MORPHOLOGICAL STUDIES OF THE IMMUNE RESPONSE TO VASECTOMY

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A thesis presented for the degree of Doctor of Philosophy in the Faculty of Medicine, University of Glasgow

VOLUME I: TEXT

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May, 1987

DEDICATED

TO MY WIFE

ACKNOWLEDGEMENTS

I would like to take this opportunity to express my sincere gratitude to Professor R.J. Scothorne for suggesting this project and supervising this work, and for his continual guidance, interest and encouragement during the course of the project.

I am also grateful to the technical staff of the Department, especially Miss Mary Fife, Mr Owen Reid, Mr James McGadey, and Mr Robert Kerr for their technical expertise and their willingness to offer assistance whenever required.

I am also indebted to Mr Gillespie for his kindly guidance and advice; Mr Neil Bennett who carried out all the operations, and cared for the animals, with his staff in the Animal House; Miss Margaret Hughes for her untiring help in preparing all the photomicrographs presented in this thesis and Miss Caroline Morris who assisted in mounting them; and finally Mrs L. Peedle for her skill and care in typing the thesis.

I also thanks Dr A.P. Payne for his kind help with the statistical analysis of the results.

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SUMMARY

1. The morphological changes in the epididymis, testes, and spleen, were studied at seven postoperative periods (6 wks and 4,6,9,12,15, and 18 mos.) after unilateral (left) vasectomy or sham operation, in a series of 72 mature Albino rats.

2. In a group of 6 additional animals, in which vasectomy was performed on the left side, with the right vas as a sham-operated control, the luminal surface of the epididymis was studied by scanning electron microscopy, at 1, 3 and 6 wks after operation.

3. Spermatic granulomas developed first at the site of vasectomy. They gradually increased in size, and in many of the rats, particularly at longer intervals after vasectomy, granulomas appeared in the epididymis, usually initially in the cauda, followed by their appearance in the corpus and caput. In such animals, the vasal granulomas became reduced in size.

4. Epididymis:

(a) in all but 3 rats, there was no distension of the epididymal duct, no thinning of the epithelium and no histological and EM changes in the epithelium. In the remaining 3 animals, all of which had a granuloma in the caput, the duct was distended with sperm and the epithelium was thinned.

(b) no evidence was found of the uptake of sperm or sperm fragments by the epididymal epithelium.

(c) Intraepithelial leucocytes in both sham-operated and vasectomized animals were identified by EM as lymphocytes, monocytes and macrophages.

(d) The numbers of intraepithelial leucocytes did not differ significantly between sham-operated and vasectomized animals at any of the time intervals after operation.

(e) A site of microscopic rupture of the epididymal duct and the early formation of a granuloma has been studied in a longitudinally sectioned epididymis. The histological appearances suggest the following sequence of events:

i. obstruction of flow of sperm and fluid along the duct;

ii. distension of the duct, with thinning and weakening of its epithelial wall;

iii. the escape of sperm into the stroma;

iv. the development of a granulomatous inflammatory reaction;

v. invasion of the duct lumen by polymorphonuclear leucocytes and macrophages.

(f) Lymphatics:

In the vicinity of a granuloma these contained a variable number of lymphocytes and macrophages. These cells were not seen in sham-operated animals or in vasectomized animals at a site remote from a granuloma.

(g) SEM appearances:

No differences were seen in the surface morphology of the epididymal epithelium of sham-operated and vasectomized animals.

5. The Testes remained histologically normal up to four months after vasectomy. However in the period between 6 to 18 months after operation, 11 rats showed patchy degenerative changes of seminiferous tubules in their left testes. This was attributed to the development of a granuloma in the caput epididymidis which caused an obstruction and led to an increase in the intraductal hydrostatic pressure. Four animals at the same period, showed bilateral testicular atrophy. No evidence was found to suggest a cause, such as infection, immunological orchitis, ischemia, or cryptorchidism. The cause remained unclear.

6. Spleen:

The spleen showed no evidence of involvement in an immune response to vasectomy, as assessed by the absence of significant changes in each of the following parameters:

weight

volume density of white pulp

volume density of marginal zone

germinal centres.

Aim of the Project

The increasing popularity of vasectomy as a method of permanent birth control has spurred investigation into the side-effects of the procedure in men and experimental animals. Numerous studies have documented diverse structural and functional sequelae elicited in various mammalian species in response to experimental vasectomy. In certain species two effects have been consistently reported: the development of spermatic granulomas in the neighbourhood of the excurrent duct system of the testis and the initiation or intensification of an immune response directed against spermatozoal components.

Vasal and epididymal spermatic granulomas may result from distension of the epididymis and vas deferens with the spermatozoa which continued to be produced after ligation of the vas deferens; when the capacity for distension of the duct is exceeded, its wall ruptures. The time interval after vasectomy when the rupture occurs, the site most prone to rupture, and the gross and histological organization of the granuloma all vary both between species and within the same species. In several studies of the rat, granulomas rapidly developed within a week in nearly all animals, usually at the sectioned end of the proximal vas segment. In rabbits on the other hand, granuloma formation is deferred for several months after operation. In man, the incidence may be as high as 20 percent. It probably represents the inflammatory reaction to extravasation of sperm. If antigens from these sperm reach the lymphoid system, they may elicit

autoimmune responses. The reason for sperm being auto-antigenic, is thought to be due to the fact that these haploid cells first appear at puberty, long after tolerance to self-antigens has been established, and express sperm-specific antigens that are foreign to the immune system.

It is well established that vasectomy is followed by the development of anti-sperm immunity as indicated by the production of anti-sperm antibodies, such antibodies have been described in men, rats, rhesus monkeys, guinea pigs and other species, detectable by agglutination, immobilization and immunofluorescence.

After accumulation of sperm is sufficient to cause disruption of the lining epithelium of the vas and development of a terminal granuloma, this is an obvious site of access of antigen to blood or lymphatic vessels, from which spermatozoa are normally isolated by the blood-testis barrier and by the barrier of the lining epithelium of the various regions of the tract. It is worth noting however (i) that circulating antibodies may appear before development of a granuloma and (ii) that they also occur in species that rarely form granulomas. The work of McDonald and Scothorne (1986) has shown that the lymphatics and regional lymph nodes of the testis, epididymis and the granuloma site are involved in the genesis of humoral immunity. These findings do not of course exclude the possibility that sperm auto-antigens might also enter the blood (as well as the lymphatics)

and stimulate antisperm antibody formation in the spleen.

The immune response may not be confined to the formation of anti-sperm antibodies. There is also evidence of the elicitation of cell-mediated immune response, although the results are far from conclusive.

There have been conflicting reports regarding the effect of vasectomy on spermatogenesis, both between species and within the same species. In the rat, most authors report no significant change, but a few investigators found varying degrees of seminiferous tubule degeneration in some of their animals.

Knowledge of the fate of spermatozoa entering the epididymis and vas deferens is important to the consideration of the sequelae of vasectomy. Some investigators have reported that, after vasectomy, sperm are disposed of by leukocytes, which invade the duct first via the granuloma and then migrate within the tract. A few believe that sperm continue to accumulate within the granuloma. A third view is that the epididymal epithelium itself resorbs significant numbers of sperm.

The main aims of this project were:

1. to analyse the morphological changes in the spleen, to determine if it is involved in the immune response.
2. to study the time of appearance, distribution, size and fate of the granulomas which develop, at the vasectomy site and in the epididymis.

3. to study the microscopic changes in the seminiferous tubules and epididymis following vasectomy.
4. to study the early histogenesis and spread of granuloma in the epididymis.

REVIEW OF LITERATURE

I. Formation and Morphology of Spermatic Granulomas:

Sperm invasion of the interstitial tissue in the epididymis was apparently first mentioned in 1898 by Beneke, cited by Simmonds (1921).

Cunningham and Cook (1922) showed that the contents of an acutely inflamed ruptured epididymal duct will be extruded into the stroma and that the consequential abscesses will contain spermatozoa.

Oberndorfer (1931) recorded granuloma with sperm invasion in the epididymis of a patient with a previous history of gonorrheal epididymitis.

Orsos (1941) reported two cases of chronic granulomatous epididymitis with sperm invasion of the interstitial tissue. Both cases were considered preoperatively to be tuberculous epididymitis, and occurred in previously normal epididymides with histologically intact epithelial lining of the epididymal duct. The author, for the first time, pointed out the possibility of sperm invasion being a cause of epididymitis.

Steinberg and Straus (1947) described sperm granulomatous inflammation after invasion of the stroma of the epididymis by sperm in a 32 year old man, diagnosed preoperatively as having tuberculous epididymitis. Microscopic examination of the epididymis showed numerous spermatozoa lying free in the interstitial tissue and surrounded by epithelioid cells. There were no areas of caseation and no giant cells, and

stains showed no acid-fast bacilli. They suggested that the interstitial tissues had responded to the extravasation of sperm with a granulomatous reaction.

In an examination of seven cases of non-specific epididymitis, Cronqvist (1949) found sperm invasion of the interstitial tissue of the epididymis. In some cases the inflammatory changes were mainly localized around the invading spermatozoa, forming granuloma-like foci. All cases showed dilated epididymal duct with flattened epithelium and signs of stagnation. In four of the cases there was direct communication between the granuloma-like foci and the epididymal duct. There was a history of a previous epididymitis in five of these cases. It was suggested that the extravasation of sperm followed rupture of the wall of the epididymal duct which was due to the simultaneous effect of increased intratubular pressure and locally decreased resistance of the wall of the epididymal duct due to an earlier or active inflammation.

Friedman and Garske (1949) studied a series of spermatic granulomas which formed in man subsequent to extravasation of sperm. Most of them followed surgical procedures or trauma to the genital ducts, and they simulated other lesions, particularly tuberculous epididymitis, even histologically.

Russell and Friedman (1951) were the first to produce spermatic granulomas in rats after vasectomy, comparable to those described in man. Thirty adult male albino rats were used. The vasa deferentia were severed and their open cut

ends implanted in either the scrotal wall or the abdominal cavity and retroperitoneum. In half of the animals the testes were removed from the scrotum, either at the time of vasectomy or in a preliminary operation 3 to 4 weeks before vasectomy, and fixed in the abdominal cavity. Six combinations of operative procedures were used with five animals in each group: 1) testis left in scrotum; vas implanted in scrotal wall, 2) testis transplanted to abdomen; vas placed in abdominal cavity, 3) testis transplanted to abdomen; vas implanted in scrotal wall at the same time, 4) testis left in scrotum; vas implanted in retroperitoneum, 5) testis transplanted to abdomen; vas implanted in scrotal wall one month later, 6) testis left in scrotum; vas placed in abdominal cavity. At the time of sacrifice, granulomatous lesions were found to develop at the resected end of the vasa deferentia in most of the animals whose testes had been left in the scrotum and whose vasa had been implanted in the scrotal wall. Some granulomas had formed about the ends of the intraperitoneal vasa, but in several of the rats whose vasa had been thus transplanted, the testes had undergone degeneration and no granuloma formation had resulted. The testicular damage was attributed to interference with the blood supply at operation. In the group in which the testes had been placed in the abdomen, only a few small granulomas were found about the cut ends of the vasa and they were all formed in the group in which the vasa had been opened at the same time that the testes were relocated and in which

the testes were not yet atrophic at the time of implantation of the vasa. No granulomas were found in the group in which the testes had been placed within the abdomen for a few weeks prior to vasectomy and in which the testes were atrophic by the time the vasa were opened. Microscopically the granulomas consisted of a central mass of sperm and other material surrounded by a wall of histiocytes or macrophages.

Reiger et al. (1953) described spermatic granuloma of the epididymis in a 22 year old Negro, who had a history of trauma to the left groin. Pathologic examination of the left epididymis showed nodular induration at one end of the structure. The cut surface showed a spongy structure with milky fluid in the dilated tubular spaces. Microscopic sections through the indurated portion showed distention of the epididymal duct and atrophy of its lining epithelium. Many of the epididymal ducts were displaced or replaced by a nodular granuloma, consisting of a central pool of spermatozoa lying free in the interstitial tissue and surrounded by epithelioid macrophages, which contained ingested spermatozoa or a granular yellow-brown pigment. Other sections through portions of the epididymis not as severely involved showed an interstitial chronic inflammatory reaction and a few collections of extruded spermatozoa. In at least two places actual rupture of the distended wall of the duct of epididymis could be seen, with extrusion of masses of spermatozoa into the interstitial tissues of the epididymis. It was suggested that there may have been some partial obstruction to the

excretion of sperm, with rupture due to increased intraluminal pressure.

Berg (1953) reported that sperm nuclei in sections of testes from a number of species were acid-fast, in contrast to other testicular elements, when stained by Putt's modification of the standard Ziehl-Neelsen technique for staining mycobacteria. In the light of this finding, and the fact that both mycobacteria and extravasated spermatozoa were capable of producing morphologically similar granulomatous tissue, Berg (1954) was led to examine the chemical composition of the acid-fast component of human sperm. He succeeded in extracting a lipid fraction from spermatozoa which was responsible for the acid-fastness previously noted in intact sperm and resembled mycolic acid in its molecular absorption spectrum. This fraction produced a reaction similar to spermatic granulomas when it was injected into the subcutaneous tissue of a hamster.

In a specially investigated series of fifty autopsies, two examples of spermatic granuloma in the epididymis were encountered by Sundarasivarao (1955), who suggested that the lesion probably results from focal inflammation, with consequent obstruction, dilatation and rupture of epididymal tubules.

Glassy and Mostofi (1956) analyzed sixty-one spermatic granulomas in the epididymides of sixty patients. Two were noted as postoperative complications of vasectomy,

and seven developed after various types of trauma. The lesions were thought to begin with an inflammatory or traumatic epididymitis which provided the conditions for the sperm to invade the stroma. The initial cellular exudate of polymorphonuclear neutrophils was replaced by macrophages and epithelioid cells that eventually surround the central accumulation of sperm and cellular debris. A bandlike zone of lymphocytes then collected around the epithelioid cells. As the granulomatous reaction healed, the centrally situated cellular debris disappeared, leaving only sperm and fibroblasts. Eventually there was hyalinization in association with a mass of sperm, and fibrosis and scarring was frequently observed in the older lesions.

Mullaney (1962) produced spermatic granulomas experimentally in rats. Of the five groups of experiments which were performed, only one was successful for examination of the cellular reaction to extravasated spermatozoa. In this group, approximately 2 cm of the proximal end of the vas deferens was resected and the cauda of the epididymis was allowed to leak into the scrotal sac. After operation extensive extravasation of spermatozoa occurred, and on the second day polymorphonuclear cells surrounded and intermingled with the spermatozoa. Four days later macrophages of epithelioid character began to arrange themselves in a palisade at the periphery of the mass of sperms. The sectional profile of the lesion gradually assumed a convoluted appearance after fourteen days and the macrophages began to fuse, to form

giant cells, at approximately thirty nine days. Seven months postoperatively the spermatozoa still appeared to be morphologically intact in the main part of the lesion but were clumped, suggesting agglutination. The cellular reaction at this stage was very striking and included numerous giant cells of Langhans type. The lesion now closely resembled that of tuberculosis, and particularly so in some foci, where spermatozoa were not obviously present, though the products of their disintegration may well have been. Similarities were found between these experimental lesions and those found in man at its various stages.

Six days after unilateral vasectomy in rats, a small cyst was observed on the cut testicular end of the vas deferens by Smith (1962). At subsequent post-operative periods, the cyst was found to become progressively larger, reaching its maximum size at 40 days; it was then extremely turgid with a mean weight of 1.4 g. The turgidity later diminished and at 90 days the cyst was reduced to a flattened slip of tissue. Histologically, the cysts were, in Smith's words, "essentially similar in structure in animals sacrificed 6-60 days after vasectomy. The wall of the cyst was somewhat thickened by fibrous tissue; the lining epithelium (sic) was extremely low and in some places discontinuous. The cavity was filled with apparently normal spermatozoa." Cysts recovered from animals killed 90 days after vasectomy had thickened walls, consisting of fibrous tissue interspersed with cells resembling those of the original

lining epithelium of the vas deferens. These cells were no longer columnar, however, and did not form an organized epithelium. The spermatozoa in the cavity had degenerated and were mingled with numerous macrophages and granulocytes". As will become clear later in this thesis, Smith (1962) was in error in her reference to a lining "epithelium". This was a misinterpretation of the epithelioid arrangement of macrophages and giant cells which come to line the granuloma.

In a series of 432 patients who underwent elective vasectomy, Schmidt (1966) reported the development of a spermatic granuloma to be the most significant complication, occurring at the testicular cut end of the vas or in the epididymis shortly after, or even years after operation. Its occurrence at the cut end of the vas could be reduced if the vas were fulgurized rather than ligated.

Lyons et al. (1967) described spermatic granuloma of the epididymis in two patients one of whom had a bilateral vasectomy, the other bilateral herniorrhaphy. The authors believed that spermatic granuloma may play a role in the development of male infertility after vasectomy and in the failure to re-establish fertility after reversal of vasectomy by vaso-vasostomy.

Flickinger (1972a, b) reported that despite the use of several different vasectomy techniques, a spermatic granuloma developed around the proximal cut end of the vas deferens in most of the rat as a result of leakage of sperm. The

granulomas were yellowish, spherical or oval nodules, with a semi-fluid centre containing sperm and a wall of rubbery consistency that showed histological evidence of chronic inflammation with mononuclear and epithelioid cells. The spermatic granulomas were found to reach their maximum size by approximately one month after operation, they were then stabilized in size. The author suggested that sperm do not escape from the duct system indefinitely.

Schmidt and Morris (1973) stated that spermatic granuloma was the most common complication of vasectomy, occurring in at least 15% of cases. They regarded it as an inflammatory reaction to the extravasation of sperm, either from the vas or within the epididymis. Granuloma of the vas was thought to occur when the proximal end of the vas was inadequately occluded, allowing leakage of sperm from the lumen. Sperm leakage was believed to be caused by the ligatures cutting through the wall of the vas. The increased intraluminal pressure that follows obstruction of the vas and the peristaltic surges accompanying ejaculation were considered to help in producing it. Infection of the vas was thought to precipitate it, but trauma rarely does so. Granuloma of the epididymis was found to occur less frequently. After vasectomy, the duct of the epididymis was observed to be markedly dilated, with a corresponding thinning of its wall. Blunt trauma, even of an insignificant nature, was thought to be capable of rupturing the dilated duct. The authors stated that spermatic granulomas were

significant from a number of points of view: 1) they may cause a vasectomy to fail by initiating a spontaneous reanastomosis of the vas, 2) conversely, they may cause obstruction and thereby prevent success of a subsequent vasovasostomy, 3) they were thought to promote the development of autoimmunity to spermatozoa.

The first long-term animal study of the incidence and mechanism of formation of spermatic granulomas in rats following vasoligation was reported by Kwart and Coffey (1973). They found that spermatic granulomas were always present six months after vasectomy performed either before or after puberty in more than 100 rats. All postpubertal vasectomized animals had granulomas at the proximal end of the vas deferens but although all prepubertal vasectomized animals also had granulomas, only 46 to 50 percent of these occurred at the site of ligation. This observation appears to eliminate leakage of sperm at the operation and suture as the predisposing factor in the prepubertal vasectomized animals. In addition, another series of postpubertal vasectomized animals had their vas occluded by fulguration or ligation with either 0000 silk, plain catgut or chromic sutures and this did not alter the 100 percent occurrence of granulomas at the site of ligation. In the prepubertal group, granulomas were frequent in the cauda epididymidis and some were found in the corpus epididymidis, whereas in the postpubertal group a few occurred in the cauda and none was observed in

the corpus. The weight of granulomas six months following vasectomy was 0.3 to 0.7 gm. In several cases these lesions were very large and approached testicular size. Histologically, a typical lesion was observed to contain massive accumulations of intact spermatozoa. The authors suggested that after vasectomy there was minimal reabsorption of sperm in the epididymis, but that a considerable portion of the testicular output of sperm was transported and accumulated in the granuloma. The state of granulomatous inflammation was found to change with time. Initially appearing as an accumulation of large masses of sperm bordered by a rim of epithelioid macrophages, this rim in turn was found to be encompassed by a perivascular accumulation of round cells. The lesion was limited by a fibrous capsule. Later polymorphonuclear neutrophils were observed invading the mass of sperm. This was accompanied by destruction of the central mass and more pronounced phagocytosis of sperm by the macrophages, resulting in diminution in size of the granuloma. At this stage the lesion contained large numbers of epithelioid macrophages with Langhans giant cells. The sperm mass was considerably diminished.

Sackler et al. (1973) performed three types of operative procedures in three groups of immature albino rats. Group A underwent vasectomy, group B, vasoligation, and group C, sham operation. Numerous "yellowish cysts" were found in the cauda epididymis and vas deferens of both the vasectomized and vasoligated animals. They ranged in size from minute

to a length of 2.5 cm in the cauda epididymis of the experimental group. A few of the animals in either group A or group B did not exhibit granulomas in either the cauda epididymis or vas deferens. Most of the group B rats showed granulomas at or near the site of the ligature. "Single or a few, small yellowish cysts were observed in the fatty tissue near the spleen, liver and lungs". The authors offered no explanation for the presence of these "cysts" in spleen, liver and lungs. They did not study them histologically and it is not possible to know whether they were in fact granulomas. If they were, it is very difficult to see how they came to be formed in the lung.

Brannen et al. (1974) compared the weight and histological appearance of spermatic granulomas in immunosuppressed and non-immunosuppressed vasectomized rats. Three methods of immunosuppression were used: 1) the administration of antilymphocytic serum (ALS); 2) neonatal thymectomy; and 3) administration of ALS and treatment with cyclophosphamide. Typical spermatic granulomas were found at the proximal cut end of vas deferens 21 days after operation. The average weight of spermatic granulomas in highly immunosuppressed rats was significantly greater than the 51 mg found in non-immunosuppressed rats and was attributed to decreased sperm destruction and absorption in the granuloma. Histologically, the granulomas were primarily composed of a central core of packed sperm and

cellular debris surrounded by a paracentral zone merging into a more peripheral fibrous zone. Polymorphonuclear leucocytes were identified in the paracentral zone, lymphocytes and plasma cells predominated in the fibrous zone. The initial histological appearance at the periphery of the granuloma was found to be not sensitive to immunosuppression suggesting that the histology of the early spermatic granuloma was not the result of an inflammatory process brought about primarily by autoimmune phenomena. The possibility that the absorption of sperm in the granuloma might initiate an immune response effecting the histological changes seen in older sperm granulomas had not been eliminated.

Freeman and Coffey (1974) reduced the incidence and size of spermatic granulomas, which invariably occur after vasectomy in rats, by suppression of spermatogenesis through a subcutaneous implant of testosterone in silastic capsules.

McGlynn and Erpino (1974) divided their rats into four groups, each containing fifteen animals of which five animals were bilaterally vasectomized, five were unilaterally vasectomized, and five were subjected to bilateral sham operation. One of the animals was sacrificed at each of the following postoperative period:12 months (Group I), 9 months (Group II), 4 months (Group III), and 2 months (Group IV). Granulomas (0.5 to 2.5 cm diam.) were found at the proximal cut end of the vas deferens in nearly all of the bilaterally and unilaterally vasectomized animals. Granulomas were occasionally found on the cauda epididymidis

of bilaterally vasectomized rats in all groups and other unilaterally vasectomized rats in group I, II and III. "Small yellowish cysts were observed in intestinal and splenic mesenteries" of three bilaterally vasectomized groups (III and IV) and two unilaterally vasectomized groups (I and III) rats and were attributed to slow leakage of sperm. Histologically these abdominal cysts had a fibrous outer wall and a central mass of spermatozoa. Amorphous necrotic material was observed in the cyst but "macrophages were not detected".

Neaves (1974) compared the effect of unilateral scrotal and abdominal vasectomy, with and without ligation of the vas deferens, in rats. Spermatic granulomas, which had an average weight of about 0.4 g, were found in all vasectomized animals three months after operation. The formation of granulomas did not appear to be influenced by ligation. In all scrotally vasectomized rats, the granulomas had formed at the surgical site near the cauda epididymidis. Two abdominally vasectomized rats had granulomas at the same location, about 3 cm proximal to the surgical site. All other abdominally vasectomized rats had granulomas near the ampulla of the vas deferens, where the operation was performed. The mean spermatic granuloma weights among the four groups were not significantly different.

Spontaneous reanastomosis of the vas on one side in a 40 year old man was reported by Girgis (1975). This was attributed to the development of spermatic granulomas,

which were believed to bridge the gap between the two cut ends of the vas deferens. Then epithelium grew from each end and completed the anastomosis.

Howards et al. (1975) succeeded in reducing the incidence of spermatic granuloma, which invariably occurs after vasectomy in Sprague-Dawley rats. Several surgical modifications were tried: "Silk, nylon, and chromic catgut sutures varying in size from 4.0 to 6.0, metallic clips, and electrocautery were all used, both with and without resection of a segment of the vas deferens" and only when the vas was left in its surrounding fat sheath, regardless of the method of interruption, were they able to reduce the incidence from 100 percent to approximately 85 percent. One week postvasectomy, the granuloma appeared as a 1 cm amorphous white mass composed of a pseudocapsule surrounding leucocytes, serum, and disintegrating spermatozoa. One month post-vasectomy, the mass had increased in diameter to 2 cm and had become highly vascularized.

Voglmayr (1975) offered an alternative method of preventing the formation of spermatic granulomas in the rats by suppressing spermatogenesis through a brief temperature elevation in the testis before vasectomy. The animals were divided into three groups, each consisting of 21 animals, which were either vasectomized ("V" rats), exposed to local heating of testis shortly before vasectomy ("HV" rats) or subjected to sham operation ("C" rats). At 30 days after

surgery the testis weight of the HV rats was found to be only half that of the V and C rats. Histologically there were varying degrees of degeneration of seminiferous tubules. All "V" rats had developed large granulomas. In two of these rats, the granulomas were found at the distal cauda epididymidis; in all others they had formed at the surgical site. In contrast, only three of the HV rats developed small granuloma at the ligature site. Initially the testes of the HV rats recovered slowly but by 60 days after operation, however, only a few of the seminiferous tubules had not regenerated. Sperm granulomas were detected only unilaterally in two of the HV rats. The weight of these granulomas was negligible when compared with that in the V rats killed at the same postoperative period. Eventually by 120 days post-operatively the testes of the HV rats were observed histologically to be completely recovered and they weighed only 20 percent less than those of the C and V rats. All HV rats had developed spermatic granulomas, which were only half the size of those found in the V rats 120 days after vasectomy. The extravasated sperm were surrounded by numerous polymorphonuclear leucocytes and macrophages which had invaded the granulomas more or less, depending on the age and size of the lesion.

Bedford (1976) studied the effects of vasectomy in the rabbit, rhesus monkey, hamster, and rat. After vasectomy the spermatozoa were initially found to accumulate in, and to distend, the vas deferens and the distal portion of the

epididymis, but the response thereafter varied from species to species and sometimes among individuals within a species. The distensibility of the distal segment of the rabbit tract seemed to ensure its integrity for 24 weeks or more, but granulomas appeared in one or more regions of the epididymis by 32 weeks in most rabbits. In the monkey, discrete granulomas developed along the vas deferens and/or in the cauda epididymidis only 5-6 weeks after vasectomy in some individuals. Spermatic granulomas developed in one or more regions of the hamster epididymis within 8 weeks and about 30 percent of these animals displayed a granuloma at the site of ligature. Ten weeks after vasectomy in rats, all animals were found to display slight distension of the ipsilateral cauda epididymidis, and they had a moderately large sperm-filled cyst at the point of ligature. Ultrastructurally, many sperm in the cyst had lost their plasma membranes and acrosomes, mitochondrial cristae and axial microtubules of the tail, and the keratinoid organelles were observed being ingested by the cells bounding the cyst cavity. Three of six rats examined 33-37 weeks after unilateral vasectomy differed from the 10 week group only in the large size of the cystic granulomas at the site of ligature. The sperm within these granulomas were not invaded by leucocytes. The other three rats of this group displayed multiple granulomas in the cauda epididymidis and two had in addition small vasal cysts. Ultrastructural examination showed sperm nuclei and tails being ingested by the leucocytes and by the epithelioid border of the granuloma. In the

group examined 60 weeks after unilateral vasectomy, only 2 out of 10 rats had both vasal and epididymal granulomas. Among the eight remaining rats of the group, five exhibited large vasal granulomas, of dimensions reaching 2.5 x 1.3 cm, extending up into the inguinal canal. The other three rats had only small vasal granulomas. The sperm content of the vasal granulomas still remains free from leucocytes.

Alexander and Schmidt (1977) found that of 77 men requesting vasovasostomies, only 17 percent had antisperm antibodies as revealed by immunofluorescence, and more than 35 percent had granulomas associated with the vas deferens or the epididymis. They reported that spermatic granulomas developed in some individuals due to the surgical technique used. Their occurrence did not indicate circulating antibodies, since the presence or absence of granulomas caused no difference in the amount of antibodies detected by immunofluorescence.

Kennedy and Heidger (1980) studied comprehensively the histology and ultrastructure of spermatic granuloma of the rat vas deferens which arise after vasectomy. Twenty-four adult male Sprague Dawley rats were bilaterally vasectomized. Groups of eight animals were killed at each of the following postoperative periods: 2, 4, and 12 weeks. Spermatic granulomas were found on the proximal cut end of the vas deferens in most of the rats. Only in a very few rats were granulomas of the vas absent. They increased in size with time after vasectomy, reaching a length of

approximately 1 cm by 12 weeks postoperatively. In such animals, the testes were found atrophied. A typical spermatic granuloma appeared as a nodular mass of firm consistency with a well vascularized wall lying within the connective tissue surrounding the vas. The content of the nodule was usually paste-like in consistency. Microscopically the granuloma consisted essentially of a wall, composed of a compactly arranged mass of cells, mainly macrophages and epithelioid, surrounding a central mass of sperm and occasional neutrophils, macrophages and lymphocytes; a transitional region between the cellular wall and the central sperm mass, which consisted of loosely arranged collections of macrophages, epithelioid cells, and occasional lymphocytes. In addition clusters of polymorphonuclear leucocytes containing phagocytosed spermatozoan remnants were interspersed within the interface region. Multinucleate giant cells were prominent at 12 weeks post-vasectomy. The outer cellular wall was supported peripherally by a layer of loosely organized highly vascular connective tissue, containing only sparse collagen and few macrophages. Numerous macrophages, epithelioid cells, lymphocytes and plasma cells were usually collected around blood vessels and within lymphatic vessels situated within the loosely organized connective tissue layer. No discrete basal lamina was observed separating the cellular wall from the more peripherally disposed, loosely organized connective tissue region. A well developed capsule composed of macrophages, collagenous bundles and smooth muscle cells surrounds the granuloma.

Lopes and Hayashi (1981) reported that after vasectomy in the rat, the spermatic granulomas which developed at the proximal cut end of the vas deferens showed a continuous growth during 280 days after operation. Histologically they consisted of an accumulation of masses of sperm bordered by a rim of epithelioid macrophages and giant cells containing identifiable sperm fragments.

The main findings which emerge from all this detail may be summarized as follows:

1. Vasal spermatic granulomas were thought to be the most common complication of vasectomy in rats and men. They occurred in most of the rats within a week after vasectomy. These are caused by a rupture occurring in the epithelial lining of the duct, as a result of increased intraductal pressure, and/or through the ligature cutting through the wall of the vas. Sperm escaped into the surrounding connective tissue and became surrounded by a layer of epithelioid macrophages, which in turn was encompassed by a perivascular accumulation of round cells, thus forming the spermatic granulomas, which were limited by a fibrous capsule.
2. They were pale, spherical or oval nodules with a rubbery wall and soft consistency.
3. Some investigators have reported that they become progressively larger, reaching their maximum size at 40 days after vasectomy, then reduced to flattened slips of tissue at 90 days after operation. A few believe that they reach their maximum size by approximately one month after surgery,

they were then stabilized in size. Others showed that they grow continuously up to 9 months after vasectomy.

4. Epididymal granulomas, on the other hand, occurred after vasectomy, due to the simultaneous increased intraductal pressure, and inflammatory or traumatic epididymitis as a result of decreased resistance of the wall of the epididymal duct. They simulated tuberculous epididymitis, even histologically.

5. It was found that spermatozoa contained a lipid fraction which, when injected into the subcutaneous tissue produced a reaction similar to a spermatic granuloma.

6. The initial histology of the spermatic granuloma was found not to be the result of an inflammatory process brought about primarily by autoimmune phenomena. The possibility that sperm absorption by the granuloma might initiate an immune response effecting the histological changes seen in older sperm granulomas had not been eliminated.

7. Spermatic granulomas were thought to be significant from a number of points of view:

a) They may initiate a spontaneous reanastomosis of the vas.

b) They may obstruct the duct and subsequently prevent a successful vasovasostomy.

c) They may cause a permanent infertility.

8. It was found that the incidence of spermatic granulomas can be reduced by suppressing spermatogenesis, by leaving the vas in its surrounding fat sheath, regardless of the method of interruption, or by fulguration of the vas rather than ligation.

II. Effect of Vasectomy on Intratubular Hydrostatic Pressure in the Testis and Epididymis:

It has been postulated that vasectomy results in an increase in the intratubular pressure in the testis and epididymis which in turn causes alteration in spermatogenesis and sperm transport (Smith, 1962; Swanson and Hafs, 1969; Sackler et al., 1973; Schmidt and Morris, 1973; Silber, 1977 and 1979). However no data were available to support this hypothesis until Howards and Johnson (1979) used micropuncture techniques to measure the intratubular hydrostatic pressure in the seminiferous tubules, caput epididymidis, proximal and distal cauda epididymidis and epididymal vas of hamsters and guinea pigs before and after vasectomy.

In unoperated hamsters, the mean values of the hydrostatic pressure measured in the seminiferous tubules, caput, proximal and distal cauda epididymidis and epididymal vas were 4.4, 5.2, 3.9, 4.3 and 5.1 cm H_2O respectively. The pressure in the seminiferous tubules was significantly lower than the pressure in the caput. The pressures in the proximal and distal caudal epididymal duct were significantly lower than that in the caput and there was a significant increase in pressure from proximal to distal cauda epididymidis.

Forty-five percent of the hamsters examined two weeks after vasectomy had formed a spermatic granuloma at the site of the vasectomy. Animals with spermatic granulomas were excluded from the pressure measurements. The mean pressures in the seminiferous tubule; caput, proximal and distal cauda epididymidis and epididymal vas were 3.3, 4.4,

10.6, 14.6 and 18.6 cm H_2O respectively. The pressure in the seminiferous tubule was significantly lower than the pressure in the caput. The seminiferous tubule pressure was also significantly lower than the pressure in the seminiferous tubules of the normal hamster. All of the pressures in the distal epididymis were higher than the pressure in the same region of the normal hamster and there was also a progressive significant increase in pressure along the length of the distal epididymis.

One month after operation, 70 percent of the hamsters developed spermatic granulomas at the site of the vasectomy and an additional 28 percent had developed leaks either in the cauda or in the caput epididymidis. Only one animal had no rupture of the ductal system. In this animal the pressures in the seminiferous tubules, caput, proximal and distal cauda, and epididymal vas were 4.0, 10.0, 12.0, 13.0 and 15.0 cm H_2O respectively. In a second animal, with only a minor leak, the hydrostatic pressure in the distal cauda epididymis was 15.0 cm H_2O. In two animals with large granulomas, the pressures in the remaining intact epididymal tubules and the seminiferous tubules ranged from 3-6 cm H_2O.

In the normal guinea pig, the mean values of the intratubular hydrostatic pressure in the seminiferous tubules, caput, proximal and distal cauda epididymidis were 2.5, 7.6, 5.1 and 10.1 cm H_2O, respectively.

As was the case in the hamster, the pressure in the seminiferous

tubules was significantly lower than in the caput epididymidis. The pressure in the proximal cauda was significantly lower than the pressure in the caput and distal cauda.

All guinea pigs examined four months after vasectomy had an intact duct system. The mean pressures in the seminiferous tubules, caput, proximal and distal cauda epididymidis were now 3.4, 10.5, 4.1, and 23.2 cm H_2O respectively. The pressures in the seminiferous tubules and the distal caudal tubules were significantly greater than the corresponding normal value.

One year after vasectomy in guinea pigs, the intratubular hydrostatic pressures in the seminiferous tubules, caput, proximal and distal cauda epididymidis were 2.0, 7.0, 6.1, and 28.6 cm H_2O, respectively. The pressure in the seminiferous tubule one year after vasectomy was lower than the pressure in the normal seminiferous tubule, while the pressure in the distal cauda was greater than the corresponding normal value.

Thus in both species the increased pressure that was found in the distal epididymis after vasectomy was not transmitted to more proximal regions.

III. Effect of Vasectomy upon the Testis and Spermatogenesis:

Published reports of the effect of vasectomy on spermatogenesis are remarkably contradictory both between species and within the same species.

Sir Astley Cooper (1823) pioneered this research, when he ligated the vas deferens of a dog. Six years later, he noted that the epididymis and proximal vas deferens were dilated with no gross testicular changes. The majority of subsequent studies on the dog and other carnivores have led to the conclusion that there may be a temporary depression of spermatogenesis, but recovery is to be expected in about three months and active spermatogenesis will persist for years (Grewal and Sachan, 1968; Derrick et al., 1974; MacDougall et al., 1975).

The results of vasectomy in the rabbit differ significantly from those in the dog, with a number of investigators reporting no changes in the testis in the first three to four months (Paufler and Foot, 1969; Horan, 1973; Jones, 1973; Flickinger, 1975b; Alexander and Tung, 1977), but with patchy tubular degeneration and impaired spermatogenesis appearing six to eight months post-operatively (Flickinger, 1975b; Bedford, 1976; Alexander and Tung, 1977).

In the rat, reduced testis weight or degeneration of seminiferous tubules has been reported following vasectomy (Jhaver and Ohri, 1960; Laumas and Uniyal, 1967; Sadi et al., 1967; Rumke and Titus, 1970; Altwein and Gittes, 1972; Thakur et al., 1972; Sackler et al., 1973;

Horan, 1973 and 1975; Neaves, 1978), while others have observed little or no abnormal changes (Oslund, 1924; Poynter, 1939; Smith, 1962; Kar et al., 1965; Lee, 1967; Kubota, 1969; Collins et al., 1972; Flickinger, 1972b; Segal, 1972; Heller and Rothchild, 1974; McGlynn and Erpino, 1974; Neaves, 1974; Hernandez-Jauregui and Olivera, 1975; Howards et al., 1975; Mock et al., 1975; Neaves, 1975b; Voglmayr, 1975; Kuwahara, 1976; Lohiya et al., 1976; Bigazzi et al., 1977).

It has been shown that an immune response to sperm antigens occurs after vasectomy in some rat strains but not in others (Bigazzi et al., 1977). However five studies were performed with Sprague-Dawley rats; in four there was no changes after vasectomy (Flickinger, 1972b; Heller and Rothchild, 1974; Neaves, 1974; Mock et al., 1975), but there was a decline in testicular weight in the other (Thakur et al., 1972). Of three studies using Wistar rats, changes were reported after vasectomy in two (Sadi et al., 1967; Sackler et al., 1973), and no alteration was found in the other (McGlynn and Erpino, 1974).

The roles of surgical technique and postoperative complications have been considered, since some workers used an abdominal and others a scrotal approach for vasectomy, and some blocked the vas deferens while others did not. According to Moore (1939) who reviewed the early literature, the reports of changes in the testis after vasectomy can be attributed to ischemia resulting from compromise of the

testicular blood supply or to elevated temperature due to retraction of the testes into the abdomen.

Plaut (1973) found slight reduction in size of the testis in a few rats examined 28-58 days after vasectomy and this was attributed either to a surgical complication, mainly a compromise of the testicular circulation. Whatever the cause it seemed to affect the left testis in the animals more frequently than the right, just as varicocele in humans is more common in the left.

Heller and Rothschild (1974) presented evidence suggesting that non-specific side effects resulting from differences in surgical technique and postoperative care are responsible for much of the earlier controversy surrounding the effect of vasectomy in outbred rats.

Neaves (1974) assigned a group of rats to one of six unilateral surgical treatments: scrotal sham operation, scrotal vasectomy without ligation, scrotal vasectomy with ligation, abdominal sham operation, abdominal vasectomy without ligation, and abdominal vasectomy with ligation. Three months after surgery, the mean testis weights of the rats in the six groups were found to be unaffected by the various operative procedures, but the variance of testis weight was significantly increased after vasectomy with ligation of the vas deferens and following operation involving the abdominal approach.

Neaves (1975b) concluded that atrophy of the seminiferous epithelium appeared to be independent of the surgical technique but was related instead to the occurrence of cryptorchidism , a non-specific side effect of rat vasectomy in which the testis is retracted and retained in the abdominal cavity, where interruption of sperm production occurs due to increased temperature. It was suggested that interference of spermatogenesis after vasectomy could also occur in response to increased temperature due to infection or as a result of ischaemia following severance of the artery of the vas deferens, which was said to be a collateral source of blood for the testis.

Fawcett (1979) reported that the surgical technique itself can account for changes in the testis after vasectomy. Although abdominal approach does not entirely avoid damage to the testis, the scrotal approach to vasectomy can be expected to result in a transient retraction of the gonad into the inguinal canal or abdominal cavity. The resulting loss of the normal thermoregulatory function of the scrotum will result in impaired spermatogenesis due to elevated testicular temperature. Other contributing factors suggested were sterile inflammation due to excessive handling and dislocation of the testis during the surgical procedure, or actual infection from failure to maintain sterility. Jeopardizing the blood supply of the testis and caput was thought to be a less common cause of misleading results.

In addition to these possible causes, which lead to cessation of spermatogenesis after vasectomy, some investigators believed that if spermatic granulomas occurred in the caput epididymis or vasa efferentia there would be testicular atrophy. The literature on this point shows more speculation than fact and is as follows.

Kwart and Coffey (1973) found that multiple granulomas in rats showed at different stages of histological evolution and suggested that as some lesions became quiescent they were succeeded by other granulomatous foci, which became the reservoir of sperm accumulation. In some cases, separate granulomas also appeared in an epididymal site. The authors considered that if granulomas were to continue to extend into the efferent ducts of the testes, they could disrupt testicular function.

Horan (1973 and 1975) reported that in two rats whose left vas deferens was doubly ligated without resection, granulomas developed in the proximal cauda epididymidis. With phase contrast microscopy the sperm were found to be completely immobile and in both cases the testes showed marked atrophy. Horan (1975) suggested that if sperm stasis occurred in the caput epididymidis, there would be a build up of hydrostatic pressure proximal to this. When seminiferous tubular pressure exceeded capillary closing pressure the blood flow would decrease resulting in ischaemic necrosis of the testicular tissue and subsequent testicular atrophy. This report was confirmed by Kuwarhara and Frick (1975).

IV. Effect of Vasectomy on the Epididymis:

As yet there is little knowledge as to the possible side-effect of vasectomy on the morphology and physiology of the epididymis. One impression that can be gained from recent research, is that there are species differences in response to vasectomy. In rats and bulls, it has been reported that vasectomy causes a spermatic granuloma to develop early at the site of ligation on the vas deferens or in the epididymis (Amann and Almquist, 1962; Igboeli and Rakha, 1970; Flickinger, 1972a; Hooker and Gilmor, 1972; Alexander, 1973; McGlynn and Erpino, 1974); in other species, notably the rabbit, this either does not occur at all (Macmillan et al., 1968; Paufler and Foote, 1969; Jones, 1973), or is deferred for several months after operation (Flickinger, 1975a; Bedford, 1976; Alexander and Tung, 1977; Fawcett, 1979). In addition different investigators have also obtained differing and apparently contradictory results on the same species of animal such as the rat (Jhaver and Ohri, 1960; Smith, 1962; Kar et al., 1965; Segal, 1972; Flickinger, 1972a; Plaut, 1973; Sackler et al., 1973; Neaves, 1973, 1974), perhaps as the result of using animals of different age or strain, varying operative procedures, or other technical differences (Flickinger, 1975a). Also within a given experiment, individual animals often vary in the particulars of their response, such as whether sperm escape from the duct system or are retained within it (Horan, 1973; Johnson and Howards, 1975). However most workers agree that, at least

in the early stage, vasectomy only affects the terminal part of the epididymis.

One of the fundamental questions concerns the influence which vasectomy might have on the normal maturation and survival of spermatozoa in the epididymis. After five years from vasectomy in man, Lyons et al. (1967) reported that the epididymal duct was found dilated to a varying degree, and often contained cellular debris and masses of packed sperm.

Alexander (1973) found that spermatozoa in the lumen of the ligated ducts began to disintegrate by 8 weeks after vasectomy in the rats.

At 6 and 24 weeks after vasectomy in rabbits, Jones (1973) observed that 60 percent to 70 percent of spermatozoa from the vas deferens were without heads, whereas from the proximal cauda epididymidis less than 18 percent were decapitated. The author stated that there was renewal and mixing of spermatozoa in the cauda epididymidis but not in the vas deferens, in addition he concluded that the normal maturation and survival of spermatozoa would be affected after vasectomy.

Kothari and Mishra (1973) reported that at intervals of one week after vasectomy in dogs, the lumen of the epididymis appeared dilated but was totally free of any spermatozoa.

During vasovasostomy in 114 vasectomized men, Pardanani et al. (1976) examined 220 epididymides. They reported that in 44 instances the epididymal duct was filled with

whitish material; the lumen of the proximal segment of the vas deferens in these cases contained a creamy white fluid. Microscopic examination of this fluid revealed agglutinated masses of spermatozoa in different stages of disintegration.

Flickinger and Charlton (1979) reported that in four of the six epididymis studied five months after vasectomy in the hamster, a translucent region appeared just proximal to a spermatic granuloma. Microscopic examination of the translucent region showed a large decrease in the numbers of sperm within the epididymal lumen.

The histological changes which occurred in the epithelial lining of the epididymal duct, seem to be variable; Flickinger (1972a) studied the fine structure of the epididymal epithelium of the rat at intervals up to nine months after unilateral vasectomy, bilateral vasectomy or sham operation. Membranous material was observed to accumulate in the cytoplasm of the light cells of the cauda epididymidis after sham operation. The membranes were found in apical vacuoles, in moderately dense membrane-bound bodies in the mid-region of the cells, and most prominently, in large infranuclear masses. The amount of the membranous material absorbed by the light cells of the cauda epididymidis was found to increase after vasectomy. Readily identifiable parts of sperm were found in apical vacuoles of epithelial cells in some instances after vasectomy. The author suggested that such changes may reflect the absorption of parts of sperm by the epithelium of the cauda epididymis after vasectomy.

Alexander (1973) compared epididymal biopsies from rats that had undergone unilateral or bilateral vasectomy from one to eight months previously with biopsies from their contralateral side or from normal controls. She reported that after five weeks from surgery, lamellar bodies began to accumulate in the supranuclear region of all principal cells of zones II and III of the caput epididymidis. At about 10 weeks after vasectomy, these bodies were smaller and more electron dense, and the lamellae became less visible. At about 8 to 10 weeks, lamellar bodies became prevalent on the contralateral side of unilaterally vasectomized animals. As time went on, these bodies became more polymorphic and resembled lysosomes and residual bodies. In the basal area of the principal cells, lipid-like granules were sometimes visible. Spermatozoal breakdown and consequently epithelial uptake of fragments appeared to be especially prevalent in zone II. Whole spermatozoa were seldom ingested by epithelial cells. No striking changes were observed in zone I and IV after vasectomy. Neither the number of "halo" cells nor their position, were found to alter after vasectomy.

Among dogs, Kothari and Mishra (1973) found that, one week after vasectomy, the epithelial cells completely lost their ciliated appearance and became very much flattened with a sharp clear cut free margin.

At intervals up to nine months after vasectomy in the rabbit, Flickinger (1975a) reported that the epididymal epithelium remained columnar and functionally active as

indicated by the persistence of the characteristic complement of cellular organelles.

Alexander and Tung (1977 , 1979) reported that vasectomy resulted in mark distension of the cauda epididymis and proximal vas deferens of the rabbits. The epithelial cells appeared flattened, active with abundant rough endoplasmic reticulum, a large Golgi apparatus, and smooth endoplasmic reticulum. Sperm ingestion was not evident in any of the epithelial cells of the epididymis.

Flickinger and Charlton (1979) detected no microscopic changes in the epididymis in any of the hamsters examined two weeks after vasectomy, but alterations were found in four of the six epididymides studied five months after vasectomy. In the initial segment of some vasectomized animals, the normally regular outline of the duct was lost, the lumen appeared collapsed, and profiles of the epithelial cells were usually narrow. There was a decrease in the height of the epithelium of the caput and corpus regions from normal tall columnar variety to a low columnar shape. In the initial segment, the abundance of apical vesicles and vacuoles was diminished, and the large apical vacuoles of the caput and corpus regions were not as numerous as normal. There was collapse of the lumen of the proximal cauda in some vasectomized animals, but in other instances, the cauda were grossly distended. The principal cells of the epithelium of the cauda epididymidis remained unaltered, but the light cells were highly vacuolated in the proximal

cauda and contained large numbers of dense granules in the distal cauda epididymidis.

Urena and Malavasi (1981) observed ultrastructural changes in the clear cells of the epididymal epithelium of the hamsters, which were examined 3 and 14 months after vasectomy. They reported the accumulation of electron dense bodies of irregular forms, which were distributed between luminal and basal zones of clear cells, the presence of large vacuoles of low electron density and a loss of stereocilia, flattening of the luminal border of the cell and emission of projections toward the lumen.

V. The Fate of Sperm after Vasectomy:

Phadke (1964) reported that phagocytosis by "spermiophages" was the chief mechanism involved in the disposal of dead intraluminal spermatozoa in the majority of cases of obstructive azoospermia and after ligation of vas deferens in man. The author presented evidence to show that these "spermiophages" were derived from the basal layer of cells lining the epididymal duct. In some instances the columnar epithelial lining of the epididymal duct was found to manifest phagocytic properties.

After long-term vasectomy in the rhesus monkeys, Alexander (1972) showed that macrophages take up residence in the epididymis particularly in the ductuli efferentes and ingest and digest spermatozoa.

Flickinger (1972a) reported that the clear cells of the cauda epididymidis of the rat normally absorbed some of the degenerate sperm, and that this process is accentuated after vasectomy, when sperm are no longer able to escape from the duct system. In some instances readily identifiable parts of sperm were found to be absorbed by the epithelial cells of the cauda epididymidis after vasectomy. The author suggested that such changes may reflect the disposal of the large numbers of sperm by the epithelium of the cauda epididymidis after vasectomy.

Alexander (1973) reported that at about 8 weeks after vasectomy in the rat, spermatozoa began to disintegrate in the lumen of the caput epididymidis and to be absorbed by the principal cells.

Flickinger (1975a) observed that sperm accumulated within the male duct system in all rabbits up to 6 months after vasectomy. In 3 of 5 animals killed 6 to 9 months after surgery, the epididymis and vas deferens appeared less distended than in other vasectomized rabbits, and in 2 of these sperm appeared to have escaped the duct system and undergone phagocytosis in a spermatic granuloma in the surrounding connective tissue. Large numbers of lysosomes which range in size from a few tenths of a micrometer to large bodies that occupied much of a cell's cytoplasm were observed in the epithelium of the caput and cauda epididymidis and the proximal vas deferens. The author stated that some sperm may also have degenerated in the lumen with subsequent uptake of their component by the epithelial cells. In addition a few round cells were found in the lumen of the ductuli efferentes and scattered among the luminal contents in the epididymis and vas deferens of all the rabbits. In the ductuli efferentes of both vasectomized and sham-operated rabbits, some of these round cells appeared to contain sperm, but the numbers of sperm involved were extremely small, and no increase in their number with time after operation was observed. The author thought that migration of phagocytes into the lumen of the duct system was not important in disposal of sperm after vasectomy.

Hoffer et al. (1975) compared the effects of ligation of an isolated loop of the ductus epididymidis in the region of the caput with those of exfoliative lesions of the epididymis that follows administration of α-chlorhydrin to rats. They reported that in both cases there was early phagocytosis of apparently normal spermatozoa by the non-ciliated cells and that this epithelial spermiophagy became increasingly evident after longer intervals. The non-ciliated cells were found to extend long irregular pseudopodia that engulfed the spermatozoa. These processes contained no organelles but seemed to be composed mainly of a filament-rich ectoplasmic layer of the cytoplasmic matrix. The ingested spermatozoa were contained in membrane-limited vacuoles in the apical or perinuclear cytoplasm and their ultrastructure soon after their ingestion appeared normal. The acrosomal content was homogeneous and of normal density and portions of the sperm plasma membrane and outer acrosomal membrane were often still present. Vacuoles containing phagocytosed sperm heads and tails were at first surrounded by a filament-rich zone of cytoplasm which was comparable in its fine structure to that in the pseudopodia that originally engulfed the spermatozoa. A few macrophages containing spermatozoa were observed early in the lumen of the duct and their number increased with time. The authors concluded that epithelial spermiophagy is a consequence of obstruction, whether mechanically or chemically induced.

Bedford (1976) studied the fate of spermatozoa following vasectomy in the rabbit, rhesus monkey, hamster and rat. He reported that spermatozoa were not ingested by the intact epithelium of the vas deferens and epididymis, nor did they undergo dissolution in the duct lumen or in the granuloma. Their disposal depends on their ingestion by leukocytes, which are first attracted to and enter the tract of the vasectomized male via the granulomata, later migrating within the intact duct, and particularly within the terminal vasal cysts, by the epithelioid border of the granuloma.

Galle and Friend (1977) examined the epididymis and vas deferens, and their luminal contents, in 23 guinea pigs up to 175 days after vasectomy, in order to ascertain the fate of spermatozoa. They found no changes in the stacking pattern of sperm in animals examined between 1 and 30 days postoperatively, but in vasectomized animals 60 to 175 days after surgery, sperm dissolution was observed in the proximal portion of the vas deferens and cauda epididymis. In four of six animals examined between 90 and 175 days post-vasectomy, sperm granulomas were observed in the corpus and cauda epididymidis and in the vas deferens. At this stage, polymorphonuclear leucocytes and macrophages containing engulfed sperm, were observed in the lumen of the epididymis and vas deferens. All the macrophages observed contained spermatozoal material and were thought to degrade sperm more effectively than did polymorphonuclear

leucocytes which failed to degranulate fully. The authors suggested that, in the early stages, sperm degradation was apparently accomplished by the sperm's own complement of hydrolytic enzymes. Later, disposal of sperm commences with the appearance of granulomas and the uptake of spermatozoal material by white blood cells.

Flickinger (1979 and 1982) examined the disposition of sperm produced at intervals up to one year after vasectomy in the hamster. He reported that spermatic granulomas were associated with the excurrent ducts of all animals examined five months or one year after vasectomy and with only one of four hamsters examined two weeks after surgery. The centres of each granuloma consisted of sperm and phagocytic cells characterized by cytoplasmic lysosomes and sperm in different stages of disruption. Phagocytic cells which had ingested sperm were seen in the proximal portions of the excurrent ducts in some animals killed five months after operation and in all hamsters examined one year after vasectomy. The efferent ducts were found to be the most frequent sites for phagocytes, which were large cells with variably contoured surfaces, dense nuclei and abundant cytoplasm contained many recognizable parts of sperm as well as numerous lysosomes with polymorphous interiors. In a few animals, phagocytes having a smaller size, lobated nucleus and in some instances a granulated cytoplasm, were also observed in the initial segment and/or caput.

Phagocytic cells in these regions were thought to resemble polymorphonuclear leucocytes. Very large numbers of partially disintegrated sperm were observed in the lumina of the majority of epididymides from animals vasectomized five months or more. The most common abnormality was leakage or loss of the plasma membrane. The author concluded that, after vasectomy in the hamster, sperm were disposed of by phagocytosis in spermatic granulomas, intraluminal phagocytosis, and dissolution in the lumen of the male ducts.

VI. Humoral Antibodies to Sperm Developed as a Result of Vasectomy

Rumke (1954) and Wilson (1954) observed spermagglutinating activity in the serum, as well as in the seminal plasma, of male patients complaining of sterility, and confirmed these observations in later works (Wilson, 1956; and Rumke, 1959).

