Urol Res (1982) 10:97–104

Urological Research

BCG-induced Orchitis: Structural Changes During the Degeneration of Seminiferous Tubules of Rats and Rabbits

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Accepted: January 26, 1982

Summary. The effect of BCG-induced orchitis on the structure of the seminiferous tubules in rats and rabbits was investigated by light and electron microscopy. The formation of cavities between Sertoli cells and the displacement of the cells of the spermatogenic cycle are the earliest changes to be observed. Individual Sertoli cells degenerate and separate from spermatocytes and spermatids. The latter form multinuclear complexes by a broadening of the intercellular bridges. The nuclei of spermatids undergo ring-like chromatin condensation in the rat and swelling in the rabbit. After the loss of spermatocytes and spermatids from the germinal epithelium, the remaining Sertoli cells have a very irregular shape and contain many residual bodies, which are probably derived from previously phagocytosed spermatids. They often contain crystalline inclusions. The nuclei of Sertoli cells show small chromatin condensations. In the rabbit, the tubular wall increases considerably in diameter. In the vicinity of a granuloma in the interstitium caused by BCG inflammatory cells accumulate around the wall of the seminiferous tubules. Although the basal lamina seems to be an obstacle, penetration of macrophages into the tubular lumen could be observed. However, this occurred only after the degeneration of the germinal epithelium.

Key words: Orchitis (induced), BCG, Germinal epithelium, Sertoli cell degeneration, Multinuclear spermatids.

Introduction

Talwar [16] reported a method for the reversible inhibition of spermatogenesis by intratesticular injection of bacterium Calmette-Guerin (BCG). The rationale behind Talwars proposition was to weaken the blood-testis barrier and thus give immunocompetent cells access to the tubular lumen.

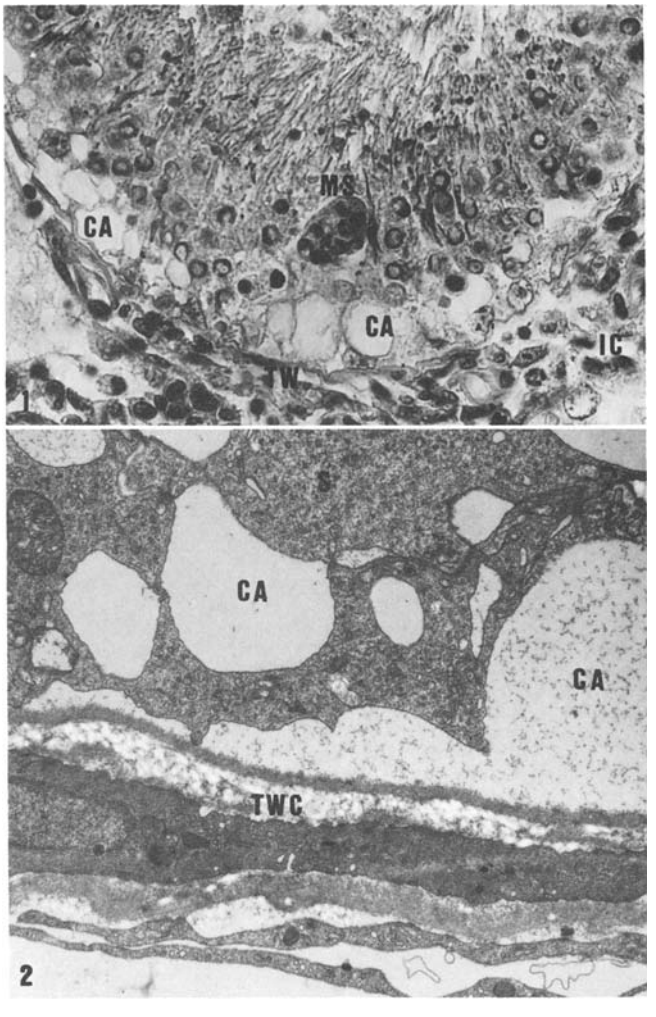

Fig. 1. Disorganization of the germinal epithelium and formation of cavities between Sertoli cells (*CA*) cap beginning of formation of multinuclear spermatids (*MS*). Inflammatory cells (*IC*) close to the tubular wall. Rat 3 days, magnif. x447

Fig. 2. Cavities (*CA*) between basal parts of Sertoli cells (*S*). Tubular wall cell (*TWC*). Rat 6 days, magnif. x13,100

