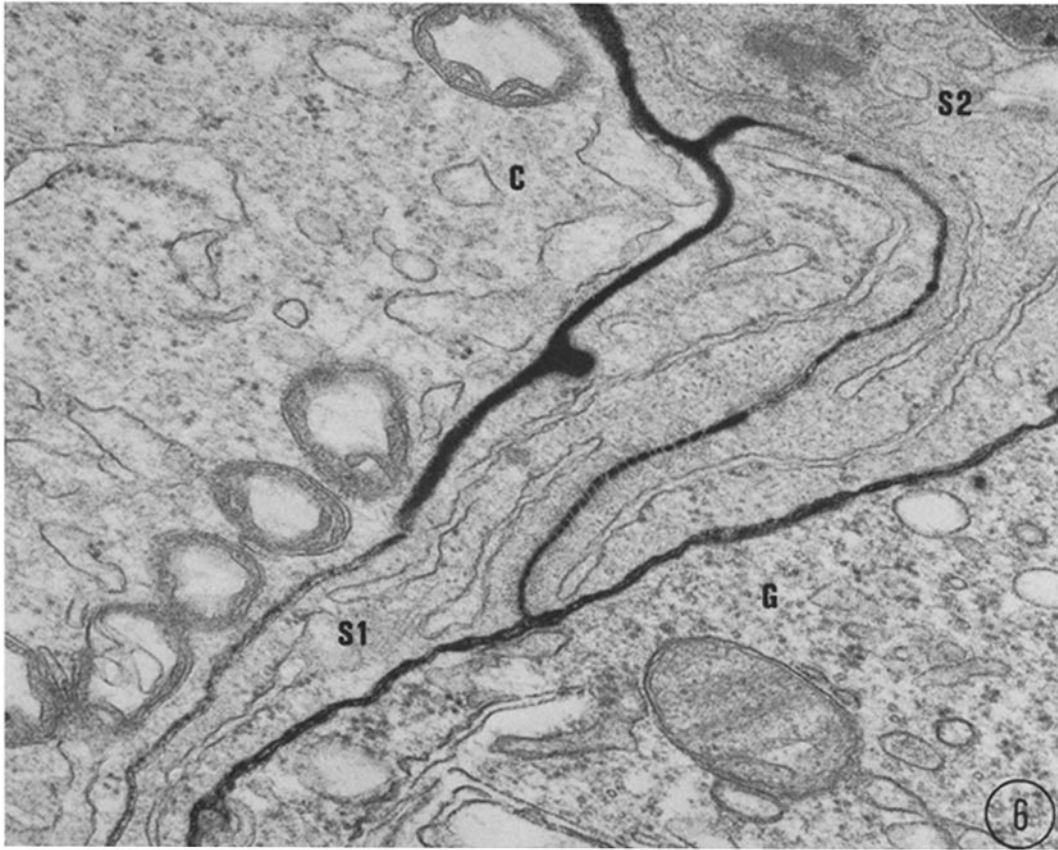
exposed to appropriate lanthanum solutions at the time of fixation.

It is possible that electron densities observed after fixation in lanthanum solutions represent staining of cell surfaces (25) rather than, or in addition to, persistence of precipitates in the intercellular spaces (11). Be this as it may, the distribution of electron densities after administration of lanthanum during fixation consistently suggests that tight junctions normally block the access of this tracer, or stain, to certain extracellular spaces, and thus, to certain cell surfaces.

Judging from previous reports, the pH of the lanthanum-containing fixative may not be critical. For example, Revel and Karnovsky (11) found no lanthanum in tight junctions exposed to a lanthanum-osmium-collidine solution at pH 7.2. They noted that this fixative, made with an aliquot of lanthanum nitrate solution brought to pH 7.6–7.8, remained cloudy at pH 7.2, indicating that colloidal lanthanum hydroxide persisted at the lower pH. This is not surprising since lanthanum hydroxide, once formed in an aqueous solution, is nearly quantitatively insoluble at neutral pH (26). Brightman and Reese (27), using a solution of lanthanum nitrate at pH 7.7 mixed with an equal volume of aldehyde fixative buffered at pH 7.1, also found that tight junctions excluded lanthanum. Goodenough and Revel (22), with a mixture of lanthanum nitrate and aldehyde fixative buffered to pH 7.7, showed again that tight junctions are normally impermeable to lanthanum. Our observations of tight junctions between Sertoli cells indicate that their permeability to lanthanum is similar regardless of whether the fixative solutions are buffered at pH 7.3 or 7.8.