Rumke and Hellinga (1959) suggested that the initiation of sperm antibody production could occur from the extravasation of spermatozoa into the interstitium, lymph vessels, or blood capillaries of the epididymis with subsequent transfer to the regional lymph nodes.

Phadke and Phadke (1961) noted large numbers of macrophage cells in the epididymal secretion in some cases of obstructive azoospermia and in semen in a few cases of severe oligozoospermia. It was then concluded that at least in some cases of obstructive azoospermia, phagocytosis was the chief mechanism utilized for the disposal of dead spermatozoa. They also thought that as the macrophages were derived from the reticulo-endothelial system of cells, and as the same system was thought to be involved in the production of antibodies, autoantibodies were to be expected in the blood sera in the selected cases of obstructuve azoospermia and in the few cases of severe oligozoospermia where the semen showed large numbers of macrophages.

Phadke and Padukone (1964) reported sperm agglutinating antibodies in the serum of 8 out of 25 men who had undergone vasectomy two to twenty years previously. This report was

confirmed by Rumke (1968) and by Zappi *et al.* (1970).

Rumke and Titus (1970) found a significant sperm agglutinin formation after unilateral vasoligation in two out of seven rats and after unilateral vasectomy in all rats. The antibody stimulation tends to cease after removal of the testis and epididymis of the operated side.

Ansbacher (1971) and Ansbacher *et al.* (1972) studied 48 men for six to twelve months after vasectomy. At six months, 26 of them (54.2%) had sperm agglutinins in their blood, whereas only one of them had such antibodies prior to operation. The sperm immobilizing antibodies had appeared in (31.3%). The antibodies (both agglutinins and immobilizing antibodies) continued to be in circulation at twelve months after the operation. In some (5 out of 27), there was a rise in titre.

Shulman *et al.* (1972) measured the titres of sperm agglutinin in the serum of 22 men, before, and at various intervals after, vasectomy. A positive result was found in approximately 55% of cases. Most titres were moderate, although one was 1:1024. The activity was thought to appear about 3 to 6 months after operation, although in some cases, it was found to develop later. Three men had pre-vasectomy antibodies to sperm, but in two of these, the titres increased after the surgery.

Halim and Antoniou (1973) reported that the pre-vasectomy incidence of sperm cytotoxic antibodies was one percent and rose to two percent six weeks after the operation. They were only detected in patients who possessed high titres of sperm agglutinating antibodies.

Howard and James (1973) noted that 32.6% of cases investigated at three to six months after vasectomy had sperm agglutinins, whereas none had these antibodies prior to the operation.

Ansbacher (1973 and 1974) demonstrated circulating sperm agglutinating antibodies in 50-62% of post-vasectomized men up to two years after surgery, sperm agglutinating activity of the serum peaked at 18 months postvasectomy both in incidence and level of the titre, with time, decay of sperm agglutinins titres took place and at three years after vasectomy they were detected in only 38.9% (7 out of 18) of cases. The sperm immobilizing antibodies had appeared in 30 percent of cases 12 months after vasectomy, but there was a dramatic fall in incidence to 9.5% at 18 months and in titre at 24 months and at 36 months after operation. Only one case out of 17 demonstrated the residual presence of these antibodies.

Alexander et al. (1974) found complement-binding antibodies in the serum of 30 men and 26 rhesus monkeys two weeks after vasectomy. In the men there was an increase over time in the average spermagglutinin titre, while although most monkeys developed and maintained a low spermagglutinin titre, a few developed a high titre.

In the series of Shulman (1974), sperm agglutinating antibodies were found in 63.7% of cases at six months after vasectomy, the prevasectomy incidence was 9 percent.

Tung (1974) was able to demonstrate two kinds of antisperm antibodies in the sera of 80 patients studied, using an immunofluorescence technique. The first kind, called "natural" antisperm antibodies, were found in 50% of patients before vasectomy and were directed to antigens in the acrosome, the equatorial segment and the postacrosomal region. They were increased in titre by two months post-operatively and showed little further change at nine months. The second kind were called "immune" antisperm antibodies and were directed to the tails or occasionally to discrete areas over the acrosome. They were found in 2-3% of patients before vasectomy and increased in incidence to 25% in two months and 50% in nine months after vasectomy. Both antibodies were present as IgG and IgM.

Samuel et al. (1975) examined sera obtained from 55 vasectomized men at 10 days, 6 weeks, 3, 6, 9 and 12 months after surgery. They reported that 60% showed agglutinating activity between 6 weeks and 6 months after vasectomy. Only 2 out of the 27 patients investigated at 1-4 months after vasectomy had sperm cytotoxic antibodies and 5 out of 23 to have them after one year. Such antibodies were only seen in patients who had high titres of sperm agglutinating antibodies.

By indirect immunofluorescent techniques, Tung (1975) has noted that antibodies in sera of vasectomized men were of seven types. Five of these reacted with accessible sperm antigens and two with antigens exposed by detergents and

proteolytic enzymes. In the former category, two reacted with acrosomes, one giving a diffused type of fluorescence and the other a speckled reaction. Other surface antigens against which antibodies were present in vasectomized men are localizable in the equatorial region of the sperm, the post-acrosomal cap and in the main piece of the tail. The two sites rendered reactive with antibodies after detergent and trypsin treatment were the nucleus and the midpiece. On the basis of observations on the localization of antibodies in non-vasectomized subjects, a hypothesis was advanced to suggest that three types of antibodies would be specifically engendered by vasectomy. These are: 1) antibodies giving speckled reaction with acrosome, 2) antibodies reacting against the main piece of the tail, and 3) antibodies reacting with the nucleus.

Ansbacher et al. (1976) reported that in a group of vasectomized patients, circulating sperm agglutination antibodies were found to have a peak incidence of 69.7% one year after the operation, with a subsequent decrease in both incidence and titre after two years. However, of the 17 patients whose serum was tested five years after surgery, the incidence was 47%.

Tung (1976) reported that the acrosome of human sperm possessed two distinct antigens which could elicit antibodies detectable by immunofluorescence. These were: 1) AC1, was diffuse in distribution and was thought to be glycoprotein, 2) AC2, was discrete in its distribution. AC1

was found to show extensive cross reaction with micro-organisms and with antigens of the human adrenal gland. The anti-AC1 was found frequently in the serum of men before vasectomy. AC2 antigen did not show cross-reaction with micro-organisms or the tissue antigens tested and its antibody was found mainly in the male and primarily after vasectomy. Thus it was suggested that anti-AC2 antibody may be more indicative of an immune response to sperm.

Linnet and Hjort (1977) reported that among forty-seven men examined one year after vasectomy, 62% had sperm agglutinins in the serum in titres from 40 to about 4000 and none of these patients had agglutinins before operation. In most cases, agglutinins were detected after 1-3 months, but in six cases they were not detectable until one year after vasectomy. The titres were found to increase during the observation period, but decreases and even disappearance were also observed. Only 4% of cases showed antibodies in the seminal fluid detectable by the gelatin agglutination test.

Bigazzi *et al.* (1977) and Bigazzi (1978) showed that different strains of rats differ in the vasectomy-elicited response to sperm antigens. A maximum of 80% of vasectomized Lewis, 47% of Brown Norway, 13% of Buffalo, 12% of Wistar-Furth, and 11% of AC1 rats were found to produce antibodies to spermatozoa after vasectomy detectable by indirect immunofluorescence, whereas no such antibodies were detected

in the serum of vasectomized Fischer, Dark Agouti, and Sprague-Dawely rats. Antibody titres were elevated in both Lewis and Brown Norway rats, but remained low in all other animals.

Hellema et al. (1978) found that agglutinins and immobilizing antibodies appeared six weeks to three months after operation. The number of positive cases kept on rising up to about six months, thereafter the pattern of subjects carrying sperm-agglutinating and/or sperm immobilizing/ cytotoxic antibodies was stabilized. The titres of antibodies, however, kept on increasing till twelve to eighteen months after vasectomy.

Hjort et al. (1978) showed that auto-immune sera of patients revealed tail to tail agglutination, and recognized two different tail antigens. This was demonstrated in blocking experiment with $F(ab)_2$ fragment of IgG fraction of several tail agglutination sera. The antibody fragments, though still revealing agglutination, were no longer able to immobilize spermatozoa in the presence of a complement. Moreover, the fragments blocked the immobilizing capacity of the whole antibody molecule of the same serum and sometimes also of other sera. But the latter was certainly not always the case. With the $F(ab)_2$ fragments of two sera revealing the same mode of tail agglutination, there was definitely no cross-blocking activity, thus disclosing the existence of two different tail antigens.

Alexander and Tung (1979) reported that 7 of 50 vasectomized rabbits had low levels of anti-sperm antibodies before operation as measured by indirect immunofluorescence; These rabbits developed higher levels after vasectomy. The prevalence of antibodies increased with time after vasectomy and at six months postoperative 30% had anti-sperm antibodies. In the group vasectomized for 8-12 months, all the animals had circulating anti-sperm antibodies and half exhibited testicular degeneration. The most common antibody type was antiacrosomal, although postacrosomal or equatorial fluorescence was observed occasionally. Most of the antibodies were of the IgG class.

Tyler et al. (1979) found the pre-vasectomy incidence of spermagglutinating antibodies was 6% with titres ranging from 1:4 to 1:8. Four months after surgery the incidence of spermagglutinating antibodies had risen to 24% with titres ranging from 1:4 to 1:32. Six of ten men possessed spermagglutinins three years after vasectomy, the titres ranging from 1:4 to more than 1:1024. Five of these six men had not possessed spermagglutinins at the earlier examination and in the six there was increases in the titre from 1:8 at 3-4 months to more than 1:1024 at 3 years.

Ansbacher (1981) followed prospectively a group of men for 10 years after their bilateral vasectomy. He reported that the sperm agglutinating antibody titre peaked around 18 to 24 months and fell slightly thereafter, whereas only one man showed a serum sperm immobilizing antibody at the low titre of 1:2, 10 years after vasectomy. None of the men had such antibodies before operation.

VII. The Maturation of Spermatozoa in the Epididymis:

Mammalian spermatozoa emerging from the testis are not yet mature, and the ability to fertilize is acquired during their passage through the duct of epididymis (Young, 1929 and 1931; Toothill and Young, 1931; Lasley and Bogart, 1944; Mukherjee and Bhattacharaya, 1949; Rao and Berry, 1949; Nishikawa and Waide, 1952; Blandau and Rumery, 1964; Bedford, 1966; Fulka and Koefoed-Johnson, 1966; Orgebin-Crist, 1967; Horan and Bedford, 1972; Bedford et al., 1973). Whether the factors responsible for such maturation are intrinsic to the sperm cells themselves or result from the environmental conditions established by the epididymal epithelium, or both, has not been clearly established (Reid and Cleland, 1957; Nicander, 1957 and 1958). A number of observations, e.g. of the movement and loss of the cytoplasmic droplet, of a change in resistance to cold shock, heat, alkaloids, acid and alkali, and an increase in specific gravity of the sperm cell, of changes in the capacity for motility, in the acrosome, and in the plasma membrane of one or more species during epididymal passage, probably reflect maturation change (Orgebin-Crist, 1969; Bedford, 1973; Bedford et al., 1973).

Blandau and Rumery (1964) noted that of 264 rat ova exposed to spermatozoa from the cauda epididymidis of rats, 245 (93%) were fertilized and were in the two-cell stage at the time of examination. By contrast only 18 (8%) of 238 ova were fertilized with spermatozoa from the caput

epididymidis. None of the 220 unfertilized ova in this group had been penetrated by spermatozoa 36 hours after insemination. The authors stated that the fertilization rate of the eggs which had been exposed to spermatozoa from the cauda epididymidis was very similar to that expected from normal matings or from females artificially inseminated with spermatozoa recovered from the vas deferens.

To determine whether spermatozoa from the caput epididymidis had reached the ampulla of the oviduct but for some reason had not been able to penetrate the ova, a number of oviducts from the experimental animals were cut above the uterotubal junctions. The periovarial sacs and ovaries were removed. The content of the oviducts were carefully flushed with a small amount of Hanks' solution and the washings examined for spermatozoa. A small number of spermatozoa were observed in only 1 of 15 oviducts which had been exposed to spermatozoa from the caput epididymidis. Literally millions of motile spermatozoa were found within the cornu of the same animal which had received spermatozoa from the same epididymal segment.

The authors compared the swimming motion of the spermatozoa from the caput epididymidis with those from the cauda, and found that the great majority of spermatozoa from the caput epididymidis, though actively motile, swam in a circular direction. This circular movement was thought to be related to a peculiar retroflexion of the head and curvature of the neck region. By contrast, only an occasional spermatozoon,

recovered from the cauda epididymidis, showed the same type of retroflexion of the head. Most of the spermatozoa from this region were found to move forward by an active bending of the head and neck, simulating a kind of "pecking" motion.

Spermatozoa from various levels of the human epididymis have been studied by Bedford et al. (1973) for acrosomal morphology, nucleus, perinuclear material and tail, the swimming activity, and characteristics of the sperm surface. They reported that with the exception of the cytoplasmic droplet, there is no change in acrosomal morphology on passage through the epididymis. However acrosomal abnormalities were found commonly during spermiogenesis, such as sharing of one acrosomal complex by two nuclei, and acrosomal cyst formation.

The nucleus of the morphologically normal human spermatozoon at spermiation appears identical at the ultrastructural level to that of normally shaped spermatozoa in the cauda epididymis or in the ejaculate, but it was found that when sperm samples from both caput and cauda epididymidis were treated, respectively, with an excess of 1% SDS (which breaks weak bonds), the nuclei of most caput spermatozoa displayed a distinct swelling, easily seen in the phase microscope, while only a very few spermatozoa from the cauda epididymidis manifested a marked swelling reaction when exposed to SDS, the majority remaining almost totally condensed. The difference in the structural quality of the nuclei of caput and cauda spermatozoa was said to

rest on the fact that the sperm nucleus becomes stabilized by -s-s crosslinks formed within the chromatin during the passage of spermatozoa along the epididymal duct.

The events which occur in human spermatozoa during their passage through the epididymis were found also to involve change in the structural quality of major organelles in the tail, and when caput spermatozoa were exposed to SDS a high proportion of the tail appeared to dissociate, and on centrifugation the outer dense fibres and the elements of the mainpiece sheath were found to segregate into large disorganized clusters or aggregates.

Subjective observations of the motility of epididymal spermatozoa were made on samples released some 15 minutes after removal of the testis and epididymis. It was observed that a significant proportion of spermatozoa released from the cauda showed sustained progressive swimming activity, while sampling of various segments traced in sequence proximal to the cauda showed a progressive decline in the capacity for flagellation or swimming activity. Spermatozoa released from the initial segment of the epididymis displayed either no vibratory movement or only a feeble one. A similar picture was observed in samples released from the caput, except that a greater proportion of the spermatozoa showed the stationary thrashing movement. This last pattern was still found to predominate among the motile spermatozoa recovered from the corpus epididymidis, but these were found to intersperse by some highly active spermatozoa

progressing efficiently and for a relatively long distance in a single direction, their tails undergoing a rapid stiff beat of limited arc - the swimming pattern of mature spermatozoa. Increasing numbers of this last type of spermatozoa were observed in samples from the upper and middle parts of the cauda epididymidis.

The character of the surface of the spermatozoal plasma membranes was studied by a modification of the technique of Gasic et al. (1968), in which the surface binding of positively charged colloidal ferric oxide particles can be visualized in the electron microscope. It was found that glutaraldehyde-fixed spermatozoa from the caput epididymidis exposed to the colloid solution at pH 1.8 failed to bind the electron-dense ferric oxide particles over both the head and tail surface. The tail region was found to exhibit significant colloid binding in the spermatozoa from the middle segment of the corpus epididymidis. On the other hand the head surface was found to acquire the capacity to bind colloid when the spermatozoa reached the lower segment of the corpus epididymidis. The author concluded that there were marked changes in the chemical conformation of the sperm cell surface during epididymal passage.

VIII. Structure and Function of the Excurrent Ducts of the Testis in the Rat:

The following account is based principally on the studies by Reid and Cleland (1957) and Hamilton (1975).

A. The ductuli efferentes

The ductuli efferentes are lined by a tall columnar epithelium, consisting of two main cell types: the principal cell is the most numerous type, with ciliated cells scattered along the length of the ductule. Occasional lymphocytes (often called "halo" cells in the older literature) are seen within the epithelium. The epithelium surrounds a stellate-shaped lumen. This varies in width along the length of the ductule. The stellate appearance of the lumen in transverse section reflects the presence of two to four epithelial ridges extending along its length. Spermatozoa are usually not seen in the lumina of efferent ductules. The ductuli efferentes are surrounded by one to three layers of elongated smooth muscle cells.

The principal cells have short apical microvilli, with invagination and micropinocytotic infolding of the plasma membrane between them. The apical cytoplasm contains numerous smooth - and rough - surfaced vesicles, together with large empty vacuoles. The Golgi apparatus is medium in size and usually surrounded by many smooth- and rough-surfaced vesicles and a few multivesicular bodies.

The nucleus is small, oblong and occasionally highly indented. It is situated in the basal half of the cell and shows marginal clumps of heterochromatin.

A variable number of large, densely stained lysosomes, numerous mitochondria, free ribosomes and polyribosomes are scattered throughout the cytoplasm.

Elongated profiles of rough endoplasmic reticulum are sparsely scattered in the cytoplasm, and are often continuous with tubules of smooth endoplasmic reticulum.

The ciliated cell is flask shaped, with the expanded apical part containing the nucleus and a stem extending to the base of the epithelium. From the basal bodies just deep to the apical plasmalemma, cilia arise and protrude into the lumen of the ductule. The apical cytoplasm may contain a few smooth- and rough-surfaced vesicles and occasional multivesicular bodies. Endoplasmic reticulum is sparse and the Golgi apparatus is small and surrounded by a few dense bodies. Mitochondria are scattered throughout the cytoplasm.

B. The ductus epididymidis is a single highly convoluted duct arranged in segments or lobules, which are demarcated by connective tissue septula. It is embedded in adipose tissue behind the testis, to which it is connected by means of a thin mesentery, to its superior, dorsal, and inferior surfaces.

As in other mammals, the rat epididymis is divided into three major parts - the caput, corpus, and cauda epididymidis. The ductuli efferentes empty into an elongated reddish process on the superior-medial aspects of the caput, called the initial segment. Its reddish colour is due to the fact that it is very highly vascular. The initial segment is separated from the caput by a deep cleft. Beyond the initial segment is the caput, composed of three to four segments. The

distal part of the caput becomes gradually narrower and extends into the corpus. This consists of several narrow segments and a single, wider distal segment, which continues into the cauda. This is a very distinctive structure which consists of a large coiled duct, milky in colour due to its content of large numbers of stored sperm.

The epididymal duct of the rat is lined by pseudo-stratified columnar epithelium, surrounding a lumen containing sperm. Proceeding from the caput to the cauda, the main changes are: a gradual increase both in the diameter of the duct and in the number of spermatozoa present in its lumen, and pari passu with this, a decrease in the height of the lining epithelium. The epithelium is composed of several cell types. The principal cells are the most numerous and are accompanied throughout the length of the duct by small basal cells with few organelles and by intraepithelial leucocytes ("halo" cells). The initial segment and the proximal part of the caput of the epididymis contain "apical" cells, and in the more distal parts of the epididymis, the so-called "clear" cells form part of the epithelium.

1) The principal cells: These are characterized by numerous microvilli or "stereocilia" which project into the lumen from the apical surfaces of the cells and by spherical or somewhat ovoid, basally situated nuclei. These contain finely granular chromatin except in the terminal part of the cauda epididymidis, where the chromatin is coarsely granular. In the caput and corpus, the nuclei of the principal cells are large and contain

one or two nucleoli. Nuclei in the principal cells of the cauda, on the other hand, resemble those of the initial segment in being smaller and having more nucleoli.

Ultrastructurally, the principal cells contain a very large Golgi apparatus in the supranuclear cytoplasm; it consists of several stacks of cisternae. Both smooth-surfaced and coated-surfaced vesicles of varying size are numerous in the vicinity of the Golgi apparatus. Autophagic vacuoles containing variable amounts of cell debris may be found anywhere in the cytoplasm, but more likely near the Golgi bodies. Lysosome-like dense bodies are frequently present in the supranuclear cytoplasm. They appear to be more common in the caudal region than elsewhere along the length of the duct. They may represent a further stage in digestion within autophagic vacuoles.

One of the most conspicuous features of the epididymal epithelium in general is the presence of multivesicular bodies. They constitute a form of lysosome and occur in two forms: the first is small, round to oval in shape, and composed of a variable number of round or elongated vesicles limited by a single thick membrane, resembling the plasmalemma more than the membranes of the endoplasmic reticulum. They are scattered throughout the apical cytoplasm. The second, less numerous, form is larger, with fewer vesicles, containing variable amounts of a flocculent, electron-dense material. This type of multivesicular body is found more frequently in the initial segment than elsewhere.

The rough endoplasmic reticulum is extensively developed and occupies much of the basal cytoplasm and also extends into the perinuclear and supranuclear regions. Elements of smooth endoplasmic reticulum, on the other hand are infrequent.

Throughout the length of the duct, the basal cytoplasm also contains free ribosomes, scattered mitochondria, and discrete droplets of lipid. Lipid may also be found as single droplets in the Golgi region.

The lateral margins of principal cells interdigitate extensively with those of adjacent cells, and true tight junctions are found between them next to the lumen of the epididymis.

2) Basal cells: This cell type is found scattered along the whole length of the duct, lying between the basal ends of the principal cells and the basement membrane. They are polygonal in sectional profile and have a relatively large nucleus and pale cytoplasm. At the electron-microscopic level, basal cells exhibit slender processes, which interdigitate with similar processes from the adjacent principal cells. The cytoplasm is relatively poor in cell organelles. The functions of these cells are still unknown.

3) "Halo" cells: "Halo" cells are also found throughout the length of the duct. They are seen insinuated between epithelial cells in all levels of the epithelium, from the base to the lumen. However they are not seen crossing the basal lamina to enter the epithelium, nor migrating into the lumen. They are most abundant in the distal part of the caput. Under

the electron microscope, they have some of the characteristics of lymphocytes. The nucleus is indented toward the cytocentrum and exhibits abundant heterochromatin. It is surrounded by a rim of pale cytoplasm, containing a small Golgi apparatus, scattered elements of endoplasmic reticulum, and a few mitochondria. Variable numbers of membrane-bounded dense granules are found within the lumen of the endoplasmic reticulum. The functional significance of this cell type is completely unknown.

4) Apical cells: These cells are confined to the initial segment of the epididymis, where the ratio of "apical" cells to principal cells is 1:3. Apart from apically situated nucleus, they closely resemble principal cells. The apical surface has fewer microvilli than principal cells.

Ultrastructurally, the "apical" cells had many features in common with principal cells, but there are qualitative differences by which they may be distinguished. There are fewer organelles than in the principal cells. The Golgi apparatus contains loosely lamellated cisternae. The mitochondria appear to be larger than those in principal cells.

Reid and Cleland (1957) suggested that "apical" cells are produced by division of principal cells. Sun and Flickinger (1980), on the other hand reported that these cells can probably be considered a form of principal cells.

5) Clear cells: Although "clear" cells are absent in the initial segment and proximal part of the caput, they are a conspicuous feature of the epithelium of the rest of the epididymal duct and are especially numerous in the cauda

epididymidis, where they make up as much as 20 percent of all the cells in the epithelium. They are characterized by a highly vacuolated apical cytoplasm, pale staining nucleus, the presence of a variable amount of granules in the infranuclear cytoplasm, and shorter and less numerous stereocilia than principal cells.

The prominent ultrastructural features of the "clear" cells are the presence of several lysosome-like dense bodies, lipofuscin material, and variable sizes of multivesicular bodies in the apical cytoplasm, and dense accumulations of lipid-like droplets in the infranuclear portion of the cell. They possess a relatively small Golgi apparatus and a variable number of mitochondria. The functions of the "clear" cells are not yet known.

After this general account of the epididymal duct, certain regional specializations are described.

B(i) Initial segment:

The epithelium of the initial segment is very tall and surrounds a small lumen, containing few sperm. It is composed of principal cells, apical cells and basal cells, with an occasional "halo" cells.

The principal cells in this region are characterized by long stereocilia, which are approximately 25 μ long at the proximal end of the segment to become 15 μ long near its distal end, the profusion in the apical cytoplasm of vesicles and small vacuoles, which occupied a thick layer of cytoplasm at the apical ends of the cells. The nuclei are small,

round to somewhat oval in shape, usually provided with more than one nucleoli.

B(ii) Caput epididymidis:

The caput epididymis is lined by a tall columnar epithelium, and the lumen contains many more sperm than does the initial segment, all of which have the cytoplasmic droplet next to the head of the cell.

In its proximal part only principal cells, basal cells, and "halo" cells comprise the epithelium. Beyond this the clear cells are a conspicuous addition to the epithelium.

In this part of the epididymis, the vesicles and vacuoles occupy a much smaller zone in the cytoplasm of the principal cells underlying the apical plasma membrane than in the initial segment. The apical vacuoles differ from those in the initial segment in being larger. The length of the stereocilia is about 12 µm at the proximal end of the caput, becoming about 6 µm near its distal end. The nuclei of the principal cells are relatively large and contain one or two nucleoli.

B(iii) Corpus epididymidis:

The epithelium is slightly shorter than in the caput and it surrounds a relatively wider lumen, containing more sperm. In this region, the cytoplasmic droplet is at the "middle piece" of the sperm.

The principal cells of this part of the epididymis differ from those of the caput mainly in that the large apically located vesicles and vacuoles are not as numerous. The stereocilia are about 5 µ long.

B(iv) Cauda epididymidis:

In the proximal part of the cauda, the epithelium has a medium columnar shape and assumes a low columnar to cuboidal appearance in its distal part. The clear cells and basal cells are most abundant in this region of the epididymis. The luminal sperm exhibit a cytoplasmic droplet attached to the tail.

The apical vesicles and vacuoles are few in number. The stereocilia are about 6 µm long and they tend to be flattened against the surface and give the impression of forming a brush border. The nuclei of the principal cells in this region resemble those of the initial segment in being smaller and having more nucleoli. The dense bodies are more numerous in this region than elsewhere.

B(v) Interstitium:

The epididymal duct is invested by smooth muscle, which is very thin over the proximal part of the duct, but thickens gradually as the duct is traced distally. Blood capillaries, small nerves, and fibroblasts are scattered among the muscular layer. Outside the muscular layer, loose connective tissue binds the adjacent loops of the duct and constitutes the interstitium of the epididymis.

Function and structure of the stereocilia:

The first description of the ciliated nature of the luminal surface of the epididymal epithelium was reported by Becker (1856), later, Aigner (1900), studying them in vivo in rats, pronounced them stationary, and Reichel (1921) named them stereocilia.

Benoit (1926) observed the progressive growth of the stereocilia during development and their regression after castration. He regarded the stereocilia simply as a differentiation of the apical cytoplasm, and thought that they played a role in the elimination of secretory products.

Shaver (1954) noted the ability of stereocilia to remove India ink particles from the lumen of the rat epididymis. The author suggested that the stereocilia having a "sifting" action on the cellular debris in the upper portion of the epididymis.

Hoffer et al. (1973) studied the fine structure of the stereocilia in the initial segment of the rat epididymis and compared them with the microvilli of the absorptive cells of the intestine. They reported that, in contrast to the microvilli, which are closely packed and extremely uniform in diameter, stereocilia are variable in diameter and are rather sparse near the cell surface. The interior of the stereocilia contains closely packed fine filaments, which extend for several microns into the apical cytoplasm, but the terminal web which is found in the apical cytoplasm, in association with the intestinal microvilli, is completely missing. The authors suggested that further investigation was necessary to determine if the thin filaments in the stereocilia are composed of actin, as they are in the intestinal microvilli.

Functions of the Epididymal Epithelium:

Several studies have shown that the epididymis is involved in absorption of fluid and particulate material from the lumen.

Young (1933) observed that the mouse testis swells after ligation of the efferent ducts but not after ligation further down the epididymal duct. He suggested that fluid produced in the testis is absorbed in the epididymis.

Volgmayr et al. (1966, 1967) reported that of the 40 ml or so of fluid which enters one epididymis of the ram each day, only 0.4 ml leaves it.

In rats, Levine and Marsh (1971) found that 50% of the fluid leaving the testis was absorbed in the caput; in bulls and boars the figure was 99% (Crabo, 1965).

Morphological studies with the light microscope showed that the epithelium of the caput epididymidis can also absorb particulate material such as India ink, or colloidal dye e.g. trypan blue, from the lumen in vivo (Gunn, 1936; Mason and Shaver, 1952; Grant, 1958) and in organ culture (Wagenseil, 1928).

More recent studies, using the electron microscope, have extended these observations to show the absorption of (1) horseradish peroxidase by the ductuli efferentes in the hamster (Sedar, 1966), and by the vas deferens of the rat (Friend and Farquhar, 1967), (2) colloidal mercuric sulphide by the caput epididymidis in the hamster (Burgos, 1964), (3) India ink by the distal part of the caput epididymidis in rabbit (Nicander, 1965), (4) iron dextran by the cauda epididymidis in the rabbit (Nicander et al., 1965).

The morphological details of absorption by the principal cells of the excretory duct do not differ significantly from similar activity in other cell types (Hamilton, 1975). Shortly after introduction of the tracer into the lumen of the duct, it was found in between the stereocilia, in the micropinocytotic infoldings present at the base of microvilli, and in apical coated vesicles of the principal cells. Later the tracer was taken into the secondary lysosomes or the multivesicular bodies, where it was presumed to be digested by hydrolytic and proteolytic enzymes of the matrix (Hamilton, 1975).

Secretion

The literature contains numerous suggestions that the epididymal epithelium synthesizes and secretes certain compounds.

1. Glyceryl phosphoryl choline (GPC)

Scott et al. (1963) demonstrated synthesis of GPC in the caput and cauda epididymidis of the rabbit and reported the secretion of the same compound in the epididymis of the ram.

Scott et al. (1963) proposed that GPC secreted in the rat epididymis was formed from lecithin or choline plasmalogen.

The physiological role of GPC in the epididymal semen is still not clear. Scott et al. (1963) believed it to play a role in the maturation of sperm.

Scott et al. (1963), Crabo and Gustafsson (1964) suggested that GPC may play a role in maintaining the osmotic pressure in the lumen of the duct, since the reduction of Na and K ions in the cauda epididymidis was compensated by an increase in GPC and protein.

The morphology of GPC synthesis and secretion is still unknown. Martan and Risley (1963), Martan and Allen (1964), Martan et al. (1964), and Martan (1969) all suggest the clear cell as the site of origin of this compound. However morphological evidence (Fahrmann and Schuchardt, 1965; Kreth, 1965; Holstein, 1969; Clermont and Flanery, 1970; Piekut and Morehead, 1971) does not support this idea.

2. Glycoproteins

Scott et al. (1963) observed incidentally the precipitation of approximately half of the orcinol-reactive carbohydrate present in the epididymal semen of the rams after the use of trichloroacetic acid. They suggested that it is protein bound.

The epithelium of the epididymis possesses several enzymes for carbohydrate metabolism and synthesis, of which many have been localized histochemically in the epithelium (Hamilton, 1972).

Bose et al. (1966), Fournier (1966), Peyre and Laporte (1966) and Rajalakshmi and Prasad (1969) all reported the presence of sialic acid in the epididymis of the rats.

Press and Porter (1966) reported that several antigenic proteins were found to be bound to sialic acid and are thus glycoprotein.

Ten minutes after the administration of ^{3}H-labelled galactose to the male rat, Neutra and Leblond (1966) localized it autoradiographically in the Golgi region of the epithelial cells of the epididymis. Twenty minutes later, the luminal

surface of the epithelial cells was covered by silver grains. They suggested direct conversion of the substrate to a complex carbohydrate by the epithelial cells.

Fleischer et al. (1969) isolated the Golgi apparatus from the epididymal epithelium of a bull. They reported that it contains highly active galactosyltransferase, which is directed toward glycoprotein production similar to other tissue rich in this enzyme.

After the staining of a thin section from rat epididymis with chromic acid-phosphotungstic acid technique, which is specific for complex carbohydrates, Rambourg et al. (1969), noted that a dense deposit was localized in the Golgi saccules of the principal cells.

The Golgi apparatus was the only morphological site in the epithelial cells of the epididymal duct, where the various investigators succeeded in showing evidence for the synthesis of glycoprotein. There is no electron microscopic evidence suggesting a mechanism for release of a secretory product from the cell.

Gottschalk and Neuberger (1966) suggested that one of the functions of glycoprotein in the semen is to reduce friction between the sperm during their passage in the duct, because of its ability to retain water, which makes it an ideal lubricant.

Johnson and Hunter (1970) reported that a portion of rabbit sperm antigens originate in the epididymis, suggesting that some of these antigens coat the sperm during their passage through the duct.

3. Carnitine

Marquis and Fritz (1965) reported the presence of a high level of carnitine in tissue from the cauda epididymis and in epididymal plasma of the rat. Carnitine could not be extracted from the epididymis of untreated castrates. Testosterone injections brought carnitine concentration in the epididymis to the normal level. The possible morphological correlates of carnitine synthesis are unknown (Fritz, 1963).

The physiological role of carnitine in the epididymis is still not clear. Pearson and Tubbs (1967) suggested that under certain conditions, it might act in acetyl CoASH buffering.

Hamilton (1972) thought that carnitine is secreted by epididymal epithelium as a source of cofactor for the enzymatic transfer of fatty acid into the mitochondria of the middle piece of the sperm for oxidation to supply energy.

4. Steroid

A number of investigators reported that the mammalian epididymis *in vitro* can (1) synthesize steroid (Frankel and Eik-Nes, 1968; Gloyna and Wilson, 1969; Inano *et al.*, 1969; Fawcett and Hamilton, 1970; Hamilton, 1971) and (2) metabolize testosterone (Gloyna and Wilson, 1969; Hamilton and Fawcett, 1970). However it is unknown whether this steroid synthesis activity exists *in vivo* or not (Hamilton, 1972).

Frankel and Eik-Nes (1968) showed that steroid production is reduced by castration and restored nearly to normal by hormone replacement therapy.

Steroid synthesis was also noted in isolated epithelium of the rat vas deferens and in the epididymis from immature rats (Hamilton, 1975).

The subcellular localization of the enzymes for synthesis of (DHEA) from pregnenolone were reported (Frankel and Eik-Nes, 1970) to reside in the mitochondria of the principal cells, while Milner and Hamilton (1971) suggested that some of the enzymes are located in the endoplasmic reticulum.

The metabolic role of steroid in the epididymis is unknown, since it is not known whether the steroid produced is secreted into the lumen of the epididymis.

IX. The Fate of Unejaculated Spermatozoa:

Foote (1962) reviewed work which showed that the production of spermatozoa by the seminiferous tubules in mammals was greater than that obtained by the exhaustive ejaculation technique. This revealed a natural loss of some spermatozoa within the male genital tract itself and efforts have been concentrated on determining the means of disposal of unejaculated spermatozoa. But confusion has arisen, since each species that has been studied seems to use different mechanisms for disposal of spermatozoa.

A. Loss in the urine

Oslund (1928) identified spermatozoa in the urethra of rats, guinea pigs and dogs. Later, in bulls, rams, and boars, in which the vas deferens has been cannulated (Amann et al., 1963; Bennett and Rowson, 1963; Wierzbowski and Wierzchos, 1969) and in men, in which radio-opaque material (iodized oil) has been injected, Wilhelm (1935) showed that spermatozoa enter the urethra as a result of the slow regular flow of luminal content toward it.

Urethral sperm would ultimately be flushed out in the urine, a mechanism that has been reported in man (Baldwin, 1928; Wilhelm and Seligmann, 1937), rams (Bielanski and Wierzbowski, 1961; Lino et al., 1967; Lino and Braden, 1972) and rabbit (Holtz and Foot, 1972), and Lino et al. (1967) showed that the mean number of the daily output of spermatozoa in the urine of rams was 88 per cent of the mean daily production of sperm estimated by exhaustive ejaculation.

B. Loss by spontaneous ejaculation

An additional phenomenon of expulsion of spermatozoa was observed in cat (Aronson, 1949), guinea pig (Martan, 1966), hamsters (Beach and Eaton, 1969) and rat (Orbach, 1961; Agmo, 1976), where spontaneous seminal ejaculations in the absence of penile stimulation, have been reported.

C. Loss by degeneration in the epididymis

In contrast to loss through the urethra, Simeone and Young (1931), observed that the vas deferens of the guinea pig contained large numbers of degenerated or liquified spermatozoa. Their observation was confirmed later in rats, mice, hamsters and guinea pigs (Martan, 1966, 1969; Cooper and Hamilton, 1977).

Disintegration or degeneration of spermatozoa have also been reported in a variety of species including rat, rabbit, guinea pig, bull, oat, cat, monkey and dog (Chang, 1943; Hancock, 1955; Glover, 1961; Roussel et al., 1967; Holtz and Foote, 1972).

Degeneration included decapitation or coiling of the tail, but not liquifaction as described by Simone and Young (1931).

D. Uptake of spermatozoa by epithelium of excurrent ducts

Some investigators have documented phagocytosis of spermatozoa by epithelial cells lining the tubuli recti of monkeys (Dym, 1974), the rete testis of fowls (Tingari, 1971; Tingari and Lake, 1972), monkeys (Burgos and Cavicchia, 1975; Dym, 1976), and rats (Dym, 1976), the ductuli efferentes of bulls (Crabo et al., 1971) and fowls (Tingari and Lake, 1972),

the caput epididymis of rabbits (Nicander, 1963, 1965), the cauda epididymis of rats (Flickinger, 1972a) and in the terminal vas deferens of rats (Cooper and Hamilton, 1977).

E. Spermiophagy by macrophages

Spermiophagy by macrophages has also been stated to occur within the lumen of the rete testes and ductuli efferentes of fowls (Tingari and Lake, 1972), and epididymis of men (Holstein, 1967), rabbits, bulls and monkeys (Roussel et al., 1967). This demonstrates another possible route for the disposal of some unejaculated spermatozoa.

MATERIALS AND METHODS

Animals:

Seventy-eight sexually mature rats (Inbred Swiss Albino rats) were used in this study. At the start of the experiment, their ages ranged from 12 to 24 weeks. They were housed without females, in a conventional animal unit, in Polycabinet cages and were fed Labsure C.R.M. diet and water.

Design of experiments:

Group I: The results were assessed macroscopically and microscopically (optical and transmission electron microscopy). Seventy-two animals were used. Both vasectomy and sham operation were performed unilaterally (left). Approximately equal numbers of vasectomized and sham-operated animals were used at each of the following intervals after operation: 6 weeks, 4, 6, 9, 12, 15, 18 months. It was thus possible to make direct comparison between the experimental and control animals. The numbers of vasectomized animals used for each postoperative period are given in Table 2.

Group II: Study by scanning electron microscopy of changes in the epididymis. Six animals in which vasectomy was performed unilaterally (left), were used for this purpose. The contralateral side of each rat was used as a control. Two animals were killed at each of the following postoperative periods: 1, 3, 6 weeks.

Experimental procedures:

1. Vasectomy Procedure: Rats were initially anaesthetized with anaesthetic ether; thereafter surgical anaesthesia was

maintained using an ether mask. The scrotum and groin of the rat were then shaved, and the animal was then laid on sterile drapes on a heated operating table, previously wiped with Hibitane in alcohol. The line of the skin incision, extending laterally from the left side of the penis for approximately 1.5 cm was marked in felt pen. The area was then wiped with Hibitane in alcohol. An incision was made in the paper drapes used to cover the rat. The incision was made in the skin with a Swan and Morton scalpel fitted with a No.15 sterile blade. The edges of the wound were clamped to the drapes and retracted. An incision was made in the cremaster muscle using scissors. The underlying fat was reflected towards the right and the left vas deferens was located. The mesentery supporting the vas deferens was punctured at the site for vasectomy and the vas was then ligated with Ethicon silk and tied with a triple knot. A second ligature was similarly tied round the vas approximately 4 mm from the first. The portion of the vas deferens between the ligatures was then excised using a scalpel fitted with a No.12 sterile blade. The segment excised was at the level of the superior pole of the testis and was approximately 1 cm from the point of origin of the vas from the epididymis. The vas deferens was returned to the scrotum. The cut edges of the cremasteric muscle were reapposed by means of 1-3 sutures of Ethicon catgut. The clamps were removed and the cut edges of the skin reapposed by 4-5 sutures of Ethicon silk. The top drapes were removed. The wound was wiped with

sterile Hibitane in alcohol and dried with a sterile swab. The wound was then sprayed with Nobecutane, care being taken to protect the penis. Anaesthesia was stopped and the rat was placed on clean paper shavings in a clean cage to recover. Recovery occurred within 10-15 minutes.

At each operation all drapes, sutures, swabs and surgical gloves were sterile and all instruments were sterilized in an autoclave. The operator wore sterile rubber gloves, a clean surgical gown, paper face-mask and paper cap while his assistant wore the latter three items.

The wound was checked each day until it healed, which required about 3 days.

2. Sham-operated procedure: The procedure for sham operation was identical to that for vasectomy except that when the mesentery supporting the vas was punctured, two Ethicon silk ligatures were looped loosely around the vas and left in place. The vas deferens between the ligatures was not cut but left intact.

Group I

Fixation:

The testis and epididymis were fixed by vascular perfusion. The rats were first weighed, then killed by ether overdose. For successful perfusions, the following steps were completed within 1 minute 15 seconds.

1. The ventral abdominal and chest walls were opened in the midline with scissors and reflected to expose the peritoneal

and thoracic cavities.

2. The incision was continued along the ventral aspect of the scrotum to expose the left testis and epididymis.

3. The splenic artery was located and clamped. The spleen was then excised.

4. A gauge 19 needle was inserted into the left ventricle.

5. Immediately afterwards, a small incision was made in the left atrium using scissors, to allow egress of the perfusion.

Mammalian Ringer solution, containing 50 ml of 2% xylocaine for every 500 ml of the solution, was run through the needle by gravity flow until the atrial return was clear. At this point, 1000 ml of 5% glutaraldehyde in 0.1M cold phosphate buffer (pH 7.4) was perfused over 35-45 minutes. For the animals used in these studies both rinsing and fixative solutions were raised to provide a pressure of 130 cm of water.

Removal of Testes, Epididymides and Granulomas:

The cut ends of the vas deferens were inspected to ensure that the ligatures were still intact. The size and location of spermatic granulomas when present, were recorded.

The left testes with the corresponding epididymides from vasectomized and sham-operated animals as well as the right testes from the vasectomized animals were then excised using scissors and fine forceps. If a granuloma had formed at the proximal cut end of the vas, it was removed separately from the testis and epididymis.

A. Epididymides:

Immediately after their removal, they were immersed in 100 ml of the phosphate-buffered glutaraldehyde fixative for

a further two hours. The tissues were then rinsed for two hours in two changes of phosphate buffer. Small pieces were cut with a razor blade from the caput and cauda of the epididymis, (and also from the corpus, if a granuloma was present in the corpus). The excised pieces were then rinsed in a third change of phosphate buffer overnight and postfixed with 1% OsO_4 in 0.1M phosphate buffer for one hour. The tissues were then rinsed for 15 minutes in phosphate buffer. The tissúes were dehydrated in a graded series of ethanols followed by propylene oxide and embedded in resin.

Dehydration and Embedding:

The tissues were dehydrated through the following sequence:

70 percent alcohol	45 minutes
90 " "	1 hour
1st absolute alcohol	" "
2nd " "	" "
3rd " "	" "
4th " "	" "
1st propylene oxide	30 minutes
2nd " "	" "
propylene oxide/Spurrs	3 hours
" " /Spurrs	overnight
Spurrs	48 hours
"	"

The materials were then embedded in Spurr's resin which was polymerized in an oven at 35°C overnight and then at 60°C in the morning until the resin became hard (usually 16 hours).

Cutting and staining:

Sections ~1.0 µm thick were cut with glass knives and stained with Azur blue. Thin sections for electron microscopy were cut with a diamond knife on a Reichert-Jung ultramicrotome, stained with double staining techniques in saturated uranyl acetate and lead citrate (Reynolds, 1963), and photographed in a Jeol 100S electron microscope.

B. Testes:

The testes, immediately after their excision, were postfixed in freshly prepared Bouin's fixative, to rinse the tissue free of glutaraldehyde and thus reduced the excessive hardening of the tissue caused by perfusion with glutaraldehyde.

After one hour the testes were removed from the fluid. Three slices, each 2 mm thick, were cut transversely through the upper pole, middle portion, and lower pole of the testis respectively, using a razor blade. The slices were fixed in fresh Bouin's fluid for a further 24 hours.

Dehydration and Embedding:

The tissues were dehydrated, cleared, and impregnated with paraffin wax by a Histokinette programmed to the following sequence.

70 per cent alcohol		2 hours
90 "	"	" "
1st absolute	"	" "
2nd "	"	" "
3rd "	"	" "
1st Amyl Acetate		" "
2nd "	"	" "
3rd "	"	" "
1st paraffin wax		3 hours
2nd "	"	" "

The materials were then embedded in paraffin wax. The testis slices were embedded in such a way that when the block was cut the testis would be seen in transverse section.

Cutting and staining:

All blocks containing the testicular slices were cut to full face before sections were taken for mounting. Sections - 5 µm thick were mounted on clean, albuminized glass slides, stretched on a hot plate and then dried in an oven at 37°C for 24 hours, before being stained with haematoxylin and eosin.

C. Vasal Granulomas:

After about 45 minutes fixation *in situ*, the vasal spermatic granuloma was excised, and immersed in phosphate buffer solution for several days before being weighed.

Two epididymides and associated granuloma were fixed after their excision in Bouin's solution for 48 hours, dehydrated and impregnated with paraffin wax using a Histokinette programmed as mentioned before. They were

then embedded in paraffin wax in such a way that when the block was cut the epididymis and granuloma would be seen in longitudinal profile. The blocks were cut serially at a thickness of 5 µm. Sections were mounted on clean albuminized slides, dried in an oven at 37°C for 24 hours, and stained with haematoxylin and eosin.

Count of the number of intraepithelial leucocytes:

This study is based on the mononuclear cells in zone 3 of the epididymal duct. These cells are most abundant in this region, where they make up as much as 25 percent of the total cells in the epithelium (Reid and Cleland, 1957).

Three animals (experimental or control) from every postoperative period were selected for this purpose. (Except for 18 mos. where 3 sham operated animals were studied but only 2 vasectomised. Material from the 3rd animal was too poor histologically for detailed counts.

An interrupted series of four sections (1 section in approximately every 8 cut) from each rat were used for these measurements. Cells were counted in the epithelium of three sectional profiles of the epididymal duct in each histological section, thus the total number of these cells were counted in twelve sectional profiles of the duct in each animal. The intraepithelial leucocytes were counted at a magnification of X400.

In order to avoid observer bias in the counts, the label of the slides (experimental or sham) was covered with a piece of paper and each series was given a reference number, so that it was not known which slides were from

experimental and which from control animals. Only when all the counts and measurements had been made were the pieces of paper removed to reveal the true identity of the slides.

Using a camera lucida attachment fitted to the Wild binocular microscope, the tubules in which the counts of intraepithelial leucocyte were performed, were drawn in outline, the tubules being viewed at a magnification of X200. Using an electronic planimeter (MOP Amo II), the length of the perimeter of each tubule was measured.

In order to estimate the number of intraepithelial leucocyte/unit length of the perimeter of each section of the duct, the total numbers of intraepithelial leucocyte measured in twelve tubules, were divided by the summed length of the perimeter of each tubule as measured by the MOP.

The mean and S.D. of intraepithelial leucocytes in each group (experimental or control) at any given postoperative period was calculated. At each of the seven postoperative periods, the estimated mean number of intraepithelial leucocytes in the experimental animals were compared with that in the control animals, to determine any significant changes.

Spleen:

After removal, the spleen was trimmed free of adipose and connective tissue and weighed. Three transverse slices, each 2 mm thick, were cut from the anterior, middle and posterior regions of the organ respectively, at approximately

regular intervals. The slices were immersed in 100 ml of freshly prepared Carnoy's fixative for 4 hours.

After fixation the materials were dehydrated, cleared, and impregnated with paraffin wax, using the standard Histokinette programme. The slices were then embedded in paraffin wax.

Cutting and staining:

Each block containing a slice from the anterior, middle, or posterior region of each spleen of the vasectomized and/or sham-operated rats, was cut serially at a thickness of 5 µm. From the complete series, an interrupted series of 14 sections (1 section in approximately every 10 cut) was mounted for microscopic study.

A group of three sections, one section from each of the three interrupted series of every animal, were mounted together on clean albuminized glass slides, in such a way that the upper, middle, and lower sections represented the anterior, middle, and posterior regions of the spleen respectively, then stretched on a hot plate. The total collection of 14 slides from each animal were then dried in an oven at 37°C for 24 hours, before being stained with 2% Methyl green, and 5% Pyronin.

Measurement of the areas of sections, white pulp, dense area, marginal zones of the spleen:

Area measurements were performed on the methyl green pyronin stained interrupted series of sections. As a pilot experiment, measurements were made in rats as follows:

Six slides selected from the original fourteen slides from each rat (vasectomized or sham-operated) were used. Every one of the three sections present on each of these slides was photographed at 20 x magnification by Wild photomicroscope M400. The photographs were used for measurement purposes. To have carried out this procedure for all the animals would have been impossibly time consuming. After analysing statistically the figures obtained in the pilot experiment it was found that a corresponding degree of accuracy could be obtained by carrying out the measurement on 1 section chosen at random from each block i.e. 3 random sections from each animal instead of 18, as in the pilot experiment.

Using the MOP Amo II the areas of the total cross section of spleen, of the white pulp, and the inner dense area of each profile of the white pulp were measured. The total area of the marginal zones in each section was calculated by subtracting the summed value of the dense areas from the total area of the white pulp in that section. After deduction of the total areas of the white pulp in every section from the area of that section, the remaining area represented the total area of red pulp, together with the trabeculae of the spleen (see Text Fig. 134).

Calculation of the mean and percentage areas of the white pulp, dense area, marginal zone, and red pulp with trabeculae of the spleen:

These various areas were therefore measured in a total of 18 sections (6 slides x 3 sections) from each animal,

in the pilot experiment, or in a total of 3 sections (3 slides x 1 section) in the remaining animals. The means for each parameter measured were then calculated. The percentage area occupied by each parameter measured was also calculated.

Counts of the germinal centres of the spleen:

Seven slides (from the original total of fourteen) from every animal (vasectomized or sham-operated) were selected for this study. The germinal centres were counted in every one of the three sections present on each slide.

Calculation of the number of germinal centres/unit area of the spleen:

The number of germinal centres per unit area of the spleen of each animal, experimental or control, was determined by dividing the total number of the germinal centres counted in 21 sections by the summed values for the area of each section.

Group II (SEM study)

Fixation:

Two animals were sacrificed at intervals of 1, 3 and 6 weeks. The rats were killed by ether overdose. Immediately afterwards, the ventral abdominal wall was reflected, the right and left epididymides were exposed and excised and placed in 5% cold glutaraldehyde in phosphate buffer (pH 7.4) for 48 hours, and then rinsed in three changes of phosphate buffer. Using the vibratome, the tissue was sliced longitudinally into 0.5 mm thick slices. The slices were then rinsed in three changes of phosphate buffer, with gentle shaking, (in an attempt to wash sperm out of the tubules), osmicated and

dehydrated using a graded series of ethanols. Amyl acetate was used as a transition fluid before critical point drying (in a Polaron critical point drying apparatus) with carbon dioxide. Prior to drying, the specimens were placed in metal baskets to minimise handling.

Dried specimens were mounted on their sides on to metal stubs using a high conductivity metal paint. They were then placed in a desiccator to keep the specimen dry. They were sputter-coated with gold using a Polaron sputter coater and viewed with a Jeol T300 scanning electron microscope.

RESULTS

I. EPIDIDYMIS OF SHAM OPERATED AND VASECTOMIZED RATS

A. Histology and Ultrastructure

i. Sham-operated rats

The caput epididymidis was lined by a high columnar epithelium, surrounding a lumen relatively filled with sperm (Figs. 1 and 2). At the proximal part of the cauda epididymidis, the epithelium was medium columnar, becoming low columnar near its distal end. The lumen was wider than that of the caput, and contained more sperm (Fig. 5). Spermatozoa in the distal part of the cauda epididymidis were interspersed with darkly-stained patches of sperm (Fig. 5). Occasional, degenerating germinal cells, and rarely macrophages, containing engulfed sperm fragments (Fig. 63), were found in the lumen of both caput and cauda.

The epithelium of the caput and cauda of the epididymis, rests on a basement membrane, encircled by a layer of smooth muscle, with the fibres circularly oriented. This muscular layer was thin in the caput (Fig. 7), but thick and distinctive in the cauda (Figs. 5 and 8). Numerous capillaries were found scattered throughout the length of the duct, between the basement membrane and the muscular layer (Fig. 8). Outside the muscular coat, the intertubular spaces were filled by a loose connective tissue containing numerous blood and lymphatic vessels and capillaries (Figs. 1, 2 and 5). This was limited by a fibrous capsule (Figs. 1 and 5).

The epithelium lining the caput and cauda epididymidis comprised: principal cells, basal cells and clear cells,

and contained intraepithelial leucocytes (Figs. 7, 8 and 9A - 22A).

1. Principal cells: They comprised the majority of the columnar cells, and were characterized as follows: The apical plasma membrane had numerous long stereocilia (Figs. 7, 8, 9A - 22A, 26, 28 and 32). The free surface of the cell between the bases of the stereocilia was highly irregular in profile and showed numerous shallow depressions or invaginations of the plasma membrane (Figs. 23 and 28).

Small vesicles of both the smooth and coated types, were scattered throughout the apical cytoplasm of the principal cells of the caput epididymidis (Fig. 23). Since the epithelium of the cauda epididymidis was short, the principal cells exhibited much less apical cytoplasm, but had more closely packed vesicles than those of the caput region (Figs. 27, 28 and 32).

The supranuclear cytoplasm contained an extremely large Golgi apparatus (Figs. 23, 26, 27 and 32), many vesicles a few multivesicular bodies (Figs. 23, 27 and 28), frequent lysosome-like dense bodies (Figs. 23, 24, 26, 29 and 32), and variable numbers of autophagic vacuoles. The multivesicular bodies were round or oval in profile and had a small number of indistinct vesicles. Frequently, they also contained a small amount of a flocculent substance. Some multivesicular bodies deeper in the cytoplasm contained more vesicles, with denser contents.

The nuclei were situated in the basal half of the cell. They were round or oval in profile, contained finely

granular chromatin, and had usually one nucleolus in the caput, or up to three nucleoli in the cauda epididymidis. Indentation of the nuclei was very frequent (Figs. 9-22, 25, 26, 29, 32 and 34).

The basal cytoplasm was occupied by anastomosing profiles of rough endoplasmic reticulum, which extended into the paranuclear and supranuclear regions (Figs. 25, 29 and 34). The smooth endoplasmic reticulum was concentrated in the apical and supranuclear parts of the cell (Figs. 23, 24, 27 and 28).

Mitochondria were scattered throughout the cytoplasm. In the caput epididymidis, they were mainly elongated and occasionally branched (Figs. 23 - 25), while in the cauda epididymidis, they were mostly round or oval in profile, though a few were slightly elongated (Figs. 26-29, 32 and 34).

Basal cells: These cells were scattered at the bases of principal and clear cells (Fig. 7). They were pyramidal in profile. The nuclei were normally flattened against the basement membrane and exhibited coarse chromatin granules. Their scanty cytoplasm contained a few profiles of endoplasmic reticulum, a small Golgi apparatus, occasional multivesicular bodies, and a few mitochondria (Fig. 36).