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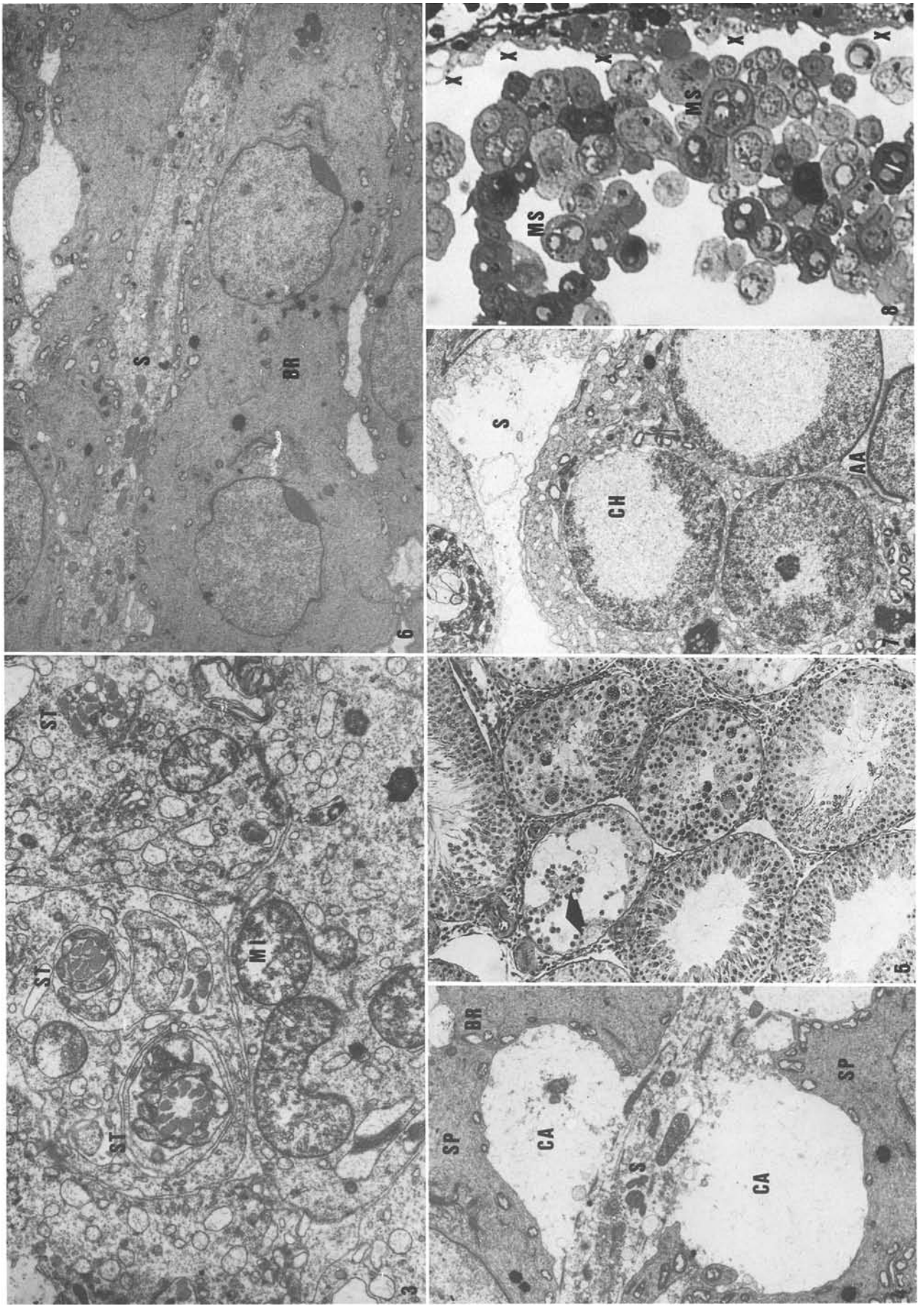
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They should elicit an autoimmune reaction against haploid spermatogenic cells being immunologically "alien" to the rest of the body cells. Removal of the stimulus of the inflammation, e.g. by antibiotics, should give the opportunity for the restoration of the barrier and the onset of spermatogenesis. Thus, no strong adjuvant should be necessary to induce autoimmune aspermatogenesis.

We have investigated the effect of BCG in the testis [18] and showed that intratesticularly injected BCG gives rise to a local granulomatous reaction, even with very low doses. At the site of deposition, many though not all the tubules degenerate and lose the spermatogenic cells without the inflammatory cells penetrating the tubular wall. As the granuloma grows, tubules are attacked by macrophages and destroyed completely. Doses of BCG in the range of those used by Talwar [16] occasionally caused total granulomatous orchitis in rabbits. Thus, we were not able to agree with Talwar [16] about the mechanism of aspermatogenesis induced by BCG nor that this could be a practicable method for male contraception. We present more, detailed observations on the mechanism of degeneration with emphasis on:

1. The early events in tubular degeneration and the changes in the Sertoli cell.
2. The degeneration of spermatids and the formation of multinuclear complexes.
3. Some features of Sertoli cells in tubules without spermatocytes and spermatids.
4. The alteration of the wall of the degenerating tubule and the importance of the basal membrane as an obstacle to penetration by inflammatory cells.