As seen in thin section, the Sertoli cell tight junction is a series of pentalaminar fusions much like those between contiguous cell membranes in tight junctions of other tissues (2). When tight junctions in other tissues are freeze-fractured, they show numerous ridges and furrows that correspond to membrane fusions and which are termed fasciae occludentes (2). Fasciae occludentes have not been demonstrated previously in the Sertoli cell tight junction, but they have been assumed to exist on the grounds that only such linearly extensive membrane fusions could effectively block extracellular diffusion (4). In the present study, en face sections of lanthanum-permeated tight junctions have provided negative images that correspond closely to the fasciae occludentes seen in freeze-fractured tight junctions in the epididymis (2). The similarity of epididymal and testicular tight junctions, composed of long fasciae occludentes in parallel array, may be related to the common role of these junctions in forming a strong immunological barrier between sperm antigens and the host environment (2, 28).

The functional significance of the blood-testis barrier has been related to the provision of a special fluid environment for spermatogenesis (5). A more specific role assigned to the barrier is immunological isolation of the alien antigens of developing spermatozoa (6). Weakening the barrier has led to autoimmune lesions of the testis (29), which suggests that similar lesions could occur in animals with ligated efferent ductules. In fact, degeneration of germ cells in rats 1 mo after efferent ductule ligation (7) closely resembles the pattern of damage to spermatogenic cells in autoallergic aspermatogenesis (29).

A surprising finding in the testes with ligated efferent ductules was the virtual absence of structural change in the Sertoli cell tight junctions as seen in thin sections in spite of their abnormal

FIGURE 6 In spite of its abnormal permeability to lanthanum, the Sertoli cell tight junction retains characteristic structural features. In this junction extending from spermatogonium (G) to spermatocyte (C), cisternae of endoplasmic reticulum are aligned in the usual manner alongside the participating membranes of contiguous Sertoli cells (S1 and S2). Membrane fusions, indicated by fine discontinuities in the lanthanum within the junction, continue to occur (see also Fig. 7). From a rat testis 1 day after efferent ductule ligation. × 43,000.