Intraepithelial leucocytes: They were more abundant in the caput than in the cauda epididymidis, and were found at all levels of the epithelium, from the lumen to the basement membrane (Figs. 7 and 9A - 22A). They were irregular in outline, with blunt processes extending between the principal

cells. They showed no form of junctional complex with epithelial cells. Three types of mononuclear cells were distinguished;

a) The first (Fig. 37) exhibited many of the features of lymphocytes. They had a relatively large nucleus surrounded by pale-staining cytoplasm. The nucleus was round or indented and provided with coarse chromatin granules, which gave it its dense appearance. The cytoplasm contained a small Golgi apparatus, scattered elements of endoplasmic reticulum, few mitochondria, and occasional multivesicular bodies.

b) The second form was less numerous and differed from the first by its larger nucleus, that varied from slightly indented to horseshoe-shaped, and a relatively more abundant cytoplasm (Fig. 38). These were regarded as monocytes.

c) A third type was large and had a polymorphous nucleus. The cytoplasm contained several large dense bodies, surrounded by a profile of endoplasmic reticulum, and numerous scattered mitochondria (Fig. 39). These cells were usually found at the basement membrane. In some sections the cell appeared entirely devoid of nucleus (Fig. 40). This was attributed to the plane of the section. These cells were regarded as macrophages.

Clear cells: The clear cells were scarce in the caput epididymidis, but in the cauda they were very numerous (Figs. 7, 8, 10A, 12A, 14A, 16A, 18A, 20A and 22A). They stained more lightly than the principal cells. The oval nucleus was pale, with a delicate chromatin network, and usually occupied the lower half of the cell (Fig. 31). The luminal

surface of the cell was very irregular and usually projected above the surface of the principal cells, although some lay at the same level or even below the general surface. Several depressions and pit-like invaginations were found at the free surface of the cell (Fig. 30). The apical cytoplasm possessed numerous vesicles and variable sized multivesicular bodies, containing variable numbers of vesicles (Figs. 30 and 31). Sometimes, in addition to the vesicles, the multivesicular bodies, exhibited a small amount of a flocculent substance and/or, rarely membranes (Fig. 30). Frequently, multivesicular bodies were also found in the upper part of the supranuclear region. These were usually larger, with more contents.

Large numbers of membrane-bound, moderately dense bodies that resembled lysosomes, were concentrated in the basal and paranuclear regions. Frequently they extended upwards to the supranuclear and apical cytoplasm (Figs. 31-33 and 34). In the caput epididymidis, the membrane-bound dense bodies, were few in number, and were mainly restricted either to the basal cytoplasm or to the supranuclear region (Fig. 30). Membranous masses were frequently found in the infranuclear region of the cell (Fig. 35). Occasionally, the basal cytoplasm contained also lipid droplets (Fig. 35).

Numerous mitochondria, and sparse profiles of endoplasmic reticulum were scattered throughout the cytoplasm. The mitochondria were round, oval or very long (Figs. 30-35).

ii. Vasectomized rats

The right epididymis of all rats, except Vas 17, 20, 21 and 22 (see below), and the left epididymis of fifteen of the vasectomized animals (Vas 1, 2, 3, 4, 6, 7, 8, 10, 12, 13, 14, 15, 16, 22 and 31), which exhibited only vasal granuloma (Table 2), were similar to those of sham-operated rats. The tubular profiles of the caput and cauda epididymidis were not engorged with sperm, nor did they show any distention (Figs. 3, 4 and 6). There was no obvious thinning in the epithelial wall (Figs. 9B - 18B, 21B and 22B), although this was not measured quantitatively. Ultrastructurally, the epithelial cells showed the same general features as those found in the sham-operated rats.

The principal cells exhibited the same features as in the sham-operated animals: long stereocilia, apical vesicles, extremely well developed supranuclear Golgi apparatus, a few supranuclear multivesicular bodies and dense bodies, scattered mitochondria, and numerous profiles of rough endoplasmic reticulum, concentrated in the basal part of the cell (Figs. 41-48 and 51).

The clear cells were also unchanged and showed a few short stereocilia, many apical vesicles and less numerous multivesicular bodies, several profiles of membrane-bound dense bodies, and frequently masses of membrane, which usually occupied the basal part of the cell (Figs. 44-46 and 50-56). These last two organelles, however, did not show any obvious increment in amount after vasectomy.

All three types of intraepithelial leucocytes detected in the sham-operated rats, were also seen in the vasectomized

animals (Figs. 42 and 56-61). Moreover the mean number and S.D. of intraepithelial leucocytes did not show any significant differences after vasectomy (Table 1). The basal cells (Fig. 52) also possessed a normal histology.

The sperm within the lumen of the caput and cauda epididymidis appeared normal. Ultrastructurally, the plasma membrane and mitochondria were intact. Microtubules and coarse fibres of the sperm tail had a normal relation to one another (Fig. 62). No cellular infiltration could be seen.

The interstitial connective tissue did not exhibit any inflammatory reaction, nor did it appear to be increased in amount (although this was not assessed quantitatively).

The left epididymis of all but three of the remaining vasectomized rats examined, possessed granulomas. The exceptions were Vas 17, 26 and 36 (Table 2). Sections of the duct near the granulomas showed intraluminal phagocytic cells. Two kinds of phagocytic cells were distinguished: macrophages and polymorphonuclear leucocytes. These were readily distinguished, by light microscopy, by their characteristic morphology (Figs. 64-67). By electron microscopy, both were seen to contain fragments of spermatozoa (Figs. 68-71).

The relative numbers of the two cell types varied. In many cases both types were present; in some, one was predominant. Fusion of macrophages to form giant cells, was sometimes seen (Figs. 64-66).

In addition to the intraluminal accumulation of phagocytes, thinning and disruption of the epithelial lining of

the ducts was sometimes seen (Figs. 72-74). Spermatozoa and phagocytes escaped into the interstitial connective tissue, leading to a new granulomatous response. Some sectional profiles of the duct were seen, in which the epithelial lining had been completely destroyed, and the lumen filled by a mass of spermatozoa, surrounded by giant cells (Fig. 75).

The interstitial tissue at the region of the granuloma seemed to have been increased in bulk.

In eleven of the vasectomized animals (Vas 11, 18, 19, 26, 27, 30, 32, 34, 35, 36 and 37), the left testes were atrophied. The left epididymal ducts contained no spermatozoa (Fig. 76). In most of these animals, a spermatic granuloma was present in the proximal part of the caput; in two, however (Vas 26 and 36), no granuloma was found.

In four vasectomized rats (Vas 17, 20, 21 and 24) both testes were atrophic. The seminiferous epithelium was degenerated, but no cellular infiltration was seen. The caput epididymidis was free of the spermatic granuloma normally seen in other animals with atrophied testes, and in Vas 17, a granuloma was not found in any part of the epididymis. The right and left epididymal ducts were empty of sperm, but contained a few sloughed germ cells (Fig. 77).

Sections through the epididymal ducts of Vas 11 and 30 distal to a caput granuloma, were devoid of spermatozoa, while those proximal to it appeared to be engorged with sperm (Fig. 78), and in Vas 11 showed marked flattening in its lining epithelium (Fig. 79). In Vas 32, the epididymis was embedded in wax and cut longitudinally. The

proximal tubules of the caput were found to be distended with sperm (Fig. 81), and there were several ruptures in its epithelial wall; in one of the sections, two ruptures were detected in the same tubule (Figs. 116 and 80). These ruptures in the lining epithelium, were associated with extravasation of sperm into the surrounding connective tissue resulting in a granulomatous lesion. By contrast, the lumen of the remaining parts of the epididymis, was empty of sperm (Fig. 81). A large granuloma was seen at the mid-region of the caput (Fig. 81). All the appearances suggested that new epididymal granulomas formed as a result of an obstruction somewhere in the epididymal duct, followed by dilatation and destruction of the epithelial lining, and rupture.

Four animals (Vas 28, 29, 33 and 38) had large epididymal granulomas confined to the caudal region only (Figs. 107 and 108). This suggested that the caudal granuloma continues to enlarge, if new granulomas do not develop in the corpus or caput regions of the epididymis.

Apart from these features, the epididymal epithelium remains normal in all regions other than in the neighbourhood of the granulomas (Figs. 19B, 20B, 76 and 77). No sperm or remnants of sperm were seen within the epithelial lining in any of the animals studied.

Scanning electron microscopy

Sections through the epididymis of the sham-operated rats, showed several sectional profiles of the coiled duct,

with an empty lumen. Most of the spermatozoa had been washed out and only occasional sperm were seen in some of them. The lining epithelium of the caput exhibited numerous long and delicate stereocilia (microvilli), which formed a dense mat which obscured the underlying principal cells (Fig. 82). The length of the stereocilia showed a gradual decrease as one proceeded distally towards the cauda epididymidis (Fig. 84). At the junction of the middle and distal thirds of the caput epididymidis (Zone III), several areas were seen which had only a few short microvilli (Figs. 82 and 86). These represented the clear cells. They became more numerous at the cauda epididymidis (Fig. 83). Most of the clear cells had their apical surfaces below the general level of the epithelium (Fig. 84). However in a few, the apical surface was dome-like and bulged above the general level (Fig. 85). These differences are consistent with those obtained by the transmission electron microscopy; they probably represent different physiological states of activity in the cell.

Many signs of secretory products were observed on the epithelial surfaces, scattered between the stereocilia (Fig. 86), and on many occasions, spermatozoa (Figs. 84 and 87) were seen.

The epididymal epithelium of the vasectomized animals showed the same histological features as mentioned above. The stereocilia did not show any obvious differences in length or density (Figs. 88, 89 and 92), but no attempt

was made to quantitate these. Both variants of the clear cells were also seen as in the controls (Figs. 89, 90 and 92). Many secretory droplets were recognized on the epithelial surfaces (Fig. 88), suggesting that the epididymal epithelial cells remain actively functional after vasectomy. Spermatozoa retained their normal histology (Figs. 91 and 92).

B. Intrinsic Lymphatics of the Epididymis

Lymphatics were studied in an interrupted series of semi-thin sections of caput and cauda of the left epididymis, from 32 sham-operated and 29 vasectomized animals. From each animal four blocks, two from the caput and two from the cauda were used. From each interrupted series from each block, approximately seven sections were selected at random. In total, therefore, twenty eight sections, fourteen of caput and fourteen of cauda portions of epididymis were studied from each animal.

Lymphatics were identified as endothelially lined vessels, containing a stained precipitate of lymph protein. Because of the use of perfusion fixation, confusion with blood vessels was avoided; the blood vessels were usually completely empty. When perfusion was incomplete, blood vessels were distinguished from lymphatics by their content of erythrocytes. Lymphatic capillaries were also characterised by their scalloped sectional profile. Collecting lymphatics had a thicker wall than capillaries and sometimes showed valves.

Lymphatics were looked for systematically in the following situations:

i. in the sparse, loose interstitial connective tissue between adjacent coils of the epididymal duct.

ii. in the more abundant connective tissue associated with larger blood vessels between lobules of the epididymis.

iii. within and immediately beneath the connective tissue capsule of the epididymis.

The frequency, size and content of cross-sectional profiles of lymphatics were compared.

1) between caput and cauda of sham-operated controls and of vasectomized animals.

2) in vasectomized animals, in the immediate vicinity of the granuloma (if present) and at some distance from it.

The main findings were:

1) In both sham-operated controls and vasectomized animals, sectional profiles of lymphatics were infrequent in the sparse interstitial connective tissue surrounding the smallest branches of the blood vessels.

2) They were more frequent and larger in the coarser interlobular connective tissue (Figs. 93 and 94).

3) Larger collecting vessels, with valves, were seen frequently within and just beneath the fibrous capsule (Fig. 95).

4) In sham-operated control animals, the lymphatic vessels of the caput and cauda of the epididymis usually contained only lymph protein (Figs. 93-95). Cells were very rarely seen.

5) The lymphatics of the caput and cauda of epididymis in all those vasectomized rats in which an epididymal granuloma had not developed appeared similar to those of the sham-operated control animals (Figs. 96 and 97).

6) In 20 of the vasectomized animals (Vas: 5,9,11,18,19,20, 21,23,24,25,27,28,29,30,32,33,34,35,37 and 38), a spermatic granuloma had formed in the epididymis. Most sectional

profiles of lymphatic vessels in the immediate neighbourhood of granulomas of almost all animals contained variable numbers of mononuclear leucocytes, the only exception being Vas 20. In some sectional profiles, only a few leucocytes were present, while in others more than twenty cells were seen (Figs. 98-103). The cells included lymphocytes, macrophages and monocytes. The lymphocytes were mainly small, with occasional medium-size ones and, rarely, large lymphoid cells (lymphoblasts).

In the exceptional case (Vas 20), the epididymal granuloma was large but apparently of long standing: its contents were mainly sperm fragments; intact sperm and inflammatory cells were sparse. The wall of the granuloma consisted almost entirely of giant cell macrophages, surrounded by a thick layer of dense fibrous tissue (Fig. 104). Lymphocytes and plasma cells were very sparse. These appearances suggested that the inflammatory response at this particular granuloma had subsided. Unlike the situation in the vicinity of 'active' granulomas, lymphatic vessels were not seen in relation to this one.

7) At sites not immediately adjacent to the epididymal spermatic granulomas, mononuclear leucocytes were not usually detected (Fig. 105).

8) Particular importance was attached to the finding that in both sham-operated and vasectomized animals, no intact spermatozoa or spermatozoal fragments were observed in any of the lymphatic vessels of the caput and cauda of the epididymis. The significance of this for the uptake of spermatozoal antigens from granulomas, and their transport to the immune system, is dealt with in the Discussion.

C. Response of Leucocytes in Epididymal Epithelium after Vasectomy

The results of the cell counts are shown in Table 1.

As described in "Materials and Methods", three animals, vasectomized or sham-operated were studied at each post-operative period, except at 18 mos. where only two vasectomized animals and three sham-operated were studied.

The total number of intraepithelial leucocytes was counted in a random sample of sections from each animal, vasectomized and sham-operated.

Semi-thin sections (about 1.0 μm) were cut from the third quarter of the caput of each animal. Four sections were chosen, each separated from its neighbour by seven sections i.e. about 7 μm apart. This spacing was chosen to avoid the possibility of any one cell being counted twice. In each section, three sectional profiles of the duct were selected. Selection was of the smallest profiles which could be conveniently included within the microscope field. Since four slides were examined, in total 4 x 3 = 12 sectional profiles of the duct were studied.

The outline of the outer perimeter of each sectional profile was drawn with the aid of a camera lucida. The total number of intraepithelial leucocytes was counted in each sectional profile, and the total number calculated (N). The length of the perimeter of each sectional profile was measured from the drawing using an electronic planimeter (MOP Amo II), and the total length of the perimeters of all

twelve tubules was calculated (L). The number of intra-epithelial leucocytes per unit length of perimeter was calculated by $\frac{N}{L}$.

The mean + S.D. of intraepithelial leucocytes was then calculated for the vasectomized rats taken as one group and similarly for the sham-operated animals. The estimated mean + S.D. of numbers of intraepithelial leucocytes in the vasectomized rats, at each given postoperative period, were compared with those in the sham-operated animals, to determine if there were any differences; no significant differences were revealed (Table 1).

II. SPERMATIC GRANULOMA AND TESTICULAR CHANGES

A. Histology of the Spermatic Granuloma

A typical vasal spermatic granuloma appeared as a soft, pale yellow, cystic mass, roughly spherical or oval in shape, with irregularly contoured surface (Figs. 106 and 107). It was rubbery in consistency, with a well-vascularized connective tissue wall. The content of the cyst was usually paste-like. Frequently the granuloma was adherent to the adjacent connective tissue.

Epididymal granulomas were usually smaller than vasal granulomas. They appeared as one or more nodular masses bulging from the surface of the epididymis (Figs. 107 and 108).

Microscopically, spermatic granulomas consisted of a central mass of sperm and cellular debris bordered by a cellular wall of epithelioid macrophages (Figs. 109-112). These were large cells and contained a pale staining nucleus, round or oval in shape, usually located at the basal area of the cell and provided usually with one deeply stained nucleolus and fine chromatin granules (Figs. 111 and 112). Their abundant cytoplasm contained numerous lysosomes with polymorphous interiors and extended irregularly toward the sperm mass (Figs. 109-112).

In many cases, polymorphonuclear neutrophils and macrophages were observed invading the central sperm mass. These phagocytes contained identifiable sperm heads and remnants (Fig. 112).

The loosely organized connective tissue layer surrounding the cellular wall (Figs. 109-111 and 115) was infiltrated by numerous mononuclear cells, mainly lymphocytes with some macrophages, plasma cells, and fibroblasts. The lymphocytes were mainly small and medium-sized with very few large lymphocytes and lymphoblast cells. Both mature and immature plasma cells were distinguished (Figs. 113 and 114). External to the highly vascular connective tissue layer, the granuloma was enclosed in its entirety by an organized connective tissue to form a well defined capsule (Figs. 111 and 115).

In Vas 32, several granulomas in an early stage of development were found at the proximal part of the caput epididymidis. The lesion included a mass of sperm that had broken through the wall of the duct into the adjacent connective tissue, surrounded by epithelioid macrophages. Numerous polymorphonuclear neutrophils and macrophages were found invading the sperm mass and ascending into the lumen of the duct (Fig. 116).

B.

Tables 2 and 3 summarize data on vasal and epididymal granulomas and on the state of the testis in each of the groups of vasectomized rats.

The following commentary analyses the findings and highlights those which seem most important.

i. Incidence of Vasal Granulomas

- They were present in all animals at 1, 3 and 6 weeks, and 4 and 6 months after vasectomy.
- they were also present in all animals at 9 months although in one (Vas 18) the granuloma was too small to weigh.
- they were present in 4/5 animals at 12 months, but in 3 they were too small to weigh.
- they were present in only 2/6 animals at 15 months.
- they were present in 3/8 animals at 18 months.

Pooling of these findings shows the following incidences of vasal granuloma:

- at 1, 3 and 6 weeks, 4 and 6 months 100%
- at 9 months ..... 100% (80% if one vasal granuloma discounted).
- at 12, 15 and 18 months .... 47.4% i.e. there is a progressive decline in the incidence and size of vasal granulomas at 1 year after operation.

ii. Weight of Vasal Granulomas

The mean weight of granulomas shows a general trend of increase to a maximum at 9 months and a decline thereafter. However the variances are very large and the changes are not statistically significant.

Some of the findings merit special comment:

- the two largest granulomas (Vas 22: 904 mgm; Vas 31: 991 mgm) were found at 12 months and 18 months respectively.
- vasal granulomas were absent, or too small to weigh, in 12/19 (63%) of the groups at 12, 15 and 18 months after vasectomy.

iii. Incidence of Epididymal Granulomas (E.G.)

Incidence	Possible Associations
. 6 wks 1/7 (14.28%):	1 EG associated with smallest vasal granuloma.
. 4 mos 1/5 (20%):	1 EG ditto
. 6 mos 1/4 (25%):	1 EG ditto
. 9 mos 2/5 (40%):	Both EG associated with smallest vasal granuloma.
.12 mos 4/5 (80%):	The 1 animal lacking an EG had the largest vasal granuloma.
.15 mos 5/6 (83.3%):	3 EG associated with no vasal granuloma. 1 EG " " small " " 1 EG " " average " " Vas 26 had neither vasal nor epididymal granuloma: (L) testis was atrophic.
.18 mos 6/8 (75%):	5 EG associated with absent or v. small vasal granuloma. 1 EG associated with average vasal granuloma. Vas 31 had no EG, but exhibited the largest vasal granuloma. Vas 36 had neither vasal nor epididymal granuloma: (L) testis atrophic.

iv. Changes in the Testes

The testes of all sham-operated rats were histologically normal. In those vasectomized animals in which the testes appeared grossly normal, no microscopic changes were detected (Figs. 117 and 118).

In several vasectomized animals (Nos: Vas 11,18,19, 26,27,30,32,34,35,36 and 37), the left testis was small, flat and flaccid. Microscopically, the seminiferous tubules were reduced in diameter and showed patchy degenerative changes of the germinal epithelium (Figs. 119 and 120). Tubules were found in various stages of degeneration. Some tubular profiles were devoid of cells except for Sertoli cells and a few spermatogonia (Fig. 121). In other instances the seminiferous epithelium contained spermatocytes but not spermatids; however it was impossible to determine the stage of meiosis (Fig. 122). In others again sectional profiles of tubules displayed all the stages of the spermatogenic cycle up to stage VII (e.g. Figs. 123 and 125), but it was not always possible to differentiate the exact stage of the cycle (Fig. 124). In some tubules, however, fibrosis had taken place (Fig. 126). Occasional groups of seminiferous tubules with normal histology, and in which spermatogenesis appeared to be still proceeding normally, were scattered in between the altered tubules (Figs. 127-129 and 133). Sloughing of immature as well as abnormal germinal cells was evident (Fig. 130). Frequently, multi-nucleated cells were observed within the lumen of some of the altered tubules. They consisted of spherical masses

of cytoplasm containing several identical nuclei within the same cell, but differed in appearance from one cell to another. They had apparently been formed by coalescence of sperm precursors (Figs. 124, 131 and 132).

In several vasectomized animals (Nos. Vas 18, 19 and 34), most of the affected tubules were lined only by Sertoli cells and some scattered spermatogonia. No multinucleated cells were detected within the lumen of the tubules (Figs. 127 and 133). Presumably these reflect old standing cases.

There was no obvious increase in the amount of the interstitial connective tissue (although this was not assessed quantitatively); nor did it show any inflammatory reaction (Figs. 119 and 120). There was however a massive increase in the extent of interstitial fluid (stained grey).

In the four vasectomized animals (Vas - 17, 20, 21 and 24), there was bilateral testicular atrophy (Table 2). Both testes showed a similar histological appearance to that described above. There was no evidence of any necrosis, inflammatory reaction or fibrosis in any of the four cases. This suggested that our vasectomies had not introduced any infection nor interfered with the blood supply of the testis in these animals. At the time when the animals were sacrificed, both testes were in the scrotum. This excludes cryptorchidism, a non-specific side-effect of rat vasectomy in which the testis is retracted and retained in the abdominal cavity, resulting in impairment of spermatogenesis, due to the loss of the normal thermoregulatory function of the scrotum, and subsequently elevated testicular temperature.

v. Possible Correlations between Site and Size of Granulomas and Testicular Changes

. In total, 11/44 (25%) showed left testicular atrophy.

. Of these 11, 9 (82%) had a granuloma in the caput.

. The two animals (Vas 26 and Vas 36) which had left testicular atrophy without a granuloma in the caput both lacked a vasal granuloma also.

. Caput granuloma had the highest incidence in the 18 mos. group (50%).

Is it possible to construct a coherent hypothesis to explain these findings, taken together with what is established in previous publications?

1. After vasectomy, sperm production continues and rising pressure in the efferent duct system of the testis leads, at least in rats, to the leakage of spermatozoa into the interstitial connective tissue and the formation of a spermatic granuloma.

2. This granuloma forms initially, in most, if not all, animals at the vasectomy site.

3. If sperm production continues and granulomas do not develop in the epididymis, the vasal granuloma continues to enlarge (e.g. Vas 22: 904 mgm at 12 mos., and Vas 31: 991 mgm at 18 mos.).

4. If sperm production continues, granulomas usually appear, sooner or later, in the epididymis, initially with a preference for the cauda (e.g. 6 wk. and 4 mos.) and then in corpus and caput.

5. Development of a granuloma in the caput usually leads to: (a) testicular atrophy on the operated side. This is presumably due to obstruction of outflow from the testis and may be regarded as equivalent to high ligation (Smith, 1962); and (b) to reduction in size of the vasal granuloma. This may be attributed to one or more of the following factors:

(i) once testicular atrophy is established, few spermatozoa are produced;

(ii) the granuloma in the caput obstructs the egress of any sperm which are produced;

(iii) the vasal granuloma, now of long standing, shrinks as the sperm which form its core are progressively phagocytosed and broken down by macrophages. The inflammatory response declines. In short, the granuloma is a "burnt out" lesion.

III. THE SPLEEN

A. Sham-operated rats

In cross-section the spleen appeared roughly triangular in shape, surrounded by a thin fibrous capsule and composed of a darkly stained area - the white pulp - and a pale area - the red pulp. Trabeculae were seen from time to time in the red pulp. Sections from the middle portion of the spleen exhibited a thickening in the capsule where it was traversed by the splenic arteries and veins (Fig. 134).

The white pulp masses were scattered throughout the red pulp. The areas of white pulp varied in size, but taken collectively they formed approximately 25% of the total amount of the splenic tissue. They consisted of irregular dense areas of diffuse lymphatic tissue (with some nodules), surrounded by a less darkly stained area (the marginal zone). The white pulp showed a network of reticular fibres, and primitive reticular cells, with fixed macrophages. The meshes of the network were filled by various sized lymphocytes. The marginal zone contained, in addition, some erythrocytes.

The red pulp displayed numerous venous sinuses, with narrow spaces in between, filled by splenic cords. The latter consisted of a network of reticular fibres, primitive reticular cells and fixed macrophages, with lymphocytes of various sizes, monocytes, free macrophages, and circulating blood cells. A few plasma cells, and occasional megakaryocytes were also seen.

Some of the nodular lymphatic tissue exhibited germinal centres, which stained red to pink with methyl green pyronin. They were mainly small to medium size but occasionally large, and contained, in addition to lymphocytes, a few lymphoblasts. The germinal centres showed a significant decrease in number and an obvious reduction in size, in the aged rats (Table 6).

B. Vasectomized animals

A reactive spleen might be expected to show:

1. increase in size.
2. changes in its morphological features:
   (a) increase in the size of the white pulp and, as a result, an alteration in the relative proportion of white and red pulps.
   (b) increase in number of the germinal centres.
   (c) increase in plasma cell population.

1. The mean weights of the experimental and control animals, at each of the seven postoperative periods, did not show any significant differences (Table 4).

2a. The areas of the white pulp, dense area (diffuse and nodular lymphatic tissue), the marginal zone and red pulp, were measured in all spleens from the vasectomized animals, and compared with those for the sham-operated rats. Again, the means of the percentage areas at any individual post-operative period showed no significant differences (Table 5).

2b. Germinal Centres

(i) When the numbers of sectional profiles of germinal centres in spleens of vasectomized and sham-operated controls were compared, at each of the intervals after operation, (i.e. vas 6 wks vs sham 6 wks; vas 4 months vs sham 4 months etc.): they did not differ significantly.

(ii) in both groups (vas and sham), the mean number of sectional profiles did not change significantly with the passage of time after operation, until 18 months, when the number of profiles declined significantly in both groups.

In assessing the significance of these results the following limitations of the method have to be borne in mind:

(a) because of considerations of time, the sample of sections of spleen from each animal was limited to 21 (i.e. 7 sections from each of 3 blocks). The variance in the number of profiles between animals was quite large, as shown by large S.D's. Pooling of the results for each group of animals gave a much larger sample of sections (e.g. 6 x 21 = 126 in sham 6 weeks). In assessing a general trend of response, it is statistically more acceptable to measure a particular parameter less frequently, but in a larger sample of animals, than to measure it more frequently, in a smaller sample. A disadvantage, however, is that this might have concealed significant changes in individual animals at any one stage. The impression was not entirely subjective however. Slides from vasectomized and control animals were assessed microscopically with their identity concealed until assessment

was completed, two observers assessing independently. A large discrepancy was evident in Vas 21 (12 mos) in which the number of profiles was twice that of the next highest member of its group and nearly 2 S.D's greater than the mean of the group and Vas 30 (15 mos) which showed a similar increase over its group. The significance of these results is uncertain, particularly in view of the fact that Sham 25 (15 mos) also showed a corresponding increase in germinal centre profiles.

(b) Although counting of sectional profiles does not give a direct measure of germinal centre diameter, the number of profiles counted in a set of random sections will clearly increase if the centres themselves are more numerous or are larger, or both more numerous and larger. This method therefore indirectly assesses number and size.

(c) the decline in numbers of profiles seen at 18 mos is probably a function of age of the animals, since it was similar in both vasectomized and sham-operated animals.

2c. Plasma cells: there was no obvious increase in numbers of plasma cells in the marginal zone in vasectomized animals as compared with sham-operated controls. This was, however, a qualitative impression: plasma cells were not counted.

If the results for all the parameters i.e. weight, volume density of white pulp, and marginal zone, and volume density of germinal centres, and the qualitative assessment of plasma cells - are taken together, it is evident that there is no significant response of the spleen to vasectomy. The implication of this conclusion is that, following

vasectomy, antigenic material is not taken up in significant amounts from the granuloma and/or genital tract, by the blood stream. This extends the finding of McDonald and Scothorne (1986) that lymphatics provide a route of uptake of sperm autoantigens from the granuloma and/or genital tract.

DISCUSSION

I. FORMATION AND HISTOLOGY OF SPERMATIC GRANULOMAS

Spermatic granulomas frequently develop in the vicinity of the excurrent duct system of the testis, after vasectomy in many species including man (Russell and Friedman, 1951; Reiger and Fuller, 1953; Glassy and Mostofi, 1956; Mullaney, 1962; Smith, 1962; Schmidt, 1966; Lyons et al., 1967; Flickinger, 1972a, b; Schmidt and Morris, 1973; Kwart and Coffey, 1973; Sackler et al., 1973; Freeman and Coffey, 1974; McGlynn and Erpino, 1974; Neaves, 1974; Howards et al., 1975; Kuwahara and Frick, 1975; Voglmayr, 1975; Bedford, 1976; Alexander and Schmidt, 1977; Kennedy and Heidger, 1980; Lopes and Hayashi, 1981; McDonald and Scothorne, 1986). Flickinger (1972a,b), however, stated that this complication was more common in rats than in other species.

In the rat, McDonald and Scothorne (1986), regarded the spermatic granuloma as the first site of leakage of sperm antigens, which might travel either via lymphatics to regional lymph nodes or via blood vessels to the spleen.

In man, Schmidt and Morris (1973) and Girgis (1975), reported that the development of a spermatic granuloma might lead to failure of vasectomy, by initiating a spontaneous reanastomosis of the cut ends of the vas; on the contrary Schmidt and Morris (1973), reported that a granuloma may cause obstruction, leading to testicular atrophy, which would frustrate a subsequent attempt to reverse the operation by vasovasostomy.

In view of these facts it is important to discuss systematically the results of this experiment and to compare them with previous studies.

A. Incidence and distribution of granulomas

In this study spermatic granulomas were present in 42 out of 44 vasectomized rats, either in the epididymis and/or the vas deferens. The two exceptional cases were Vas 26 and 36, at 15 and 18 mos. respectively.

The incidence and distribution of granulomas at various intervals after vasectomy have already been summarized in Tables 2 and 3 and their significance has been briefly analysed (Results, pp.109-110,113-114). The main points are repeated here and discussed in relation to the findings of other workers.

The incidence and percentage of animals with vasal and epididymal granulomas were as follows:

	Vasal granuloma	Epididymal granuloma
- at 1 and 3 wks.	2/2 (100%)	-
- at 6 wks.	7/7 (100%)	1/7 (14.28%)
- at 4 mos.	5/5 (100%)	1/5 (20%)
- at 6 mos.	4/4 (100%)	1/4 (25%)
- at 9 mos.	4/5 (80%)	2/5 (40%)
- at 12 mos.	2/5 (40%)	4/5 (80%)
- at 15 mos.	2/6 (33.3%)	5/6 (83%)
- at 18 mos.	3/8 (37.5%)	6/8 (75%)

A number of studies have stated that, irrespective of the surgical method used, sperm granulomas invariably form in rats following vasectomy, either at the site of vasectomy or in the epididymis or both (Kwart and Coffey, 1973; Freeman and Coffey, 1974; Kennedy and Simon, 1974; Neaves, 1974; Howards et al., 1975; Voglmayr, 1975; Bedford, 1976; McDonald and Scothorne, 1986). Some authors, e.g. Flickinger (1972a,b), Alexander (1973), Sackler et al. (1973), McGlynn and Erpino (1974), and Kennedy and Heidger (1980), reported the development of spermatic granulomas in most but not all of their animals. There are several possible explanations for this variability in incidence of vasal and epididymal granulomas:

(i) The state of sexual maturity at the time of operation

Kwart and Coffey (1973) vasectomized two groups of rats, one prepubertal, the other postpubertal. Six months later, all postpubertal rats had a vasal granuloma; in addition some animals had a granuloma in the cauda, but none had one in the corpus epididymidis.

By contrast, only 50% of the prepubertal group had developed vasal granulomas; the remainder frequently had granulomas in the cauda, and occasionally in the corpus epididymidis.

The authors concluded that "this observation appears to eliminate leakage of sperm at the operation and suture as the predisposing factor in granuloma formation in the prepubertal ~~rats~~ animals". This argument as applied to

the response in prepubertal animals does not seem logical to us; Our own interpretation of their findings is as follows:

(a) that in the prepubertal group, the interval between operation and the time of onset of sperm production allows for firm healing at the site of ligation of the vas in about 50% of the animals.

(b) the development of a vasal granuloma may indicate those animals in which firm healing has not occurred.

(c) when firm healing has occurred, the build-up of pressure occurs in the epididymis, leading to rupture and the production of a granuloma there.

(ii) The state of the testis

Kennedy and Heidger (1980), reported that of 108 rats studied, only 3 failed to show granuloma, and in these 3, the testes were atrophic. In the present study, the two cases (Vas 26 and 36), which had neither vasal nor epididymal granulomas, also showed disruption of the seminiferous tubules in the left testis.

(iii) The immediate environment of the vasectomy site (regardless of the method of interrupting continuity of the vas)

Howards et al. (1975), reported that the incidence of vasal granuloma developing after vasectomy, was reduced from 100 percent to 85 percent, when the vas was left within its surrounding fat sheath, regardless of the method of

interrupting the continuity of the vas. The present experiments, in which the vas was not surrounded by a fat sheath, do not throw any further light on this finding.

(iv) The length of the postoperative period

Bedford (1976), reported that 10 weeks after vasectomy, all rats showed a granuloma at the site of ligature. At 33-37 weeks, postoperatively, 3 out of 6 (50%) had only vasal granulomas. The other 3 animals (50%), displayed multiple granulomas in the epididymis, although two were associated with the smallest vasal granuloma. All 10 animals (100%) examined 60 weeks after operation had granulomas at the vasectomy site, but only 2 out of the 10 (20%) exhibited epididymal granulomas.

In the present investigation, a vasal granuloma was found less frequently at intervals of 9 and more months after vasectomy (at 12 months, absent in 3/5, at 15 months, in 4/6, at 18 months, in 5/8). On statistical grounds it seems very likely that vasal granulomas had been present in all earlier stages after operation and had regressed to the point where they could no longer be recognized. The evidence for this statement is that vasal granulomas were present in 18/19 animals examined at the earlier intervals of 6 weeks to 9 months.

The question one has to answer is therefore "what is the cause of regression and disappearance of vasal granulomas at longer time intervals after vasectomy?" One or both of two factors may be involved:

(a) the development of granulomas in the epididymis, which would prevent sperm passing to the vasectomy site. (b) atrophy of the testis, preventing new sperm being added to the granuloma site.

From these observations, it appears that one or more of several factors may determine the presence or absence of a granuloma.

B. Time of appearance of granuloma

In this study both rats vasectomized for one week exhibited a pin-head sized granuloma at the proximal cut end of the vas deferens. This confirms the observation of Smith (1962), Howards et al. (1975), Kennedy and Heidger (1980), and McDonald and Scothorne (1986), where spermatic granulomas were detected at the site of vasectomy in some or all of their animals, within a week after operation.

C. Size of the vasal granulomas

There is an extensive literature on the sizes of vasal granulomas at various intervals after vasectomy: Smith (1962), reported that vasal spermatic granulomas enlarged gradually, reaching their maximum size at the 40th post-operative day, after which they started to reduce in size and by 90 days after vasectomy, they appeared as "flattened slips of tissue". Flickinger (1972a), on the other hand stated that granulomas of the vas continue to grow until 40 days after surgery, then they stabilized in size throughout the remaining period of the nine months of the study.

Bedford (1976), found that 3 out of 6 rats killed at 33-37 weeks after vasectomy differed from the 10 weeks group only in the larger size of the vasal granulomas. The other 3 animals exhibited several epididymal granulomas, associated in two rats with small vasal granulomas. At 60 weeks after operation, 2 out of 10 animals displayed granulomas in the epididymis associated with average vasal granulomas. Five of the remaining rats showed a large vasal granuloma reaching 2.5 x 1.3 cm, while the other 3 animals possessed a small granuloma at the site of vasectomy, associated with an ipsi-lateral testicular atrophy. Thus a correlation was found between the size of the vasal granuloma and the presence of granulomas in the epididymis and/or testicular atrophy.

Kwart and Coffey (1973), reported that the weight of vasal granulomas at 6 months after vasectomy, was 300-700 mgm.

Voglmayr (1975) stated that the weight of vasal granulomas at 120 days was double that at 30 days after vasectomy.

Kennedy and Heidger (1980), observed an increase with time after vasectomy in the size of the spermatic granuloma at the site of ligature and by 3 months they were approximately 1 cm in length.

Lopes and Hayashi (1981), noted continuous growth of the spermatic granuloma forming at the proximal cut end of the vas deferens, throughout the 280 days of their experiment.

These various findings can now be compared with those of the present study, in which the mean weight of the vasal spermatic granulomas showed a continuous increase up to 9 months after vasectomy, but decreased thereafter. However, the size of the vasal granulomas, in general, continued to increase during the eighteen months of the experimental period in all those rats which did not exhibit testicular atrophy and/or epididymal granulomas.

By contrast, in those rats which had epididymal granulomas, and/or testicular atrophy, the vasal granulomas regressed gradually, even to the point of disappearance. The general conclusion is that growth or maintenance of the size of a vasal granuloma depends upon continuing production of spermatozoa and their escape at the vasectomy site.

D. General morphology

Grossly, the vasal granulomas examined in this study appeared as a spherical or oval pale yellow cystic mass, rubbery in consistency, and with an irregularly contoured surface. This confirmed the observations of Russell and Friedman (1951), Mullaney (1962), Flickinger (1972a,b), Sackler *et al.* (1973), and Kennedy and Heidger (1980). Howards *et al.* (1975), however, described them as white in colour.

Kennedy and Heidger (1980), observed that in some of their experimental rats, the vasal spermatic granulomas were adherent to the muscular wall of the inguinal canal

or eroded through the wall of the urinary bladder, so that the granulomas became a urine filled cyst. In a few animals in the present study, granulomas of the vas were adherent to the fat pad, but none were seen adherent to muscular tissue or to the bladder wall.

Microscopically, in the present study, granulomas were seen to consist of a mass of sperm surrounded by a cellular wall of epithelioid macrophages. This supports the reports of Russell and Friedman (1951), Mullaney (1962), Flickinger (1972a,b), Kwart and Coffey (1973), Brannen et al. (1974), Bedford (1976), Kennedy and Heidger (1980), Lopes and Hayashi (1981), in rats, and Friedman and Garske (1949), Reiger and Fuller, (1953), Glassy and Mostofi (1956), in men. Smith (1962), however, described the epithelioid layer as a "discontinuous low epithelium", and was clearly mistaken.

In many animals of this study, the central sperm mass was invaded by polymorphonuclear neutrophils and macrophages, similar to the findings of Mullaney (1962), Kwart and Coffey (1973), Voglmayr (1975), Bedford (1976), Kennedy and Heidger (1980).

Glassy and Mostofi (1956), studied the spermatic granulomas which developed in the epididymis of men after vasectomy; they reported that after extravasation of sperm into the interstitial tissue, there was initially a cellular exudate of polymorphonuclear neutrophils and macrophages, which was replaced later by epithelioid macrophages. In the present study, however, neutrophils were found among

the extravasated sperm after the appearance of the epithelioid wall. This agrees with the findings of Kwart and Coffey (1973).

Brannen et al. (1974), reported the presence of polymorphonuclear neutrophils actually within the cellular wall, of epithelioid macrophages but this was not seen in the present study.

The epithelioid macrophages in this study had abundant cytoplasm, which contained numerous lysosomes and fragments of spermatozoa. Identifiable spermatozoal remnants within the cytoplasm of the epithelioid cell of the granuloma were also mentioned in the rat by Bedford (1976) and Kennedy and Heidger (1980), and in man by Reiger and Fuller (1953) and Chapman and Heidger (1979). However, Reiger and Fuller (1953) reported that these cells contained also a granular yellow pigment, but this was not a finding of the present project.

The most striking feature of the granuloma in the present investigation was the development of the highly vascularized connective tissue layer, that surrounded the epithelioid layer. Large numbers of mononuclear cells, including lymphocytes, macrophages, plasma cells accumulated around the blood vessels. Of particular interest and importance was the presence of variable numbers of lymphocytes and/or macrophages within the lymphatic vessels, which were abundant in this layer. These findings support those of Kennedy and Heidger (1980). Flickinger (1972a), also reported the presence of mononuclear cells in this area, while

Brannen et al. (1974) described this layer as a fibrous zone containing lymphocytes and plasma cells. In human epididymal granulomas, Glassy and Mostofi (1956) and Chapman and Heidger (1979), also mentioned the presence of a lymphocyte rich layer.

The presence of mononuclear cells within the lumen of the lymphatic vessels of the connective tissue layer of the granuloma is regarded as a significant finding, since these cells could be one vehicle for transport of sperm auto-antigens to the immune system.

The granulomas of this research were limited by a fibrous capsule, agreeing with the work of Smith (1962), Kwart and Coffey (1973), McGlynn and Erpino (1974), Robbins (1974), and Kennedy & Heidger (1980) in rats, and Schmidt and Morris (1973) and Chapman and Heidger (1979), in men.

E. Etiology

Spermatic granulomas have long been known to develop in the epididymis of men, after trauma or focal inflammation such as gonorrheal epididymitis and have been assumed to be the result of extravasation of sperm into the interductal connective tissue (Oberndorfer, 1931; Orsos, 1941; Steinberg and Straus, 1947; Cronqvist, 1949; Friedman and Garske, 1949; Reiger and Fuller, 1953; Sundarasivarao, 1955; Glassy and Mostofi, 1956).

This assumption has been repeatedly supported by many subsequent studies in animals and is fully confirmed in the present study.

In patients with non-specific epididymitis or history of trauma to the left pubic area, Cronqvist (1949), Reiger and Fuller (1953), and Sundarasivarao (1955) noted dilatation of the epididymal duct with attenuation and rupture of its epithelial wall, and the formation of spermatic granuloma. They concluded that spermatic granulomas formation was consequent upon a rupture in the wall of the duct due to the increased intraluminal pressure as a result of obstruction to the tract. Howards and Johnson (1979), measured the intratubular pressure at various levels along the male reproductive tract of hamsters and guinea-pigs before and after vasectomy. They found that in both species, there was a great increase in the pressure in the distal cauda epididymis, and part of the vas deferens proximal to the site of vasectomy. However, at least in the hamster, this pressure was relieved by the development of a spermatic granuloma.

In the rat, Russell and Friedman (1951) and Bedford (1976), reported dilatation of the epididymal duct. The epididymal duct of all but three of the vasectomized rats examined in this study, showed no signs of distension. This general absence of distension may perhaps be attributed to the development of spermatic granulomas at the vasal site and/or epididymis, which, by providing an escape route, prevented a rise in the intraductal hydrostatic pressure.

In the three exceptional cases (Vas 11, 30 and 32), the tubular profiles of the proximal caput epididymidis were engorged with sperm and dilated, and their epithelium was flattened, although, by contrast the distal caput, the corpus and the cauda were empty of spermatozoa. In Vas 32 a large granuloma was present in the mid-caput region, with several ruptures in the wall of the epididymal duct at a site nearer to the testis. This was accompanied by the escape of sperm into the surrounding connective tissue and the formation of new granulomas. The presence of an old granuloma, with signs of stagnation and rupture, and of newly formed granulomas, proximal to it, agrees with the work of Sundarasivarao (1955), where granuloma was thought to form as a result of an obstruction, which was followed by dilatation, attenuation of the epithelium, and rupture. It also supports the suggestion of Kwart and Coffey (1973), that as granulomas become quiescent, they are replaced by new lesions in more proximal locations, which become the reservoir of sperm accumulation; and perhaps might explain the development of multiple granulomas in the epididymis, in addition to the vasal ones, in some of the rats, of the present experiment, particularly of the long term. If a granuloma causes obstruction of the epididymis, this may lead to the development of a new epididymal granuloma nearer to the testis, the older one would then become isolated from further contributions of sperm from the testis and as a result, become reduced in size. This explained why some of the vasal cysts were smaller than others, at

the same postoperative period, when they were accompanied by epididymal granulomas.

Schmidt and Morris (1973), believed that spermatic granuloma of the vas was formed as a result of the sperm leakage, caused by the ligature cutting through the vasal wall. This was helped by the increased intravasal pressure, and the peristaltic surges accompanying ejaculation. In the present study the ligature was not seen cutting through the wall of the vas. Kwart and Coffey (1973) found that all mature rats had granuloma at the site of vasectomy, six months after operation. The authors tried several methods for occlusion of the vas including fulguration, ligature with 000 silk, plain catgut, and chromic sutures, but granulomas developed at the cut end of the vas in all animals. This was confirmed by Howards et al. (1975). These observations showed that the type of suture material is not necessarily a predisposing factor in the formation of granulomatous lesion developed at the vasectomy site.

Schmidt (1966), reported that the incidence of the vasal granuloma in men could be reduced if the vas were fulgurized rather than ligated. Schmidt (1973), concluded that fulguration of the mucosal lining of the lumen leaves the muscular layer with sufficient tone to resist distension; he also suggested that these layers provide a source of fibrocytes, which invade the lumen and form a fibrous plug. Although, the method of fulguration was used, in some of the experimental rats of Kwart and Coffey (1973), and

Howards et al. (1975), and all of the rats utilized in the studies of Kennedy and Heidger (1980), granulomas consistently formed at the site of vasectomy. Indeed differences exist between species utilized.

II. CHANGES IN THE TESTES

Degenerative changes in the testes were found in 15 out of the 44 (34.09%) vasectomized rats examined in this study. The testes appeared normal in all rats up to 4 months after operation; however, testicular atrophy, ipsilateral or bilateral, was found in 1/4 (25%) at 6 mos., 3/5 (60%) at 9 mos., 3/5 (60%) at 12 mos., 3/6 (50%) at 15 mos., and 5/8 (62.5%) at 18 mos. after vasectomy. Degenerative changes were restricted to the left testis only in eleven animals, while the other four rats exhibited testicular atrophy on both sides.

A number of studies have reported no change in the rat testis after vasectomy (Oslund, 1924; Moore and Quick, 1924; Young, 1933; Poynter, 1939; Moore, 1939; Amann, 1962; Smith, 1962; Kar et al., 1965; Lee, 1967; Kubota, 1969; Paufler and Foote, 1969; Flickinger, 1972b; Segal, 1972; Plant, 1973; Heller and Rothchild, 1974; McGlynn and Erpino, 1974; Neaves, 1974,; Howards et al., 1975; Neaves, 1975b; Voglmayr, 1975; Kuwahara, 1976; Lohiya et al., 1976; Reinke and Stohs, 1977; Lopes and Hayashi, 1981). It is important to note, however, that in the majority of these works, the effects were studied for a period of less than six months after surgery. In the present study also the testes were mostly found to be normal up to 6 months after operation.

Impaired spermatogenesis or reduction in testicular weight, on the other hand, were reported by Myers (1916),

Steinach (1920), Jhaver and Ohri (1960), Laumas and Uniyal (1967), Sadi *et al.* (1967), Rumke and Titus (1970), Altwein and Gittes (1972), Thakur *et al.* (1972); Sackler *et al.* (1973), Mock et al. (1975), Bedford (1976), and Neaves (1978). Several explanations have been offered for these contradictory reports.

Oslund (1924) noted that in those cases in which the testis had undergone degeneration it was retained in the abdominal cavity by adhesions (artificial cryptorchidism). He suggested that such a surgical complication, occurring after vasectomy, was responsible for the degenerative changes found in the testis due to the loss of the normal thermo-regulatory function of the scrotum. This postoperative complication was also reported by Moore (1939), and Neaves (1975a). However, in the present studies, all the atrophied testes detected were present in the scrotum at the time when the animals were sacrificed.

Testicular degeneration has also been attributed to other surgical complications such as ischaemia of the testis, due to ligation of the artery of the vas deferens, which was regarded as giving a collateral supply of blood to the testis (Moore, 1939; Neaves, 1975a). However, in the present study, none of the degenerated testes examined showed any signs of ischaemic necrosis.

Oslund (1924), considered the effects of differences in the surgical procedures. Neaves (1974), compared four different surgical methods of vasectomy in the rat: abdominal division with and without ligation and, scrotal division with

and without ligation. Three months after surgery all testes were normal. The author suggested that the differences in the results found in the literature must involve factors other than procedural differences.

Steinach (1920), noted degeneration of the testes of rats killed shortly after vasectomy, while in those animals that had lived for a longer time following surgery, the testes were normal; this was attributed to a subsequent regeneration. If occlusion of the duct is sufficient to cause degeneration, it seems unlikely that regeneration would take place until the causal factor had been removed. In his description of the surgical technique, he not only drew the testes into the abdominal cavity, but while examining them he fully exposed them.

Myers (1915), reported on a group of rats in which he observed that the longer the interval after operation, the more animals showed degenerated testes. The present work would support this finding, as would that of Bedford (1976).

Kwart and Coffey (1973), noted the development of spermatic granulomas in the cauda and corpus epididymidis of some of the rats killed at four months after vasectomy. They suggested that if granulomas continued to extend up in the epididymis, they could disrupt the seminiferous tubules, similar to the effect of ligation of the ductuli efferentes (Smith, 1962). Nine of the eleven degenerated left testes found in this study, and sacrificed between six and eighteen months after vasectomy (i.e. longer than the

postoperative period of Kwart and Coffey, 1973) were accompanied by granuloma of the caput. In eight rats, these capital granulomas were associated with those in the corpus and/or cauda. Bedford (1976), also observed capital granuloma in some of the hamsters and rabbits; such animals exhibited degeneration of the testes.

Horan (1975), suggested that when sperm stasis occurred in the caput epididymidis, there would be a subsequent testicular atrophy as a result of an increase in the intraluminal pressure proximal to the obstruction. The present results would confirm this, since in three animals (Vas 11, 30 and 32) with left testicular degeneration, there was distension in the duct proximal to an obstruction caused by the caput granuloma. However, Horan (1975), attributed degenerative changes in the testes, to ischaemia which occurred when the increased intraluminal pressure which built up proximal to the sperm stasis, exceeded that within the testicular blood vessels, and occluded them. However, in this work, the atrophied testes did not show any feature of ischaemic necrosis, but showed appearances suggesting that the degenerative changes were the result of pressure atrophy similar to that described by Smith (1962), after ligation of the ductuli efferentes.

In four of the rats, both testes were atrophied. Granulomas were detected in the vas and/or epididymis, but none of these epididymal granulomas occurred in the caput. Microscopic examination of the testes revealed no

signs of ischaemic necrosis, inflammatory reaction or fibrosis. At the time when the animals were sacrificed, all testes were found in the scrotum.

Mancini (1976), reported that two weeks after sensitization of guinea pigs with autoclaved testis, there was patchy degeneration of the testes. Cellular infiltration including macrophages, fibroblast and mononuclear cells, but mainly lymphocytes, were sometimes present. The epididymis contained sperm and some degenerated germ cells. During the third week there was almost complete loss of cells in some seminiferous tubules and infiltration of inflammatory cells in the interstitium. The proximal portion of the epididymis was devoid of sperm, but sperm were still found in the distal segment. By the sixth week there were no germinal cells in the tubules. The intertubular infiltration was absent. Regeneration of germinative epithelium could be detected after the fourth month.

It is possible that the four rats examined in this study had suffered an immunologic orchitis, but we do not have the evidence either for or against this, as all cases were at long intervals after vasectomy, so that inflammatory lesions could have subsided without residual trace.

In this work the degenerative changes in the testes were patchy in distribution and included exfoliation of immature as well as abnormal germ cells and the formation of multinucleate giant cells possibly formed by coalescence of sperm precursors. Many tubules were left with only Sertoli cells and a few scattered spermatogonia. These observations are in agreement with those of Smith (1962) and Neaves (1978).

III. EPIDIDYMIS

A. Sham-operated rats

The epithelial lining, and the luminal contents of the caput and cauda epididymidis were examined by light and electron microscopy, and showed features similar to those described by Reid and Cleland (1957), and by Hamilton (1975).

(a) The principal cells showed characteristic features, including pinocytotic invagination of the apical surface, the apical cytoplasm being occupied by vesicles, multivesicular bodies and smooth endoplasmic reticulum, and a large supranuclear Golgi. These findings are in agreement with reports that principal cells are involved in two functions: (i) absorption of fluid from the lumen (Mason and Shaver, 1952; Macmillan, 1957; Grant, 1958; Burgos, 1964; Nicander, 1965; Sedar, 1966; Friend and Farquhar, 1967; Hoffer et al., 1973; Hamilton, 1975; Moore and Bedford, 1979), and (ii) secretion (Rambourg et al., 1969; Fawcett and Hamilton, 1970; Hamilton, 1972; Hoffer et al., 1973; Moore and Bedford, 1979).

(b) The clear cells have been thought to be holocrine secretory cells and the source of glycerylphosphorylcholine (Martan and Risley, 1963; Martan and Allen, 1964; Martan et al., 1964; Martan, 1969), but autoradiographic and

ultrastructural investigations disproved this hypothesis (Kreth, 1965; Holstein, 1969; Clermont and Flannery, 1970; Hamilton, 1972). During the technical preparation for this study, several staining methods were tried. It was found that the granules (dense bodies) of the clear cells, were PAS-positive and stained intensely with toluidine blue, confirming the findings of Martan and Risley (1963) and Fahrmann and Schuchardt (1966). Such staining properties were attributed to lysosomes (Gahan, 1967) or secretory granules (Martan and Risley, 1963; Fahrmann and Schuchardt, 1966). In addition, these granules were found to be acid phosphatase positive (Martan and Risley, 1963; Martan and Allen, 1964; Wise and Flickinger, 1970).

Flickinger (1972a) observed membranous materials in the multivesicular bodies, dense bodies, and infranuclear region of the clear cells. He suggested that the dense bodies represented lysosomes, while the infranuclear membranous masses, which had a polymorphic interior composed of granules and amorphous material, and showed a positive acid phosphatase reaction, were residual bodies. The present study agrees with that, since similar membranes were detected by E.M. mainly in the dense bodies and infranuclear region of the cells. However, Flickinger (1972a) attributed this membranous material to the absorption of sperm by the clear cells, although he detected recognizable sperm fragments only rarely. The report of Calvin and Bedford (1971), that because of their keratinoid nature sperm heads and tails are unlikely to disintegrate completely,

suggests that if sperm fragments were to be absorbed in this way, the various visible parts would be found frequently in the cytoplasm of the cells. They have not been found at all in the present study.

Hamilton (1975) found lipid droplets at the base of the clear cells. In the present study, also, lipid-like droplets were detected in the infranuclear region of some of the clear cells. This does not exclude the possibility of these cells being also secretory. However the exact function of these cells cannot be established on the basis of data presently available and this needs further investigation.

(c) The intraepithelial leucocytes were first described by Reid and Cleland (1957) as surrounded by a very lightly staining area and for this reason they were given the name of "halo" cells. However in the present study in which the animals were fixed by vascular perfusion, this zone was not seen, and is concluded to be an artifact.

Hoffer et al. (1973) reported that the intraepithelial leucocytes were typical granulocytes, monocytes, and lymphocytes, while Dym and Romrell (1975) stated that they closely resembled the intraepithelial lymphocytes of the gastrointestinal tract or those found in lymphoid organs and blood. In the present ultrastructural study, it was possible to recognize three kinds of intraepithelial leucocytes: lymphocytes, monocytes and macrophages. The

significance of these cells in the epididymal epithelium is unknown. Although absent in the seminiferous tubules, they have been found in the epithelium of the tubuli recti, rete testis, vasa efferentia, epididymis and vas deferens (Dym and Romrell, 1975). Migratory cells bearing a close resemblance to the intraepithelial leucocytes have been observed in the epithelium of the prostate and seminal vesicle of ageing rats (Allison and Cearley, 1972). They were found to become more numerous in older rats, and the authors suggested that the presence of these cells in the epithelium of the prostate and seminal vesicle reflects some sort of age-related immune response. However, in the present study, the number of the epididymal intraepithelial leucocytes did not show any significant differences between young and old rats. Neither in the present study, nor in previous reports were epididymal intraepithelial leucocytes seen to enter or to leave the epithelium.