Material and Methods

Thirty adult male Wistar rats of the same age received 2.5 units of BCG (freeze dried vaccine was kindly supplied by G. P. Talwar, All India Institute of Medical Sciences, New Delhi, India) in 0.1 ml of sterile physiological saline into the right testis under light ether anaesthesia. The vaccine was deposited at various sites while moving the needle from one pole of the testis to the other. Six animals received 0.1 ml of saline. After 3, 6, 10, 20 and 56 days both testes of five rats were excised under light ether anaesthesia, and one rat was sacrificed after 230 days. The testes were prefixed in 1% glutaraldehyde and divided longitudinally. One part was fixed in Bouin's solution and prepared for light microscopy. Sections were stained with hematoxylin-eosin. The other part was cut into small pieces and fixed for 2 h in 1% glutaraldehyde and after washing in cacodylate buffer transferred to 1% OsO_4. Semi-thin sections were stained with toluidine blue and ultra-thin sections were stained with uranyl acetate and lead citrate and photographed in a Philips EM 300.

After semen analysis of 14 one years old rabbits from a local inbred strain, which showed no abnormalities, nine of them received 12.5 units of BCG in 0.5 ml sterile physiological saline into both testes under light pentobarbital anaesthesia. Five animals received 0.5 ml of saline into both testes. After 70 days, the testes were excised under pentobarbital anaesthesia, fixed in Bouin's solution or in 1% glutaraldehyde followed by 1% OsO_4 and produced for light or electron microscopy respectively.

Results

1. Three days after the administration of a small dose of BCG an apparent disorganization of the germinal epithelium had already taken place in the rat testis. The arrangement of the spermatocytes and spermatids of a given stage in the spermatogenic cycle was disturbed (Fig. 1). The phenomenon was accompanied by the formation of clefts and cavities between the basal parts of the Sertoli cells, containing flocculent electron translucent material and occasionally membrane remnants (Fig. 2). In the cells, the cisternes of the endoplasmic reticulum were widened, giving rise to larger vacuoles. Phagocytosed spermatozoa and spermatids in all stages of degradation were frequently observed. Degradation of flagella occured apparently with a loss of the 9 + 2 pattern, followed by the destruction of the mitochondrial sheath and the coarse fibres (Fig. 3). The cytoplasm of individual Sertoli cells was very electron translucent. Dense bodies were observed regularly in these cells. Frequently the plasma membrane was interrupted and even total vesiculisation of the cytoplasma occurred (Fig. 4). Finally, a gap in the germinal epithelium arose (Fig. 5), which was sometimes closed by the neighbouring Sertoli cells later on (however, tubules without Sertoli cells were also observed, see Fig. 8). The control testes showed no abnormalities compared to normal testes.

2. Adjacent to apparently undamaged areas with Sertoli cells appearing normal and with preserved spermatids and spermatocytes, germinal cells surrounded by Sertoli cells with very electron translucent and vesiculated cytoplasm underwent degeneration. The close relation between different cells was lost, and empty cavities were observed between spermatids and projections of Sertoli cells (Fig. 4). Only at very few points the plasma membranes remained as close together as they were in the undamaged epithelium. Spermatids gained highly variable shapes. The intercellular bridges broadened, preparing the formation of multi-

◀ **Fig. 3.** Sertoli cytoplasma: Enlarged mitochondria (*MI*), remnants of spermatozoal tails undergoing degradation (*ST*) lacking 9 + 2 pattern. Rat 6 days, magnif. x18,500