FIGURE 7 Even after the tight junction becomes permeable to lanthanum, pentalaminar fusions (between arrows) persist throughout the junctional zone. In portions of the junction where participating membranes are sectioned obliquely, the fusion sites, which exclude lanthanum, appear as light lines. Cross sections of filaments occur as usual in Sertoli cell cytoplasm adjacent to the tight junction. From a rat testis 2 days after efferent ductule ligation. × 126,000.
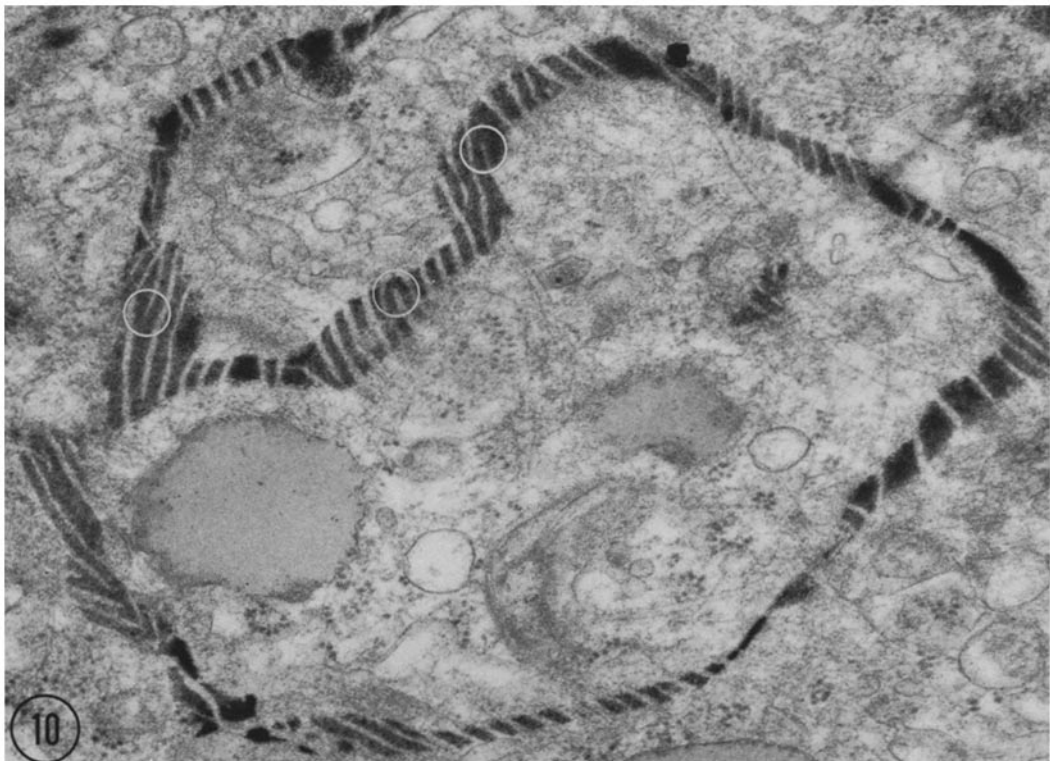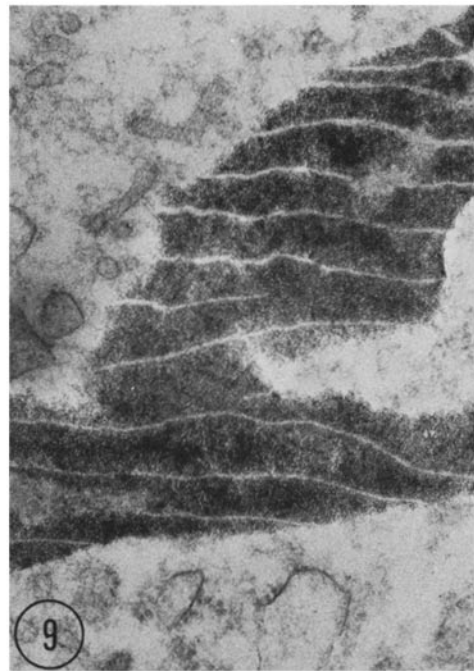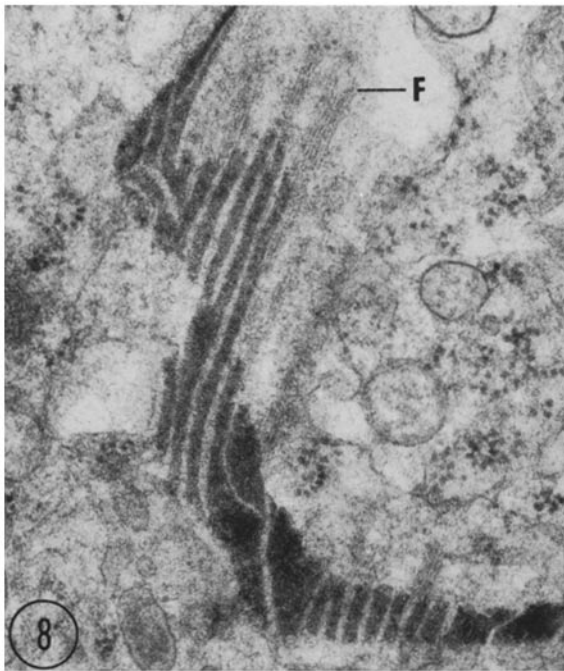
permeability to lanthanum. A critical but very subtle change in these junctions could have involved an increased incidence of free-ending fasciae occludentes. Such an occurrence might have permitted lanthanum to circumvent the bands of obliterated extracellular space and traverse the entire junction. Unfortunately, detection of free-ending fasciae occludentes in this study required thorough penetration of the junction by lanthanum, a condition that did not occur in normal testes, thereby preventing an assessment of change after efferent ductule ligation. Freeze-fracturing would be necessary to determine the incidence of free-ending fasciae occludentes in normal Sertoli cell tight junctions.

The high degree of structural integrity in Sertoli cell tight junctions after the relatively damaging efferent ductule ligation points to the remarkable resistance of the blood-testis barrier to induced change. Experimental weakening of this barrier has previously relied on such drastic procedures as mechanical trauma of the seminiferous tubules and pharmacological induction of testicular ischemia (28). Even an immunological assault as vigorous as isoimmunization with testis in adjuvant results in only partial and temporary damage to the tubule barrier in guinea pigs (30). Less severe immunization resulting from exposure to sperm antigens after unilateral vasectomy has no effect on fertility in rats (19), suggesting that the blood-testis barrier protects developing germ cells from circulating spermagglutinins. The present study has shown that Sertoli cell tight junctions remain impermeable to lanthanum after vasoligation, a procedure which apparently fails to produce the physical or immunological impact necessary to alter the blood-testis barrier.

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FIGURE 8   When tight junctions permeated with lanthanum are sectioned in the plane of the participating membranes, membrane fusions appear as electron-translucent lines in lakes of opaque tracer. The extent of these lines indicates that membrane fusions travel long distances over the surfaces of contiguous Sertoli cells. Cytoplasmic filaments (F) associated with the tight junction appear in longitudinal array. From a rat testis 1 wk after efferent ductule ligation. × 43,000.

FIGURE 9   When lanthanum-filled tight junctions are exposed by en face sectioning, it is apparent that some electron-translucent lines end blindly. From a rat testis 1 mo after efferent ductule ligation. × 43,000.

FIGURE 10   When larger areas of lanthanum-filled tight junctions are exposed, numerous blind-ending lines (circles) become evident. These discontinuities in the network of membrane fusion may facilitate permeation of the tight junction by lanthanum. From a rat testis 1 wk after efferent ducutle ligation. × 38,000.

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