B. Vasectomized animals

In the present study granulomas developed in the epididymis of several rats, particularly at longer intervals after vasectomy. Epididymal granulomas have also been mentioned in the rat (Kwart and Coffey, 1973; Bedford, 1976; McDonald and Scothorne, 1986), and in man (Pardanani, 1976) after vasectomy.

In the present study, phagocytic cells containing many intact sperm or sperm fragments were observed in the lumen

of sectional profiles of the duct adjacent to spermatic granulomas; and according to their morphological features, it was possible to distinguish two kinds of phagocytes, namely macrophages and polymorphonuclear leucocytes. Phagocytes in this location were also detected in some of the experimental rats by Bedford (1976), who suggested that they enter the duct via the spermatic granuloma; while Flickinger (1982) also classified the intraluminal spermiophages observed in the lumen of the excurrent duct of the hamster after vasectomy into macrophages and polymorphonuclear leucocytes.

The majority of the epididymides examined in this project showed no obvious dilatation. This agrees with the findings of Alexander (1973), although Bedford (1976) reported a slight distension of the cauda epididymis. In the human on the contrary, the epididymidis appeared turgid and distended (Pardanani, 1976). This difference was perhaps due to the fact that the resistance of the rat excurrent duct to the increased intraluminal pressure is less than in the human, leading to early rupture which resulted in a temporary relief of the pressure; the development of a granuloma at the vasal site within a week after surgery would support this. Also in three of the rats there were signs of stagnation caused by granuloma which developed in the mid-region of the caput, with distension in the duct on the testicular side of the

obstruction. In one of these animals, several ruptures were detected in the wall of the duct, presumably caused by the increased intratubular hydrostatic pressure.

In some rats there was disruption of the seminiferous tubules; in these animals, the epididymal duct was empty; presumably spermatozoa which had been there had been moved onwards by contraction of smooth muscle of the epididymal duct, and they had not been replaced because the testis was inactive.

The epididymal epithelium showed the normal characteristic complement of cellular organelles found in the controls suggesting that it remained functionally active after vasectomy. No recognizable sperm or sperm fragments were seen in the cytoplasm of the epithelial cells in the present study. This agrees with the findings of Bedford (1976) and suggests that the epididymal epithelium of the rat plays no part in the removal of sperm after vasectomy.

An important finding of the present study was the absence of any change in the number and position of intra-epithelial leukocytes at any interval after vasectomy. This places on a firm quantitative basis the general impression gained by Alexander (1973).

The finding is important because it suggests strongly that vasectomy does not lead to <u>increased</u> uptake of sperm antigens by the epididymal epithelium, and that peripheral sensitisation of intraepithelial lymphocytes is unlikely to contribute to the development of the immune response to vasectomy.

IV. THE FATE OF SPERM AFTER VASECTOMY

The mechanism by which the sperm were disposed of after vasectomy was studied carefully. The possibility of resorption of degenerating sperm by the epithelial cells of the epididymis was considered, since some investigators reported this phenomenon to occur in their animals (Flickinger, 1972a; Alexander, 1973; Urena and Malavasi, 1981), but the present results provide no evidence suggesting that the intact epididymal epithelium plays a role in the disposal of sperm or their fragments after ligation of the duct.

Flickinger (1972a) observed the presence of a membranous material in the cytoplasm of the clear cells of the cauda epididymis in the sham-operated rats. These were detected in the apical vacuoles, the membrane limited dense bodies, and infranuclear regions of the cell. After vasectomy, the membranous materials were reported to increase in amount, and although this observation was not supported by quantitative studies, the author suggested that it represents the disposal of the large number of trapped sperm by the epididymal epithelium after vasectomy. In another study, on the hamster, Urena and Malavasi (1981), also noted the accumulation of electron dense bodies in the cytoplasm of these cells. In the present study, membranous material was found mainly in the membrane-bounded dense bodies, and infranuclear regions, and rarely in the apical multivesicular bodies of the clear cells of both sham-operated and vasectomized rats and no obvious differences were detected in the amount between the two groups.

Alexander (1973), reported the breakdown of spermatozoa in the lumen of the caput epididymis of the rats after vasectomy. This was accompanied by the appearance of lamellar bodies in the supranuclear region of the principal cells. These bodies were thought to resemble lysosomes and residual bodies and were attributed to the absorption of the spermatozoal fragments. In the present study, lysosome-like dense bodies were mainly found at the Golgi area of the principal cells of both sham-operated and vasectomized rats. However, they were few in number and did not show any obvious increment after vasectomy.

Calvin and Bedford (1971), reported that the high concentration of-S-S crosslinks in head and tails of the mature mammalian sperm makes them unlikely to disintegrate completely. Therefore if sperm are disposed of in this way, one would expect to find a significant amount of recognizable sperm remnants in the cytoplasm of these cells. However visible sperm fragments were rarely seen by Flickinger (1972a), while Alexander (1973) and Urena and Malavasi (1981), did not mention them. Thus it seems unlikely that the epididymal epithelium constitutes a significant route for the removal of sperm after vasectomy.

Hoffer et al. (1975) noted the uptake of intact sperm by the non-ciliated cells of the vasa efferentia after ligation of the initial segment of the epididymis in the rat. This was followed by intraluminal phagocytosis of sperm by macrophages. The vasa efferentia were not included in the present study.

Phadke (1964) observed a large number of macrophages containing phagocytosed spermatozoa in the lumen of the epididymis of men after vasectomy, and concluded that epididymal macrophages played an important role in the disposal of spermatozoa in men; but this was not a finding in the present study in the rat.

Kwart and Coffey (1973), noted that in several rats the vasal spermatic granuloma approached the size of the testis. They suggested that after vasectomy the sperm were transformed and accumulated in the granuloma. Neaves (1975a) reported that the expanding spermatic cyst of the vas was of sufficient size to accommodate total sperm production through at least seven months after vasectomy. In the present experiment, vasal spermatic granulomas were found to increase in size with age after vasectomy in all rats, which failed to show an epididymal granuloma, and in two animals of the long term, they reached the size of the testis.

Several investigators presented evidence suggesting that the cellular wall of the spermatic granuloma was involved in the uptake of sperm or its fragments from the lumen of the granuloma (Reiger and Fuller, 1953; Flickinger, 1975a; Bedford, 1976; Chapman and Heidger, 1979; Kennedy and Heidger, 1980). Sperm phagocytosis were not restricted only to the epithelioid cells, but numerous macrophages and polymorphonuclear neutrophils were found interspersed within the central sperm mass (Mullaney, 1962; Kwart and

Coffey, 1973; Voglmayr, 1975; Bedford, 1976; Kennedy and Heidger, 1980). In the present study, the epithelioid macrophages of the cellular wall of the granuloma, showed many remnants of sperm in their cytoplasm, while macrophages and polymorphonuclear neutrophils were detected among the sperm mass in the lumen of the granulomas and in the tubular profiles of the duct adjacent to the spermatic granulomas. Thus, it seems likely that after vasectomy, at least in the rat, the removal of sperm, which continue to be produced, by the testis, occurs (i) by their passive escape into the granuloma, which as a result showed a continuous growth, and (ii) by the phagocytic activity of cells in the wall and the lumen of the granuloma.

A third route of removal has been reported by Ball and Setchell (1983), who studied vasectomized rams and boars and found large numbers of free spermatozoa in the testicular lymphatics intermittently between 1 week and 2-3 months after operation. They found phagocytosis of spermatozoa by sinus macrophages in the regional testicular lymph nodes and, in some cases, the development of a granulomatous reaction. In rats, on the other hand, McDonald and Scothorne (1987) found very few, if any, spermatozoa in the regional testicular lymph nodes of vasectomized and sham operated animals (of the same strain as that used here) at intervals of 1-10 weeks after operation. This result is fully confirmed by the present study, which failed to find free sperm or sperm fragments in epididymal

lymphatics of vasectomized rats. There seem therefore to be species differences in the mode of presentation of sperm autoantigens to the regional nodes after vasectomy.

REFERENCES

AGMO, A. (1976)
The number of spermatozoa in spontaneous ejaculates of rats.
Journal of Reproduction and Fertility 48, 405-407.

AIGNER, A. (1900)
Cited by Shaver (1954).

ALEXANDER, N.J. (1972)
Vasectomy: Long term effect in the rhesus monkey.
Journal of Reproduction and Fertility 31, 399-406.

ALEXANDER, N.J. (1973)
Ultrastructural changes in rat epididymis after vasectomy.
Zeitschrift fur Zellforschung und Mikroskopische Anatomie (Berlin) 136, 177-182.

ALEXANDER, N.J., WILSON, B.J. and PATTERSON, G.D. (1974)
Vasectomy: Immunological effects in rhesus monkeys and men.
Fertility and Sterility 25, 149-156.

ALEXANDER, N.J. and SCHMIDT, S.S. (1977)
Incidence of antisperm antibody levels and granulomas in men.
Fertility and Sterility 28, 655-657.

ALEXANDER, N.J. and Tung, K.S.K. (1977)
Immunological and morphological effects of vasectomy in the rabbit.
Anatomical Record 188, 339-350.

ALEXANDER, N.J. and Tung, K.S.K. (1979)
Vasectomy in the rabbit: Immunological and morphological effects. in "Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man". edited by I.H. Lepow and R. Crozier. Academic Press pp. 355-377.

ALLISON, V.F. and CEARLEY, G.W. (1972)
Electron microscopic study of cells within the epithelium of the seminal vesicle of ageing rats.
Anatomical Record 172, 262 (Abstract).

ALTWEIN, J.E. and GITTES, R.F. (1972)
Vasektomic und hodenfunktion.
Tierexperimentelle Untersuchungen Actuelle Urologie 3, 235-237.

AMANN, R.P. (1962)
Reproductive capacity of dairy bulls. III. The effect of ejaculation frequency, unilateral vasectomy, and age on spermatogenesis.
American Journal of Anatomy 110, 49-67.

AMANN, R.P. and ALMQUIST, J.O. (1962)
Reproductive capacity of dairy bulls. VI. Effect of unilateral vasectomy and ejaculation frequency on sperm reserves: aspects of epididymal physiology.
Journal of Reproduction and Fertility 3, 260-268.

AMANN, R.P., HOKANSON, J.F. and ALMQUIST, J.O. (1963)
Cannulation of the bovine ductus deferens for quantitative recovery of epididymal spermatozoa.
Journal of Reproduction and Fertility 6, 65-69.

ANSBACHER, R. (1971)
Sperm-agglutinating and sperm-immobilizing antibodies in vasectomized men.
Fertility and Sterility 22, 629-632.

ANSBACHER, R., KEUNG-YEUNG, K. & WURSTER, J.C. (1972)
Sperm antibodies in vasectomized men.
Fertility and Sterility 23, 640-643.

ANSBACHER, R. (1973)
Vasectomy: Sperm antibodies.
Fertility and Sterility 24, 788-792.

ANSBACHER, R. (1974)
Bilateral vas ligation: Sperm antibodies.
Contraception 9, 227-237.

ANSBACHER, R., HODGE, P., WILLIAMS, A. & MUMFORD, D.M. (1976)
Vas ligation: Humoral sperm antibodies.
International Journal of Fertility 21, 258-260.

ANSBACHER, R. (1981)
Humoral sperm antibodies: A 10 year follow-up of vas-ligated men.
Fertility and Sterility 36, 222-224.

ARONSON, L.R. (1949)
Behavior resembling spontaneous emissions in the domestic cat.
Journal of Comparative and Physiological Psychology 42, 226-227.

BALDWIN, B.T. (1928)
The determination of sex maturation in boys by a laboratory method.
Journal of Comparative Psychology 8, 39-43.

BALL, R.Y. and SETCHELL, B.P. (1983)
The passage of spermatozoa to regional lymph nodes in testicular lymph following vasectomy in rams and boars.
Journal of Reproduction and Fertility 68, 148-153.

BEACH, F.A. and EATON, G. (1969)
Androgenic control of spontaneous seminal emission in hamsters.
Physiology and Behavior 4, 155-156.

BECKER, O. (1856)
Uber flimmerzellen in nebenhoden des menschen.
Wiener Medizinische Wochenschrift 6, 184-193.

BEDFORD, J.M. (1966)
Development of the fertilizing ability of spermatozoa in the epididymis of the rabbit.
Journal of Experimental Zoology 163, 319-330.

BEDFORD, J.M. (1973)
Maturation, transport and fate of spermatozoa in the epididymis. In: "Handbook of Physiology, Male Reproduction", Vol. III, Section 7. Edited by R.O. Greep and E.B. Astwood, American Physiological Society, Washington, D.C.

BEDFORD, J.M., CALVIN, H. and COOPER, G.W. (1973)
The maturation of spermatozoa in the human epididymis.
Journal of Reproduction and Fertility 18, 199-213.

BEDFORD, J.M. (1976)
Adaptations of the male reproductive tract and the fate of spermatozoa following vasectomy in the rabbit, rhesus monkey, hamster and rat.
Biology of Reproduction 14, 118-142.

BENEKE (1898)
Cited by Simmonds (1921).

BENNETT, J.P. and ROWSON, L.E. (1963)
A fistula for the collection of epididymal semen from the bull.
Journal of Reproduction and Fertility 6, 61-64.

BENOIT, J. (1926)
Recherches anatomiques, cytologiques et histophysiologiques sur les voies excretices du testicle chez les mammiferes.
Archives D Anatomie D Histologie Et D. Embryologie 5, 175-183.

BERG, J.W. (1953)
Differential staining of spermatozoa in sections of testis.
American Journal of Clinical Pathology 23, 513-515.

BERG, J.W. (1954)
An acid-fast lipid from spermatozoa.
Archives of Pathology 57, 115-120.

BIGAZZI, P.E., KOSUDA, L.L. and HARNICK, L.L. (1977)
Sperm autoantibodies in vasectomized rats of different inbred strains.
Science 197, 1282-1283.

BIELANSKI, W. and WIERZBOWSKI, S. (1961)
Attempts at determination of the daily output of spermatozoa in rams on the basis of the so-called "Depletion Test" carried out at varying intervals.
Proceedings of the 4th International Congress on Animal Reproduction 2, 274-279.

BIGAZZI, P.E. (1978)
Autoimmune responses to spermatozoa in vasectomized rats and mice of different strains. In: "Genetic Control of Autoimmune Disease" edited by N.R. Rose, P.E. Bigazzi and N.L. Warner, New York: Elsevier, pp.455-462.

BLANDAU, R.J. and RUMERY, R.E. (1964)
The relationship of swimming movements of spermatozoa to their fertilizing capacity.
Fertility and Sterility 15, 571-579.

BOSE, A.R., KAR, A.B. and DAS GUPTA, P.R. (1966)
Sialic acid in the genital organs of the male rat.
Current Science India 35, 336-337.

BRANNEN, G.E., KWART, A.M. and COFFEY, D.S. (1974)
Immunological implications of vasectomy: I. Cell-mediated immunity.
Fertility and Sterility 25, 508-514.

BURGOS, M.H. (1964)
Uptake of the colloidal particles by cells of the caput epididymidis.
Anatomical Record 148, 517-525.

BURGOS, M.H. and CAVICCHIA, J.C. (1975)
Phagocytic activity in the epithelium of the rete testis.
Extended Abstracts presented at the 10th International Congress of Anatomists (Tokyo).

CALVIN, H.I. and BEDFORD, J.M. (1971)
Formation of disulphide bonds in the nucleus and accessory structures of mammalian spermatozoa during maturation in the epididymis.
Journal of Reproduction and Fertility Supplement 13, 65-75.

CHANG, M.C. (1943)
Disintegration of epididymal spermatozoa by application of ice to the scrotal testis.
Journal of Experimental Biology 20, 16-22.

CHAPMAN, E.S. and HEIDGER, P.M. (1979)
Spermatic granuloma of the vas deferens after vasectomy in rhesus monkeys and men: Light and electron microscopic study.
Urology 13, 629-639.

CLERMONT, Y. and FLANNERY, J. (1970)
Mitotic activity in the epithelium of the epididymis in young and old adult rats.
Biology of Reproduction 3, 283-292.

COLLINS, P.M., BEDE, J.B.G. and TSANG, W.N. (1972)
The effects of vasectomy on steroid metabolism by the seminiferous epithelium and interstitial tissue of the rat testis: A comparison with the effects of ageing.
Journal of Endocrinology 55, 18-19.

COOPER, T.G. and HAMILTON, D.W. (1977)
Observations on destruction of spermatozoa in the cauda epididymidis and proximal vas deferens of non-seasonal male mammals.
American Journal of Anatomy 149, 93-110.

CRABO, B. and GUSTAFSSON, B. (1964)
Distribution of sodium and potassium and its relation to sperm concentration in the epididymal plasma of the bull.
Journal of Reproduction and Fertility 7, 337-345.

CRABO, B. (1965)
Studies on the composition of epididymal content in bulls and boars.
Acta Veterinaria Scandinavica 5, 1-94.

CRABO, B., GUSTAFSSON, B., NICANDER, L. and RAO, A. (1971)
Subnormal testicular function in a bull concealed by phagocytosis of abnormal spermatozoa in the efferent ductules.
Journal of Reproduction and Fertility 26, 393-396.

CRONQVIST, S. (1949)
Spermatic invasion of the epididymis.
Acta Pathologica et Microbiologica Scandinavica 26, 786-794.

CUNNINGHAM, J.H. and COOK, W.H. (1922)
The operative treatment and pathology of acute epididymitis.
Journal of Urology 7, 139-152.

DERRICK, F.C., GLOVER, W.L., KANJUPARAMBAN, Z., JACOBSON, C.B., McDOUGALL, M., McCOWIN, K., MERCER, H.D. and ROLLINS, L.D. (1974)
Histologic changes in the seminiferous tubules after vasectomy.
Fertility and Sterility 25, 649-658.

DUNNETT, C.W. (1964)
New tables for multiple comparisons with a control.
Biometrics 20, 482-491.

DYM, M. (1974)
The fine structure of monkey Sertoli cells in the transitional zone at the junction of the seminiferous tubules with the tubuli recti.
American Journal of Anatomy 140, 1-26.

DYM, M. and ROMERELL, L.J. (1975)
Intraepithelial lymphocytes in the male reproductive tract of rats and rhesus monkeys.
Journal of Reproduction and Fertility 42, 1-7.

DYM, M. (1976)
The mammalian rete testis - a morphological examination.
Anatomical Record 186, 493-524.

FAHRMANN, W. and SCHUCHARDT, E. (1965)
Nebenzellen in epithel des ductus epididymidis.
Naturwissenschaften 52, 620-621.

FAHRMANN, W. and SCHUCHARDT, E. (1966)
Licht- und electronenmikroskopische untersuchungen am nebenhodengan-gepithel der ratte vor und nach geschlechtsreifung.
Zeitschrift fur Mikroskopisch-Anatomische Forschung 74, 337-362.

FAWCETT, D.W. and HAMILTON, D.W. (1970)
Electron microscopic and biochemical evidence for steroid synthesis in the epididymis. In: "Morphological Aspects of Andrology", edited by A.F. Holstein and E. Horstman. Berlin: Grosse. pp.119-121.

FAWCETT, D.W. (1979)
Interpretation of the sequelae of vasectomy. In: "Vasectomy: Immunologic and Pathophysiologic Effects in Animal and Man". edited by I.H. Lepow and R. Crozier. Academic Press pp.3-21.

FLEISCHER, B., FLEISCHER, S. and OZAWA, H. (1969)
Isolation and characterization of Golgi membranes from bovine liver.
Journal of Cell Biology 43, 59-79.

FLICKINGER, C.J. (1972a)
Alteration in the fine structure of the rat epididymis after vasectomy.
Anatomical Record 173, 277-300.

FLICKINGER, C.J. (1972b)
Ultrastructure of the rat testis after vasectomy.
Anatomical Record 174, 477-483.

FLICKINGER, C.J. (1975a)
I. Fine structure of the rabbit epididymis and vas deferens after vasectomy.
Biology of Reproduction 13, 50-60.

FLICKINGER, C.J. (1975b)
II. Fine structure of the rabbit testis after vasectomy.
Biology of Reproduction 13, 61-67.

FLICKINGER, C.J. and CHARLTON, J.S. (1979)
Ultrastructure of the testis and different regions of the epididymis in normal and vasectomized hamsters.
In: "Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man". edited by I.H. Lepow and R. Crozier. Academic Press pp.25-49.

FLICKINGER, C.J. (1982)
The fate of sperm after vasectomy in the hamster.
Anatomical Record 202, 231-239.

FOOTE, R.H. (1962)
The use of radioactive isotopes to study spermatogenesis and transport of spermatozoa in mammals.
Symposia Genetica et Biologica Italica 9, 55-59.

FOURNIER, S. (1966)
Distribution of sialic acid in the genital system of adult normal and castrated Wistar rats.
Comptes Rendus De La Societe de Biologie 160, 1087-1090.

FRANKEL, A.I. and EIK-NES, K.B. (1968)
Steroidogenesis in vitro of the epididymis of the rabbit (Abstract).
Federation Proceedings 27, 624.

FRANKEL, A.I. and EIK-NES, K.B. (1970)
Testosterone and dehydroepiandrosterone in the epididymis of the rabbit.
Journal of Reproduction and Fertility 23, 441-445.

FREEMAN, C. and COFFEY, D.S. (1974)
A new method for suppressing sperm granuloma formation in the vasectomized rat.
Fertility and Sterility 25, 276-279.

FRIEDMAN, N.B. and GARSKE, G.L. (1949)
Inflammatory reactions involving sperm and the seminiferous tubules: Extravasation, spermatic granulomas and granulomatous orchitis.
Journal of Urology 62, 363-374.

FRIEND, D. and FARQUHAR, M.G. (1967)
Functions of coated vesicles during protein absorption in the rat vas deferens.
Journal of Cell Biology 35, 357-376.

FRITZ, I.B. (1963)
Carnitine and its role in fatty acid metabolism.
Advances in Lipid Research 1, 285-334.

FULKA, J. and KOEFOED-JOHNSON, H.H. (1966)
The influence of epididymal passage in rabbits on different spermatozoan characteristics including fertilizing capacity.
Report: Royal Veterinary and Agricultural College 213-227.

GAHAN, P.B. (1967)
Histochemistry of lysosomes.
International Review of Cytology 21, 1-63.

GALLE, J. and FRIEND, D.S. (1977)
The fate of sperm after vasectomy in the guinea-pig: Fine structure, freeze-fracture and cytochemistry.
Laboratory Investigation 37, 79-95.

GASIC, G.J., BERWICK, L. and SORRENTINO, M. (1968)
Positive and negative colloidal iron as cell surface electron stains.
Laboratory Investigation 18, 63-71.

GIRGIS, S.M. (1975)
Spontaneous reanastomosis of the vas deferens after surgery: A case report.
Fertility and Sterility 26, 182-185.

GLASSY, F.J. and MOSTOFI, F.K. (1956)
Spermatic granulomas of the epididymis.
American Journal of Clinical Pathology 26, 1303-1313.

GLOVER, T.D. (1961)
Disintegrated spermatozoa from the epididymis.
Nature 190, 185-186.

GLOYNA, R.E. and WILSON, J.D. (1969)
A comparative study of the conversion of testosterone to 17β-hydroxy-5α-androstan-3-one(dihydrotestosterone) by prostate and epididymis.
Journal of Clinical Endocrinology and Metabolism 29, 970-977.

GOTTSCHALK, A. and NEUBERGER, A. (1966)
Retrospect and outlook. In "Glycoproteins", edited by A. Gottschalk. Amsterdam: Elsevier pp.594-597.

GRANT, J.H. (1958)
The passage of Trypan blue through the epididymis and its uptake by this organ.
Proceedings of the Society for the Study of Fertility 10, 95-101.

GREWAL, R.S. and SACHAN, M.S. (1968)
Changes in testicle after vasectomy. An experimental study.
International Surgery 49, 460-462.

GUNN, R.M. (1936)
Fertility in sheep. Artificial production of seminal ejaculation and the characters of the spermatozoa contained therein.
Bulletin. Council for Scientific and Industrial Research 94, 1-116.

HALIM, A. and ANTONIOU, D. (1973)
Autoantibodies to spermatozoa in relation to male infertility and vasectomy.
British Journal of Urology 45, 559-562.

HAMILTON, D.W. and FAWCETT, D.W. (1970)
In vitro synthesis of cholesterol and testosterone from acetate by rat epididymis and vas deferens.
Proceedings of the Society for Experimental Biology and Medicine 133, 693-695.

HAMILTON, D.W. (1971)
Steroid function in the mammalian epididymis.
Journal of Reproduction and Fertility Supplement 13, 89-97.

HAMILTON, D.W. (1972)
The mammalian epididymis. In: "Reproductive Biology" edited by H. Balin and S. Glasser. Amsterdam "Excerpta Medica Foundation". pp.268-337.

HAMILTON, D.W. (1975)
Structure and function of the epithelium lining the ductuli efferentes, ductus epididymidis and ductus deferens in the rat. In: "Handbook of Physiology, Male Reproduction" Volume V, Section 7. edited by R.O. Greep and E.B. Astwood, American Physiological Society, Washington, D.C.

HANCOCK, J.L. (1955)
The disintegration of bull spermatozoa.
Veterinary Record 67, 825-826.

HELLEMA, H., SAMUEL, T. and RUMKE, P. (1978)
'Sperm autoantibodies after vasectomy'.
In Proceedings 3rd International Symposium on Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia. pp.387-396.

HELLER, G.V. and ROTHCHILD, I. (1974)
The influence of the surgical technique used for vasectomy on testis function in rats.
Journal of Reproduction and Fertility 39, 81-82.

HERNANDEZ-JAUREGUI, P. and OLIVERA, J. (1975)
A study of the morphology of the Leyding cells of the rat after unilateral vasectomy.
Journal of Reproduction and Fertility 45, 405-407.

HJORT, T., HANSEN, K.B. and POULSEN, F. (1978)
The reactivity of $F(ab)_2$ fragment of sperm antibodies and their use in the investigation of antigen antibody system. In: "Spermatozoa, Antibodies and Infertility". edited by J. Cohen and W.F. Hendry. Blackwell, Oxford pp.101-115.

HOFFER, A.P., HAMILTON, D.W. and FAWCETT, D.W. (1973)
The ultrastructure of the principal cells and intraepithelial leucocytes in the initial segment of the rat epididymis.
Anatomical Record 175, 169-202.

HOFFER, A.P., HAMILTON, D.W. and FAWCETT, D.W. (1975)
Phagocytosis of spermatozoa by epithelial cells of the ductuli efferentes after epididymal obstruction in the rat.
Journal of Reproduction and Fertility 44, 1-9.

HOLSTEIN, A.F. (1967)
Spermiophagen in nebenhoden des menschen.
Naturwissenschaften 54, 95-99.

HOLSTEIN, A.F. (1969)
Morphologische studien am nebenhoden des menschen.
Zwanglose Abhandlungen aus dem gebiet der Normalen und Pathologische Anatomie 20, 1-91.

HOLTZ, W. and FOOTE, R.H. (1972)
Sperm production, output and urinary loss in the rabbit.
Proceedings of the Society for Experimental Biology and Medicine 141, 958-962.

HOOKER, R.H. and GILMORE, D.P. (1972)
Obstruction of the Vasa Deferentia of the male rat and rabbit with silastic medical adhesive.
International Journal of Fertility 17, 14-16.

HORAN, A.H. and BEDFORD, J.M. (1972)
Development of the fertilizing ability of spermatozoa in the epididymis of the Syrian hamster.
Journal of Reproduction and Fertility 30, 417-423.

HORAN, A.H. (1973)
The pathogenesis of hydro-testis after ligation of the vas deferens: A study in several species.
Journal of Urology 110, 317-321.

HORAN, A.H. (1975)
When and why does occlusion of the vas deferens affect the testis?
Fertility and Sterility 26, 317-328.

HOWARDS, P.J. and JAMES, L.P. (1973)
Immunological implications of vasectomy.
Journal of Urology 109, 76-78.

HOWARDS, S.S., JESSEE, S. and JOHNSON, A.L. (1975)
Micropuncture and microanalytic studies of the effect of vasectomy on the rat testis and epididymis.
Fertility and Sterility 26, 20-28.

HOWARDS, S.S. and JOHNSON, A.L. (1979)
Effects of vasectomy on intratubular hydrostatic pressure in the testis and epididymis.
In: "Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man" edited by I.H. Lepow and R. Crozier
Academic Press pp.55-66.

IGBOELI, G. and RAKHA, A.M. (1970)
Bull testicular and epididymal functions after long-term vasectomy.
Journal of Animal Science 31, 72-75.

INANO, H., MACHINO, A. and TAMAOKI, B.I. (1969)
In vitro metabolism of steroid hormones by cell-free homogenates of epididymides of adult rats.
Endocrinology 84, 997-1003.

JHAVER, P.S. and OHRI, B.B. (1960)
The history of experimental and clinical work on vasectomy.
Journal of the International College of Surgeons 33, 482-486.

JOHNSON, A.L. and HOWARDS, S.S. (1975)
Intratubular hydrostatic pressure in testis and epididymis before and after vasectomy.
American Journal of Physiology 228, 556-564.

JOHNSON, W.C. and HUNTER, A.G. (1970)
Immunofluorescent changes associated with maturation of rabbit sperm (Abstract).
Proceedings of the Society for the Study of Reproduction 3, 8-15.

JONES, R. (1973)
Epididymal function in the vasectomized rabbit.
Journal of Reproduction and Fertility 36, 199-202.

KAR, A.B., CHANDRA, H. and KAMBOJ, V.P. (1965)
Long-term effect of vasectomy on the gonad-pituitary system of rats.
Acta Biologica et Medica Germanica 15, 381-385.

KENNEDY, S.W. and HEIDGER, J.R. (1980)
Fine structure of the spermatic granuloma of the rat vas deferens following vasectomy.
Anatomical Record 198, 461-474.

KENNEDY, S.W. and SIMON, B.W. (1974)
Spermatic granuloma in vasectomized rats.
Texas Reports on Biology and Medicine 32, 857-861.

KOTHARI, L.K. and MISHRA, P. (1973)
Histochemical changes in the testis and epididymis after vasectomy.
International Journal of Fertility 18, 119-125.

KRETH, H.W. (1965)
Uber die hellen zellen in ductus epididymidis der ratte.
Zeitschrift fur Zellforschung und Mikroskopische Anatomie (Berlin) 68, 28-42.

KUBOTA, R. (1969)
Electron microscopic studies after vasectomy in rats and men.
Japanese Journal of Urology 60, 373-397.

KUWAHARA, M. and FRICK, J. (1975)
The ligation of the male reproductive organs and the role of the spermatic cyst.
Andrologia 7, 1-14.

KUWAHARA, M. (1976)
The effects of ligation of vas deferens, corpus epididymidis and vasa efferentia on the testicular function in rats.
Tohoku Journal of Experimental Medicine 120, 251-257.

KWART, A.M. and COFFEY, D.S. (1973)
Sperm granulomas: an adverse effect of vasectomy.
Journal of Urology 110, 416-422.

LASLEY, J.E. and BOGART, R.A. (1944)
Some factors affecting the resistance of ejaculated and epididymal spermatozoa of the boar to different environmental conditions.
American Journal of Physiology 141, 619-624.

LAUMAS, K.R. and UNIYAL, J.P. (1967)
Disintegration of spermatozoa and testicular atrophy by a silastic block of vas deferens in rats.
Indian Journal of Experimental Biology 5, 199-202.

LEE, H.Y. (1967)
Studies on vasectomy. IV. Experimental studies on biological, histologic and histochemical effects of vasectomy.
Journal of the Korean Medical Association 10, 893-899.

LEVINE, N. and MARSH, D.J. (1971)
Micropuncture studies of the electrochemical aspects of fluid and electrolyte transport in individual seminiferous tubules, the epididymis and the vas deferens in rats.
Journal of Physiology 213, 557-570.

LINNET, L. and HJORT, T. (1977)
Sperm agglutinins in seminal plasma and serum after vasectomy: Correlation between immunological and clinical findings.
Clinical and Experimental Immunology 30, 413-420.

LINO, B.F., BRADEN, A.W. and TURNBULL, K.E. (1967)
Fate of unejaculated spermatozoa.
Nature 213, 594-595.

LINO, B.F. and BRADEN, A.W. (1972)
The output of spermatozoa in rams. I. Relationship with testicular output of spermatozoa and the effect of ejaculations.
Australian Journal of Biological Sciences 25, 351-358.

LOHIYA, N.K., DIXIT, V.P. and ARYA, M. (1976)
Long-term effects of vasectomy on the pituitary-gonadal system of rat and mouse.
Endokrinologie 67, 152-159.

LOPES, C.M. and HAYASHI, H. (1981)
Fertility of unilaterally vasectomized rats and ultrastructure of sperm granuloma.
Revista Brasileira De Biologia 41, 867-871.

LYONS, R.C., PETRE, J.H. and LEE, C.N. (1967)
Spermatic granuloma of the epididymis.
Journal of Urology 97, 320-323.

MacDOUGALL, M.K., McCOWIN, K., DERRICK, F.C., GLOVER, W.L. and JACOBSON, C.B. (1975)
The effects of vasectomy on spermatogenesis in the dog, canis familiaris: a meiotic analysis.
Fertility and Sterility 26, 786-790.

MACMILLAN, E.W. (1957)
The caput epididymidis and trypan blue.
Journal of Anatomy 91, 616 (Abstract).

MACMILLAN, K.L., DESJARDINS, C., KIRTON, K.T. and HAFS, H.D. (1968)
Gonadal and extragonadal sperm reserves after unilateral vasoligation in rabbits.
Fertility and Sterility 19, 982-990.

MANCINI, R.E. (1976)
Immunologic aspects of testicular function
edited by F. Gross, Heidelberg, M.M. Crumbach, San Francisco, A. Labhart, Zurich, M.B. Lipestt, Cleveland. T. Mann, Cambridge. L.T. Samuels, Salt Lake City. J. Zander, Munchen.

MANEELY, R.B. (1959)
Epididymal structure and function: a historical and critical review.
Acta Zoologica 40, 1-21.

MARQUIS, N.R. and FRITZ, I.B. (1965)
Effects of testosterone on the distribution of carnitine, acetylcarnitine and carnitine acetyltransferase in tissues of the reproductive system of the male rat.
Journal of Biological Chemistry 240, 2197-2200.

MARTAN, J. and RISLEY, P.L. (1963)
Holocrine secretory cells of the rat epididymis.
Anatomical Record 146, 173-190.

MARTAN, J. and ALLEN, J.M. (1964)
Morphological and cytochemical properties of the holocrine cells in the epididymis of mouse.
Journal of Histochemistry and Cytochemistry 12, 628-639.

MARTAN, J., RISLEY, P.L. and HRUBAN, Z. (1964)
Holocrine cells of the human epididymis.
Fertility and Sterility 15, 180-187.

MARTAN, J. (1966)
Occurrence of the intact spermatozoa in spontaneous ejaculates of isolated male guinea pigs.
Transactions of the Illinois State Academy of Science 59, 78-80.

MARTAN, J. (1969)
Epididymal histochemistry and physiology.
Biology of Reproduction 1, 134-154.

MASON, K.E. and SHAVER, S.L. (1952)
Some functions of the caput epididymis.
Annals of the New York Academy of Sciences 55, 585-593.

McDONALD, S.W. and SCOTHORNE, R.J. (1986)
On the response of the regional testicular lymph nodes after unilateral vasectomy in rats.
Journal of Anatomy 144, 49-59.

McDONALD, S.W. and SCOTHORNE, R.J. (1987)
On the mode of sperm autoantigen presentation to the regional lymph node of the testis after vasectomy in rats.
Journal of Anatomy (in Press).

McGLYNN, J.M. and ERPINO, M.J. (1974)
Effects of vasectomy on the reproductive system and sexual behaviour of rats.
Journal of Reproduction and Fertility 40, 241-247.

MILNER, A.J. and HAMILTON, D.W. (1971)
Ultrastructural criteria for assessing the functional integrity of endocrine cells in vitro.
Acta Endocrinologica Supplementum 153, 62-80.

MOCK, E.J., KAMEL, F., WRIGHT, W.W. and FRANKEL, A.I. (1975)
Plasma testosterone levels in vasectomized rats.
Journal of Reproduction and Fertility 44, 575-578.

MOORE, C.R. and QUICK, W.J. (1924)
Properties of the gonads as controllers of somatic and physical characteristics. VII. Vasectomy in the rabbit.
American Journal of Anatomy 34, 317-336.

MOORE, C.R. (1939)
Biology of the testes. In: "Sex and Internal Secretions" edited by E. Allen, C.H. Danforth and E.A. Doisey.
Baltimore, pp.353-451.

MOORE, H.D. and BEDFORD, J.M. (1979)
The differential absorptive activity of epithelial cells of the rat epididymis before and after castration.
Anatomical Record 193, 313-328.

MUKHERJEE, D.P. and BHATTACHARAYA, P. (1949)
Study of the spermatozoa from different levels of the male reproductive tract of the sheep, goat and buffalo.
Proceedings of the Zoological Society 2, 149-156.

MULLANEY, J. (1962)
Experimental production of spermatic granuloma in rats.
Nature 194, 487-488.

MYERS, B.D. (1916)
Histological changes in testes following vasectomy.
Anatomical Record 10, 228-229.

NEAVES, W.B. (1973)
Permeability of Sertoli tight junctions to Lanthanum after ligation of ductus deferens and ductuli efferentes.
Journal of Cell Biology 59, 559-572.

NEAVES, W.B. (1974)
The rat testis after vasectomy.
Journal of Reproduction and Fertility 40, 39-44.

NEAVES, W.B. (1975a)
Biological aspects of vasectomy. In: "Handbook of Physiology", Section 7 "Endocrinology" Volume V "Male Reproductive System"
American Physiological Society, Washington D.C.
pp.383-404.

NEAVES, W.B. (1975b)
The androgen status of vasectomized rats.
Endocrinology 96, 529-534.

NEAVES, W.B. (1978)
The effect of vasectomy on the testis of inbred Lewis rats.
Journal of Reproduction and Fertility 54, 405-411.

NEUTRA, M. and LEBLOND, C.P. (1966)
Radioautographic comparison of the uptake of galactose-^{3}H and glucose-^{3}H in the Golgi region of various cells secreting glycoproteins or mucopolysaccharides.
Journal of Cell Biology 30, 137-150.

NICANDER, L. (1957)
On the regional histology and cytochemistry of the ductus epididymis in rabbits.
Acta Morphologica Neerlando-Scandinavica 1, 99-118.

NICANDER, L. (1958)
Studies of the regional histology and cytochemistry of the ductus epididymis in stallions, rams and bulls.
Acta Morphologica Neerlando-Scandinavica 1, 337-362.

NICANDER, L. (1963)
Resorption phenomena in the epididymis.
International Journal of Fertility 8, 866-887.

NICANDER, L. (1965)
An electron microscopical study of absorbing cells in the posterior caput epididymidis of rabbits. Zeitschrift fur Zellforschung und Mikroskopische Anatomie (Berlin) 66, 829-847.

NICANDER, L., PALSSON, S. and SELANDER, U. (1965)
An electron microscopical study of iron absorption in the epididymal tail of rabbits. Scandinavian Congress on Cell Research, 4th, 1965, pp.51-52.

NISHIKAWA, Y. and WAIDE, Y. (1952)
Studies on the maturation of spermatozoa. 1. Mechanisms and speed of transition of spermatozoa in the epididymis and their functional changes. Bulletin of the National Institute of Agricultural Sciences, Tokyo 3, 68-74.

OBERNDORFER, S. (1931)
Die inneren mannlichen geschlechtsorgane In: "Handbuch der Speziellen Pathologischen Anatomie and Histologie". edited by F. Henke and O. Lubarsch. Berlin; Julius Springer, Vol. 6, pp.427-865.

ORBACH, J. (1961)
Spontaneous ejaculation in the rat. Science 134, 1072-1073.

ORGEBIN-CRIST, M.C. (1967)
Sperm maturation in rabbit epididymis. Nature (London) 216, 816-818.

ORGEBIN-CRIST, M.C. (1969)
Studies on the function of the epididymis. Biology of Reproduction Supplement 1, 155-175.

ORSOS, F. (1941)
Die spermainvasion. Virchows Archiv fur Pathologische Anatomie und Physiologie und fur Klinische Medizin 307, 352-361.

OSLUND, R.M. (1924)
A study of vasectomy on rats and guinea pigs. American Journal of Physiology 67, 422-443.

OSLUND, R.M. (1928)
The physiology of the male reproductive system. Journal of the American Medical Association 90, 829-830.

PARDANANI, D.S., PATIL, N.G. and PAWAR, H.N. (1976)
Some gross observations of the epididymides following vasectomy: A clinical study.
Fertility and Sterility 27, 267-270.

PAUFLER, S.K. and FOOTE, R.H. (1969)
Spermatogenesis in the rabbit following ligation of the epididymis at different levels.
Anatomical Record 164, 339-345.

PEARSON, O.J. and TUBBS, P.K. (1967)
Carnitine and derivatives in rat tissues.
Biochemical Journal 105, 953-963.

PEYRE, A. and LAPORTE, P. (1966)
Evolution des acides sialiques epididymaires chez ie rat impubere et adulte.
Comptes Rendus Hebdomadaires des Seances de I'Academie des Sciences 263, 1872-1875.

PHADKE, A.M. and PHADKE, G.M. (1961)
Occurrence of macrophages cells in the semen and in the epididymis in cases of male infertility.
Journal of Reproduction and Fertility 2, 400-404.

PHADKE, A.M. (1964)
Fate of spermatozoa in cases of obstructive Azoospermia and after ligation of vas deferens in man.
Journal of Reproduction and Fertility 7, 1-12.

PHADKE, A.M. and PADUKONE, K. (1964)
Presence and significance of autoantibodies against spermatozoa in the blood of men with obstructed vas deferens.
Journal of Reproduction and Fertility 7, 163-170.

PIEKUT, D.T. and MOREHEAD, J.R. (1971)
Radioautographic study of the "holocrine cell cycle" in the rat epididymis. (Abstract)
Anatomical Record 169, 402.

PLAUT, S.M. (1973)
Testicular morphology in rats vasectomized as adults.
Science 181, 554-555.

POYNTER, H. (1939)
Testis hormone secretion in the rat under conditions of vasectomy or isolation.
Anatomical Record 74, 355-379.

PRESS, E.M. and PORTER, R.R. (1966)
Immunoglobulins. In: "Glycoproteins", edited by A. Gottschalk. Amsterdam: Elsevier pp.395-412.

RAJALAKSHMI, M. and PRASAD, M.R. (1969)
Changes in sialic acid in the Testis and Epididymis of the rat during the onset of puberty.
Journal of Endocrinology 44, 379-385.

RAMBOURG, A., HERNANDEZ, W. and LEBLOND, C.P. (1969)
Detection of complex carbohydrates in the Golgi apparatus of rat cells.
Journal of Cell Biology 40, 395-414.

RAO, C.K. and BERRY, R.O. (1949)
The cytoplasmic drop and the cytoplasmic cap in the development of boar spermatozoa.
American Journal of Veterinary Research 10, 357-361.

REICHEL, H. (1921)
Die saisonfunktion des nebenhodens vom maulwurf.
Anatomischer Anzeiger 54, 129-135.

REID, B.L. and CLELAND, K.W. (1957)
The structure and function of the epididymis. 1. The histology of the rat epididymis.
Australian Journal of Zoology 5, 223-246.

REIGER, I.T. and FULLER, R.H. (1953)
Spermatic granuloma of epididymis: Case report.
Journal of Urology 69, 819.

REINKE, L.A. and STOHS, S.J. (1977)
Lack of effect of vasectomy on hepatic mixed function mono-oxygenase enzymes in the rat.
Contraception 15, 535-542.

REYNOLDS, E.S. (1963)
The use of lead citrate at high pH as an electron-opaque stain in electron microscopy.
Journal of Cell Biology 17, 208-212.

ROBBINS, S.L. (1974)
Pathologic Basis of Disease.
edited by W.B. Saunders Co., Philadelphia, pp.55-88.

ROUSSEL, J.D., STALLCUP, O.T. and AUSTIN, C.R. (1967)
Selective phagocytosis of spermatozoa in the epididymis of bulls, rabbits and monkey.
Fertility and Sterility 18, 509-516.

RUMKE, P. (1954)
The presence of sperm antibodies in the serum of two patients with oligozoospermia.
Vox Sanguinis 4, 135-140.

RUMKE, P. (1959)
Auto-antibodies against spermatozoa in sterile men. In: "Immunopathology". 1st International Symposium, Base 1/Seelisberg, 1958. edited by P. Grabar and P. Miesch, Benno.

RUMKE, P. and HELLINGA, G. (1959)
Autoantibodies against spermatozoa in sterile men. American Journal of Clinical Pathology 32, 357-363.

RUMKE, P. (1968)
Sperm-agglutinating autoantibodies in relation to male infertility.
Proceedings of the Royal Society of Medicine 61, 275-278.

RUMKE, P. and TITUS, M. (1970)
Spermagglutinin formation in male rats by subcutaneously injected syngeneic epididymal spermatozoa and by vasoligation or vasectomy.
Journal of Reproduction and Fertility 21, 69-79.

RUSSELL, M. and FRIEDMAN, N.B. (1951)
Studies in general biology of sperm: Experimental production of spermatic granuloma.
Journal of Urology 65, 650-654.

SACKLER, A.M., WELTMAN, A.S., PANDHI, V. and SCHWARTZ, R. (1973)
Gonadal effects of vasectomy and vasoligation.
Science 179, 293-295.

SADI, A., CHIORBOLI, E. and SAAD, F. (1967)
Vasectomia em ratos.
Hospital (Rio de Janeiro) 72, 267-272.

SAMUEL, T., KOLK, A.H.J., RUMKE, P. and VAN LIS, J.M.J. (1975)
Autoimmunity to sperm antigens in vasectomized men.
Clinical and Experimental Immunology 21, 65-74.

SCHMIDT, S.S. (1966)
Technics and complications of elective vasectomy: The role of spermatic granuloma in spontaneous recanalization.
Fertility and Sterility 17, 467-482.

SCHMIDT, S.S. (1973)
Prevention of failure in vasectomy.
Journal of Urology 109, 296-297.

SCHMIDT, S.S. and MORRIS, R.R. (1973)
Spermatic granuloma: The complication of vasectomy.
Fertility and Sterility 24, 941-947.

SCOTT, T.W., DAWSON, R.M. and ROWLANDS, I.W. (1963)
Phospholipid interrelationships in rat epididymal tissue and spermatozoa.
Biochemical Journal 87, 507-512.

SCOTT, T.W., WALES, R.G., WALLACE, J.C. and WHITE, I.G. (1963).
Composition of ram epididymal and testicular fluid and the biosynthesis of glycerylphosphorylcholine by the rabbit epididymis.
Journal of Reproduction and Fertility 6, 49-59.

SEDAR, A.W. (1966)
Transport of exogenous peroxidase across the epithelium of the ductuli efferentes (Abstract).
Journal of Cell Biology 31, 102A.

SEGAL, R.J. (1972)
Effect of vasoligation of 14-day-old and 21-day-old rats on spermatogenesis.
International Journal of Fertility 17, 33-34.

SHAVER, S.L. (1954)
The role of stereocilia in removing India ink particles from the lumen of the rat epididymis.
Anatomical Record 119, 177-185.

SHULMAN, S., ZAPPI, E., AHMED, U. and DAVIS, J.E. (1972)
Immunologic consequences of vasectomy.
Contraception 5, 269-278.

SHULMAN, S. (1974)
In Advances in Voluntary Sterilization:
Proceedings of the 2nd International Conference, edited by M.E. Schima, I. Lubell, J.E. Davis, E. Connell and D.W.K. Cotton. Excerpta Medica, Amsterdam. pp.86-91.

SILBER, S.J. (1977)
Sperm granuloma and reversibility of vasectomy.
Lancet 2, 588-589.

SILBER, S.J. (1979)
Epididymal extravasation following vasectomy as a cause for failure of vasectomy reversal.
Fertility and Sterility 31, 309-315.

SIMEONE, F.A. and YOUNG, W.C. (1931)
A study of the function of the epididymis.
IV. The fate of non-ejaculated spermatozoa in the genital tract of the male guinea pig.
Journal of Experimental Biology 8, 163-175.

SIMMONDS, M. (1921)
Uber das verhalten des menschlichen hodens bei narbigem verschluss des samenleiters.
Verhandlungen der Deutschen Pathologischen Gesellschaft 18, 201-203.

SMITH, G. (1962)
The effects of ligation of the vasa efferentia and vasectomy on testicular function in the adult rat.
Journal of Endocrinology 23, 385-399.

STEINACH, E. (1920)
Verjungung durch experimentelle neubelebung der alternden pubertatsdruse.
Archiv Entwicklungsmechanik Organismen 46, 557-619.

STEINBERG, J. and STRAUS, R. (1947)
Sperm invasion of the epididymis.
Journal of Urology 57, 498-503.

SUN, E.L. and FLICKINGER, C.J. (1980)
Morphological characteristic of cells with apical nuclei in the initial segment of the adult rat epididymis.
Anatomical Record 196, 285-293.

SUNDARASIVARAO, D. (1955)
Spermatozoal granuloma of the epididymis.
Journal of Pathology and Bacteriology 69, 324-326.

SWANSON, L.V. and HAFS, H.D. (1969)
Testicular and epididymal sperm numbers of unilaterally vasoligated rabbits.
Proceedings of the Society for Experimental Biology and Medicine 131, 763-768.

THAKUR, A.N., SHETH, A.R. and RAO, S.S. (1972)
Biochemical studies on rat testes and sex accessory organs after vasoligation operation.
Fertility and Sterility 23, 834-837.

TINGARI, M.D. (1971)
On the structure of the epididymal region and ductus deferens of the domestic fowl (Gallus domesticus).
Journal of Anatomy 109, 423-435.

TINGARI, M.D. and LAKE, P.E. (1972)
Ultrastructural evidence for resorption of spermatozoa and testicular fluid in the excurrent ducts of the testis of the domestic fowl, Gallus domesticus.
Journal of Reproduction and Fertility 31, 373-381.

TOOTHILL, M.C. and YOUNG, W.C. (1931)
The time consumed by spermatozoa in passing through the epididymis of the guinea-pig determined by India ink injections.
Anatomical Record 50, 95-107.

TUNG, K.S.K. (1974)
Immunopathologic responses following vasectomy.
Federation Proceedings 33, 814.

TUNG, K.S.K. (1975)
Human sperm antigens and antisperm antibodies.
I. Studies on vasectomy patients.
Clinical and Experimental Immunology 20, 93-104.

TUNG, K.S.K. (1976)
Human sperm antigens and antisperm antibodies.
III. Studies on acrosomal antigens.
Clinical and Experimental Immunology 24, 292-299.

TYLER, J.P.P., RICHARDSON, D.W. and NEWTON, J.R. (1979)
The hormonal and immunological status of vasectomized men.
Contraception 19, 599-611.

URENA, F. and MALAVASI, J. (1981)
Estudio ultraestructural en epididimo de hamster vasectomizado (Mesocricetus auratus).
Revista De Biologia Tropical 29, 237-249.

VOGLMAYR, J.K., WAITES, G.M. and SETCHELL, B.P. (1966)
Studies on spermatozoa and fluid collected directly from the testis of the conscious ram.
Nature 210, 861-863.

VOGLMAYR, J.K., SCOTT, T.W., SETCHELL, B.P. and WAITES, G.M. (1967)
Metabolism of testicular spermatozoa and characteristics of testicular fluid collected from conscious rams.
Journal of Reproduction and Fertility 14, 87-99.

VOGLMAYR, J.K. (1975)
Suppression of sperm granulomas in vasectomized rats by local heating of the testis.
Biology of Reproduction 13, 453-460.

WAGENSEIL, F. (1928)
Experimentaluntersuchungen am nebenhoden der maus.
Zeltschrift fur Zellforschung und Mikroskopische Anatomie 7, 141-176.

WIERZBOWSKI, S. and WIERZCHOA, E. (1969)
Cannulation of the vas deferens in the boar.
Journal of Reproduction and Fertility 19, 173-175.

WILHELM, S.F. (1935)
Observation on the emptying of the vasa deferentia and seminal vesicles.
Journal of Urology 34, 284-287.

WILHELM, S.F. and SELIGMANN, A.W. (1937)
Spermatozoa in urine.
American Journal of Surgery 35, 872-873.

WILSON, K. (1954)
Spermagglutinins in human semen and blood.
Proceedings of the Society for Experimental Biology and Medicine 85, 652-655.

WILSON, L. (1956)
Sperm agglutinins due to autoantibodies.
Fertility and Sterility 7, 262-267.

WISE, G.E. and FLICKINGER, C.J. (1970)
Cytochemical staining of the Golgi apparatus in Amoeba proteus.
Journal of Cell Biology 46, 620-626.

YOUNG, W.C. (1929)
A study of the functions of the epididymis.
II. The importance of the ageing process in spermatozoa.
Journal of Morphology 48, 475-491.

YOUNG, W.C. (1931)
A study of the function of the epididymis.
III. Functional changes undergone by spermatozoa during their passage through the epididymis and vas deferens in the guinea pig.
Journal of Experimental Biology 8, 151-162.

YOUNG, W.C. (1933)
Die resorption in den ductuli efferentes der maus und ihre bedeutung fur das problem der unterbindung in hoden nebenhoden system.
Zeitschrift fur Zellforschung und Mikroskopische Anatomie (Berlin) 17, 729-759.

ZAPPI, E., AHMED, U., DAVIS, J. and SHULMAN, S. (1970)
Immunologic consequences of vasectomy.
Federation Proceedings 29, 728.

MORPHOLOGICAL STUDIES OF THE IMMUNE RESPONSE TO VASECTOMY

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VOLUME II: TABLES AND PHOTOGRAPHS

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May, 1987

Table 1: The mean number and S.D. of the intraepithelial leucocytes per unit length of the perimeter of sectional profiles of duct of epididymis at seven postoperative intervals, after vasectomy or sham-operation.

Time after operation	Vasectomy		Sham-operation		
	Mean	S.D.	Mean	S.D.	
6 wks.	0.087	± 0.008	0.087	± 0.004	n.s.
4 mos.	0.098	± 0.007	0.102	± 0.003	n.s.
6 mos.	0.108	± 0.025	0.095	± 0.004	n.s.
9 mos.	0.094	± 0.003	0.091	± 0.01	n.s.
12 mos.	0.099	± 0.005	0.101	± 0.005	n.s.
15 mos.	0.099	± 0.006	0.095	± 0.003	n.s.
18 mos.	0.092	± 0.078	0.099	± 0.004	n.s.
	F = 0.98 n.s.		F = 6.08 $p < 0.01$*		

*Figures are significantly less at 6 wks. than at
4 mos $p < 0.01$
6 mos. $p < 0.05$
12 mos. $p < 0.01$
15 mos. $p < 0.05$
18 mos. $p < 0.01$

Table 2: Presence and site of the spermatic granuloma; size, shape and length of the vasal granuloma; and condition of the testes.

Time after vasectomy	Rat Serial No.	Vasal Granuloma	Weight of Vasal Granuloma (mgm)	Means & SD of the weight of vasal granuloma	Shape of Vasal Granuloma	Length of Vasal Granuloma (mm)	Site of granuloma in epididymis	Right Testis	Left Testis
6 weeks	V_1	+	435	165 ± 152	Spherical	12.5	None	Normal	Normal
	V_2	+	106		Cylindrical Narrow	13.0	None	Normal	Normal
	V_3	+	126		Thin Irregular	10.0	None	Normal	Normal
	V_4	+	97		Thin Irregular	11.5	None	Normal	Normal
	V_5	+	63		Cylindrical Narrow	8.0	Cauda and corpus	Normal	Normal
4 months	V_6	+	327	279 ± 99	Spherical	11.0	None	Normal	Normal
	V_7	+	211		Spherical	14.0	None	Normal	Normal
	V_8	+	355		Oval	16.0	None	Normal	Normal
	V_9	+	139		Spherical	11.0	Cauda and corpus	Normal	Normal
	V_{10}	+	361		Oval	15.0	None	Normal	Normal

Table 2 (contd.)