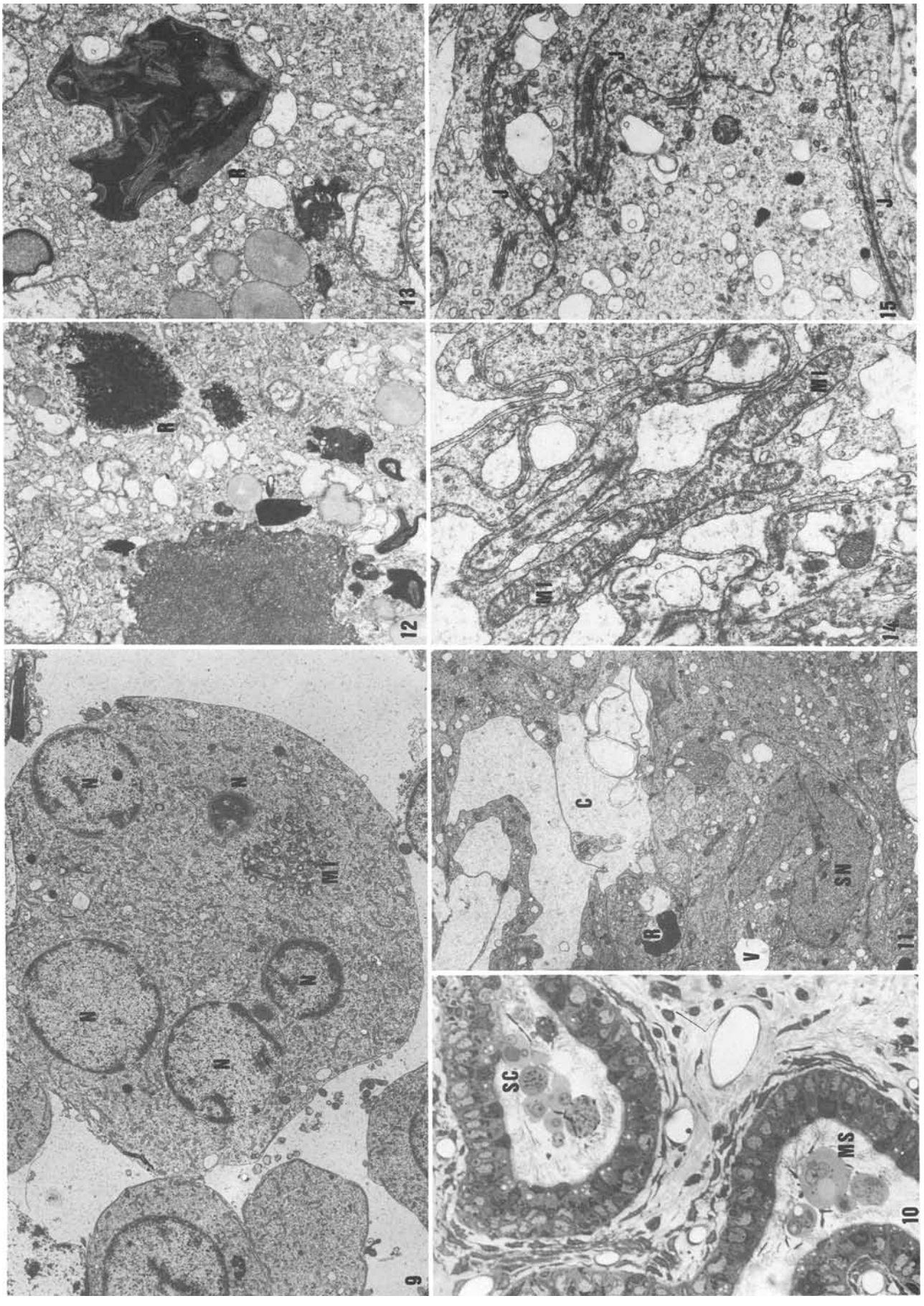
Fig. 4. Vesiculated electron transparent Sertoli cell (*S*) losing contact to spermatids (*SP*) with enlarging intercellular bridges (*BR*). Formation of cavities (*CA*). Rat 230 days, magnif. x5,350

Fig. 5. Degeneration of the germinal epithelium: Loss of Sertoli cells leading to a gap (*arrow*). Rat 6 days, magnif. x120

Fig. 6. Broadening of the intracellular bridges between spermatids (*BR*). Sertoli cell with electron translucent cytoplasma (*S*) Rat 230 days, magnif. x4,400

Fig. 7. Condensation of chromatin (*CH*) in a degenerating multinuclear spermatid. Common acrosomal anlage (*AA*) of two nuclei, translucent Sertoli cell (*S*). Rat 6 days, magnif. x5,800

Fig. 8. Multinuclear spermatids (*MS*) with ring-like chromatin condensations. Loss of Sertoli cells (*XXX*). Rat 6 days, magnif. x600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nuclear complexes (Fig. 6). In the rat testis, a marked condensation of chromatin beneath the nuclear membrane left a less electron dense area in the centre of the nucleus (Fig. 7). Under the light microscope, these nuclei appeared ring-like with a light refracting border (Fig. 8). In the rabbit testis, degenerating spermatids showed a tendency to swell, the nuclei underwent an extension while the nucleoplasm became more electron translucent. In the rat testis, a similar swelling process could not be observed.

After the confluence of spermatids, the multinuclear complexes contained up to 30 nuclei in the rat. The parts of the cytoplasm derived from individual cells were not discernible and the mitochondria of rabbit multinuclear spermatid complexes accumulated at one place (Fig. 9). Acrosomal anlages were often vacuolised, sometimes two or more nuclei (Fig. 7) showing a common giant acrosomal cistern. The multinuclear complexes were degraded after approximately two weeks, or they left the tubule by the rete testis. Occasionally, they could be observed in the epididymis (Fig. 10) together with other remnants of the germinal epithelium. However, the formation of multinuclear aggregates seemed to be a long-lasting process, since after 230 days there were still signs of confluence of spermatids (see Figs. 5 and 6).

After the disappearance of the germinal elements (except a few spermatogonia with homogenous dark cytoplasm and few organelles) only Sertoli cells remained. They had numerous long and thin protrusions surrounding frequently smaller or larger cavities (Fig. 11). There were only small differences in the electron density of the cytoplasm between individual cells. Lysosomes and residual bodies of all sizes were frequent. Crystalline inclusions either consisted of filamentous electron dense asterisk-like accumulations (Fig. 12), or they were composed of unstained rods in parallel arrays embedded in a dense matrix (Fig. 13). Mitochondria were long and sometimes branched (Fig. 14). In the protrusions there were numerous filaments, microtubules and cisterns of the endoplasmic reticulum. Junctional specialisations were present (Fig. 15), although they seemed to be unevently distributed. In the rat, the Sertoli nucleus was round or lobulated as it was in the undamaged epithelium (Fig. 16), in the rabbit being more polygonal. Numerous small chromatin accumulations were located mainly beneath the nuclear membrane (Fig. 17).

The diameter of the tubules diminished during the degeneration of the germinal epithelium. The peritubular cells shortened, their extensions branch and the nuclei became more rounded, finally the cell appeared in a more asterisk shape (Figs. 18 and 19). The contacts of the cell's protrusions were lost. In the rabbit, an enormous addition of ground substance of the outer lamella increased the diameter of the tubular wall up to 15 um. The layer of the basal membrane-like material around the tubular wall cells was interrupted. Beneath the basal membrane, collagen fibrils appeared (Fig. 20). The layers of the basal membrane in the rabbit were frequently altered to a mass of medium density. In the vicinity of a granuloma, macrophages and lymphocytes accumulated around the tubules (Fig. 21). Occasionally, the invasion of inflammatory cells could be observed. In these cases, the germinal epithelium had undergone degeneration previously. Around tubules which were not invaded, the inflammatory cells were located adjacent to the basal membrane. Remnants of the other parts of the tubular wall were sometimes seen interspersed between them (Fig. 22). The diameter of the basal membrane was unevently increased at sites which were in contact with the granuloma.

Discussion

All the features described could be observed during the first week after the application of BCG, although degenerative changes of the same types were detected during the whole course of the study in the periphery of the slowly growing granulomas, or even after their disappearance during the formation of naevus-like connective tissue replacing the destroyed parenchyma, after 230 days. We think therefore that it is justified to speak of a sequence of degenerative changes which occurs in all tubules losing active spermatogenesis during the inflammatory process and the subsequent events. However, the penetration of the basal membrane and the invasion of inflammatory cells into the tubular lumen occurred only in the presence of an actively growing granuloma.

Differences in the reaction to BCG between rats and rabbits were partly due to the different doses. In the rabbit, relatively higher doses caused the complete destruction of larger areas of the testis. However, the alteration of the tubular wall was more pronounced in rabbits even in the sorroundings of a granuloma (comparable to sites in the rat testis). The ring-like chromatin condensations in spermatid

◀**Fig. 9.** Aggregate of spermatids with 5 nuclei (*N*) at the plane of section and accumulation of mitochondria (*MI*). Rabbit 56 days, magnif. x4,700

Fig. 10. Multinuclear spermatids (*MS*) and spermatocytes (*SC*) in the epididymis. Rabbit 56 days, magnif. x460

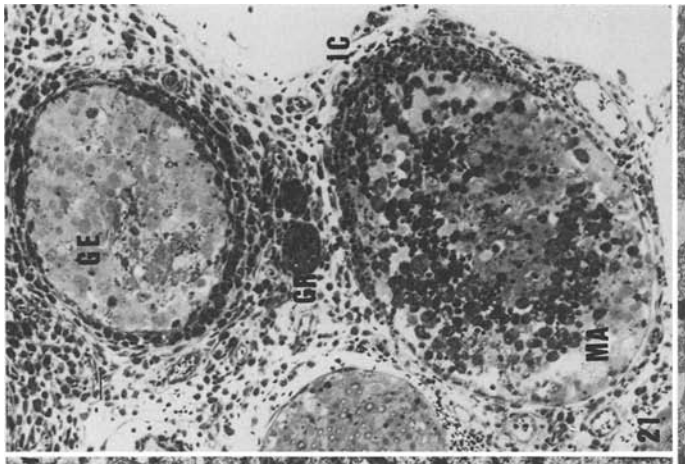
Fig. 11. Degenerated germinal epithelium: Sertoli cells containing vacuoles (*V*), residual bodies (*R*) and lobulated nucleus (*SN*). Cellular protrusions surrounding cavities (*C*). Rat 56 days, magnif. x1,400

Fig. 12. Degenerated germinal epithelium: Sertoli cell with residual bodies containing crystalline asterisk inclusions (*R*). Rat 10 days, magnif. x9,650

Fig. 13. Sertoli cell with residual bodies containing crystalline parallel rods (*R*). Rats 10 days, magnif. x16,300

Fig. 14. Sertoli cell with a long branched mitochondrium (*MI*) in a cellular protrusion. Rat 230 days, magnif. x19,500