Time after vasectomy	Rat Serial No.	Vasal Granuloma	Weight of Vasal Granuloma (mgm)	Means & SD of the weight of vasal granuloma	Shape of Vasal Granuloma	Length of Vasal Granuloma (mm)	Site of granuloma in epididymis	Right Testis	Left Testis
6 months	V_{11}	+	37	306 ± 201	Cylindrical Narrow	11.0	Cauda,corpus and caput	Normal	Atrophic
	V_{12}	+	338		Oval	20.0	None	Normal	Normal
	V_{13}	+	526		Oval	17.0	None	Normal	Normal
	V_{14}	+	324		Spherical	14.0	None	Normal	Normal
9 months	V_{15}	+	619	329 ± 281	Oval	23.0	None	Normal	Normal
	V_{16}	+	595		Oval	21.0	None	Normal	Normal
	V_{17}	+	332		Oval	19.0	None	Atrophic	Atrophic
	V_{18}	+	-		Pinhead	-	Cauda,corpus and caput	Normal	Atrophic
	V_{19}	+	99		Thin Irregular	12.0	Cauda,corpus and caput	Normal	Atrophic
12 months	V_{20}	-	-	221 ± 391	-	-	Cauda and corpus	Atrophic	Atrophic
	V_{21}	+	-		Pinhead	-	Cauda and corpus	Atrophic	Atrophic
	V_{22}	+	904		Oval	24.0	None	Normal	Normal
	V_{23}	+	201		Oval	10.0	Cauda	Normal	Normal
	V_{24}	+	-		Pinhead	-	Cauda	Atrophic	Atrophic

Table 2 (Contd.)

Time after vasectomy	Rat Serial No.	Vasal Granuloma	Weight of Vasal Granuloma (mgm)	Means & SD of the weight of vasal granuloma	Shape of Vasal Granuloma	Length of Vasal Granuloma (mm)	Site of granuloma in epididymis	Right Testis	Left Testis
15 months	V_{25}	+	300	64 ± 120	Oval	13.0	Cauda	Normal	Normal
	V_{26}	-	-		-	-	None	Normal	Atrophic
	V_{27}	+	82		Cylindrical Narrow	9.0	Cauda and caput	Normal	Atrophic
	V_{28}	-	-		-	-	Cauda (large)	Normal	Normal
	V_{29}	-	-		-	-	Cauda (large)	Normal	Normal
	V_{30}	-	-		-	-	Cauda and caput	Normal	Atrophic
18 months	V_{31}	+	991	164 ± 347	Oval	23	None	Normal	Normal
	V_{32}	-	-		-	-	Cauda,corpus and caput	Normal	Atrophic
	V_{33}	-	-		-	-	Cauda (large)	Normal	Normal
	V_{34}	-	-		-	-	Cauda and caput	Normal	Atrophic
	V_{35}	+	47		Narrow Cylindrical	8.0	Cauda,corpus and caput	Normal	Atrophic
	V_{36}	-	-		-	-	None	Normal	Atrophic
	V_{37}	-	-		-	-	Caput	Normal	Atrophic
	V_{38}	+	276		Oval	13.0	Cauda (large)	Normal	Normal

Table 3: Presence and site of the spermatic granuloma, and condition of the testes.

Time after vasectomy	Rat serial no.	Vasal Granuloma	Epididymal Granuloma	Right Testis	Left Testis
One week	V_{39}	+	-	Normal	Normal
	V_{40}	+	-	Normal	Normal
Three weeks	V_{41}	+	-	Normal	Normal
	V_{42}	+	-	Normal	Normal
Six weeks	V_{43}	+	-	Normal	Normal
	V_{44}	+	-	Normal	Normal

Table 4: The mean weights (gms) and S.D. of the spleens of the sham-operated and vasectomized animals calculated at each of the seven postoperative periods.

Time after operation	Sham-operation		Vasectomy		
	Animal Serial No.	Spleen weight (gms)	Animal Serial No.	Spleen weight (gms)	
6 wks.	S1 S2 S3 S4 S5 S6	0.92 1.06 2.06 0.99 0.85 0.95	V1 V2 V3 V4 V5	0.82 1.13 0.92 0.99 0.96	
	Mean and S.D.	1.14 ± 0.45	Mean and S.D.	0.96 ± 0.11	n.s.
4 mos.	S7 S8 S9 S10 S11a	0.92 0.92 0.82 0.95 1.13	V6 V7 V8 V9 V10	0.91 1.19 1.15 0.81 0.94	
	Mean and S.D.	0.95 ± 0.11	Mean and S.D.	1.0 ± 0.16	n.s.
6 mos.	S11b S12 S13 S14	1.21 0.95 1.08 1.11	V11 V12 V13 V14	0.85 1.02 Excluded (Abnormal) 1.11	
	Mean and S.D.	1.09 ± 0.11	Mean and S.D.	0.99 ± 0.13	n.s.
9 mos.	S15 S16 S17 S18	1.20 1.44 1.12 1.33	V15 V16 V17 V18 V19	1.25 1.18 1.36 1.22 1.04	
	Mean and S.D.	1.27 ± 0.14	Mean and S.D.	1.21 ± 0.12	n.s.

Table 4 (Contd.)

Time after operation	Sham-operation		Vasectomy		
	Animal Serial No.	Spleen weight (gms)	Animal Serial No.	Spleen weight (gms)	
12 mos.	S20 S21 S22 S23 S24	1.32 1.30 1.47 1.38 1.40	V20 V21 V22 V23 V24	1.49 1.52 1.41 1.28 1.26	
	Mean and S.D.	1.37 ± 0.06	Mean and S.D.	1.39 ± 0.12	n.s.
15 mos.	S25 S26 S27 S28 S29	1.14 1.51 1.37 1.41 1.27	V25 V26 V27 V28 V29 V30	1.60 1.67 1.48 1.34 1.68 1.28	
	Mean and S.D.	1.34 ± 0.14	Mean and S.D.	1.51 ± 0.17	n.s.
18 mos.	S31 S32 S33 S34	1.04 1.51 2.11 1.33	V31 V32 V33 V34 V35	1.29 1.05 1.13 0.89 1.64	
	Mean and S.D.	1.5 ± 0.45	Mean and S.D.	1.2 ± 0.29	n.s.
	F = 2.25 n.s.		F = 7.93 $p < 0.001$ Figures are significantly greater at 12 mos. than at 6 wks. $p < 0.01$ 4 mos. $p < 0.01$ 6 mos. $p < 0.05$ Figures are significantly greater at 15 mos. than at 6 wks. $p < 0.01$ 4 mos. $p < 0.01$ 6 mos. $p < 0.01$		

Table 5: The means and S.D. of the percentage areas of the white pulp, dense area, marginal zone and red pulp of the spleens of the sham-operated and vasectomized animals, at the seven postoperative periods.

Time after operation	Animal Serial No.	% White Pulp		% Dense Area		% Marginal Zone		% Red Pulp		
		Sham-operation	Vasectomy	Sham-operation	Vasectomy	Sham-operation	Vasectomy	Sham-operation	Vasectomy	
6 wks.	S1, V1	26.1	19.9	14.0	11.5	12.1	8.3	73.9	80.1	
	S2, V2	26.1	24.5	13.2	13.9	12.9	10.6	73.9	75.5	
	S3, V3	15.5	21.3	8.5	11.5	7.0	9.8	84.5	78.6	
	S4, V4	27.1	28.5	15.6	14.4	11.5	14.1	72.9	71.5	
	S5, V5	20.0	22.7	11.2	12.7	8.8	10.0	80.0	77.3	
	S6	26.8	-	14.9	-	11.9	-	73.2	-	
	Mean and S.D.	23.6 ± 4.8	23.4 ± 3.3	12.9 ± 2.6	12.8 ± 1.3	10.7 ± 2.3	10.6 ± 2.2	76.4 ± 4.8	76.6 ± 3.3	n.s.
4 mos.	S7, V6	23.2	19.7	12.1	10.9	11.1	8.7	76.8	80.3	
	S8, V7	24.1	26.2	12.8	15.1	11.2	11.1	75.9	73.8	
	S9, V8	25.2	24.5	13.7	13.8	11.5	10.7	74.8	75.5	
	S10,V9	21.0	22.1	12.8	11.7	8.2	10.4	79.0	77.9	
	S11a,V10	22.4	23.9	11.3	12.2	11.1	11.7	77.6	76.1	
	Mean and S.D.	23.2 ± 1.6	23.3 ± 2.5	12.5 ± 0.9	12.7 ± 1.7	10.6 ± 1.4	10.5 ± 1.1	76.8 ± 1.6	76.7 ± 2.5	n.s.
6 mos.	S11b,V11	29.8	28.0	16.0	14.5	13.8	13.6	70.2	72.0	
	S12,V12	30.0	28.2	15.5	15.3	14.6	12.9	70.0	71.8	
	S13	23.7	-	12.6	-	11.0	-	76.3	-	
	S14,V14	29.0	29.0	15.6	16.2	13.3	12.8	71.0	71.0	
	Mean and S.D.	28.1 ± 3.0	28.4 ± 0.5	14.9 ± 1.6	15.3 ± 0.9	13.2 ± 1.5	13.1 ± 0.4	71.9 ± 3.0	71.6 ± 0.5	n.s.
9 mos.	S15,V15	24.3	25.8	13.6	13.2	10.7	12.6	75.7	74.2	
	S16,V16	19.8	21.3	11.1	11.8	8.7	9.4	80.2	78.7	
	S17,V17	22.0	26.1	11.9	15.2	10.1	10.9	78.0	73.9	
	S18,V18	23.1	24.3	12.7	14.4	10.4	9.9	76.9	75.7	
	V19	-	25.6	-	14.3	-	11.3	-	74.4	
	Mean and S.D.	22.3 ± 1.9	24.6 ± 2.0	12.3 ± 1.1	13.8 ± 1.3	10.0 ± 0.9	10.8 ± 1.3	77.7 ± 1.9	75.4 ± 2.0	n.s.
12 mos.	S20,V20	25.4	21.6	14.8	11.8	10.6	9.7	74.6	78.4	
	S21,V21	22.0	27.8	13.5	16.5	8.4	11.3	78.0	72.2	
	S22,V22	26.7	30.6	15.4	16.9	11.3	13.7	73.3	69.4	
	S23,V23	28.0	23.7	14.6	12.6	13.2	11.1	72.0	76.3	
	S24,V24	29.9	31.3	15.5	18.2	14.1	13.1	70.1	68.7	
	Mean and S.D.	26.4 ± 3.0	27.0 ± 4.2	14.8 ± 0.8	15.2 ± 2.8	11.5 ± 2.2	11.8 ± 1.6	73.6 ± 3.0	73.0 ± 4.2	n.s.

Table 5 (Contd.)

Time after operation	Animal Serial No.	% White Pulp		% Dense Area		% Marginal Zone		% Red Pulp		
		Sham-operation	Vasectomy	Sham-operation	Vasectomy	Sham-operation	Vasectomy	Sham-operation	Vasectomy	
15 mos.	S25,V25 S26,V26 S27,V27 S28,V28 S29,V29 V30	27.0 21.0 26.6 26.1 30.4 -	26.3 22.7 25.7 30.1 27.5 28.0	14.6 12.4 14.7 14.7 17.3 -	15.2 13.1 14.8 16.0 15.0 15.9	12.4 8.5 11.9 11.4 13.1 -	11.1 9.6 10.9 14.1 12.4 12.1	73.0 79.0 73.4 74.0 69.6 -	73.7 77.3 74.3 69.9 72.5 72.0	
	Mean and S.D.	26.2 ± 3.4	26.7 ± 2.5	14.7 ± 1.7	15.0 ± 1.0	11.5 ± 1.8	11.7 ± 1.5	73.8 ± 3.4	73.3 ± 2.5	n.s.
18 mos.	S31,V31 S32,V32 S33,V33 S34,V34 V35	29.8 25.4 26.6 28.7 -	23.6 24.7 29.4 29.5 25.6	15.6 15.0 17.0 16.5 -	12.9 13.6 16.2 17.3 14.5	14.2 10.4 9.6 12.2 -	10.7 11.1 13.3 12.2 11.1	70.2 74.6 73.4 71.3 -	76.4 75.3 70.6 70.5 74.4	
	Mean and S.D.	27.6 ± 2.0	26.6 ± 2.7	16.0 ± 0.9	14.9 ± 1.8	11.6 ± 2.0	11.7 ± 1.1	72.4 ± 2.0	73.4 ± 2.7	n.s.
		F = 2.38 n.s.	F = 2.10 n.s.	F = 3.59 p < 0.01 *Figures are significantly greater at 18 mos. than 6 wks. p< 0.05 4 mos. p< 0.05 9 mos. p< 0.05	F = 2.11 n.s.	F = 1.29 n.s.	F = 1.53 n.s.	F = 2.38 n.s.	F = 2.09 n.s.	

Table 6: The mean number of the sectional profiles of germinal centres per unit sectional areas of spleens of seven postoperative intervals, after sham-operation and vasectomy.

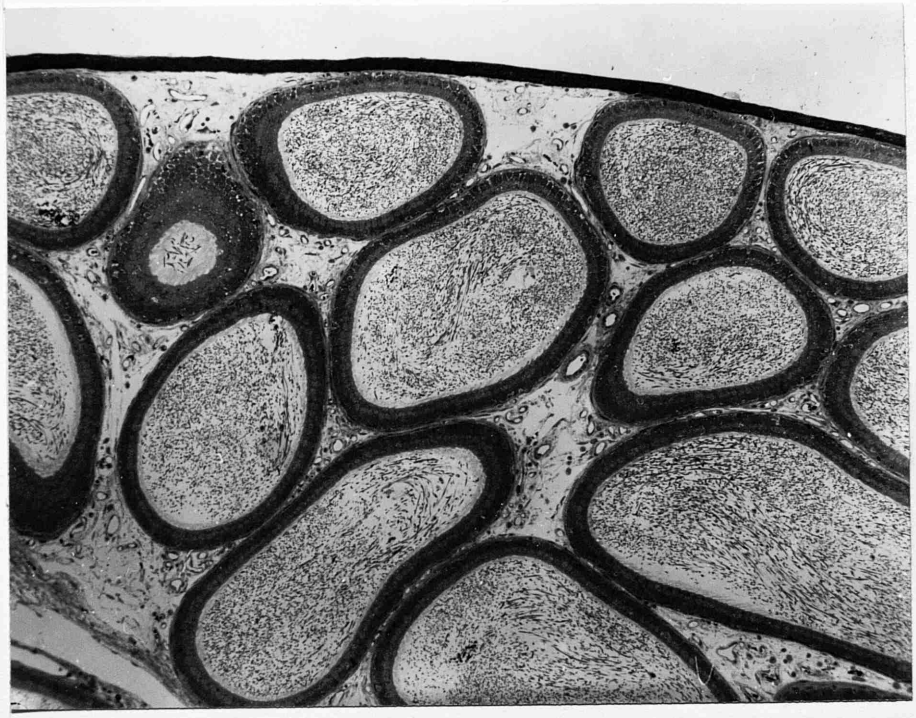
Time after operation	Vasectomy		Sham-operation		
	mean $\times 10^7$	S.D.	mean	S.D.	
6 wks.	1750	± 486	1834	± 455	n.s.
4 mos.	1381	± 235	1443	± 112	n.s.
6 mos.	1618	± 587	1911	± 457	n.s.
9 mos.	1475	± 334	1602	± 331	n.s.
12 mos.	1968	± 1031	1545	± 319	n.s.
15 mos.	1680	± 1496	1927	± 1202	n.s.
18 mos.	696	± 496	613	± 442	n.s.
	F = 1.76 n.s.		F = 2.51 $p < 0.05$ Germinal centre profiles are <u>less</u> numerous at 18 mos. than at 6 wks. $p < 0.05$ 6 mos. $p < 0.05$ 15 mos. $p < 0.05$		

Fig. 1: Low power light micrograph of caput epididymidis of a rat, four months after sham operation. The lumens of the tubular profiles are filled with sperm. Loose connective tissue occupied the intertubular spaces. The outer perimeter of the caput is limited by a fibrous capsule.

(Sham 7 x 90)

Fig. 2: Medium power light micrograph of the caput epididymidis of a rat, four months after sham operation, illustrating its general morphological features.

(Sham 7 x 162.5)



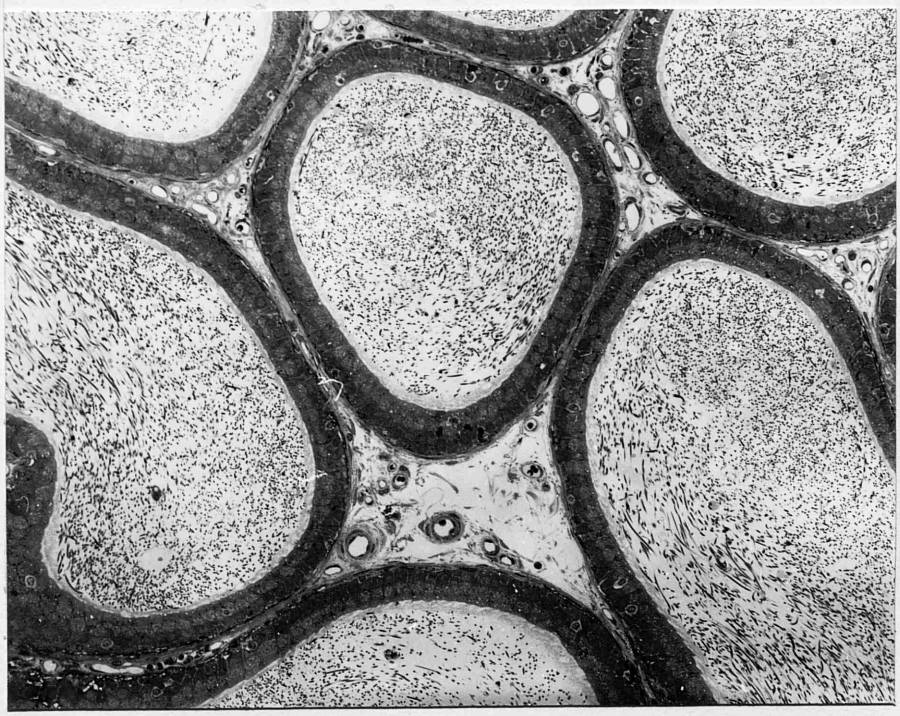
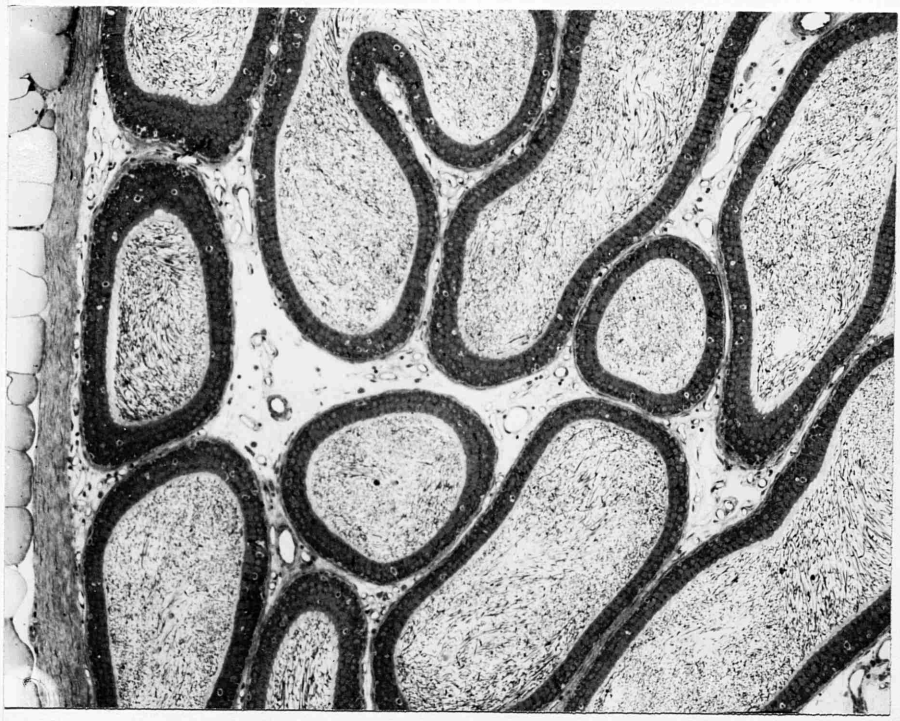


Fig. 3: Low power light micrograph of caput epididymidis four months after vasectomy.

(Vas 6 x 90)

Fig. 4: Medium power light micrograph of caput epididymidis, four months after vasectomy.

(Vas 6 x 162.5)



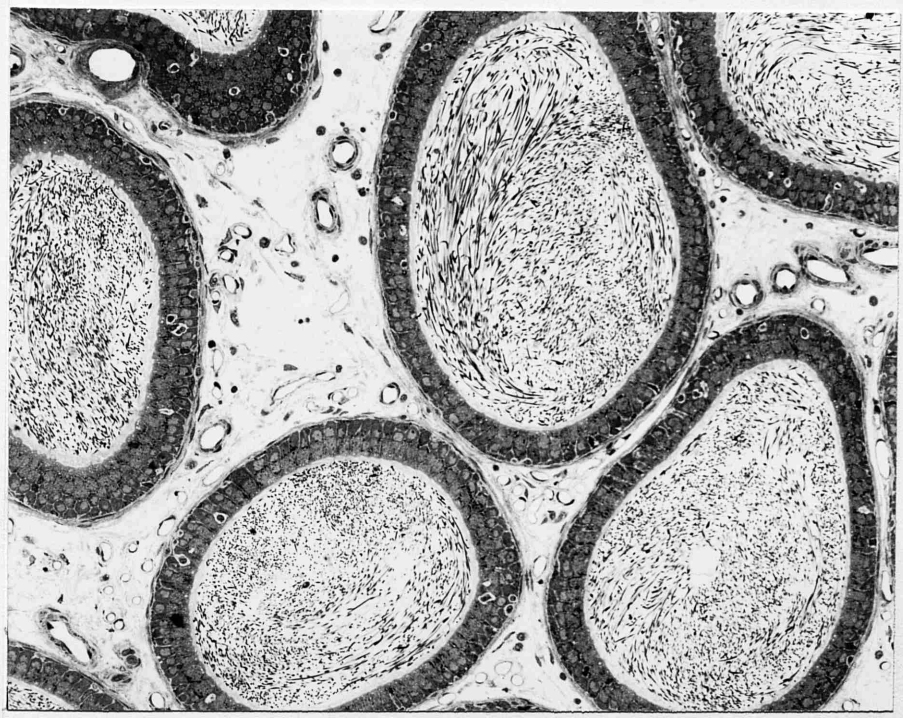
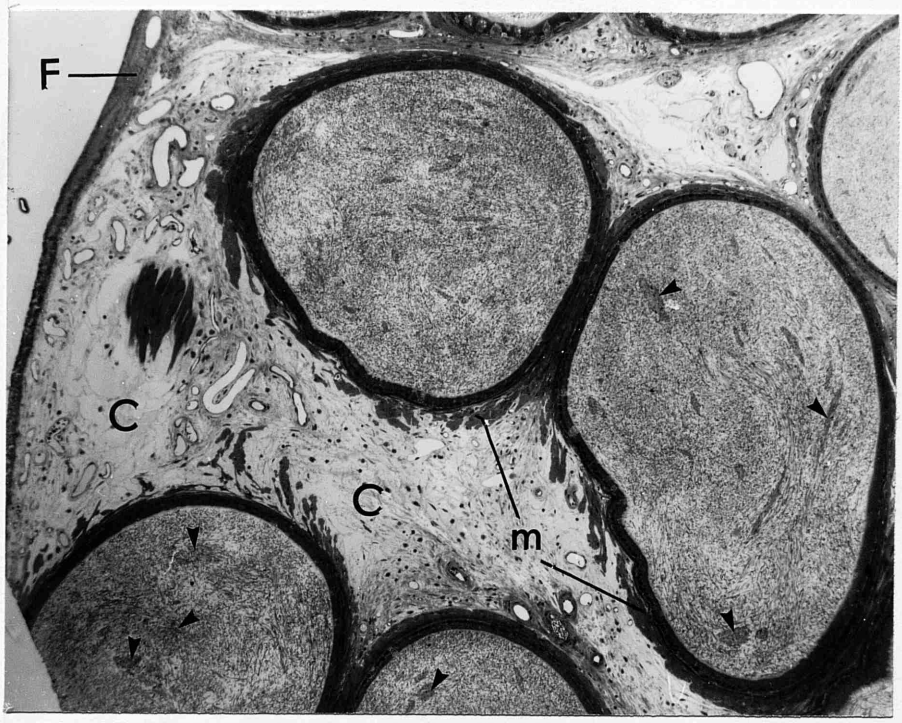


Fig. 5: Low power light micrograph of the cauda epididymidis four months after sham operation. The lumens of the tubular profiles are wider and contain more sperm than those in the caput. Several clumps of sperm (arrows) are seen scattered within the lumen. Note the prominent muscular layer surrounding the tubules (m), the inter-tubular connective tissue (C), and the outer fibrous capsule (F).

(Sham 11a x 65)

Fig. 6: Low power micrograph of the cauda epididymidis four months after vasectomy.

(Vas 6 x 65)


F
C
C
m

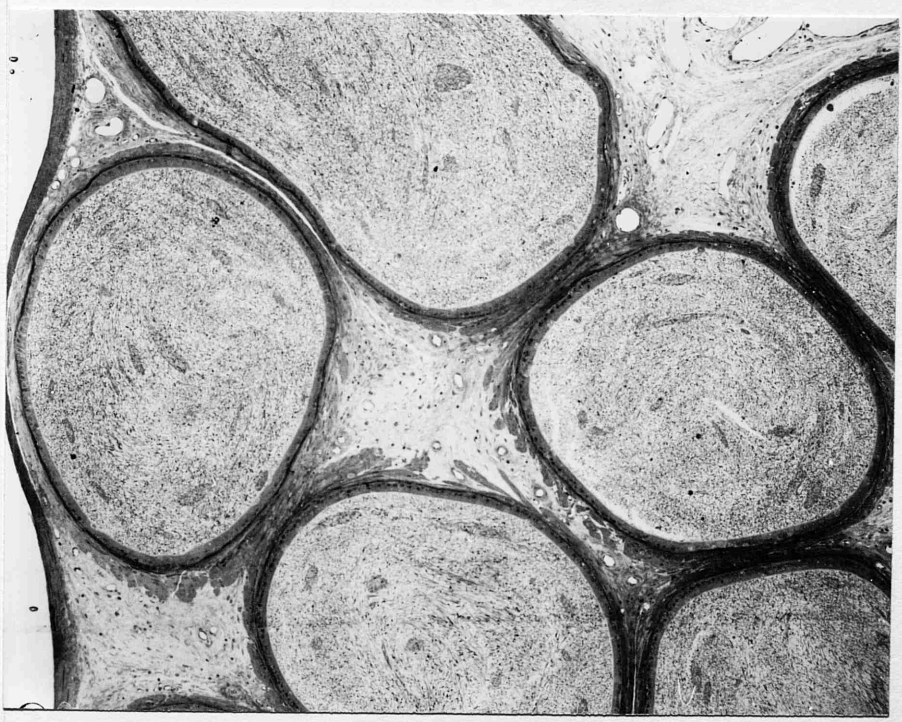
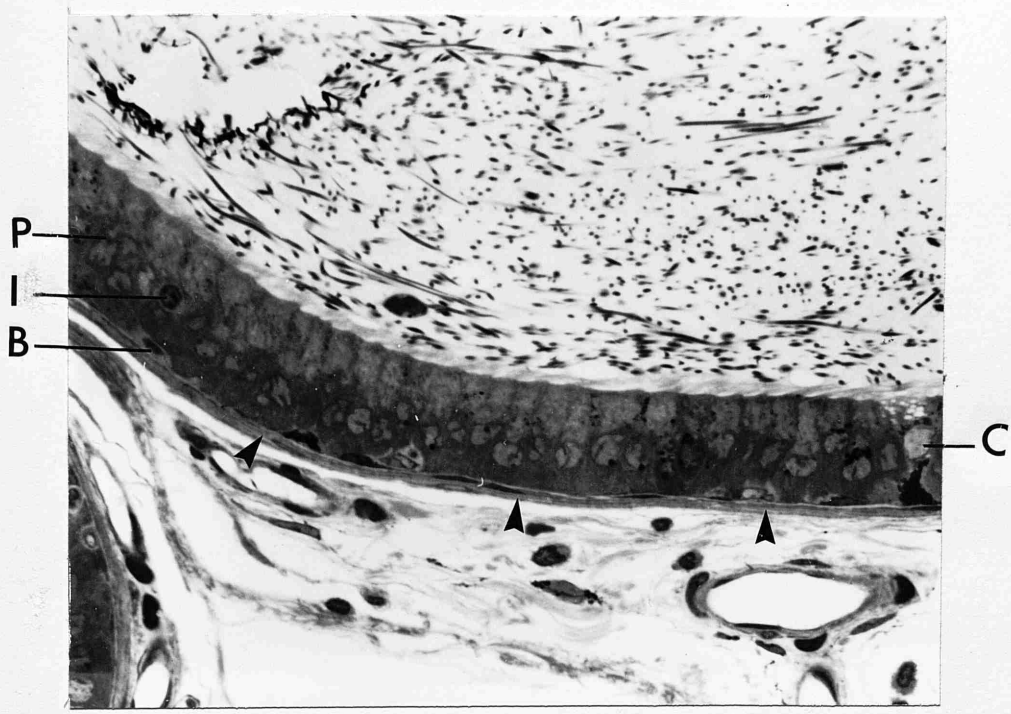


Fig. 7: Light micrograph of the caput epididymidis in a sham-operated rat. The cell population in the epithelium comprises principal cells (P), basal cells (B), and intraepithelial leucocytes (I), with an occasional clear cell (C). Note the thin muscular layer (arrows) outside the basement membrane.

(Sham 2 x 500)

Fig. 8: Light micrograph of the cauda epididymidis in a sham-operated rat. The epithelial cells are less tall than those in the caput, the clear cells (C) are more abundant and the muscular coat (arrows) appear more prominent. Note the blood capillaries (V), between the basement membrane and the muscular layer.

(Sham 1 x 500)


P
I
B
C

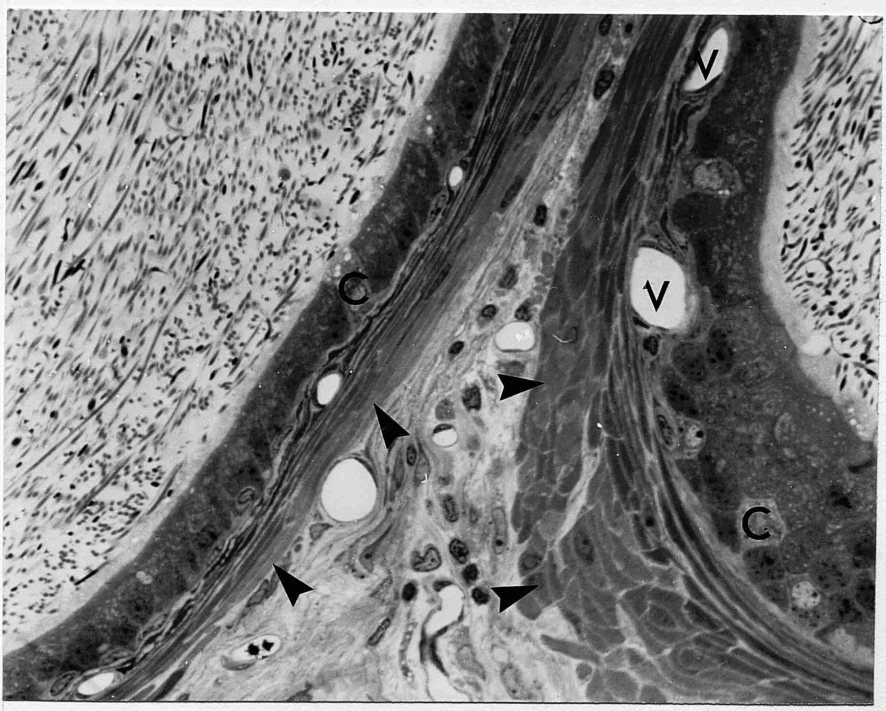
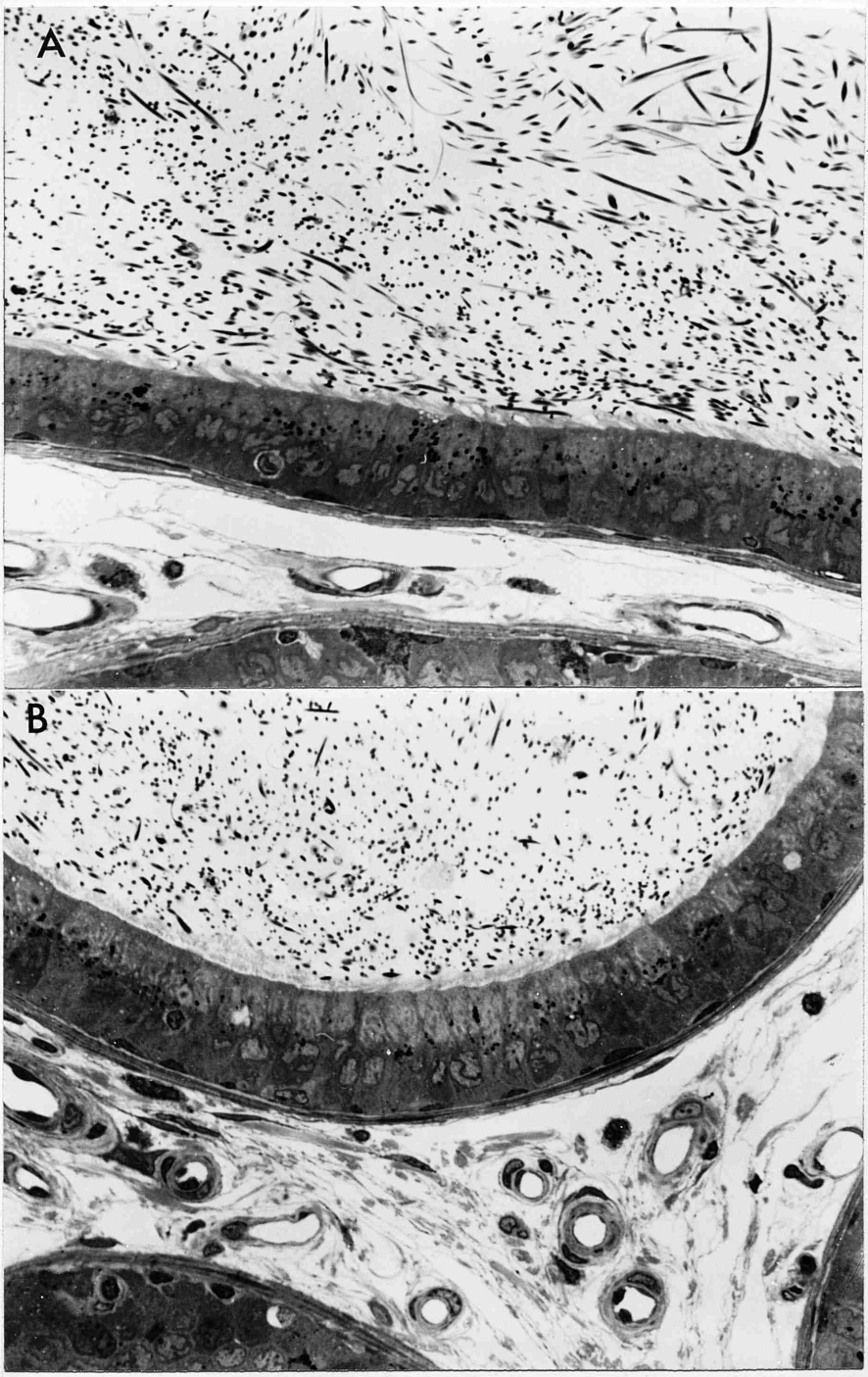

V
C
V
C

Fig. 9: High power micrographs of the caput epididymidis six weeks after (A) sham operation, (B) vasectomy.

(Sham 2, Vas 4 x 500)


A
B

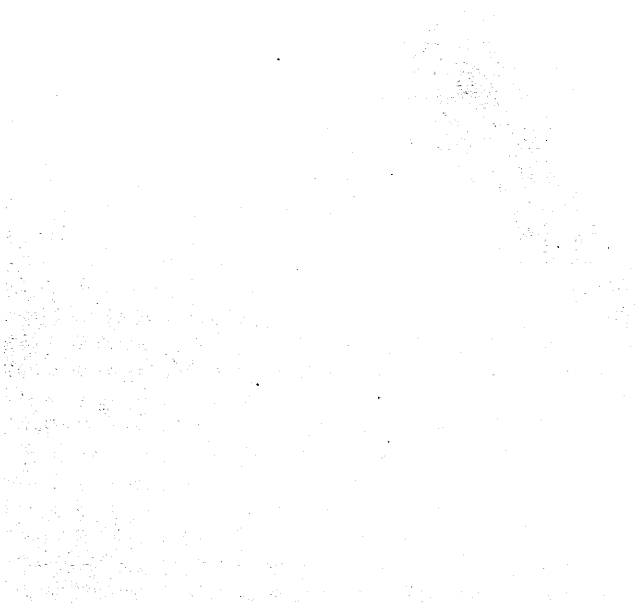


Fig. 10: High power micrographs of the cauda epididymidis six weeks after (A) sham operation, (B) vasectomy.

(Sham 2, Vas 1 x 500)

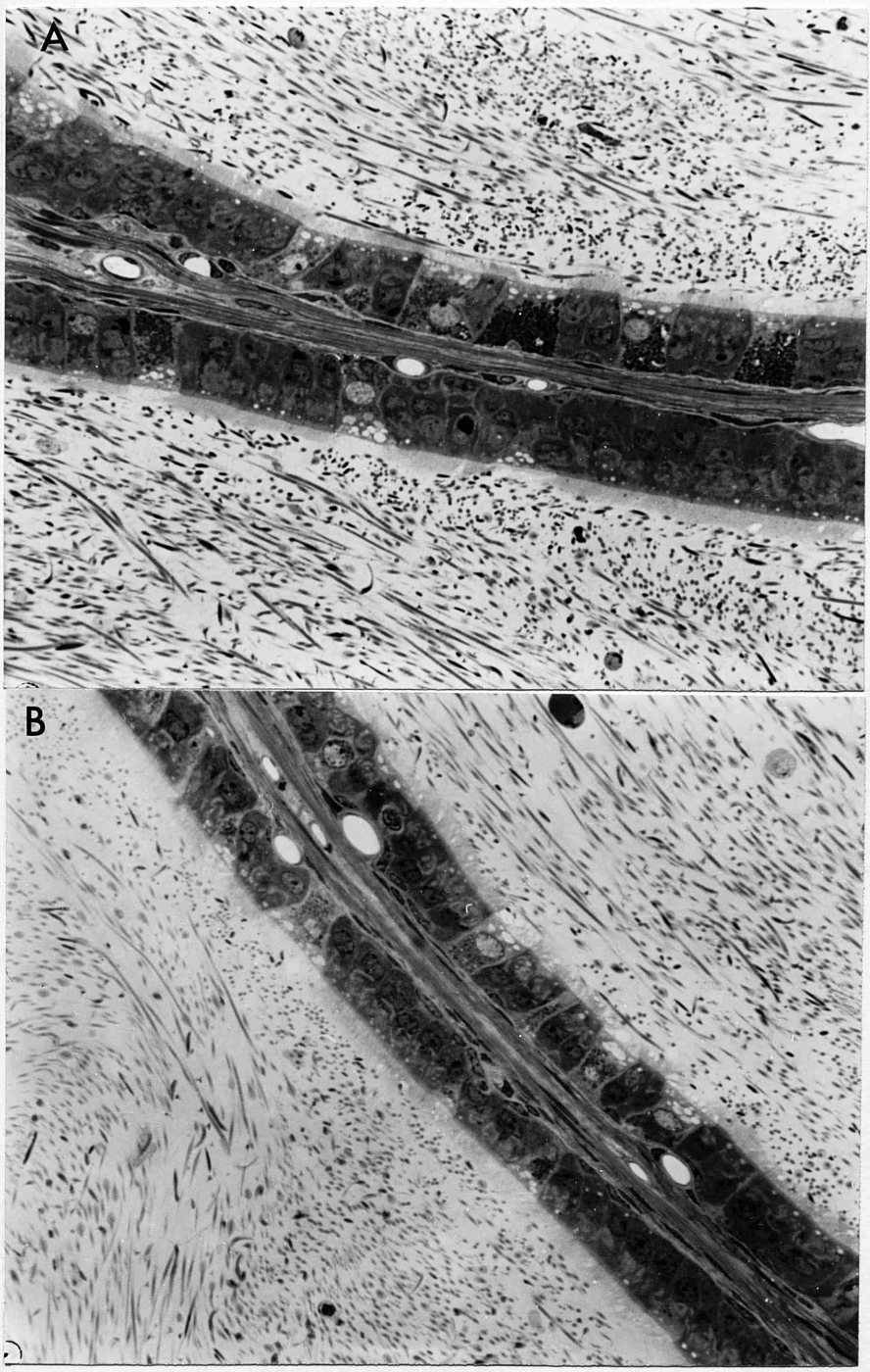

A
B

Fig. 11: High power micrographs of the caput epididymidis four months after (A) sham operation, (B) vasectomy.

(Sham 8, Vas 6 x 500)

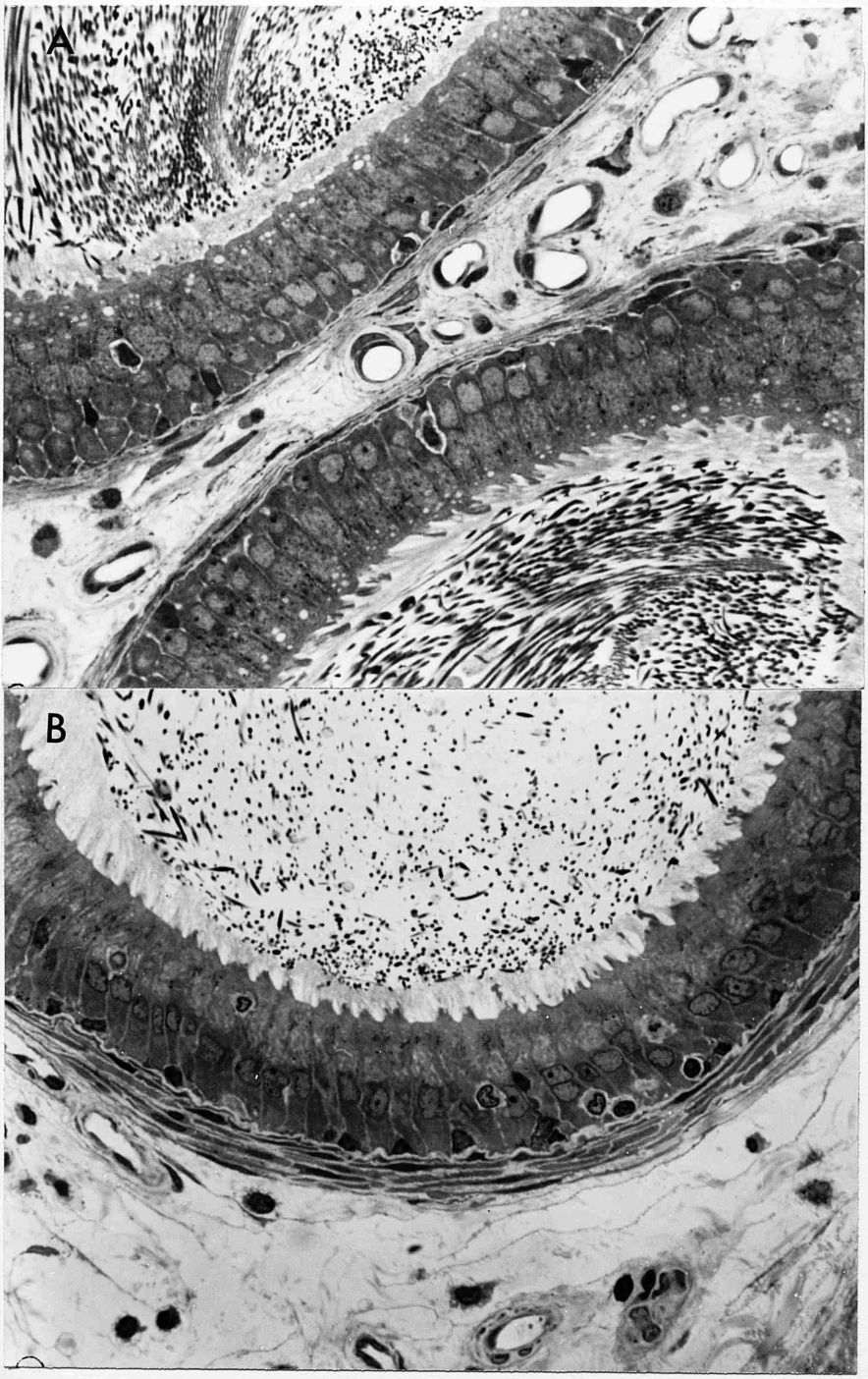

A
B

Fig. 12: High power micrographs of the cauda epididymidis four months after (A) sham operation, (B) vasectomy.

(Sham 10, Vas 6 x 500)

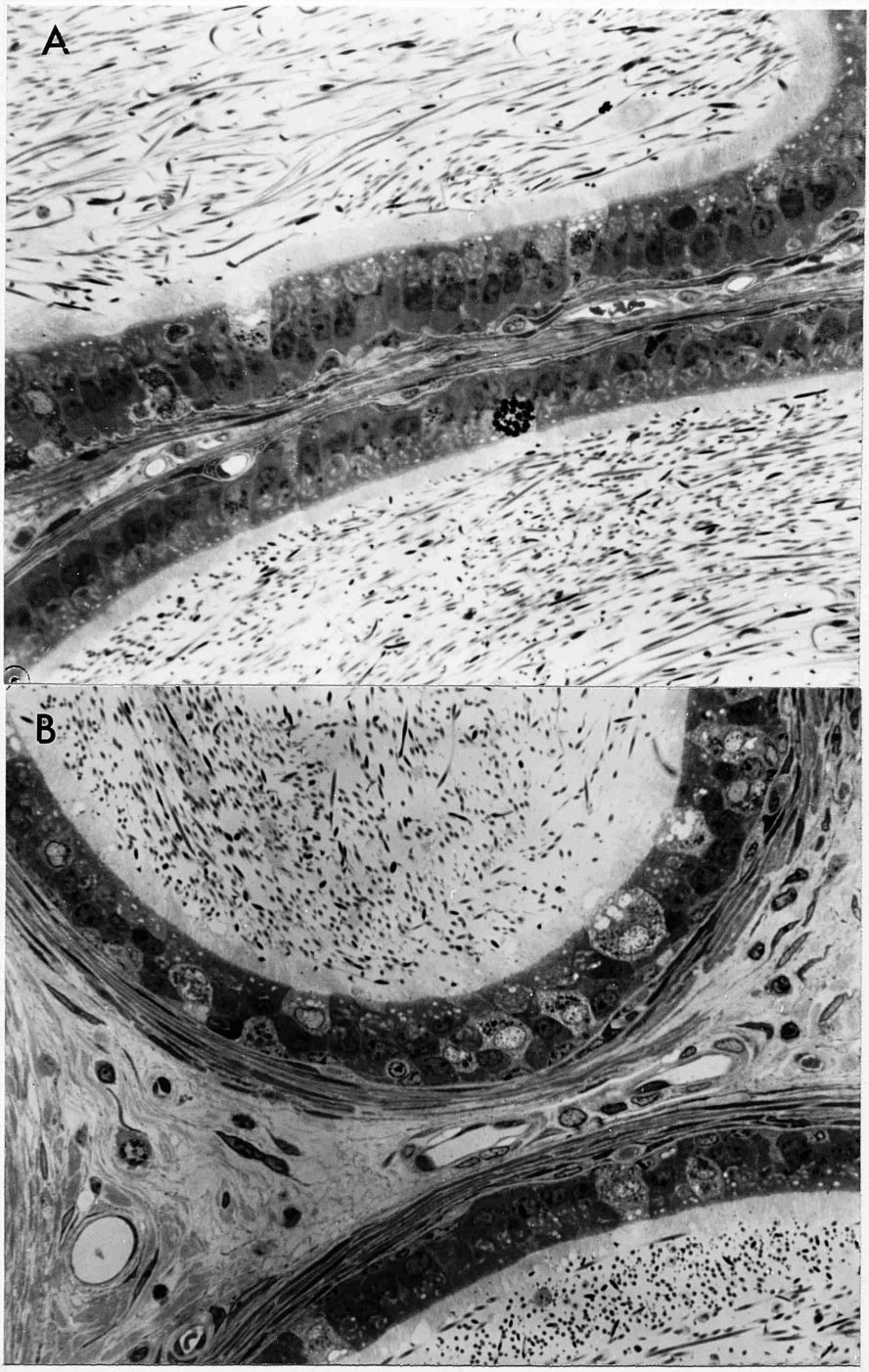

A
B

Fig. 13: High power micrographs of the caput epididymidis six months after (A) sham operation, (B) vasectomy.

(Sham 11, Vas 12 x 500)

A
B

Fig. 14: High power micrographs of the cauda epididymidis six months after (A) sham operation, (B) vasectomy.

(Sham 13, Vas 13 x 500)

A
B

Fig. 15: High power micrographs of the caput epididymidis, nine months after (A) sham operation, (B) vasectomy.

(Sham 17, Vas 15 x 500)

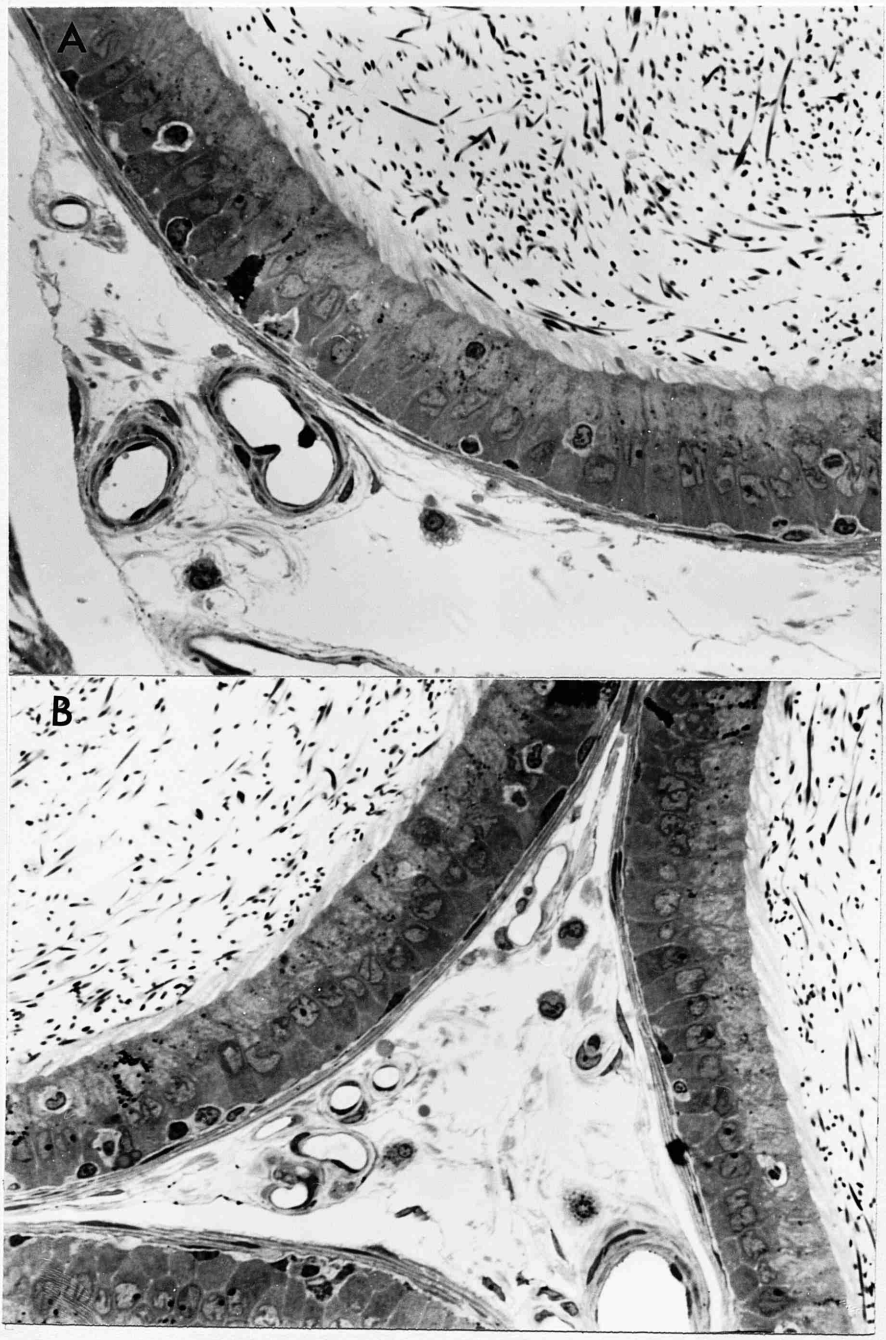

A
B

Fig. 16: High power micrographs of the cauda epididymidis, nine months after (A) sham operation, (B) vasectomy.

(Sham 16, Vas 16 x 500)

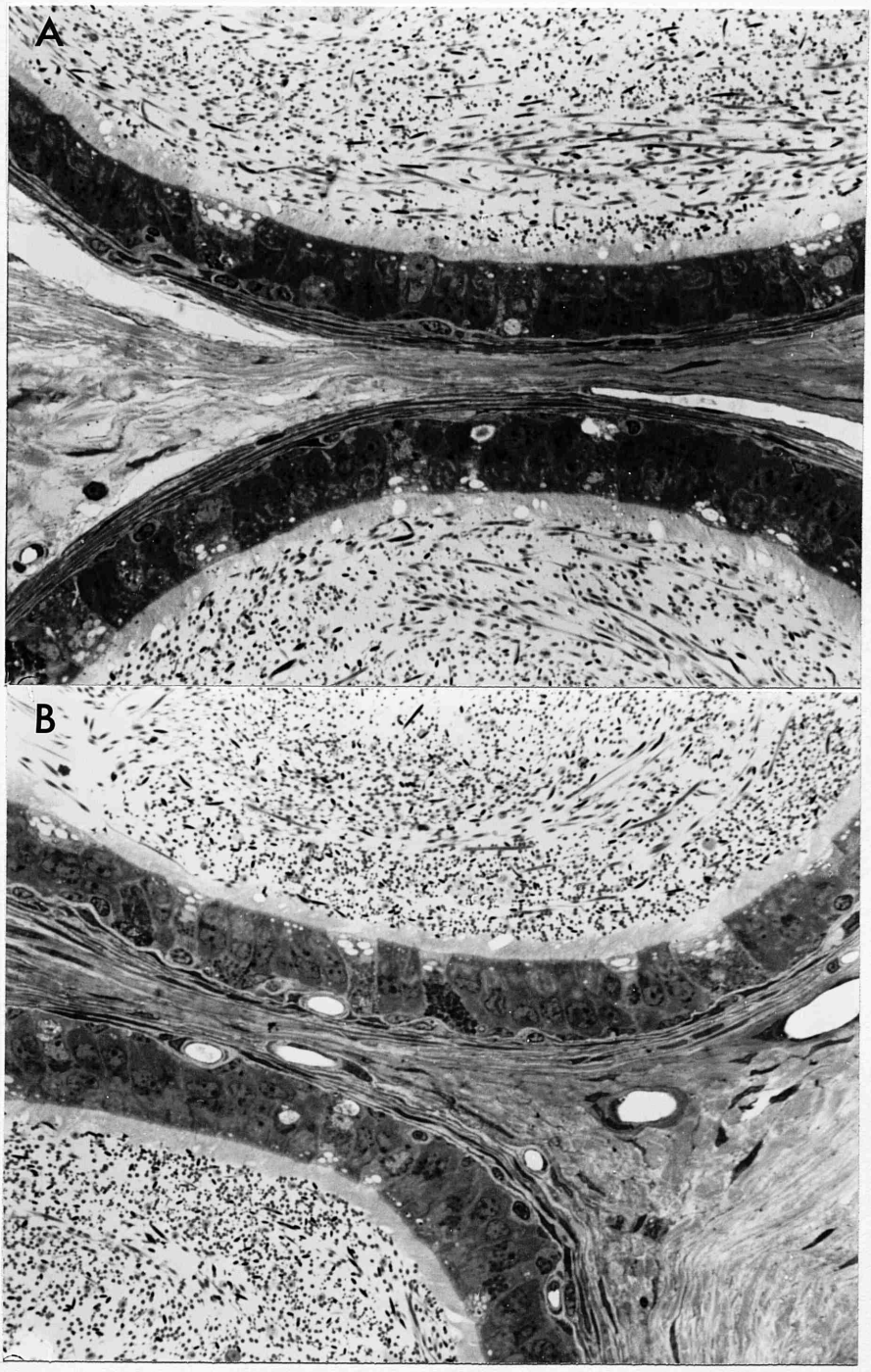

A
B

Fig. 17: High power micrographs of the caput epididymidis, twelve months after (A) sham operation, (B) vasectomy.