Fig. 15. Sertoli cell with intracellular junctional specialisations (*J*) Rat 230 days, magnif. x12,000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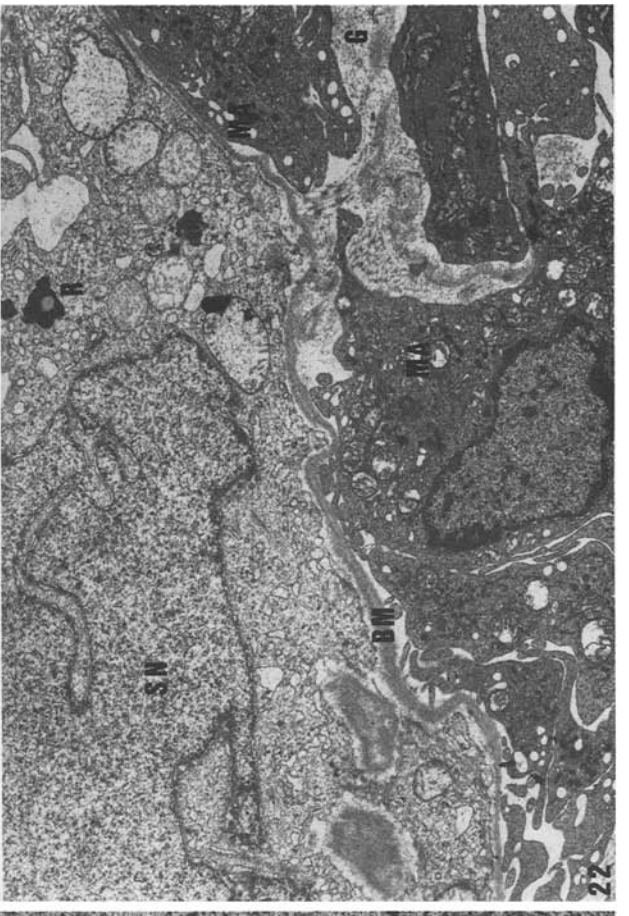
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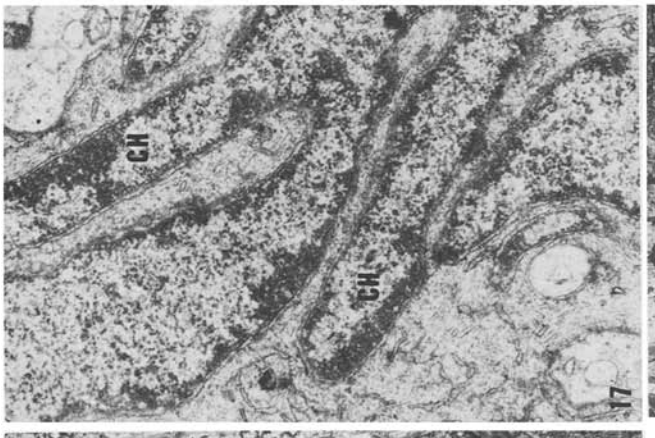
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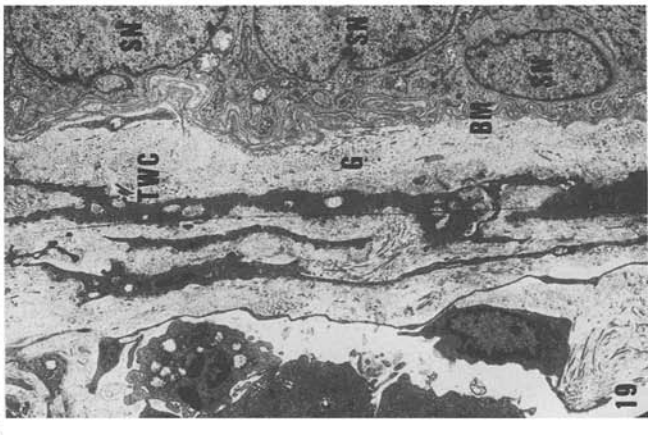
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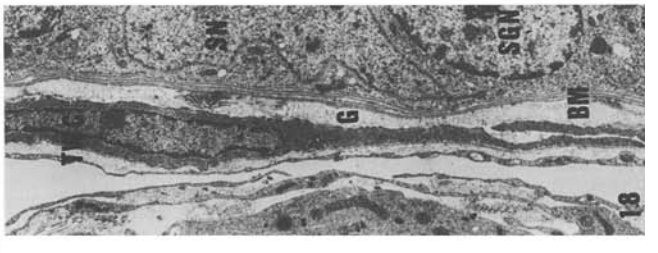
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nuclei could mainly be observed in rats, whereas in rabbits, a swelling of the nuclei occurred.

The features of testicular degeneration observed in the present study are not confined to the reactions during inflammation, but seem to be similar, at least in part, after the administration of various noxious agents.

A vacuolisation of Sertoli cells is commonly reported after inflammations of various kinds [11, 12], whereas Tedde [17] reported intercellular cavities. Mikuz [12] reported an increase in electron density of individual degenerating Sertoli cells during bacterial orchitis in guinea pigs. This is in apparent contrast to the loss of density observed in our material, but it indicates a similar action on individual Sertoli cells preceding the degeneration of the whole tubule. Thus, we believe, that the loss of Sertoli cells, and consequently the loss of spermatogenic cells within their "reach". is to be considered as one of the first events in tubular degeneration. A breakdown of the blood-testis barrier may be associated with this process. The barrier is probably re-established later on as indicated by the presence of junctional specialisations in tubules with Sertoli cells only. The degeneration of spermatids leads to a remarkable alteration of their nuclear structure. Similar nuclear ring-like chromatin condensations to those found in the rat testis during inflammation have been reported by Ellis [5] after irradiation, and by Parvinen and Parvinen [15] after the application of Thio-Tepa, an alkylating substance. It seems to be a common feature of degenerating spermatids in the rat. This applies also to the multinuclear "giant cells", which are more aggregates of dying spermatids than true cells. A variety of noxious agents, cryptorchidism as well as nutritional deficiencies [1], or treatment with the antimetabolite 5-thio-D-glucose [13] produce the same phenomenon. The latter suppose a loss of cytoplasmic bridges linking cells of one generation in the germinal epithelium [3] occurring prior to the formation of multinuclear complexes. On the contrary, in our material a broadening of the cytoplasmic bridges seemed to be the reason for the formation of the complexes, associated with, or even following a loss of the close association of spermatids (and spermatocytes) and the Sertoli cells.

Spermatids with two nuclei sharing one common acrosome, which may be vacuolised, were found by Holstein [10] in human testes with altered spermatogenesis and are not a sign of secondary confluence, although there is no proof that this could not have happened. Multinucleated spermatocytes have been observed only rarely in our material, though they may exist to a greater extent.

The remaining Sertoli cells in a tubule with degenerated epithelium have a complex outer shape, an altered nucleus with chromatin condensation and many lysosomes and residual bodies in the cytoplasm, including crystalline structures. A more or less random array of microfibrils and microtubules is accompanied by the appearence of unusually long and branched mitochondria. Chromatin condensations in Sertoli cells have been observed after anti-LH administration which leads to a decrease of androgen [4], after immunisation against pachytene spermatocytes and Sertoli cells constituents [19] and after autoimmune orchitis [11]. A relation to a high phagocytic activity of the Sertoli cells was suspected by Mancini [11]. Remarkably, the structure of the Sertoli cells in the transitional zone [14] is somewhat similar to the one observed after degeneration of the germinal epithelium.

The special intercellular junctions which are the morphological equivalent to the blood-testis barrier [6] are present even if spermatocytes and spermatids are lacking and the Sertoli cells show the alterations described. Since some of the Sertoli cells have been lost early in the degenerative process, new junctions had to be established between "new neighbours". Whether the blood-testis barrier is tight under conditions where most of the germinal cells are lacking could only be proven by lanthanum diffusion experiments. During epinephrine application [7], artificial cryptorchidism [8] or nitrofurazone application [9] the barrier remained tight.

The wall of the seminiferous tubules of rats and rabbits differs considerably in structure under normal conditions [2]. These differences increase during the degeneration of the tubules, since in the rabbit considerable amounts of ground substance seem to be incorporated. However, this does not prevent various inflammatory cells (mostly macrophages) from crossing the tubular wall. The basal membrane, altered and often thickened, seems to be the main obstacle for the inflammatory cells to reach the tubular lumen and to destroy its content. Spermatids and spermatocytes are very seldom observed in infiltrated tubules, whereas the morphology of the Sertoli cells is always altered. An invasion of inflammatory cells into a tubule is always associated with the presence of a growing granuloma in the neighbour-

◀ **Fig. 16.** Sertoli cells with nuclei showing chromatin condensations (*SN*). Rabbit 56 days, magnif. x2,470

Fig. 17. Chromatin condensations (*CH*) beneath the membrane in branches of a Sertoli nucleus. Rat 230 days, magnif. x19,000

Fig. 18. Normal tubular wall: Tubular wall cells (*TWC*), ground substance (*G*), basal membrane (*BM*), germinal epithelium with Sertoli cell nucleus (*SN*) and spermatogonial nuclus (*SGN*). Rabbit 56 days, magnif. x2,100

Fig. 19. Wall of a degenerated tubule: Tubular wall cells with branched extensions (*TWC*), augmented ground substance (*G*), folded basal membrane, Sertoli cell nuclei (*SN*). Rabbit 56 days, magnif. x2,850

Fig. 20. Degenerated tubular wall: Collagen fibrils (*CO*) in ground substance (*G*) beneath the altered and folded basal membrane (*BM*). Sertoli cells (*S*). Rabbit 56 days, magnif. x11,700

Fig. 21. Inflammatory cells (*IC*) surrounding a tubule with degenerated germinal epithelium (*GE*). Invasion of macrophages into the adjacent tubule (*MA*). Formation of small granulomatous accumulations (*GR*). Rat 6 days, magnif. x130

Fig. 22. Macrophages (*MA*) adjacent to the unevently thickened basal membrane (*BM*) between the remnants of the tubular wall ground substance (*G*). Altered Sertoli cell with lobulated nucleus (*SN*) and residual bodies (*R*). Rat 10 days, magnif. x7,100

ing interstitium, creating a harmful milieu to the sensitive seminiferous tubules.