(Sham 23, Vas 22 x 500)

A
B

Fig. 18: High power micrographs of the cauda epididymidis, twelve months after (A) sham operation, (B) vasectomy.

(Sham 23, Vas 22 x 500)

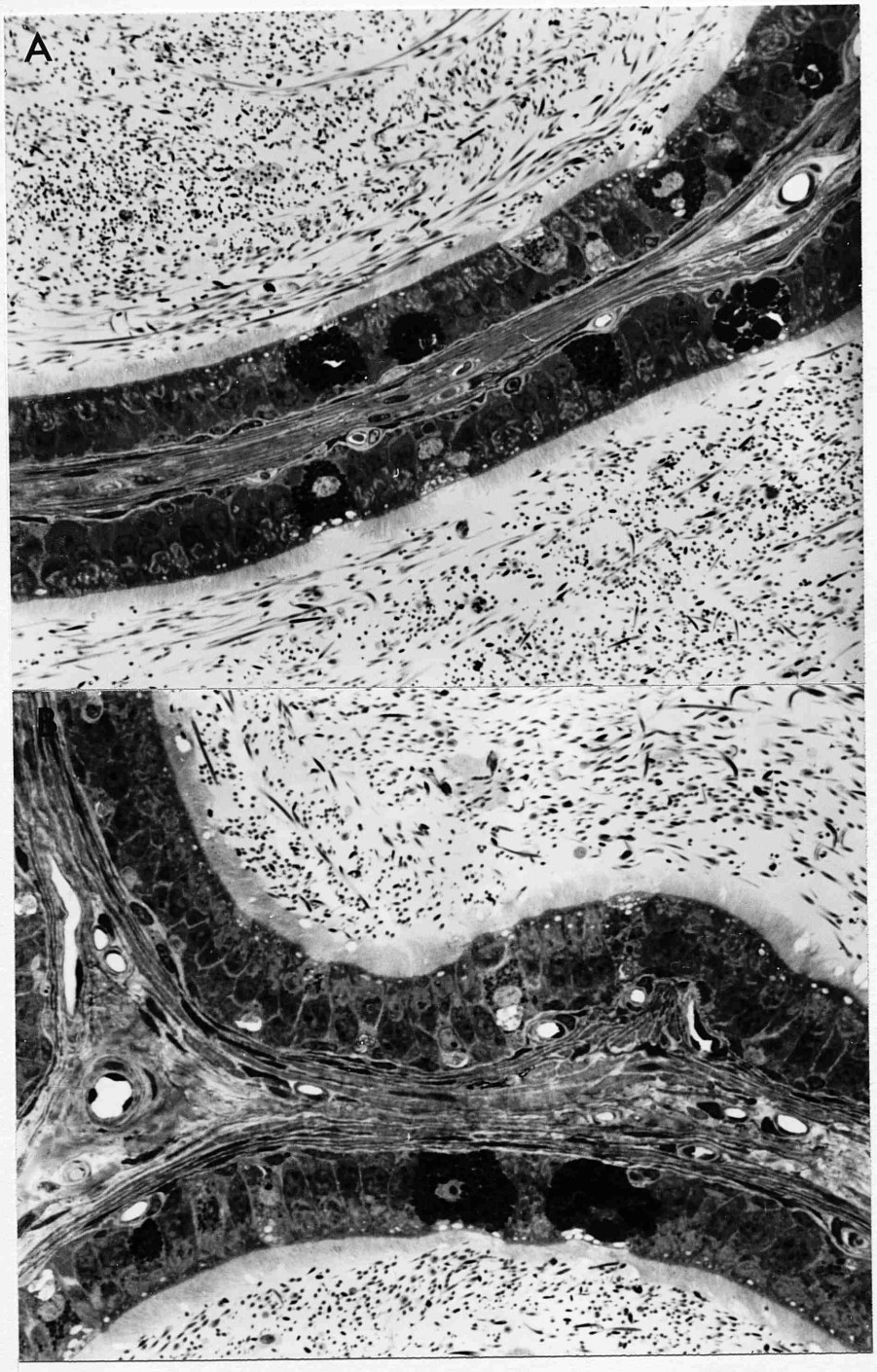

A
B

Fig. 19: High power micrographs of the caput epididymidis, fifteen months after (A) sham operation, (B) vasectomy.

(Sham 29, Vas 29 x 500)

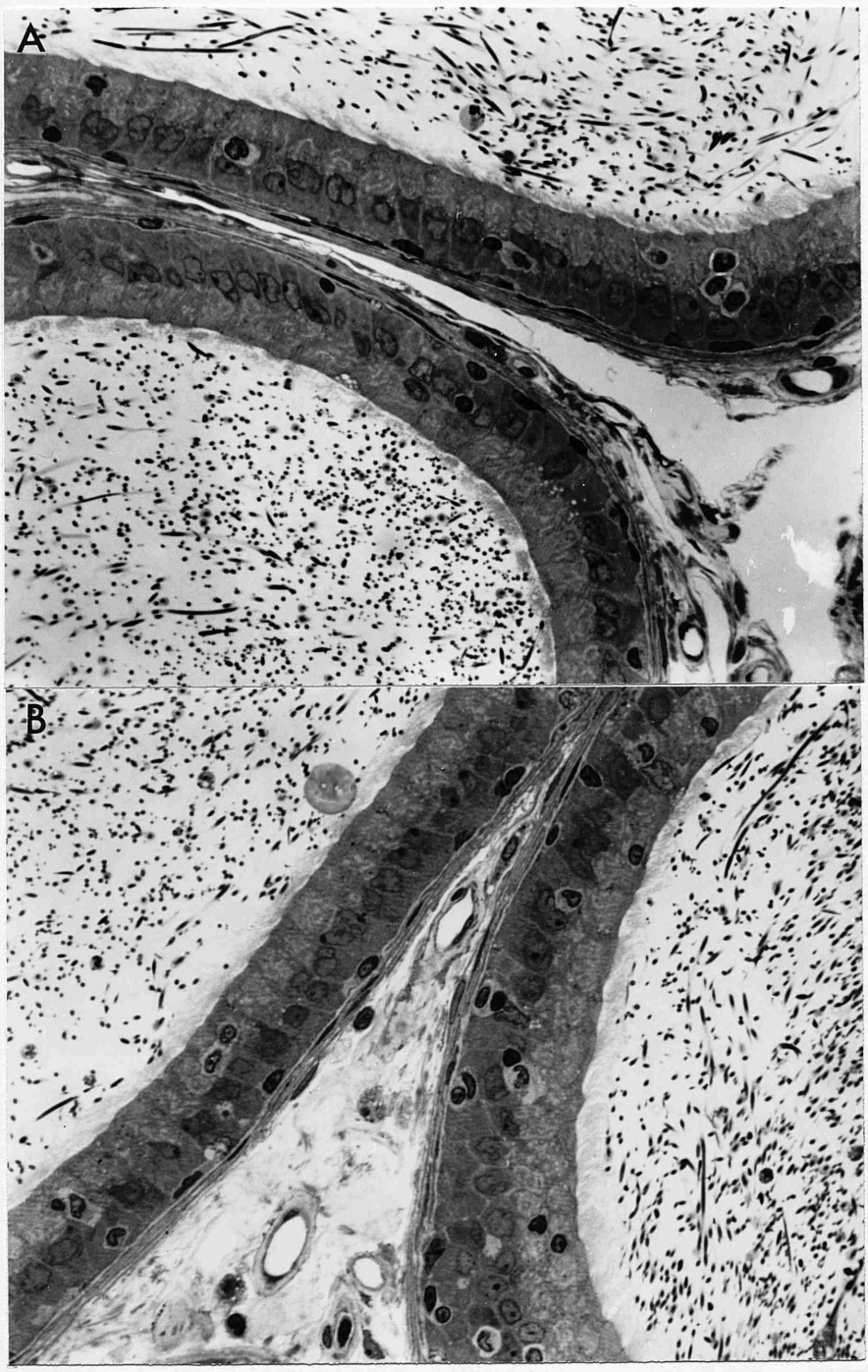

A
B

Fig. 20: High power micrographs of the cauda epididymidis, fifteen months after (A) sham operation, (B) vasectomy.

(Sham 28, Vas 25 x 500)

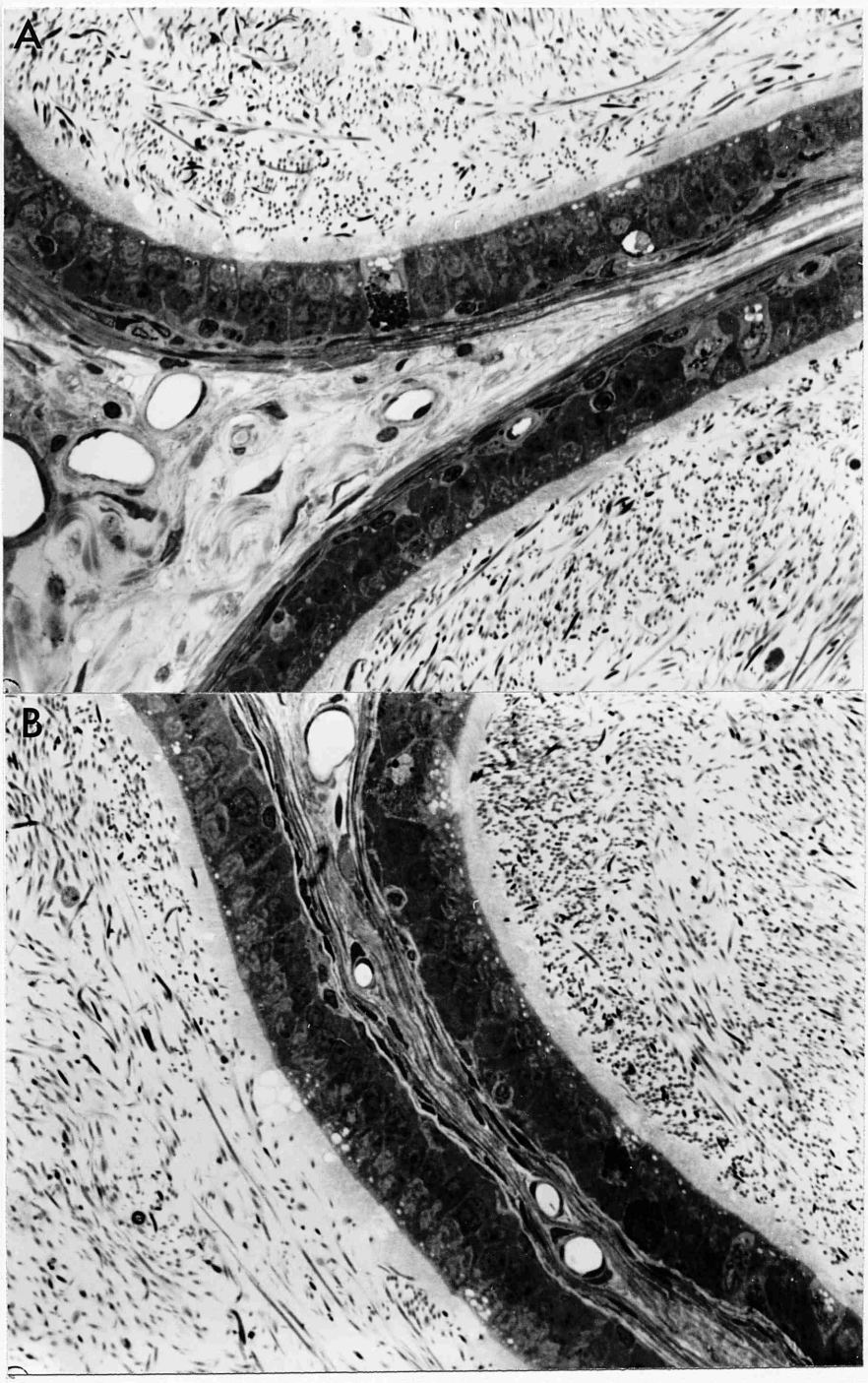

A
B

Fig. 21: High power micrographs of the caput epididymidis, eighteen months after (A) sham operation, (B) vasectomy.

(Sham 31, Vas 31 x 500)

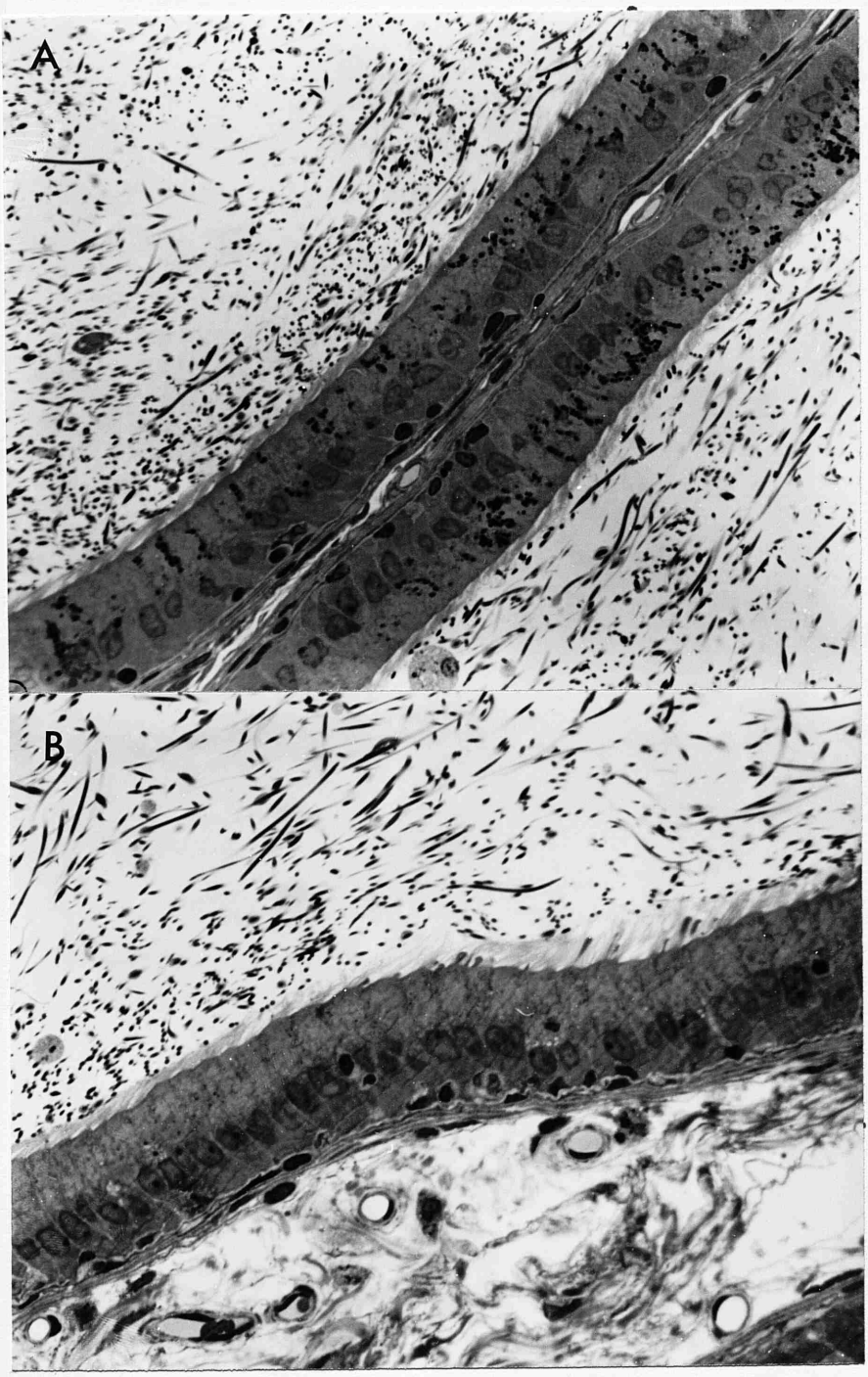

A
B

Fig. 22: High power micrograph of the cauda epididymidis, eighteen months after (A) sham operation, (B) vasectomy.

(Sham 34, Vas 31 x 500)

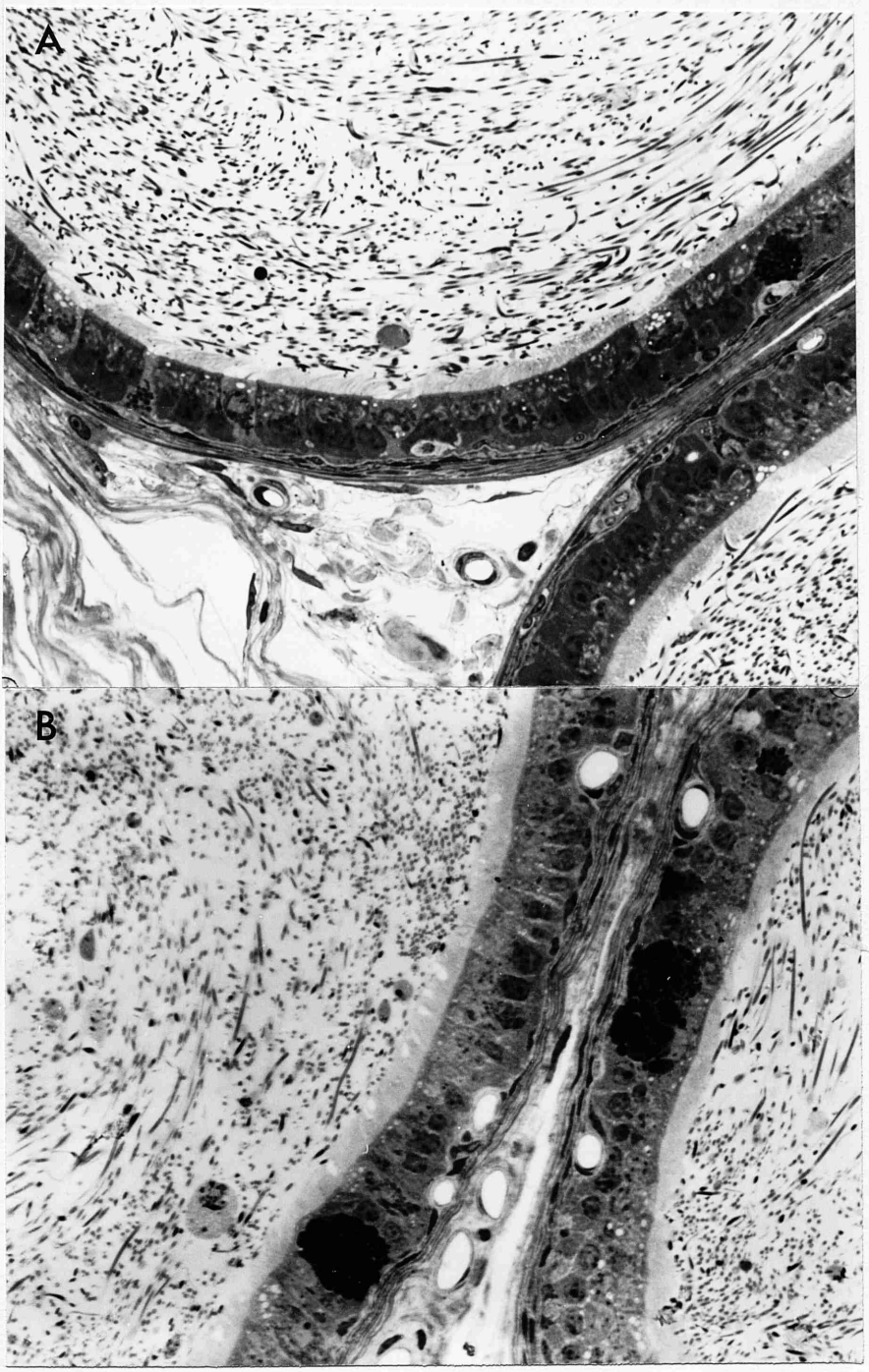

A
B

Fig. 23: Electron micrograph of the supranuclear cytoplasm of three principal cells from the caput epididymidis of a sham-operated rat. The free surface between the stereocilia exhibits numerous coated invaginations of the plasma membrane (arrow). Vesicles (V), multivesicular bodies (MVB), extensive Golgi apparatus (G), scattered mitochondria (m), membrane limited dense bodies (d), and numerous profiles of endoplasmic reticulum are visible in the underlying cytoplasm.

(Sham 20 x 12,600)

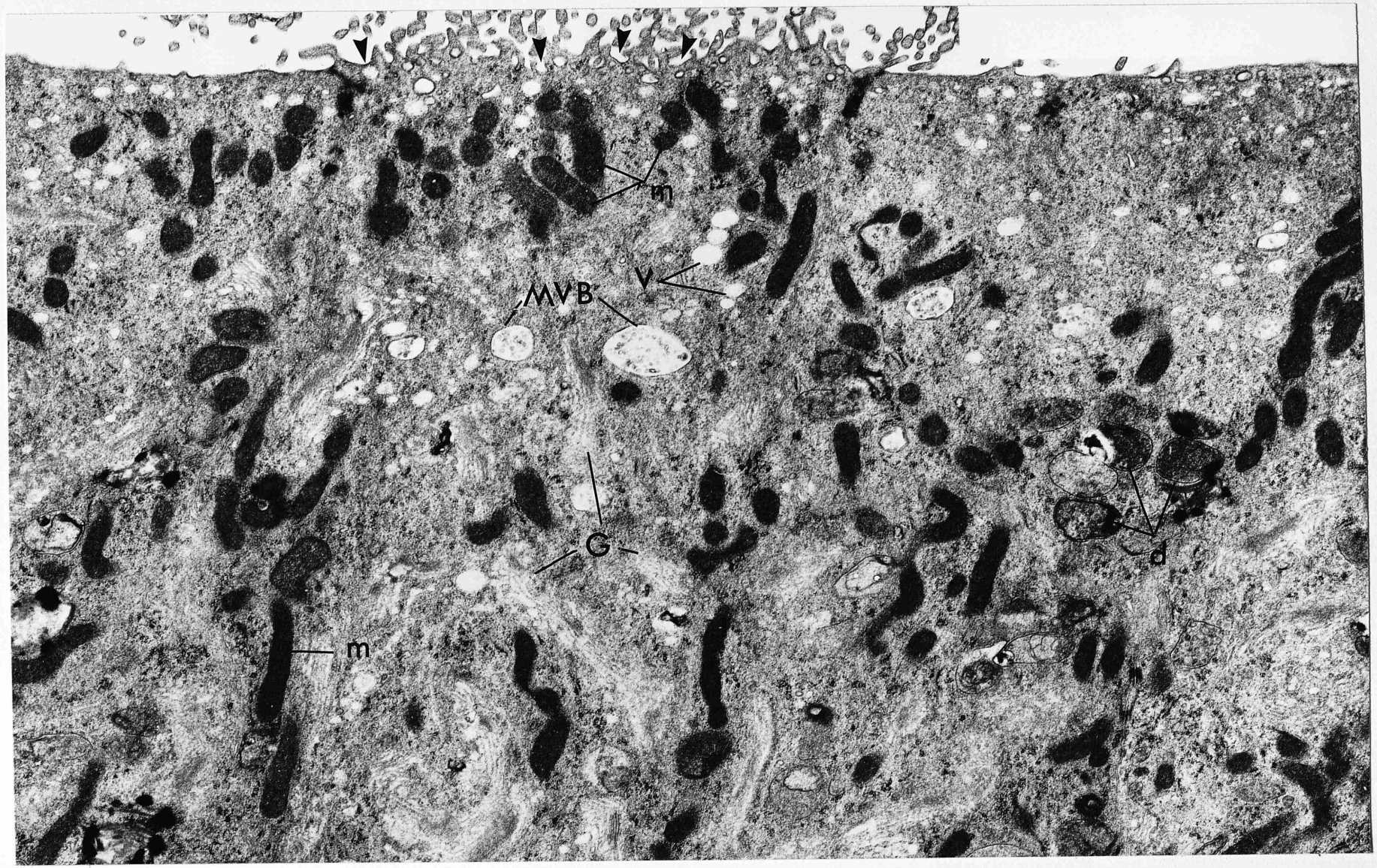

m
V
MVB
G
d
m

Fig. 24: This electron micrograph illustrates the various forms of dense bodies (d), found adjacent to Golgi apparatus in the principal cells of the caput epididymidis of the sham-operated rats.

(Sham 2 x 16,800)

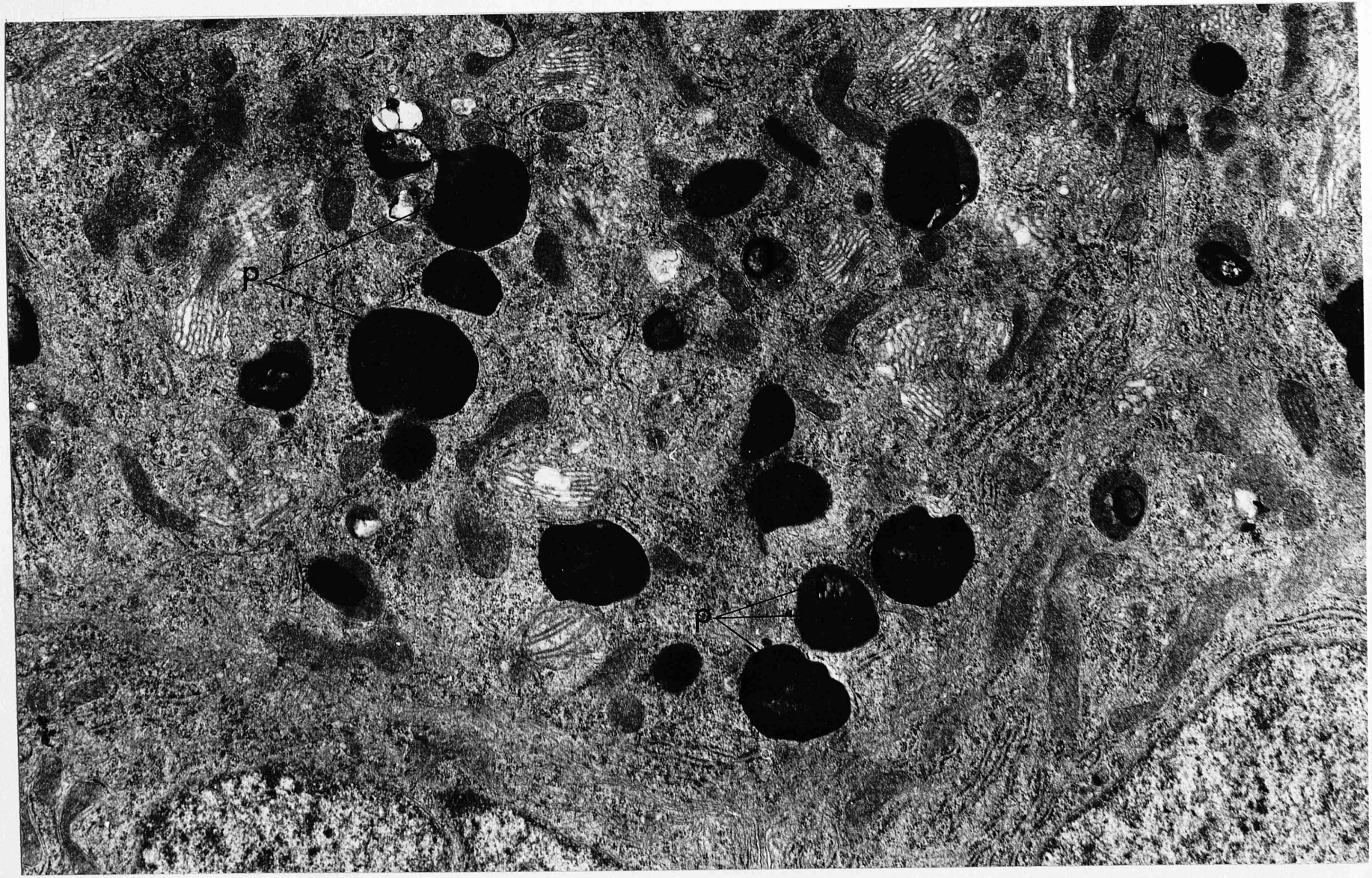

p
p

Fig. 25: The basal cytoplasm of a principal cell in the caput epididymidis of a sham-operated rat. The rough surfaced endoplasmic reticulum is extensively developed in this region of the cell.

(Sham 20 x 21,000)

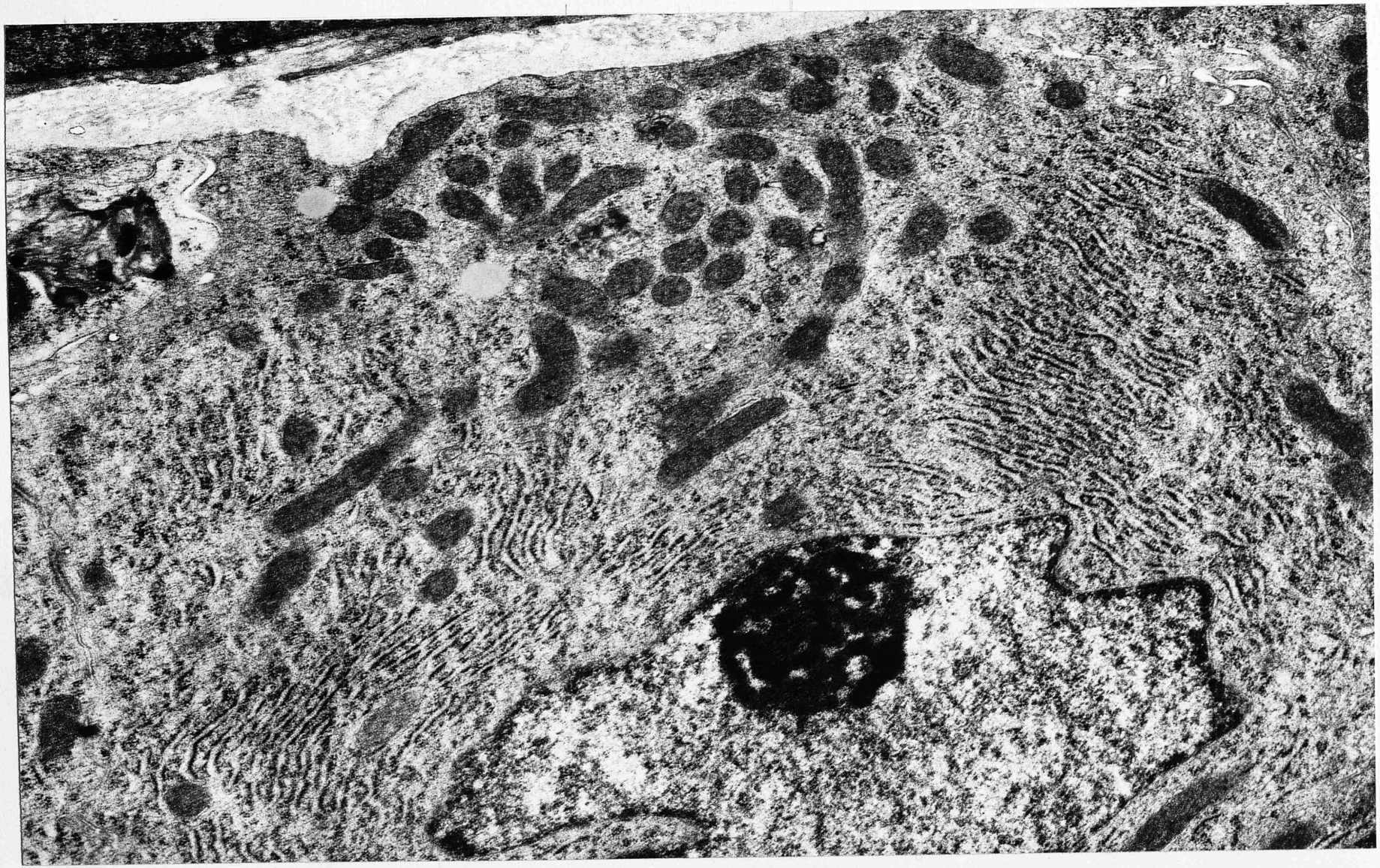


Fig. 26: Micrograph illustrating the general features of the principal cell in the cauda epididymidis of the sham-operated rats. Note the long stereocilia and the extremely well developed Golgi complex (G).

(Sham 14 x 12,600)

G

Fig. 27: Electron micrograph of the apical and supranuclear region of a principal cell from the cauda epididymidis of a sham-operated rat, illustrating the numerous vesicles (V) that occupy the apical part of the cytoplasm, and the extensive supranuclear development of the Golgi apparatus (G). Note the two forms of multivesicular bodies (MVB), A and B.

(Sham 3 x 21,000)

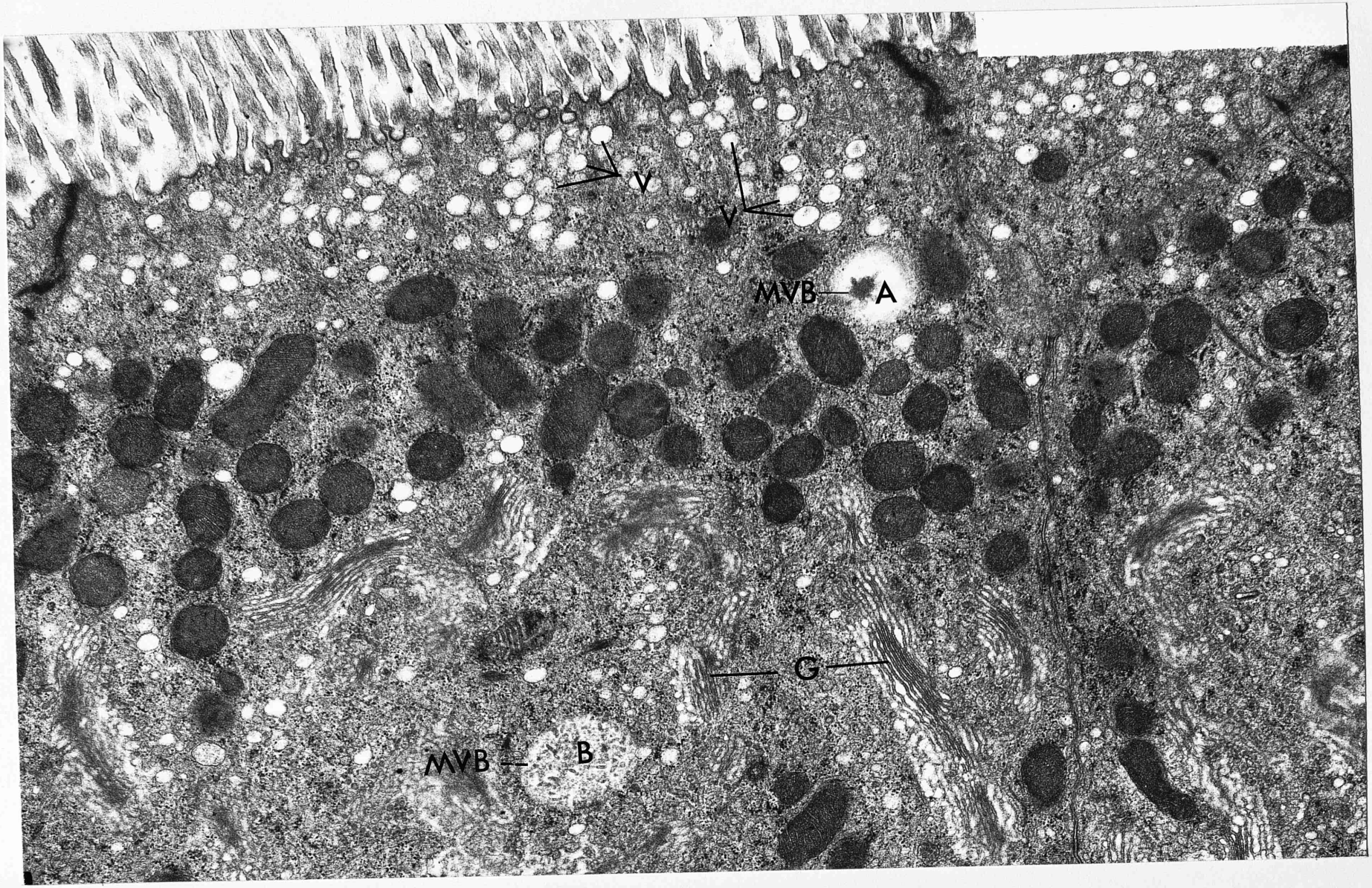

V
V
MVB
A
G
MVB
B

Fig. 28: High power electron micrograph of the apical part of a caudal principal cell from a sham-operated rat. The apical plasma membrane between the roots of the stereocilia is irregular and shows numerous invaginations (arrow). The underlying cytoplasm is filled by vesicles (V), mitochondria (m) and multivesicular bodies (MVB).

(Sham 3 x 30,000)

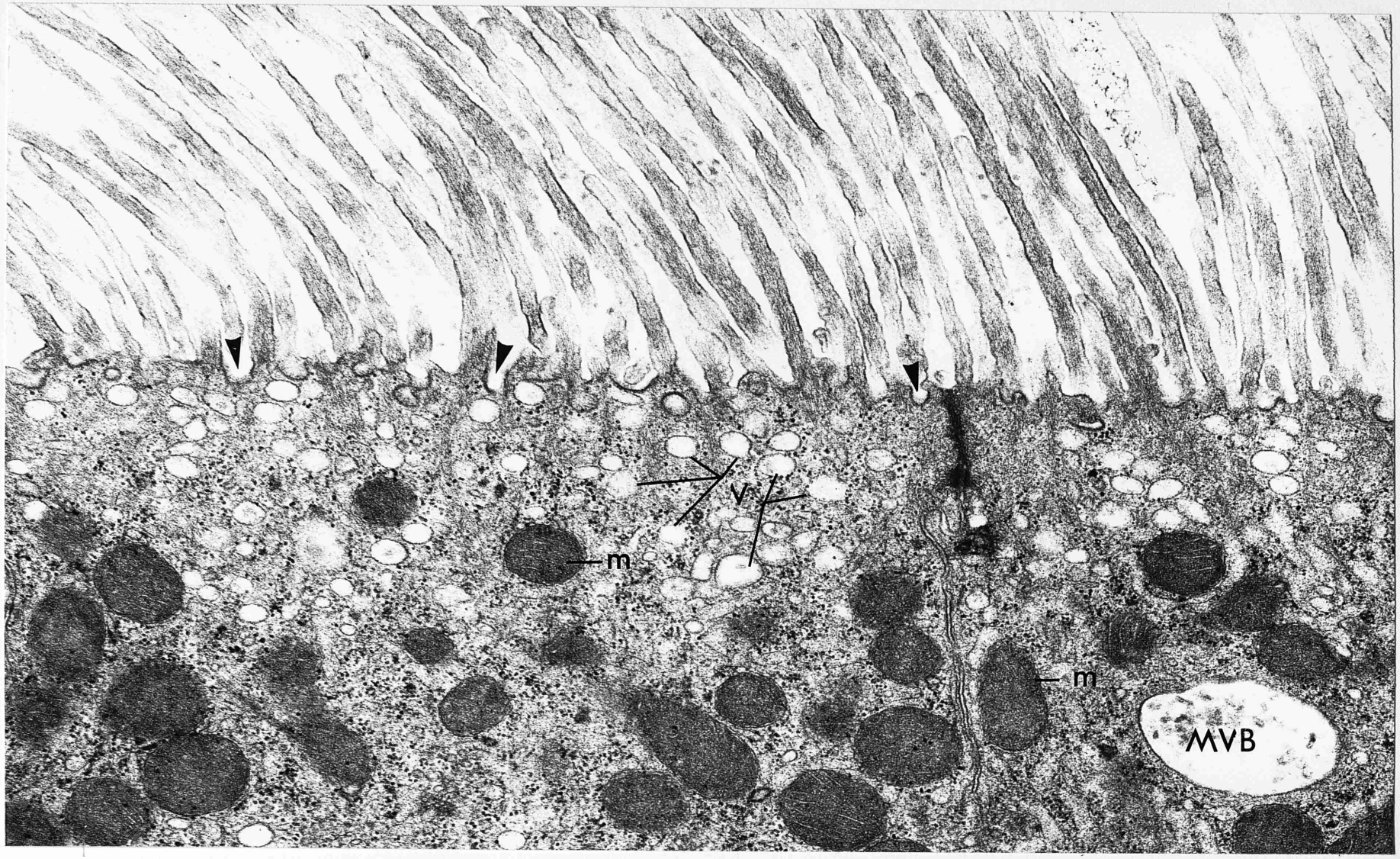

V
m
m
MVB

Fig. 29: Electron micrograph of principal cells from the cauda epididymidis of a sham-operated rat. Note the membrane-bounded dense bodies (d), present at the supranuclear aspect of the cell.

(Sham 3 x 12,600)

Fig. 30: In the caput epididymidis of the sham-operated rats, the clear cell is characterized by numerous vesicles, multivesicular bodies of variable size, and mitochondria. The membrane-bounded dense bodies are few in number, and usually seen at the basal part of the cell. Note the irregularity of the apical surface of the cell.

(Sham 2 x 10,500)

Fig. 31: Electron micrograph of the cauda epididymal epithelium from a sham-operated rat. The clear cell (C) has fewer and much shorter stereocilia than the principal cells. The apical part of the cytoplasm contains many vesicles and multivesicular bodies. In the mid-region of the cell, there are profiles of membrane-bounded bodies.

(Sham 3 x 10,500)

C

Fig. 32: Low power electron micrograph of several cells from the cauda epididymidis of a sham-operated rat. The principal cells (P) exhibit long stereocilia, numerous apical vesicles, and extremely well developed Golgi apparatus. The clear cells (C) are characterized by many membrane-bounded dense bodies that fill the cytoplasm of the middle and basal part of the cell.

(Sham 3 x 6,300)

C
P
P
C
C

Fig. 33: Higher power view of the supranuclear part of the left clear cell, in the previous micrograph, showing the membrane-limited dense bodies, that filled its cytoplasm.

(Sham 3 x 21,000)

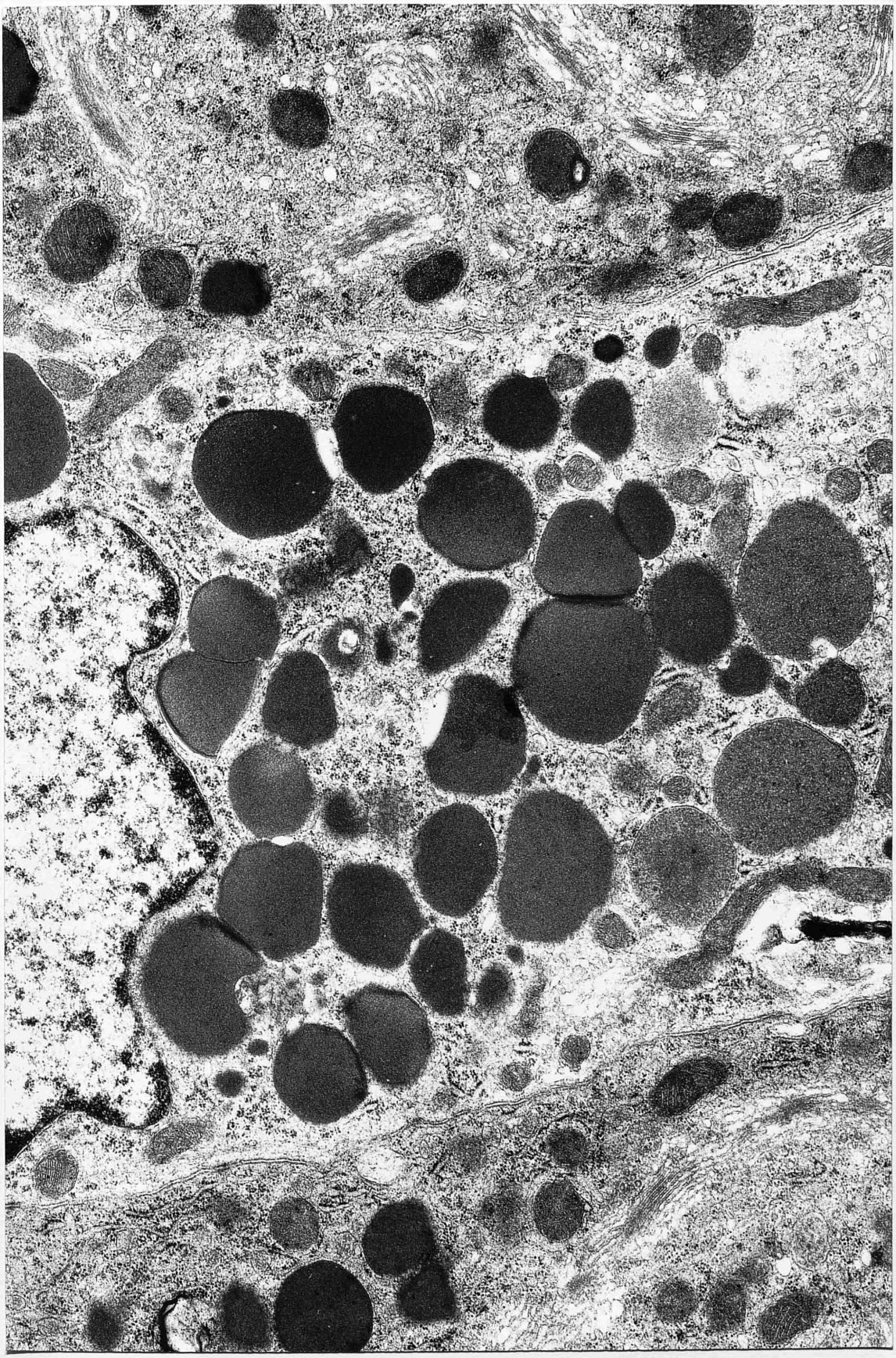


Fig. 34: Electron micrograph of the cauda epididymal epithelium from a sham-operated rat. Note the profiles of membrane-bounded bodies (d) that fill the cytoplasm of the mid and basal region of the clear cell (C).

(Sham 3 x 12,600)

P
P
P
P
C

Fig. 35: This electron micrograph of the infranuclear portion of one clear cell from the caput epididymidis of a sham-operated rat, showing the membranous masses and lipid droplets, filling its cytoplasm.

(Sham 14 x 12,600)

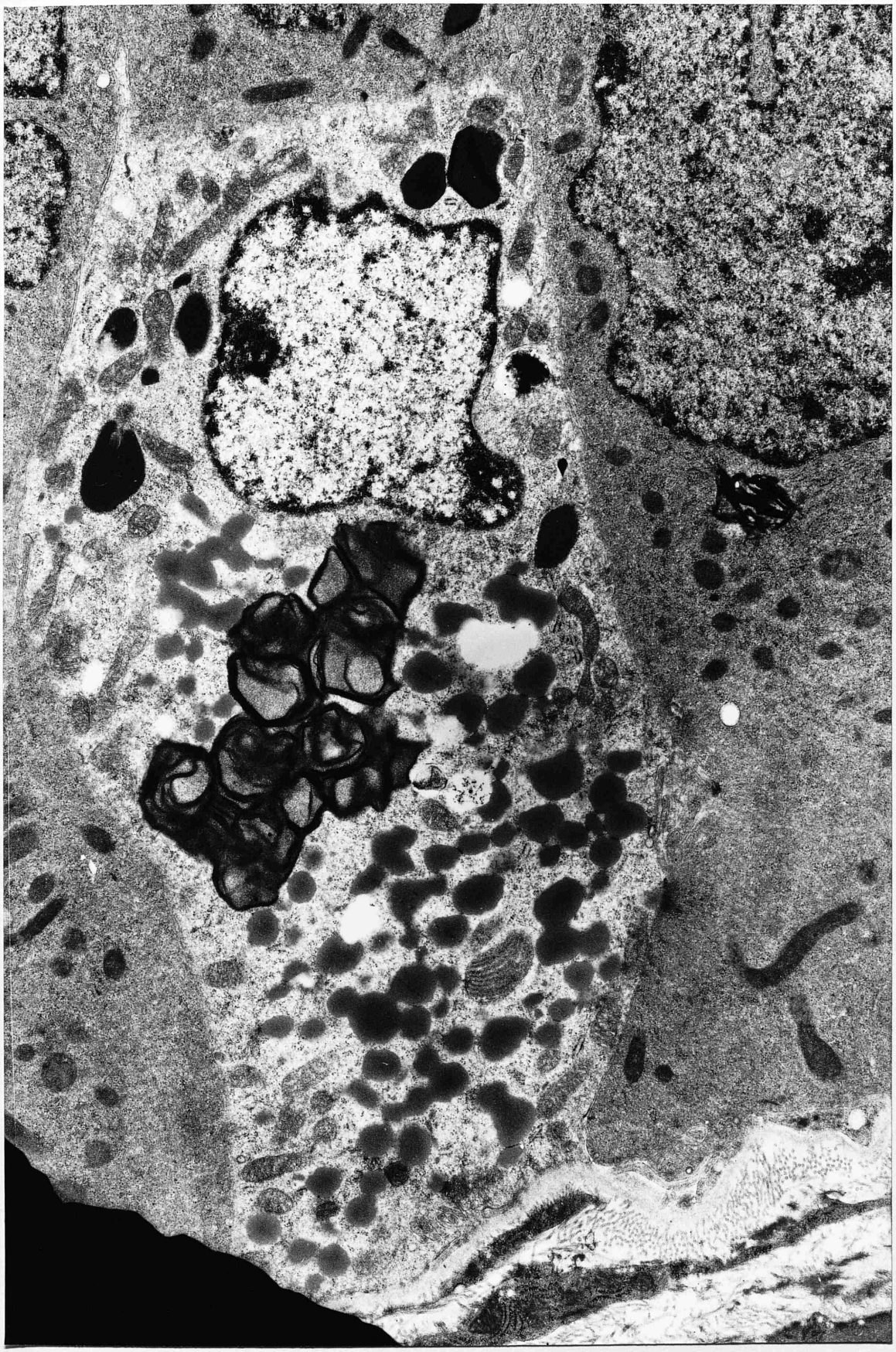


Fig. 36: In the sham-operated rats, the bases of principal cells (P) interdigitate with the plasma membrane of basal cells (B), as shown in this electron micrograph of the cauda epididymidis. The basal cell nucleus is flattened against the basement membrane (arrows), and contain coarse chromatin granules. The cytoplasm contains relatively few organelles.

(Sham 20 x 12,600)

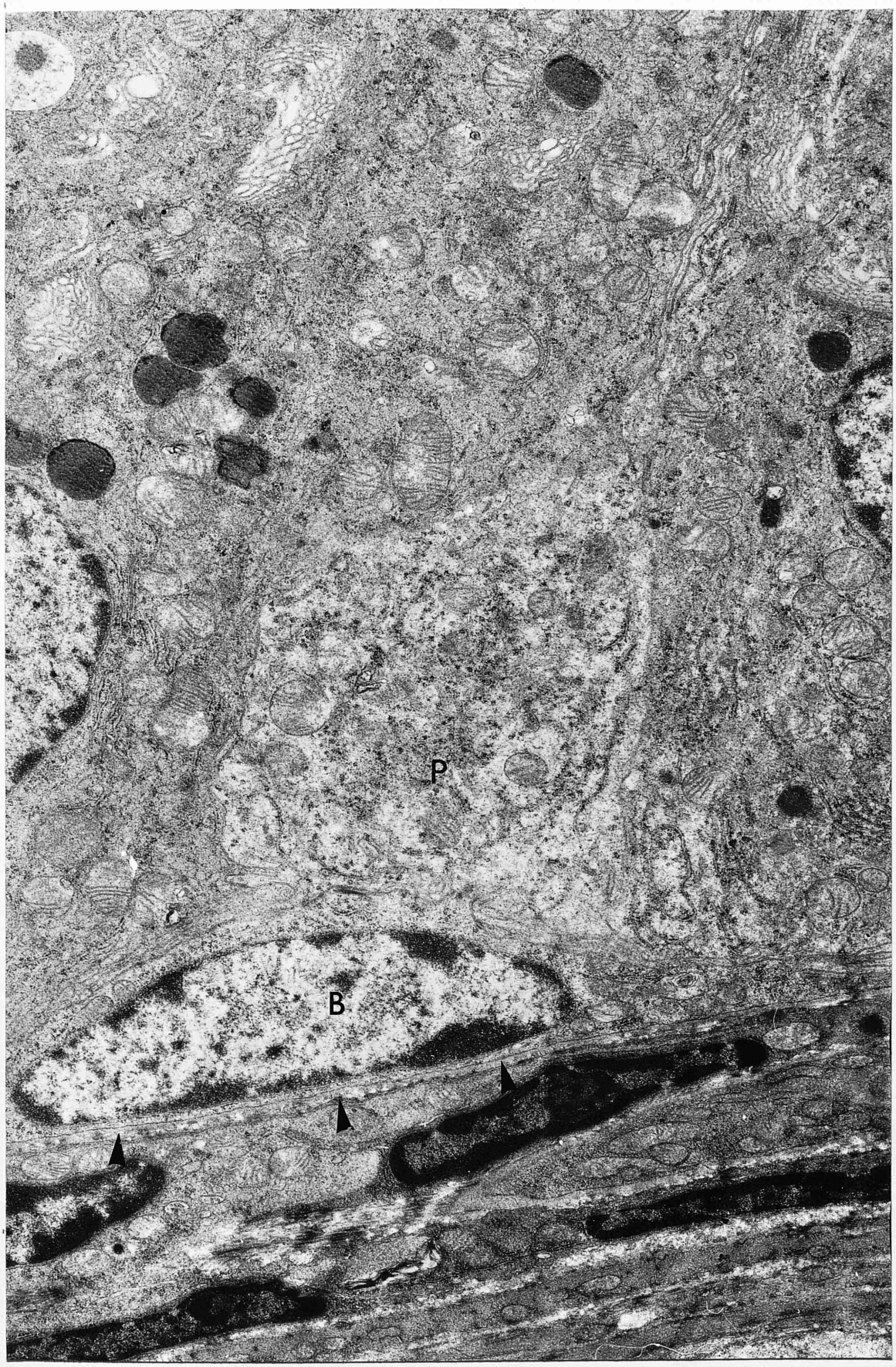

P
B

Fig. 37: An electron micrograph of the caput epididymidis from a sham-operated rat, showing intraepithelial cell bearing a close resemblance to lymphocytes. The nucleus contains peripheral clumps of chromatin, and prominent central chromatin clumps. The sparse cytoplasm contains several large mitochondria, and infrequent elements of rough endoplasmic reticulum.