Thus, in our opinion the degeneration of the germinal epithelium precedes the invasion and contact of immunocompetent cells and the immunologically "alien" haploid cells in the seminiferous tubule.

Acknowledgement. This work was supported in part by the Austrian Society for the Promotion of Scientific Research (project no. 3380) and the Foundation of the Paris Lodron Society (project no. 4070).

References

1. Benitz KF, Dambach G (1965) The toxological significance of multinucleated giant cells in dystrophic testes of laboratory animals and man. Arzneim Forsch 15:391–404
2. Bustos-Obregon (1976) Ultrastructure and function of the lamina propria of mammalian semiferous tubules. Andrologia 8:179–185
3. Dym M, Fawcett DW (1971) Further observations on the number of spermatogonia, spermatocytes and spermatides connected by intracellular bridges in the mammalian testis. Biol Reprod 4:195–215
4. Dym M, Raj HGM, Chemes HE (1977) Response of the testis to the selective withdrawal of LH or FSH using antigonadotropic sera. In: Troen P, Nank HE (eds) The testis in normal and infertile men. Raven, New York pp 97–124
5. Ellis LC (1970) Radiation effects. In: Johnson AD, Gomes WR, Van Demark NL (eds). The testis, vol III: influencing factors. Academic Press, New York, pp 333–376
6. Fawcett DW (1975) Ultrastructure and function of the Sertoli cell. In: Hamilton DW, Greep RO (eds) Handbook of physiology, vol. V. Am Physiol Soc, Washington DC, pp 7, 21–55
7. Gravis CJ, Chen I, Yates RD (1977) Stability of the intraepithelial component of the blood-testis barrier in epinephrine-induced testicular degeneration in Syrian hamster. Am J Anat 148:19–31
8. Hagenäs L, Plöen L, Ritzen EM, Ekwall H (1977) Blood-testis barrier: Maintained function of inter-Sertoli cell functions in experimental cryptorchidism in the rat, as judged by a simple lanthanum immersion technique. Andrologia 9:3–7
9. Hagenäs L, Plöen L, Ritzen EM (1977) The effect of nitrofurazone on the endocrine, secretory and spermatogenic function of the rat testis. Andrologia 7:1–22
10. Holstein AF (1975) Morphologische Studien an abnormen Spermatiden and Spermatozoen des Menschen. Virchows Arch A 367:93–112
11. Mancini RE (1976) Immunological aspects of testicular function. In: Gross F et al. (eds) Monographs on endocrinology. Springer, Berlin Heidelberg New York
12. Mikuz G (1978) Orchitis. Morphologische und funktionelle Untersuchungen bei Versuchstieren und beim Menschen. In: Bargmann W, Doerr W (eds) Normal and pathological anatomy, 36. Thieme, Stuttgart
13. Neumann F, Schenk B (1978) Pharmakologische Beeinflussung der Spermatogenese. In: Senge Th, Neumann F, Schenck B (eds) Physiologie und Pathophysiologie der Hodenfunktion. Georg Thieme Verlag, Stuttgart, pp 105–131
14. Nykänen M (1979) Fine structure of the transitional zone of the rat seminiferous tubule. Cell Tissue Res 198:441–454
15. Parvinen LM, Parvinen M (1978) A "living cell method" for testing the early effects of antispermatogenic compounds: Model experiments with two alkylating agents, Thio Tepa and nitrogen mustard. In: Hansson V, Ritzen M, Purvis K, French KS (eds) Endocrine approach to male contraception. Transactions of the 5th annual workshop on the testis. Scriptor, Copenhagen, pp 523–537
16. Talwar GP, Naz RK, Das DC, Das RP (1979) A practical immunological approach to block spermatogenesis without loss of androgen. Proc Soc Natl Acad Sci USA 76:5882–5885
17. Tedde G, Pala AM, Tedde-Piras A, Giunti M, Succi A (1973) Structural and ultrastructural observations of the rat testicles in experimental allergic orchitis. Acta Eur Fertil 4, 1:31–35
18. Torgersen HM, Rovan E, Steiner M, Frick J, Adam H (1981) The use of BCG (bacterium Calmette-Guerin) as an antispermatogenic agent. Structural and hormonal changes. J Andrology
19. Tung P S, Fritz JB (1978) Histophatoholigical changes in testes or Sertoli Cells. In: Hansson V, Ritzen M, Purvis K, French FS (eds) Endocrine approach to male contraception. Transactions of the 5th annual workshop in the testis, pp 459–481

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