(Sham 20 x 21,000)

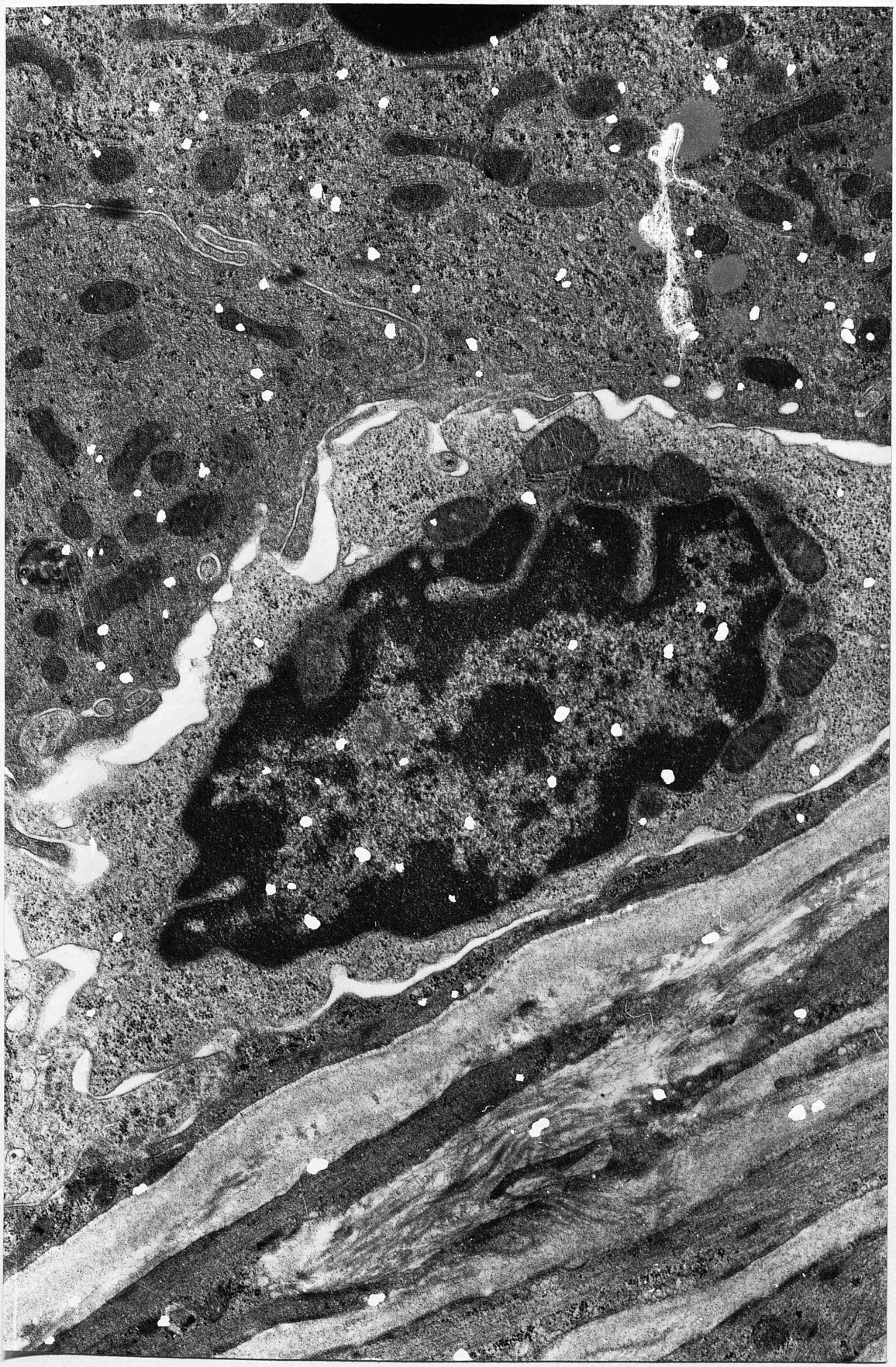


Fig. 38: An electron micrograph of intraepithelial leucotyes (monocyte), from the caput epididymidis of a sham-operated rat. The nucleus is slightly indented toward the cytocentrum. The cytoplasm contains few large mitochondria, multivesicular bodies, some element of rough endoplasmic reticulum, and numerous scattered free ribosomes.

(Sham 20 x 21,000)

Fig. 39: This electron micrograph at the basal part of the epididymal epithelium from the caput after sham operation, showing a large phagocyte containing numerous dense bodies, surrounded by profiles of rough surfaced endoplasmic reticulum.

(Sham 20 x 21,000)

Fig. 40: The intraepithelial leucocyte (phagocyte) shown in this electron micrograph from the caput epididymidis after sham operation, exhibits two large membrane-limited dense bodies surrounded by well developed elements of rough endoplasmic reticulum.

(Sham 20 x 21,000)

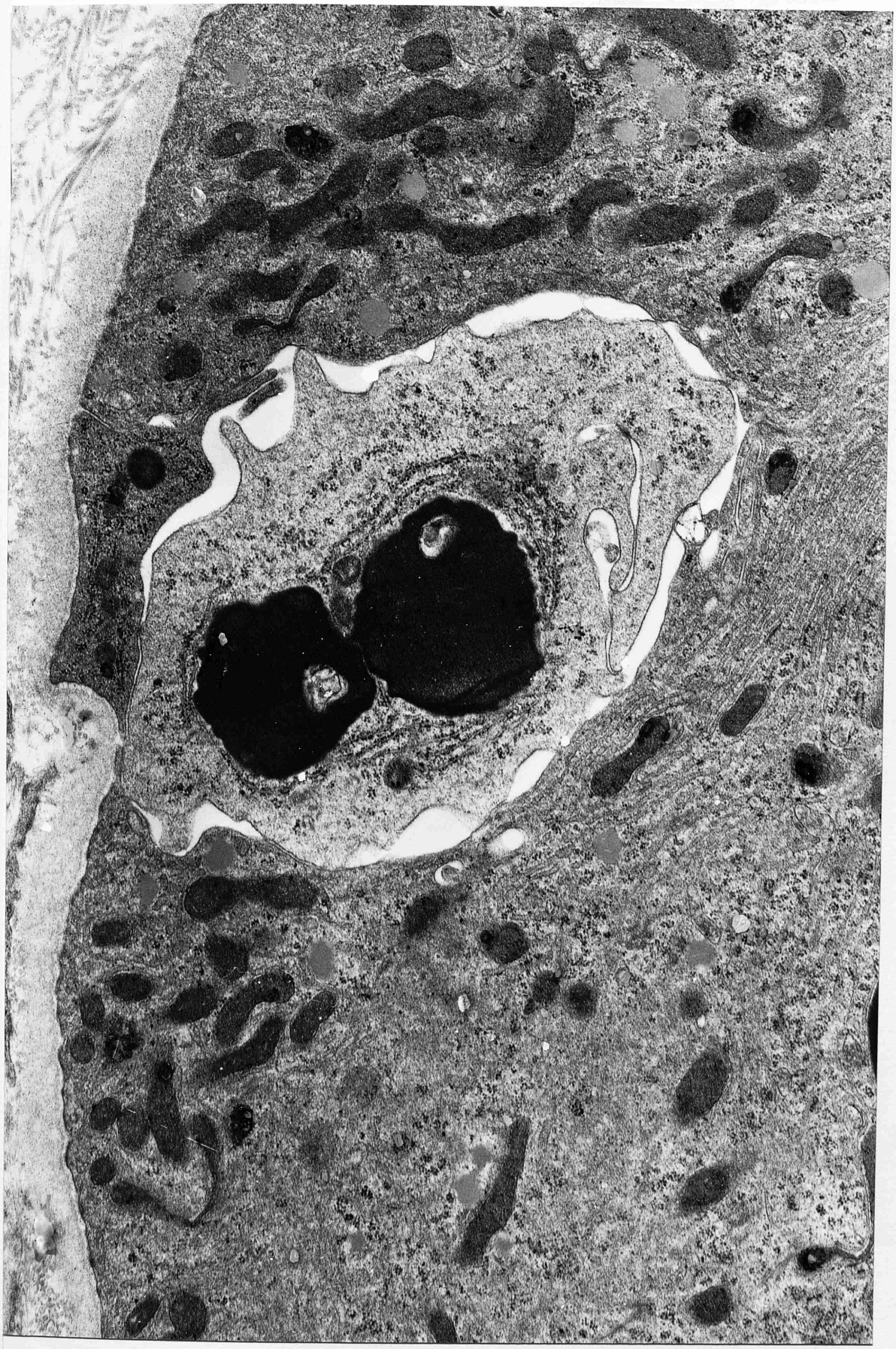


Fig. 41: Electron micrograph illustrating the apical and supranuclear part of principal cells from caput epididymidis of a rat vasectomized for six weeks. The apical cytoplasm contains numerous vesicles. The supranuclear region of the cell exhibits extensive Golgi apparatus, vesicles, scattered mitochondria and few dense bodies and autophagic vacuoles (A).

(Vas 3 x 12,600)

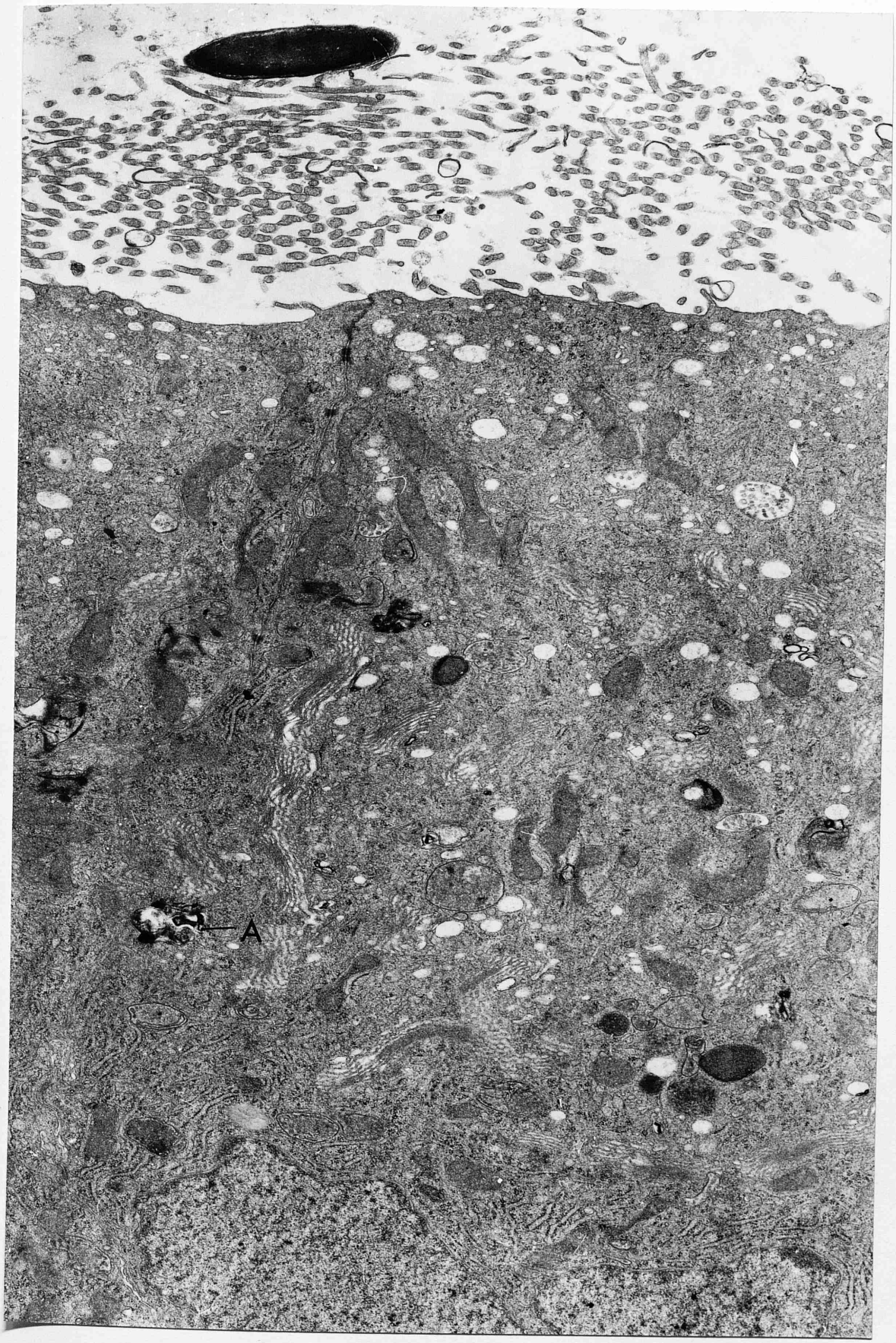

A

Fig. 42: Electron micrograph of the basal part of the epithelium of the caput epididymidis from a rat killed six weeks after vasectomy. Note the intraepithelial lymphocyte present at the base of the principal cell.

(Vae 3 x 12,600)

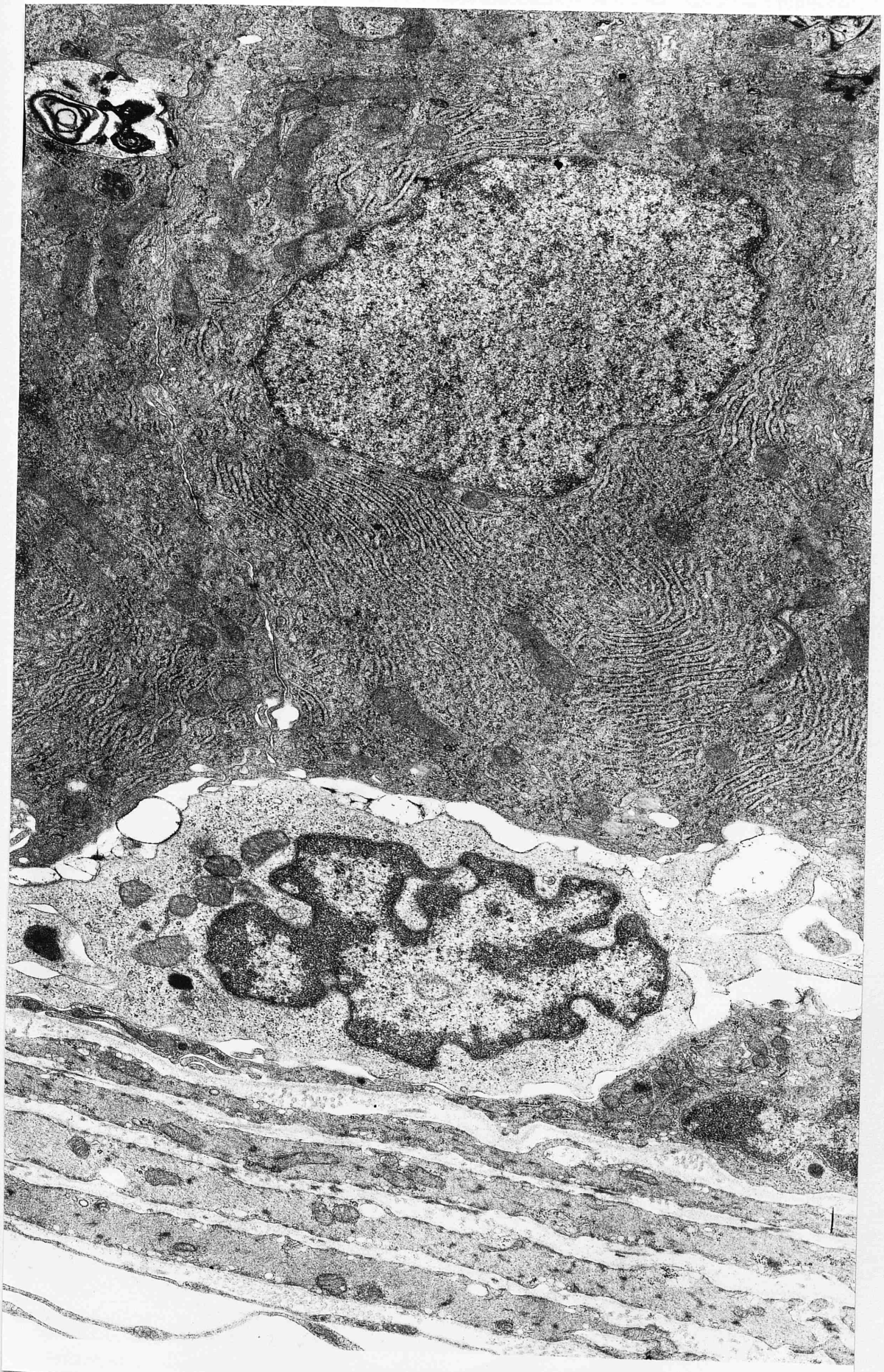


Fig. 43: Electron micrograph of principal cells from the caput epididymidis of a rat vasectomized for six months. Note the stereocilia.

(Vas 14 x 12,600)

Fig. 44: An electron micrograph of the epididymal epithelium of the caput epididymidis six weeks after vasectomy, showing the same histological features as found in the sham-operated rats.

(Vas 3 x 12,600)

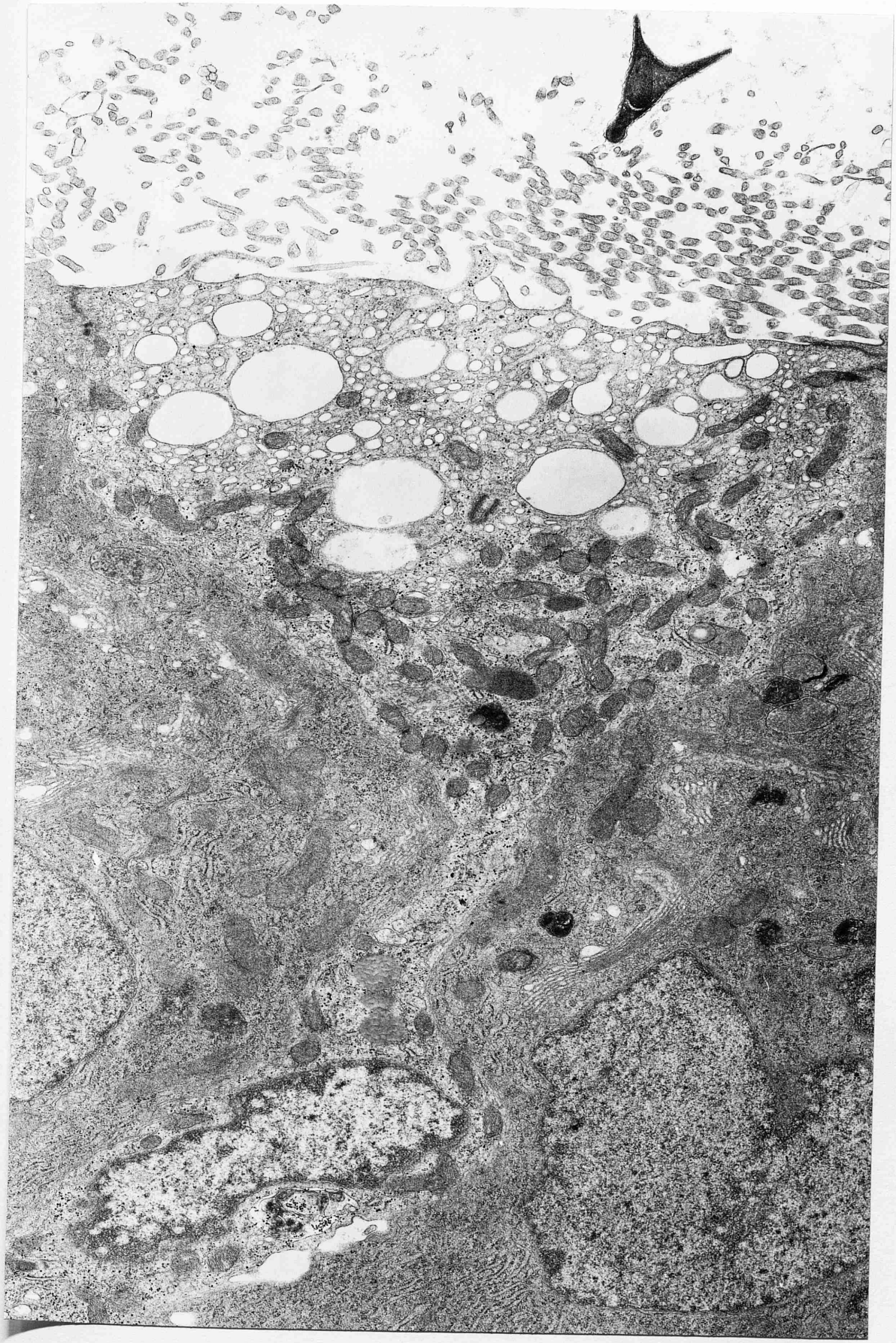


Fig. 45: The epithelium of the caput epididymidis from a rat, vasectomized for six months. Note the large number of vesicles (V), and the less numerous multivesicular bodies (MVB) present in the apical and supranuclear part of the clear cell (C).

(Vas 14 x 12,600)

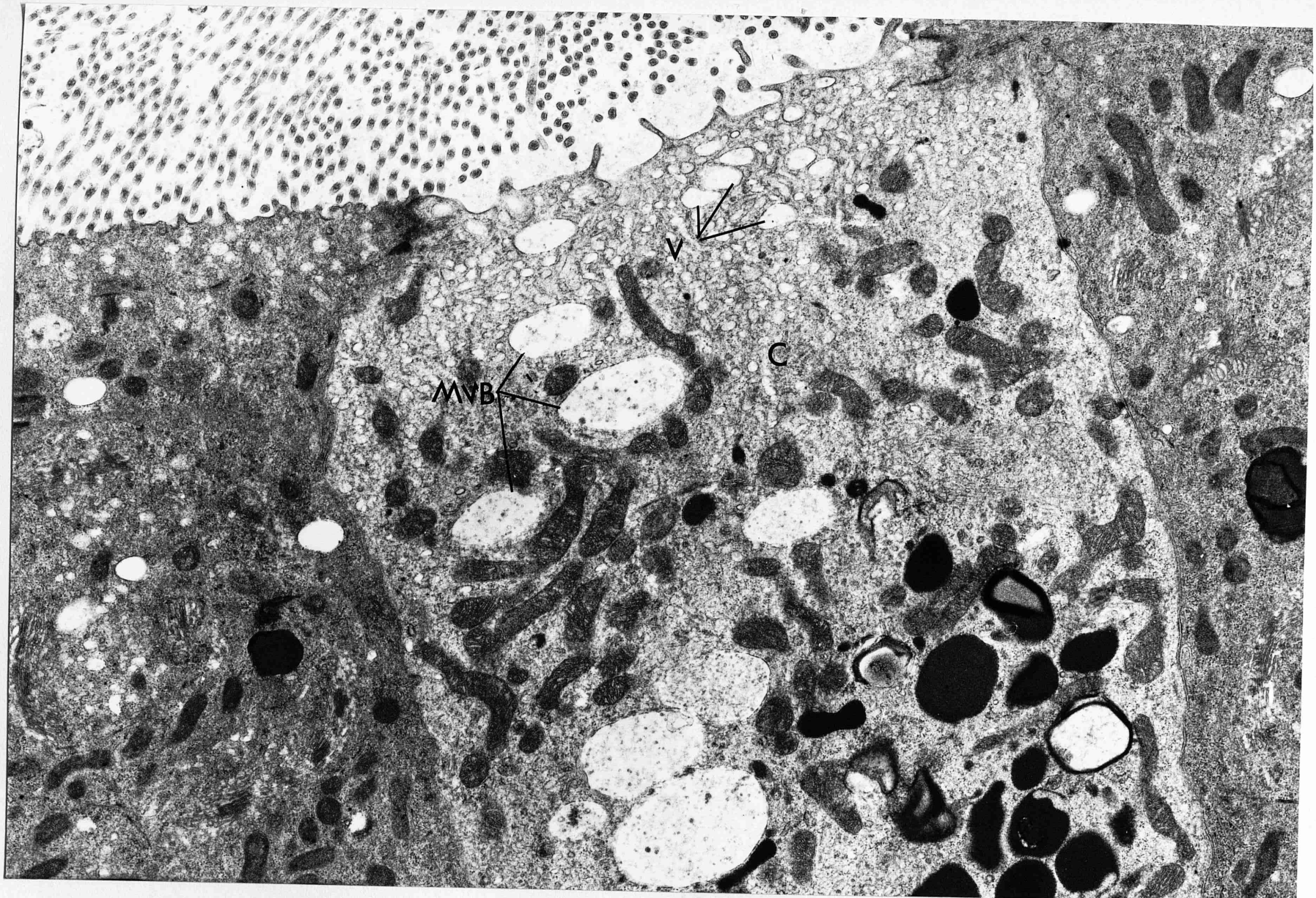

V
C
MVB

Fig. 46: This electron micrograph showing two clear cells (C) on the lateral side, and one principal cell (P) from the cauda epididymidis of a rat, killed six weeks after vasectomy. Note the extremely well developed Golgi apparatus present in the supranuclear part of the principal cell.

(Vas 2 x 12,600)

C
P
C

Fig. 47: Electron micrograph of principal cells from the cauda epididymidis of a vasectomized rat, for six weeks, showing the same histological features as found in the sham-operated animals.

(Vas 2 x 12,600)

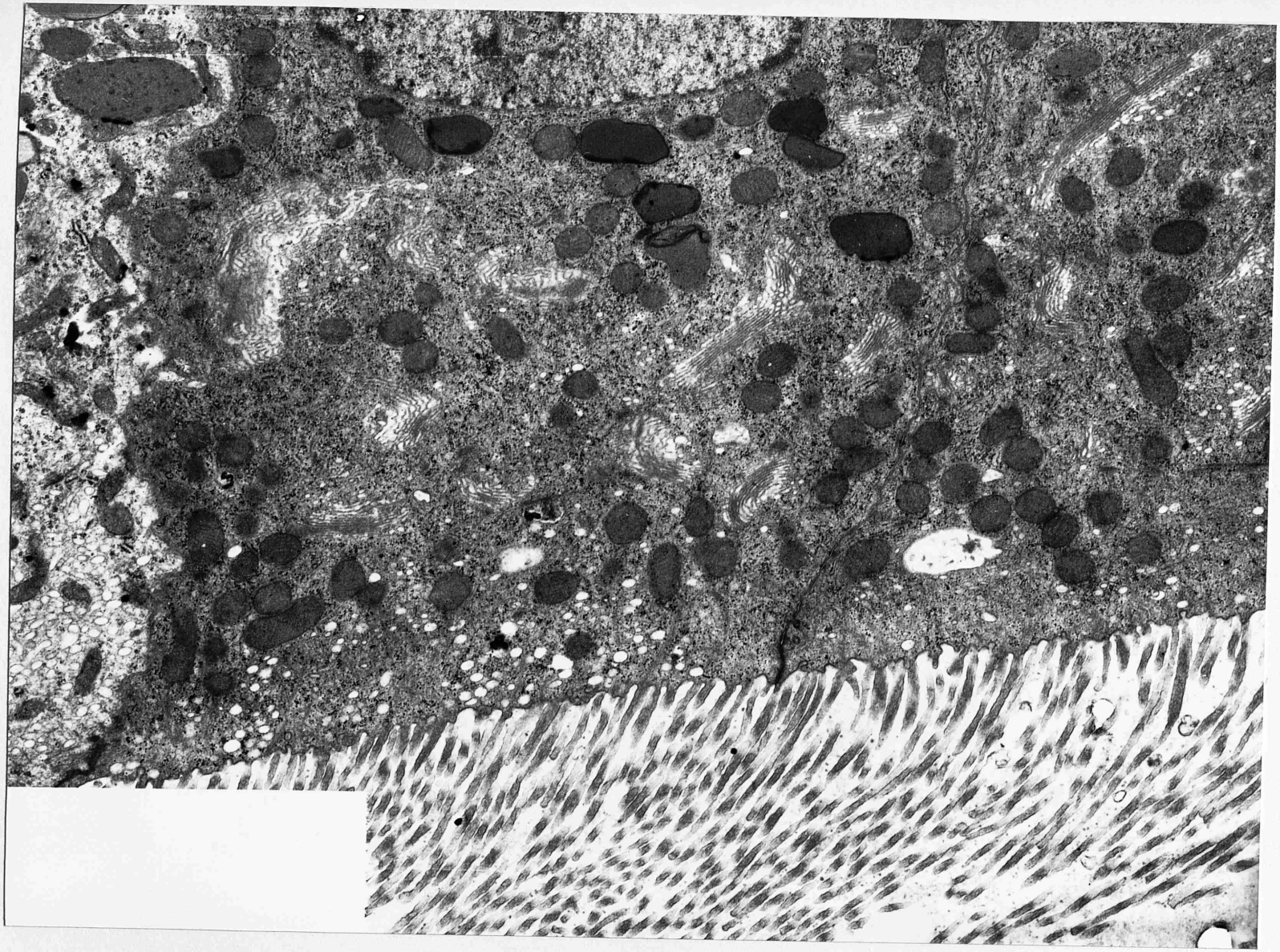


Fig. 48: Electron micrograph of the apical and supranuclear region of the epithelial cells of the cauda epididymidis from a rat, twelve months after vasectomy. Note the numerous vesicles (V), occupied the apical cytoplasm of the principal cells (P), and the extensive Golgi apparatus (G), present in the supranuclear part of the same cell.

(Vas 22 x 21,000)

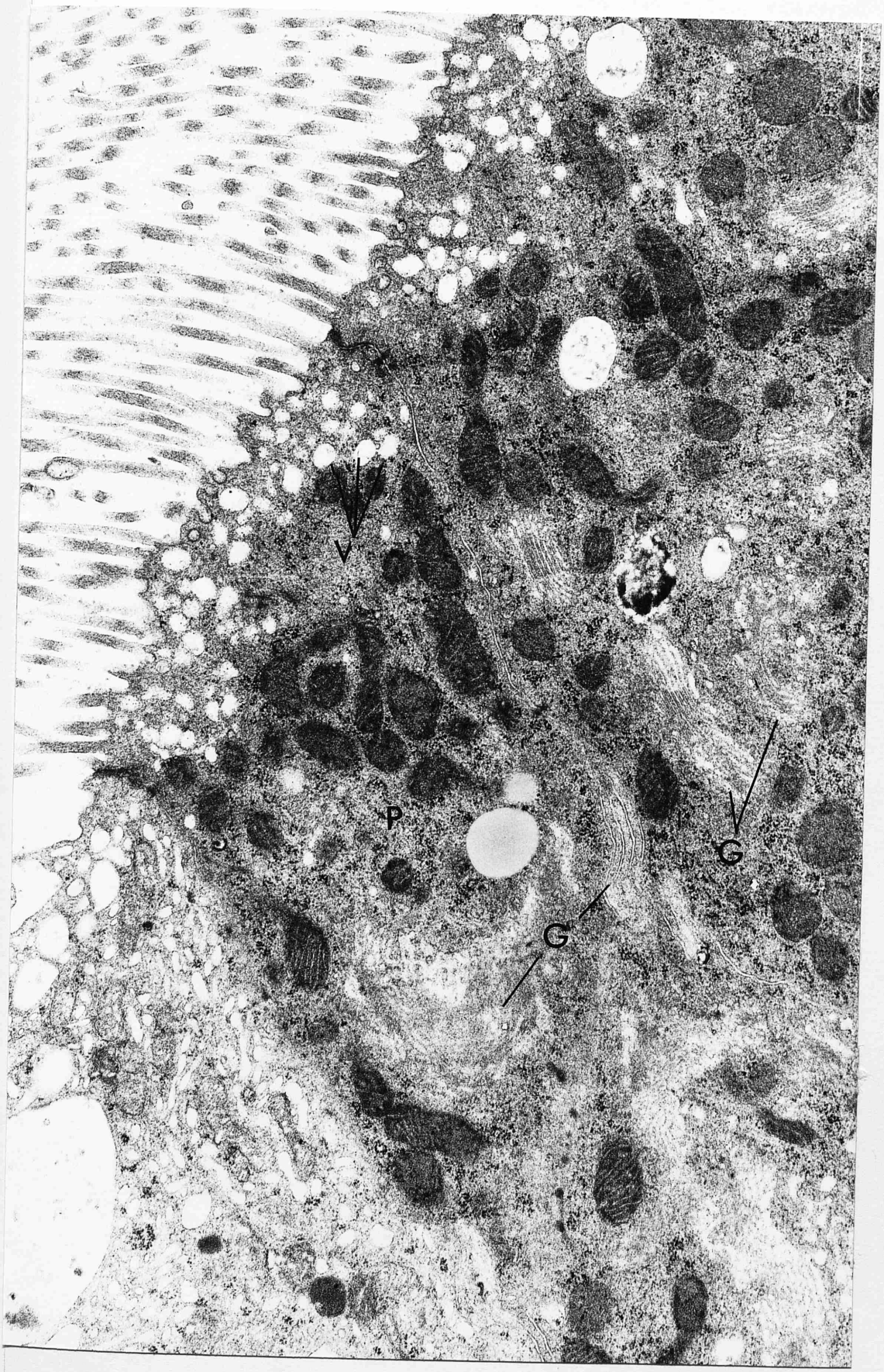

V
P
G
G

Fig. 49: The basal part of the epithelium of the cauda epididymidis of a rat, vasectomized for six weeks. Note the basal cell (B), present between the bases of the principal cells (P) and the basement membrane (M).

(Vas 2 x 16,800)

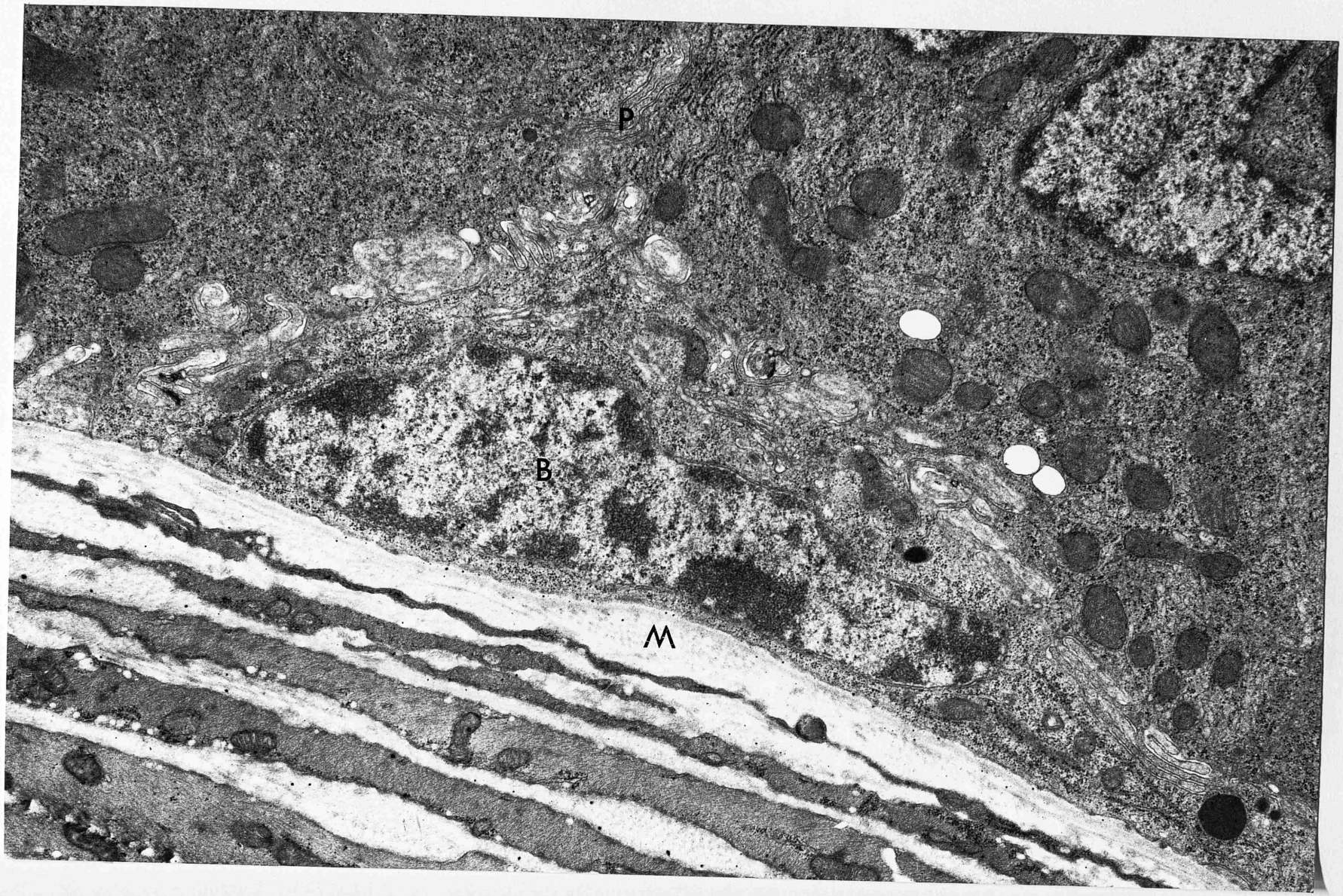

P
B
M

Fig. 50: Electron micrograph of the epididymal epithelium of the cauda of a rat with vasectomy for six weeks. The clear cell (C) showed fewer and shorter microvilli than the principal cells (P). The underlying cytoplasm contains numerous vesicles (V), multivesicular bodies (MVB), and scattered mitochondria. The supranuclear part of the cell exhibit numerous profiles of membrane-limited dense bodies (d).

(Vas 2 x 10,500)

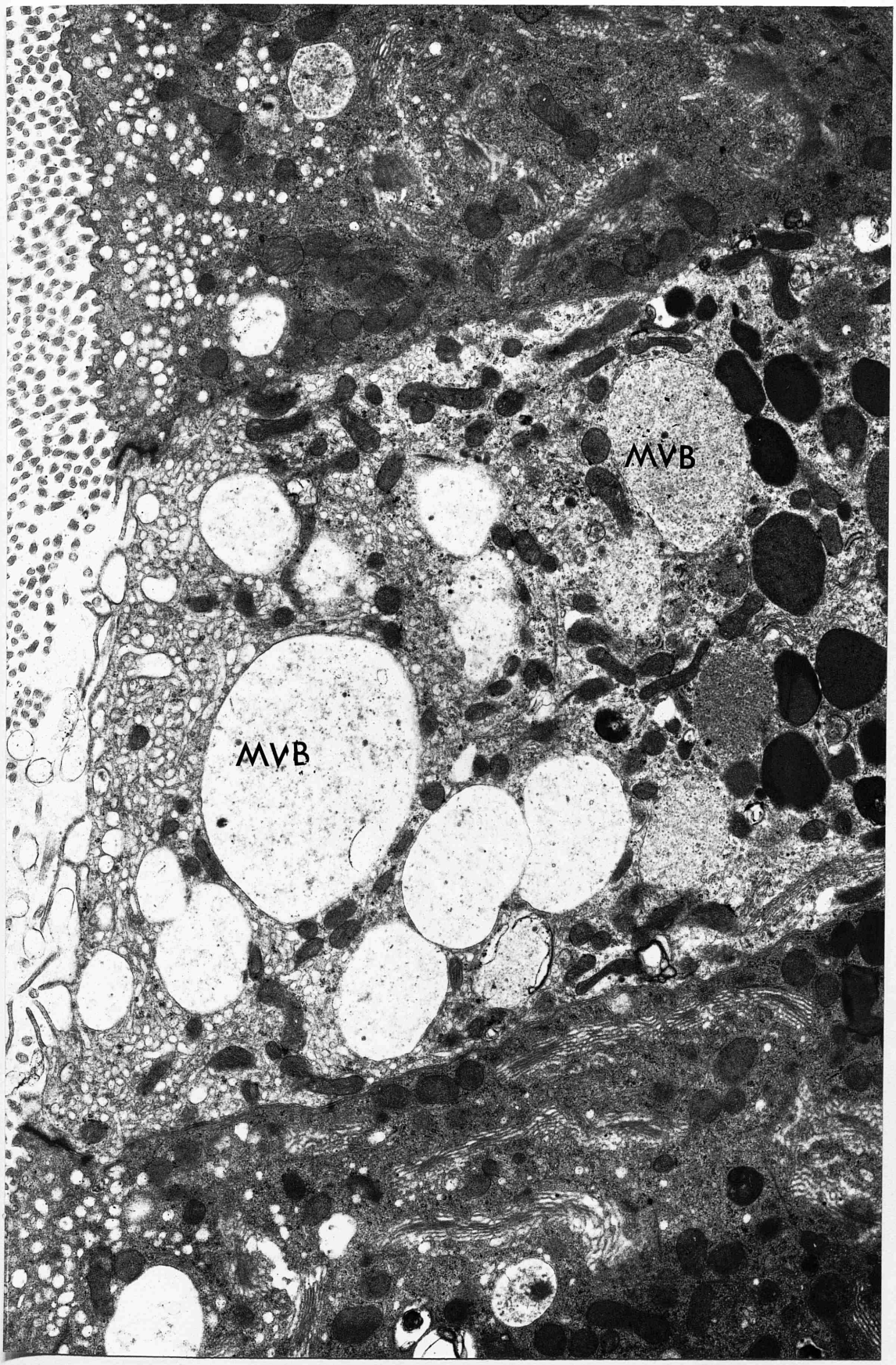

MVB
MVB

Fig. 51: Electron micrograph illustrating the apical part of the epithelium of the cauda epididymidis of a rat, six weeks after vasectomy. Note the long stereocilia.

(Vas 2 x 12,600)

Fig. 52: Electron micrograph of the basal part of the epithelium of the cauda epididymidis from a rat, six weeks after vasectomy. The supra and infra nuclear cytoplasm of the clear cell (C) are filled by membrane-bounded dense bodies. Note the basal cell (B) present between the basal surface of the clear cell and the basement membrane (M).

(Vas 2 x 10,500)

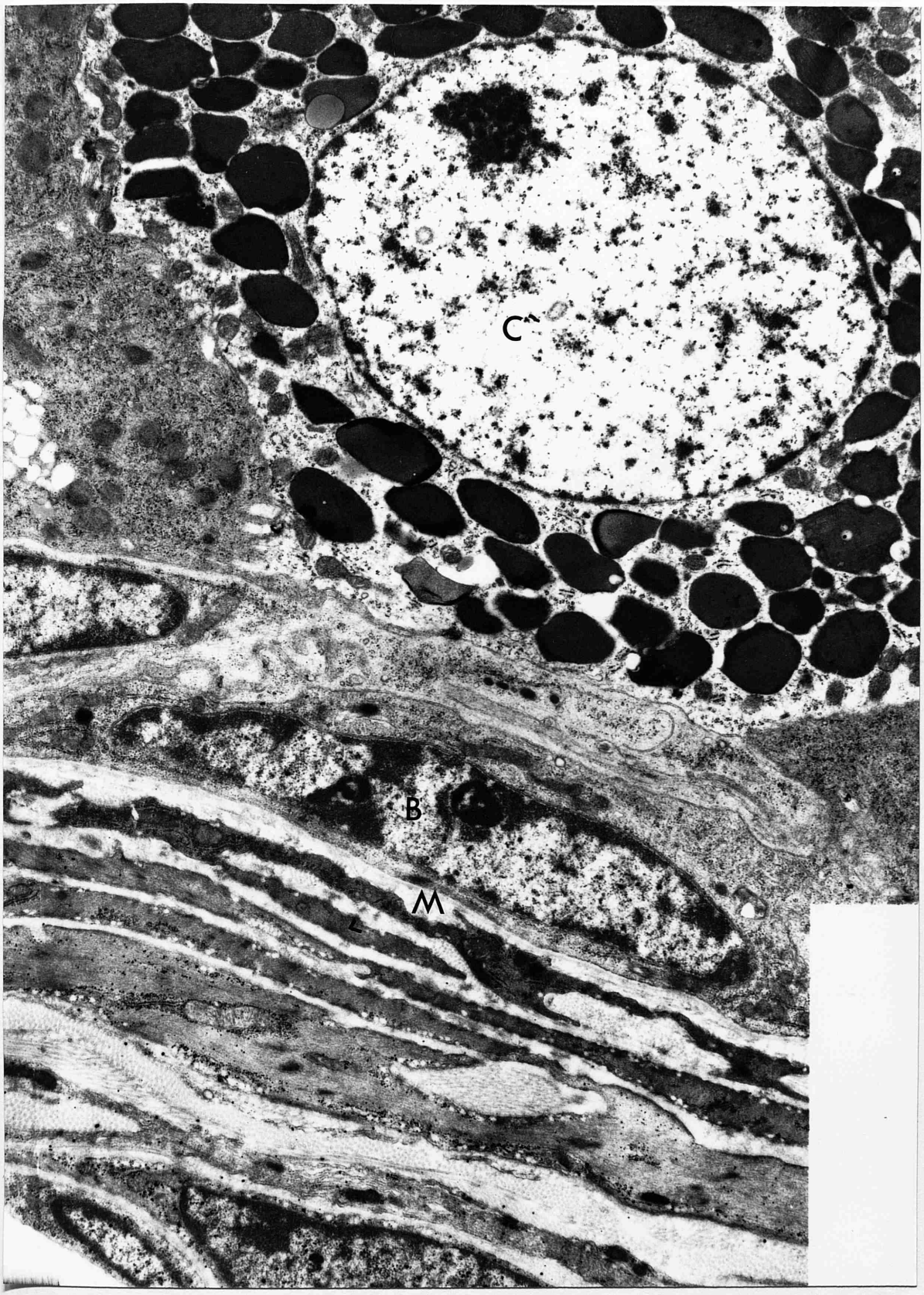

C^
B
M
Z

Fig. 53: An electron micrograph of a clear cell from the cauda epididymidis of a rat, twelve months after vasectomy, showing the various sizes of dense bodies present in the supranuclear region of the cytoplasm. Note the masses of membranes that accumulate in the infranuclear region of the cell.

(Vas 22 x 12,600)

Fig. 54: A low magnification electron micrograph of a clear cell from the cauda epididymidis of a rat, four months after vasectomy. The apical part of the cytoplasm contains many vesicles and multivesicular bodies. The supranuclear, perinuclear and infranuclear regions of the cell are filled by the accumulation of dense bodies. Note that the cytoplasm matrix of clear cells is less dense than the principal cells.

(Vas 6 x 8,400)

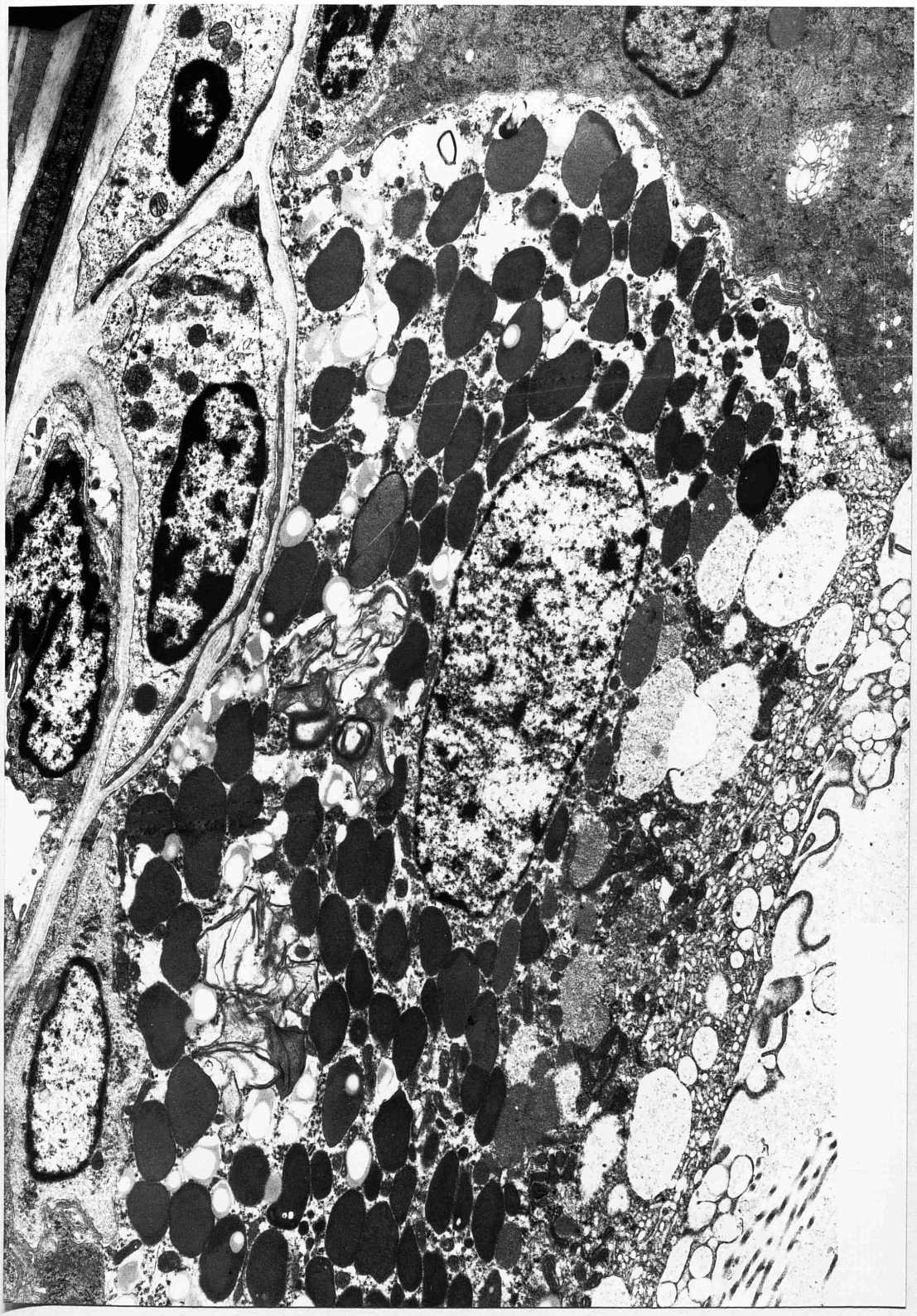


Fig. 55: Electron micrograph of the infranuclear cytoplasm of a clear cell, from the cauda epididymidis of a rat, four months after vasectomy, showing the profile of dense bodies that fill its cytoplasm. Note the membranous masses present at the base of the cell.

(Vas 6 x 21,000)

Fig. 56: Electron micrograph of the basal part of the epithelium of the cauda epididymidis from a rat, six weeks after vasectomy. Note the intraepithelial lymphocyte (L) seen between the clear cell (C) and the principal cell (P).

(Vas 2 x 10,500)

C
P
L

Fig. 57: Electron micrograph of an intraepithelial leucocyte (monocyte) present at the supranuclear region of principal cells from the caput epididymidis of a rat, six weeks after vasectomy. The large nucleus is indented toward the cytocentrum. The cytoplasm contains several dense granules, lysosomes, mitochondria, scattered elements of rough endoplasmic reticulum, and free ribosomes.

(Vas 3 x 12,600)

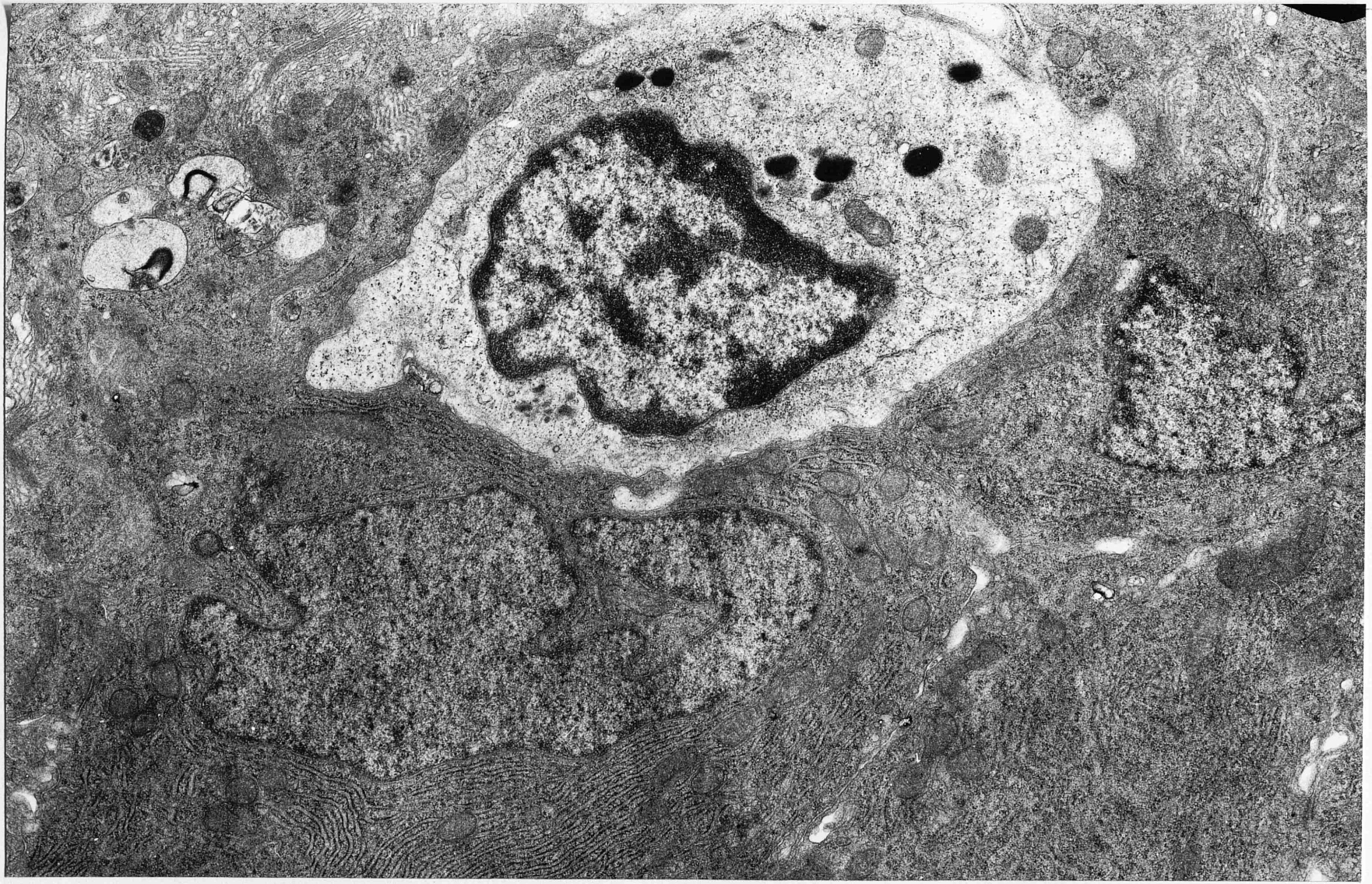


Fig. 58: The intraepithelial leucocyte (monocyte) shown in this electron micrograph at the basal part of an epithelium from the caput epididymidis of a rat, six weeks after vasectomy, exhibits a large horseshoe-shaped nucleus, a multivesicular body, mitochondria, dense bodies, infrequent elements of rough endoplasmic reticulum, and free ribosomes.

(Vas 3 x 12,600)

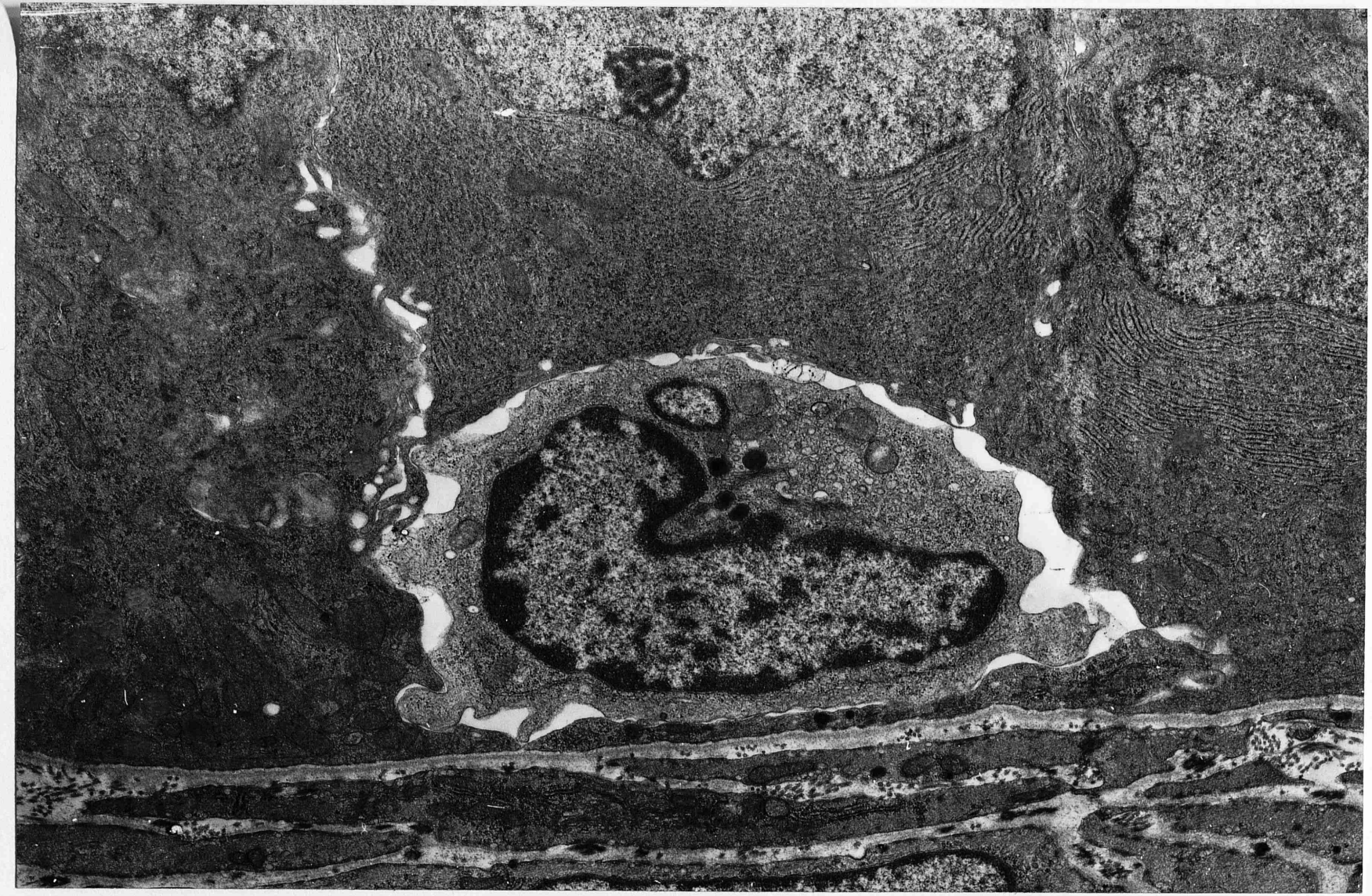


Fig. 59: Electron micrograph showing an intraepithelial leucocyte (monocyte) present in the epithelium of the caput epididymidis of a rat, six months after vasectomy. Note the large horseshoe-shaped nucleus.

(Vas 14 x 21,000)

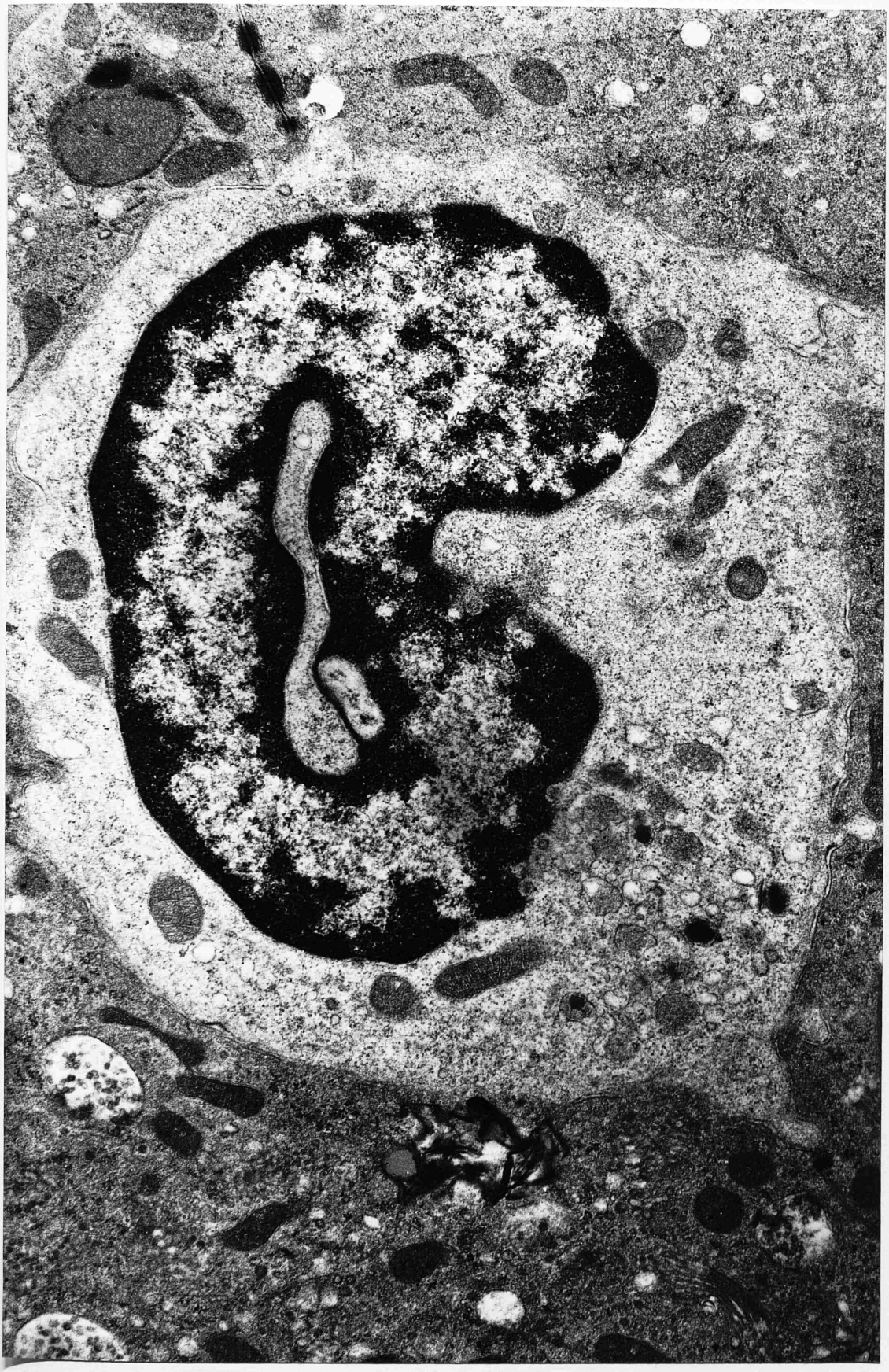


Fig. 60: Electron micrograph of the basal part of an epithelium from the cauda epididymidis of a rat, six weeks after vasectomy. Note the intraepithelial phagocytic cell (ph) present at the basal region of the principal cell (P).

(Vas 2 x 16,800)

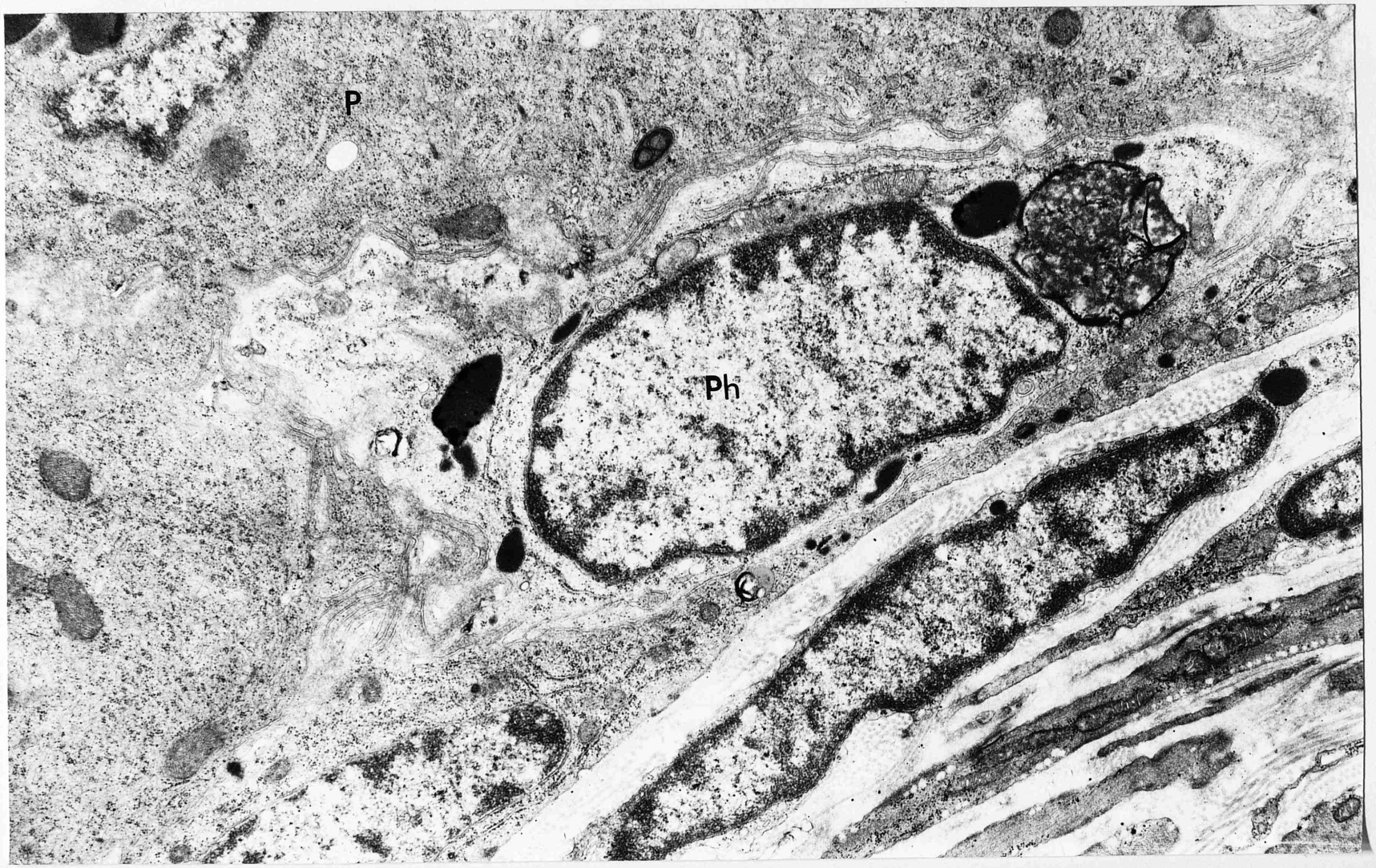

P
Ph

Fig. 61: Electron micrograph of the basal part of epididymal epithelium from a rat caput, twelve months after vasectomy, showing phagocytic cell with many lysosomes, surrounded by rough surfaced endoplasmic reticulum.

(Vas 20 x 16,800)

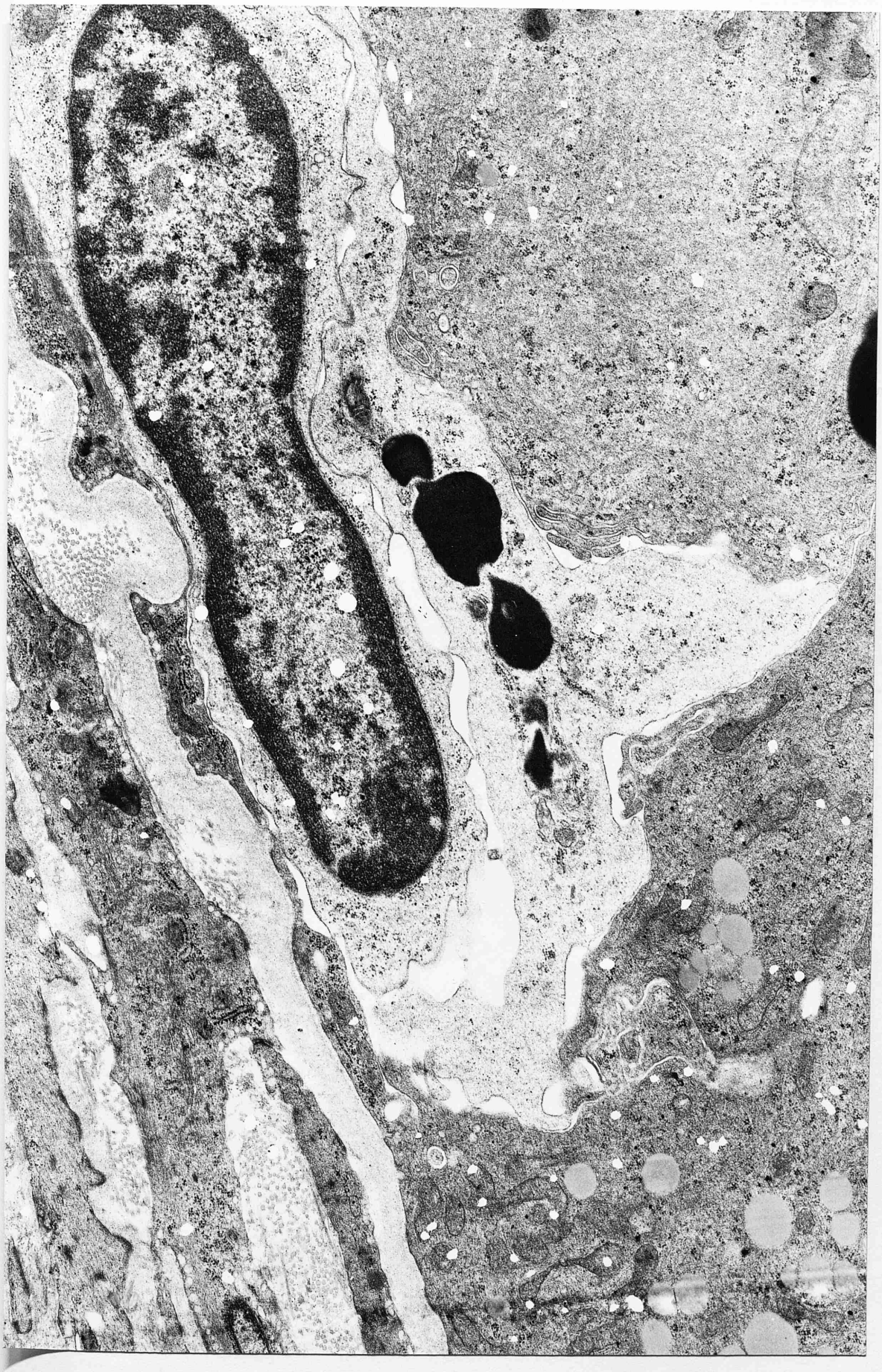


Fig. 62: Sperm in the lumen of the caput epididymidis of a rat, six months after vasectomy. Plasma membrane and mitochondria are intact. Microtubules and coarse fibres of the sperm tails have a normal relation to one another.

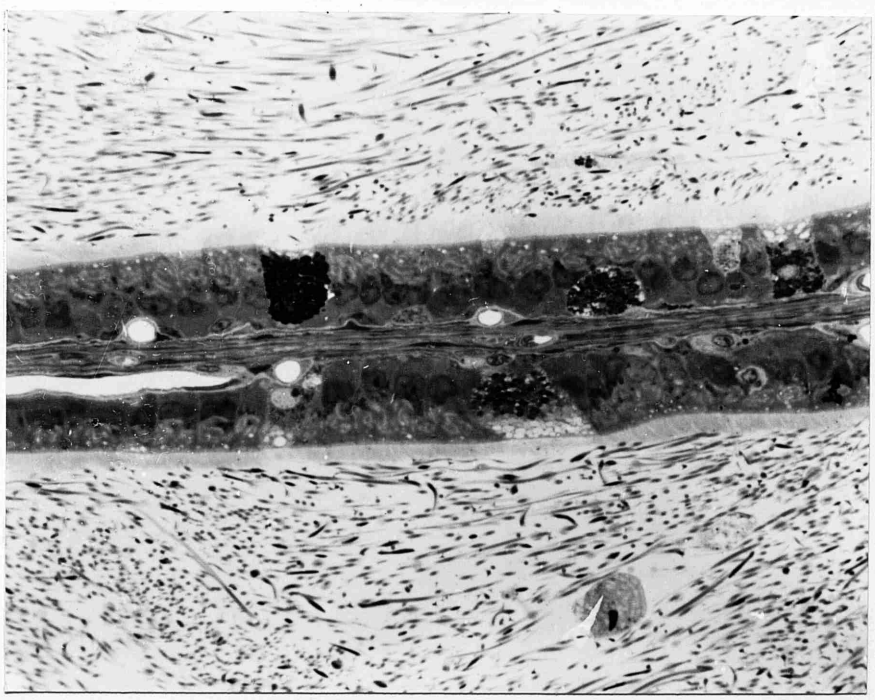
(Vas 14 x 8,400)

Fig. 63: The cauda epididymis from a sham-operated rat, showing a macrophage seen within the lumen of the duct.

(Sham 10 x 500)

Fig. 64: Light micrograph of a tubular profile adjacent to a caput granuloma of a rat, fifteen months after vasectomy. The epithelium surrounds a lumen containing sperm and large phagocytes.

(Vas 30 x 500)



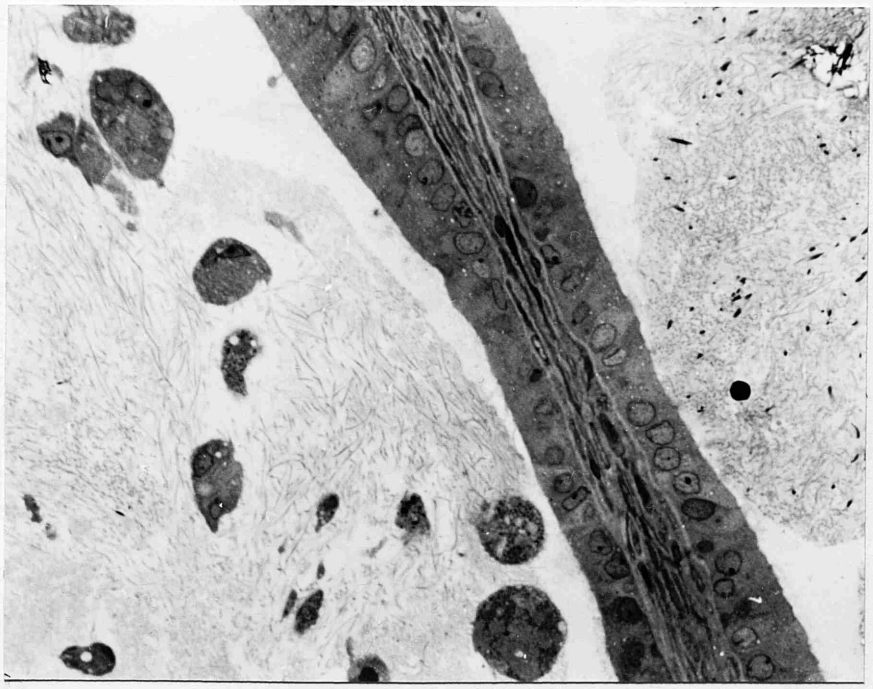
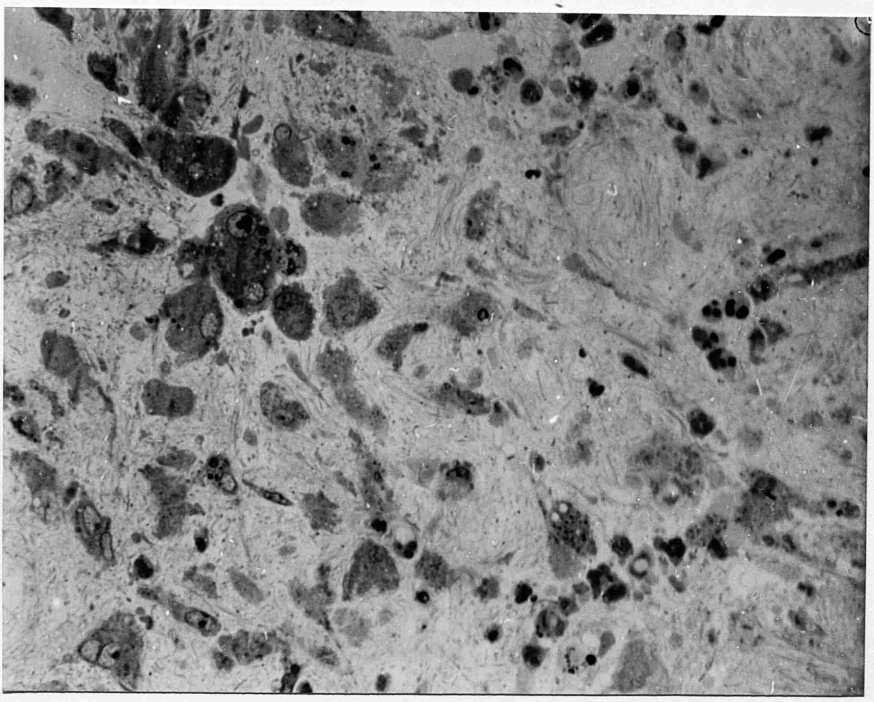


Fig. 65: Numerous macrophages and polymorphonuclear leucocytes are present in the lumina of the duct at the immediate neighbourhood of a caput granuloma from a rat, six months after vasectomy.

(Vas 11 x 500)

Fig. 66: Light micrograph of the cauda epididymidis in the immediate neighbourhood of a caudal granuloma from a rat, twelve months after vasectomy. Note the polymorphonuclear leucocytes, macrophages, and multinucleated giant cells, seen within the lumen of the duct.

(Vas 21 x 440)



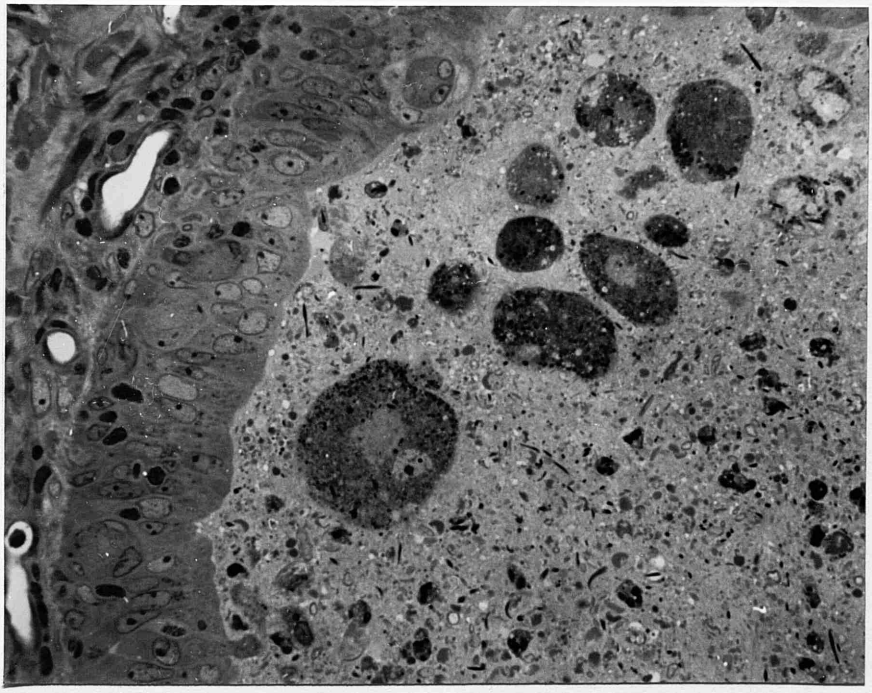
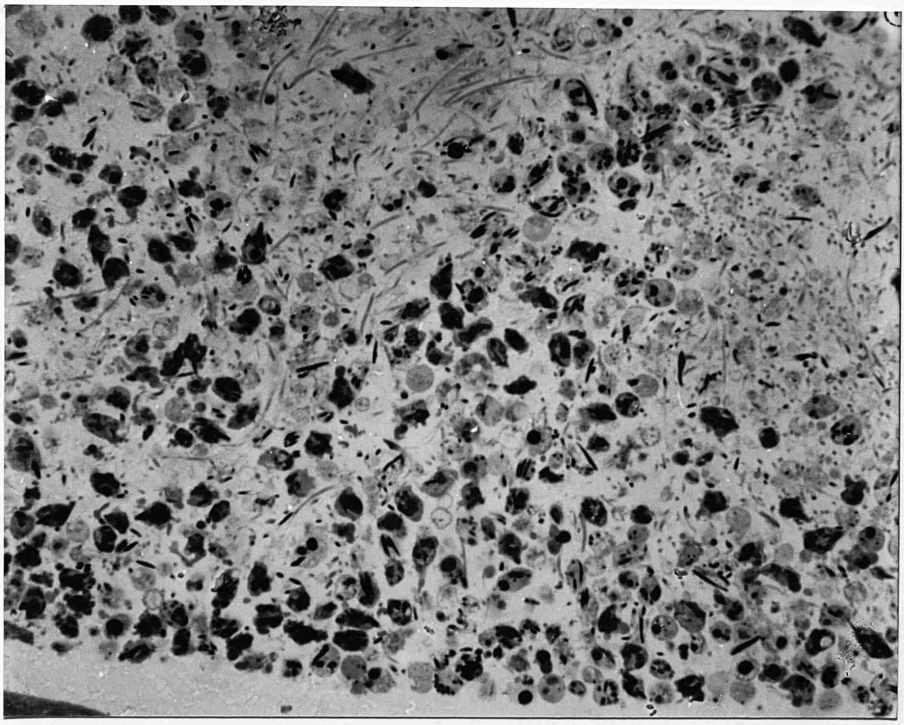


Fig. 67: Light micrograph of the lumen of the epididymal duct, adjacent to a caudal granuloma of a rat, four months after vasectomy. Note the large number of polymorphonuclear leucocytes, with numerous sperm heads and tails in the cytoplasm.

(Vas 9 x 500)

Fig. 68: Electron micrograph of a large phagocyte found within the lumen of the epididymal duct in the immediate neighbourhood of the caudal granuloma from a rat, twelve months after vasectomy. Note the head of sperm present within the cytoplasm of the cell.

(Vas 21 x 7000)



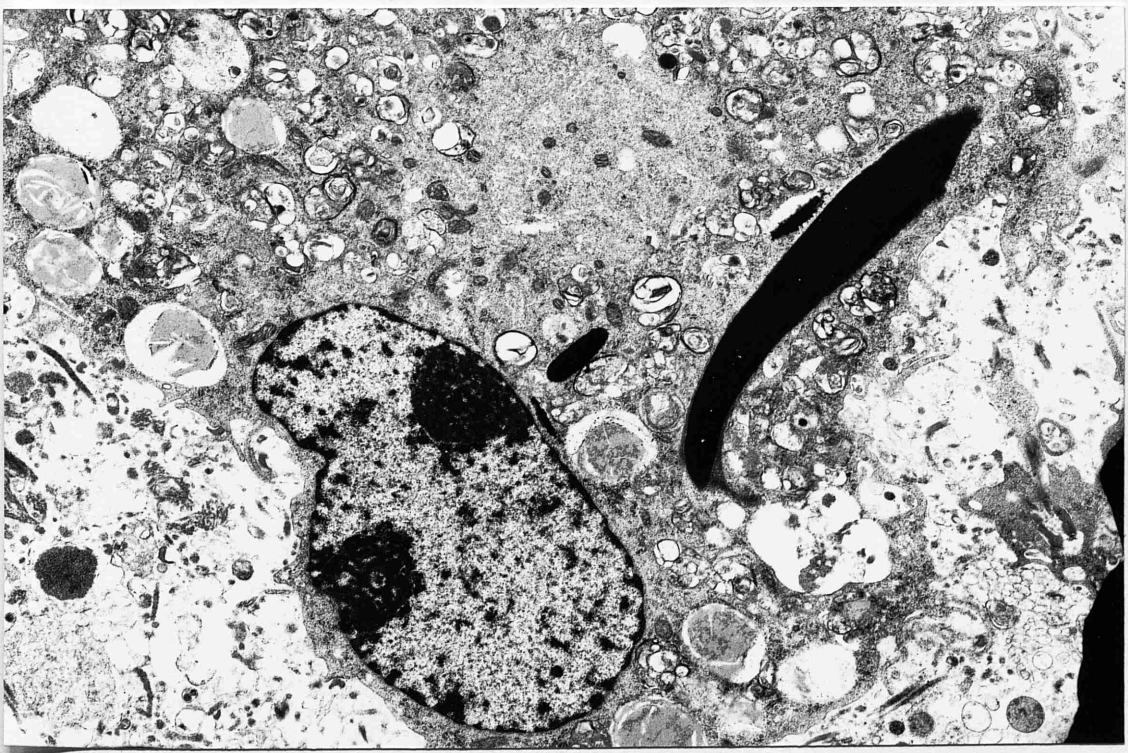
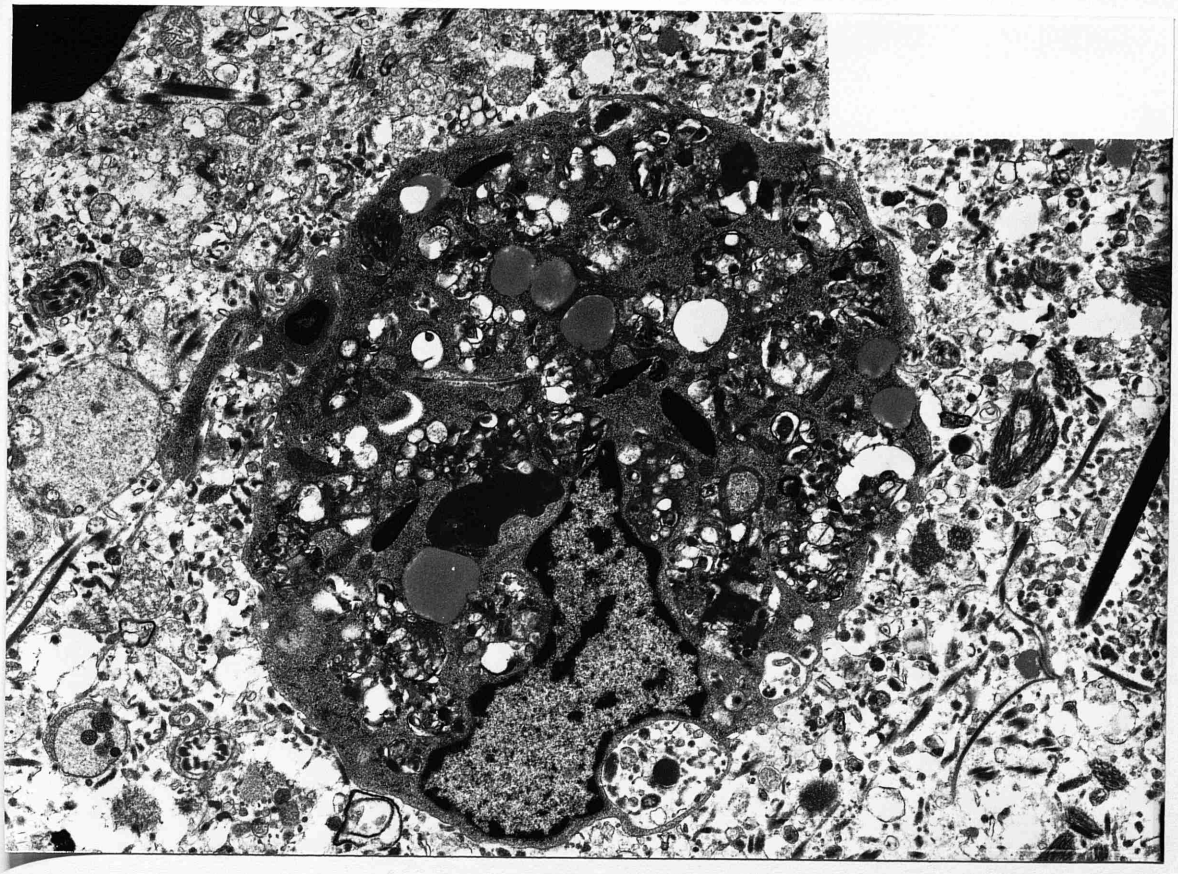


Fig. 69: Electron micrograph of a macrophage present within the lumen of the tubular profiles adjacent to a spermatic granuloma of the cauda epididymidis from a rat, twelve months after vasectomy.

(Vas 21 x 7000)

Fig. 70: Electron micrograph of a polymorphonuclear leucocyte, found within the lumen of the tubular profiles, adjacent to a spermatic granuloma of the cauda from a rat, twelve months after vasectomy. Portions of sperm heads and tails lie within the cytoplasm.

(Vas 21 x 14000)



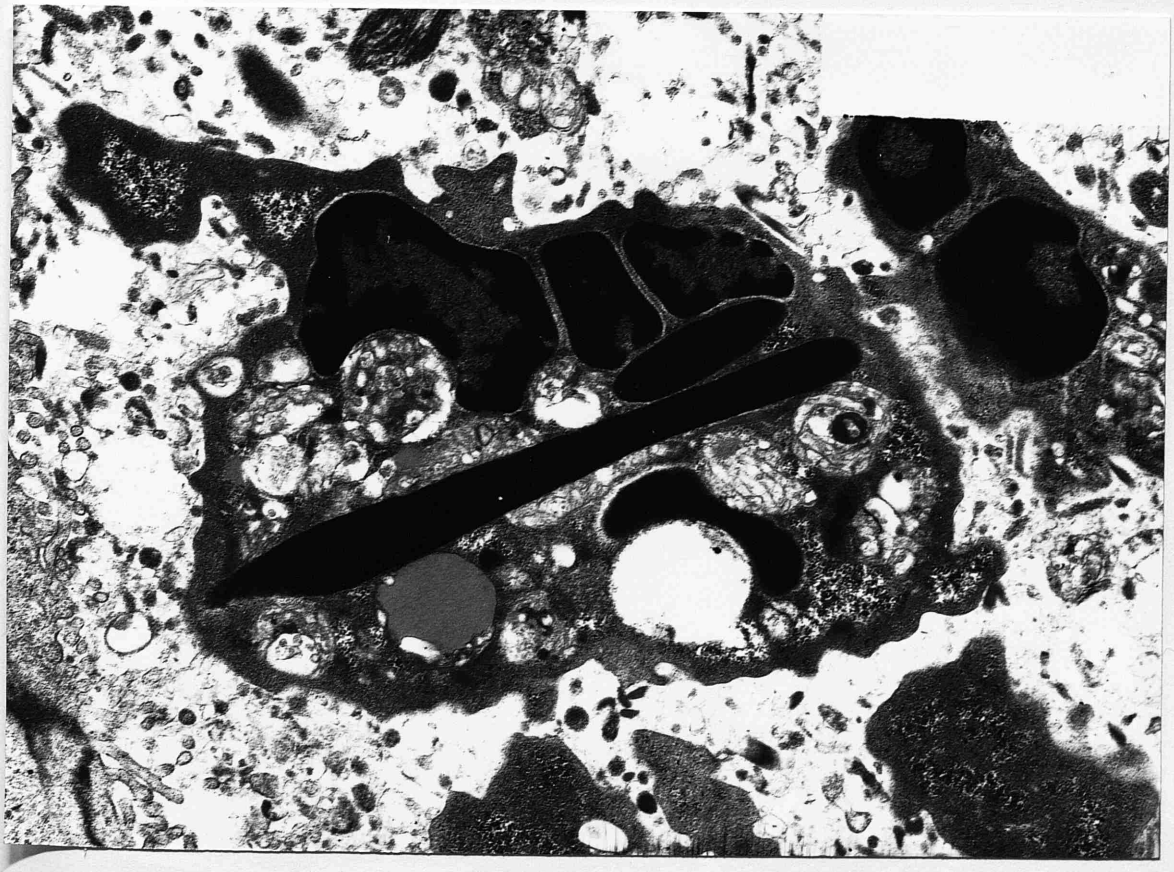
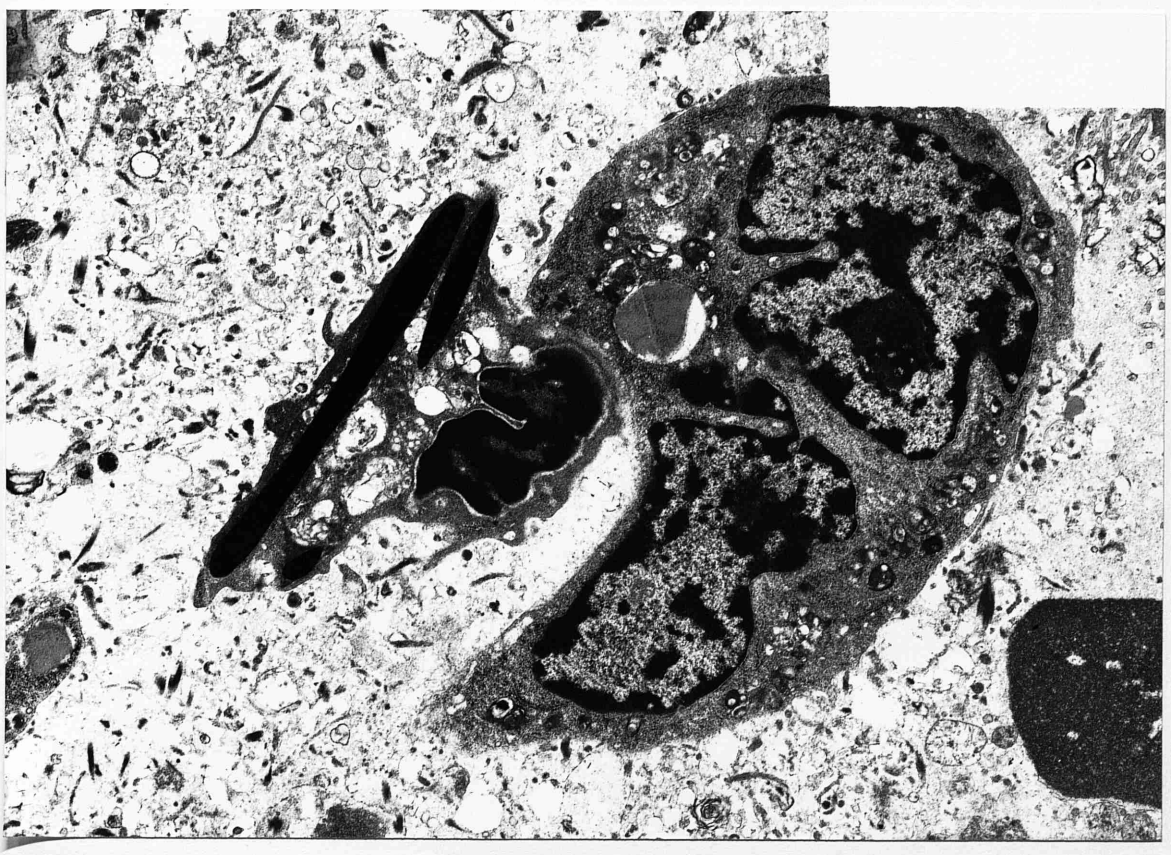


Fig. 71: Electron micrograph of a polymorphonuclear leucocyte and macrophage in the lumen of a tubular profile adjacent to a caudal granuloma of a rat, twelve months after vasectomy. Note the sperm fragments present within the cytoplasm of the polymorphonuclear leucocyte.

(Vas 21 x 7000)

Fig. 72: This light micrograph of the cauda epididymis of a rat four months after vasectomy, showing thinning and disruption in the epithelial lining of the duct adjacent to a spermatic granuloma, with massive intraluminal accumulation of spermophages.

(Vas 9 x 440)



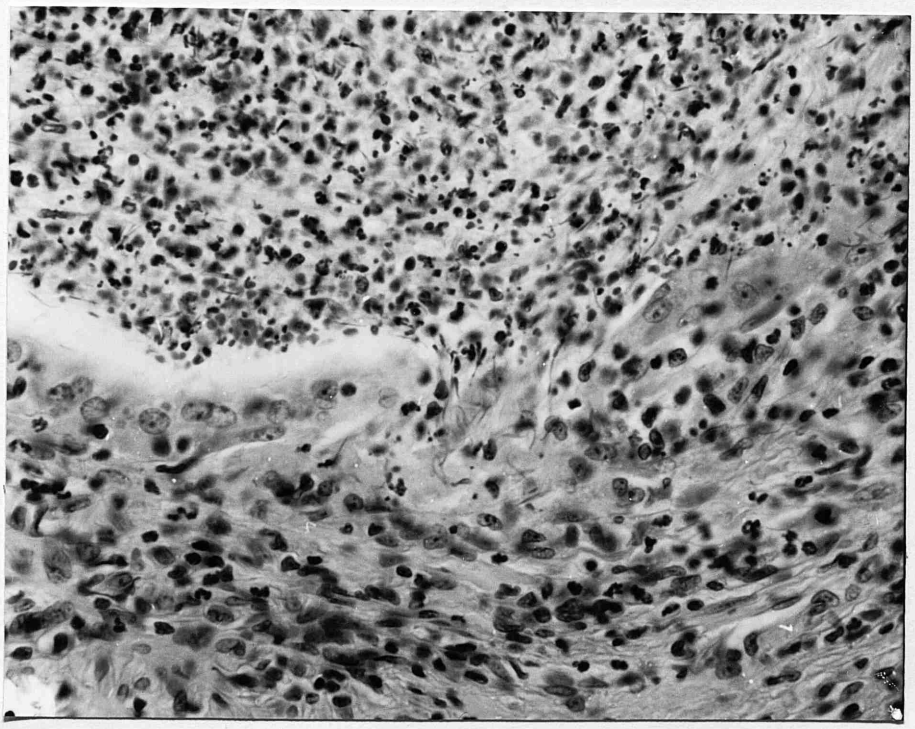


Fig. 73: Medium power light micrograph of the caput epididymis of a rat eighteen months after vasectomy, showing severe disruption in the wall of one of the sectional profiles of the duct adjacent to a spermatic granuloma.

(Vas 32 x 252)

Fig. 74: Sectional profile of an epididymal duct adjacent to a caput granuloma from a rat eighteen months after vasectomy. The lumen was infiltrated by small and large phagocytes. Only small portion of the lining epithelium was left intact, the remaining part was disrupted.

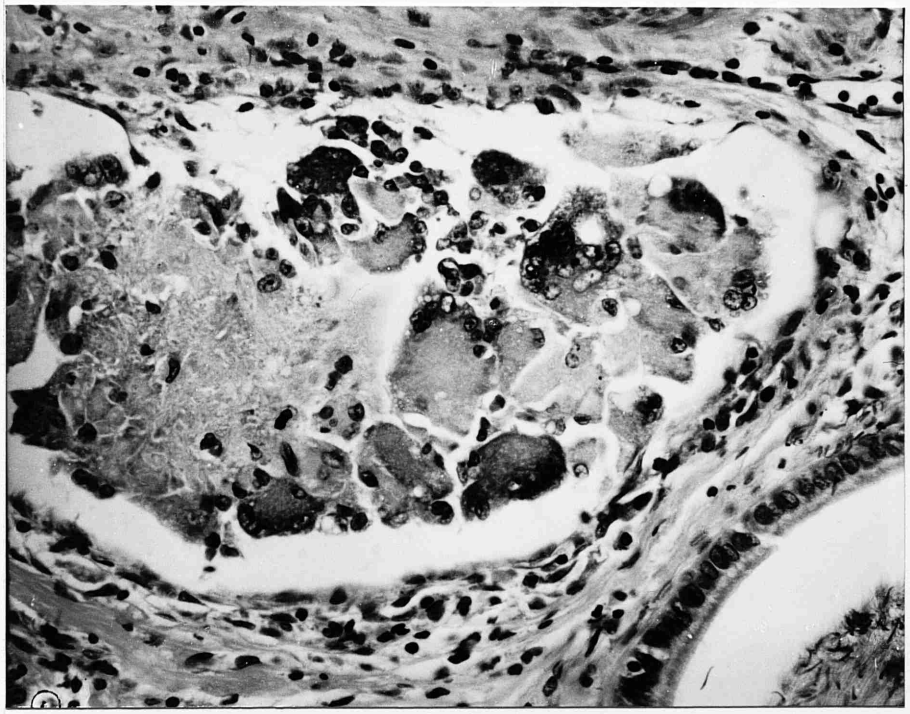
(Vas 32 x 260)

Fig. 75: Sectional profile of an epididymal duct adjacent to a spermatic granuloma of the caput, from a rat eighteen months after vasectomy, showing complete disruption of the lining epithelium. The luminal sperm were surrounded by giant cells.

(Vas 32 x 320)

Fig. 76: The epididymal epithelium of all vasectomized animals with ipsi-lateral testicular atrophy remained normal, but the lumen appeared empty of sperm.

(Vas 19 x 500)



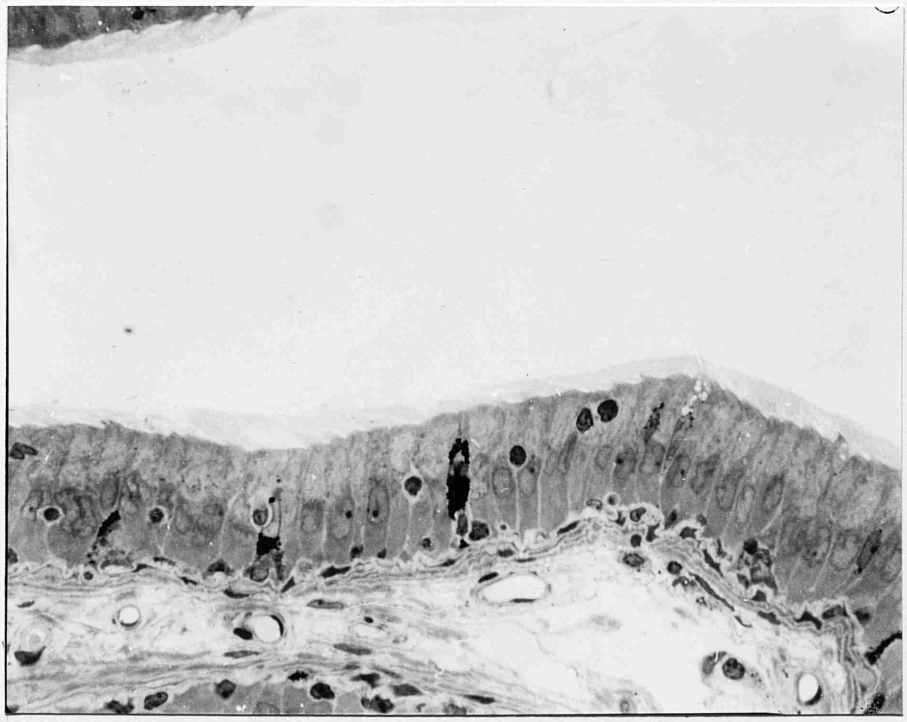


Fig. 77: Micrograph of an epididymal duct at the region of the caput, from a rat with bilateral testicular atrophy, twelve months after vasectomy, showing empty lumen except for few sloughed testicular cells.

(Vas 21 x 500)

Fig. 78: Section through the caput epididymis of a rat with a caput granuloma fifteen months after vasectomy, showing some tubular profiles with a completely empty lumen. Others, nearer to the testis, were engorged with sperm.

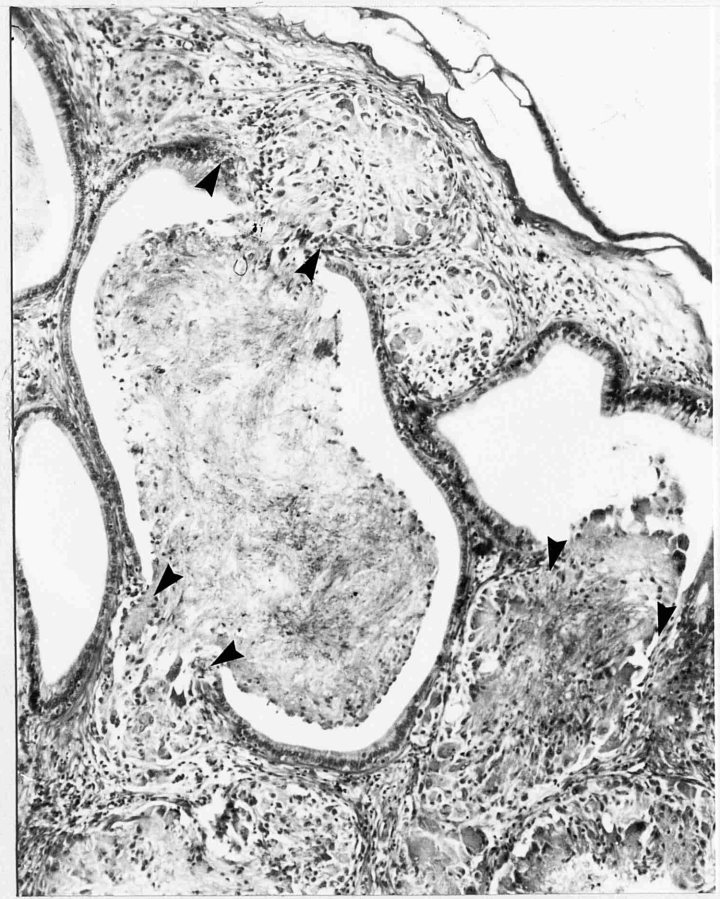
(Vas 30 x 50)

Fig. 79: An epididymal duct proximal to a caput granuloma from a rat six months after vasectomy, showing signs of obstruction including distension with flattening in its epithelial lining.

(Vas 11 x 312.5)

Fig. 80: This micrograph at the caput epididymis of a rat eighteen months after vasectomy, showing several ruptures (arrows) in the wall of the duct with extravasation of spermatozoa into the adjacent connective tissue and new granulomatous formation.

(Vas 32 x 95)



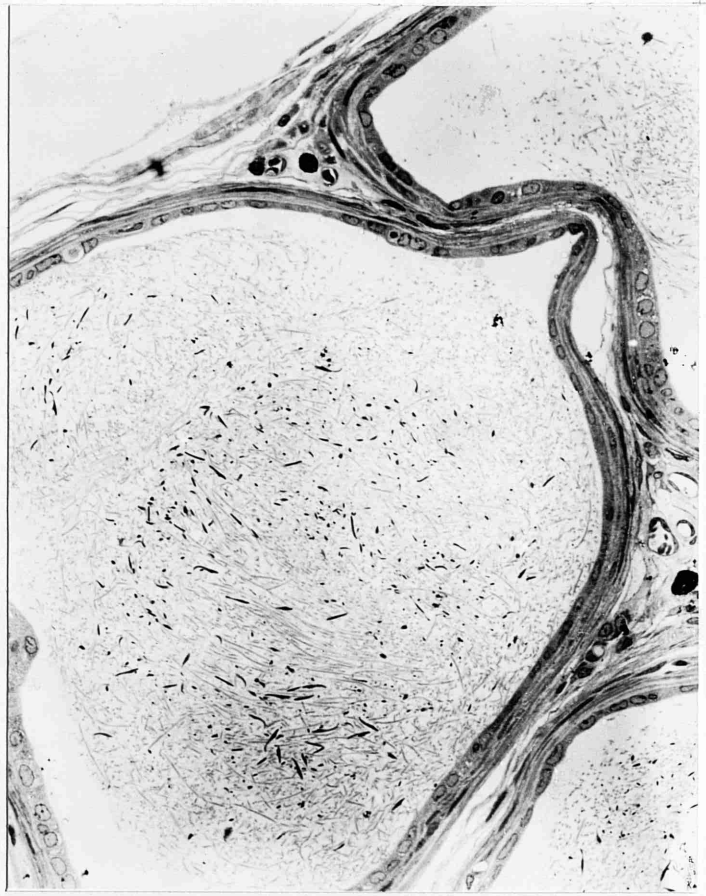


Fig. 81: Longitudinal section through the caput and part of corpus epididymidis of a rat eighteen months after vasectomy showing the lumen of that part nearer to the testis filled with sperm while the distal part was empty. Note the granulomatous lesions developed at the mid caput region (G1) and corpus (G2).

(Vas 32 x 12.5)

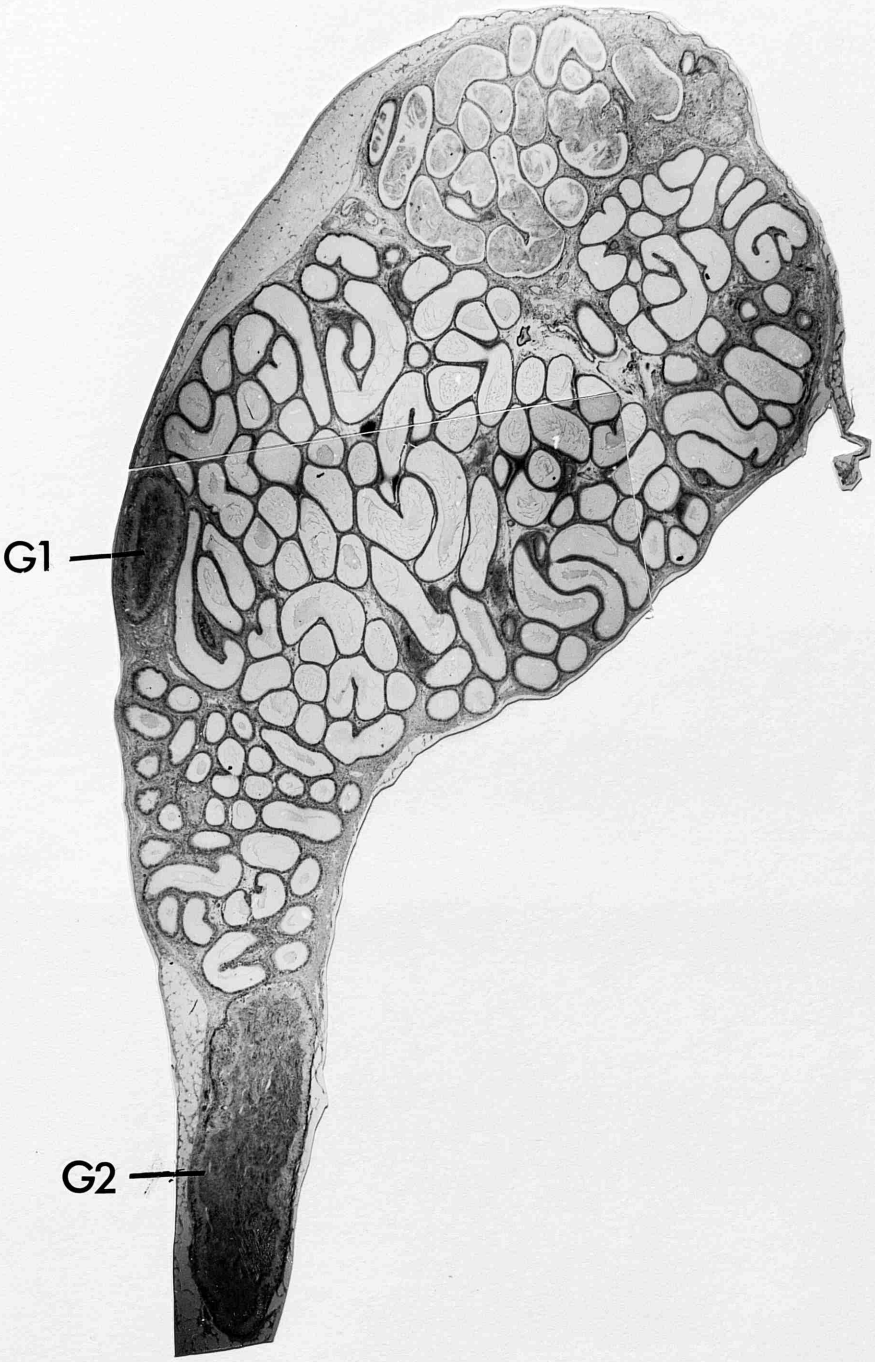
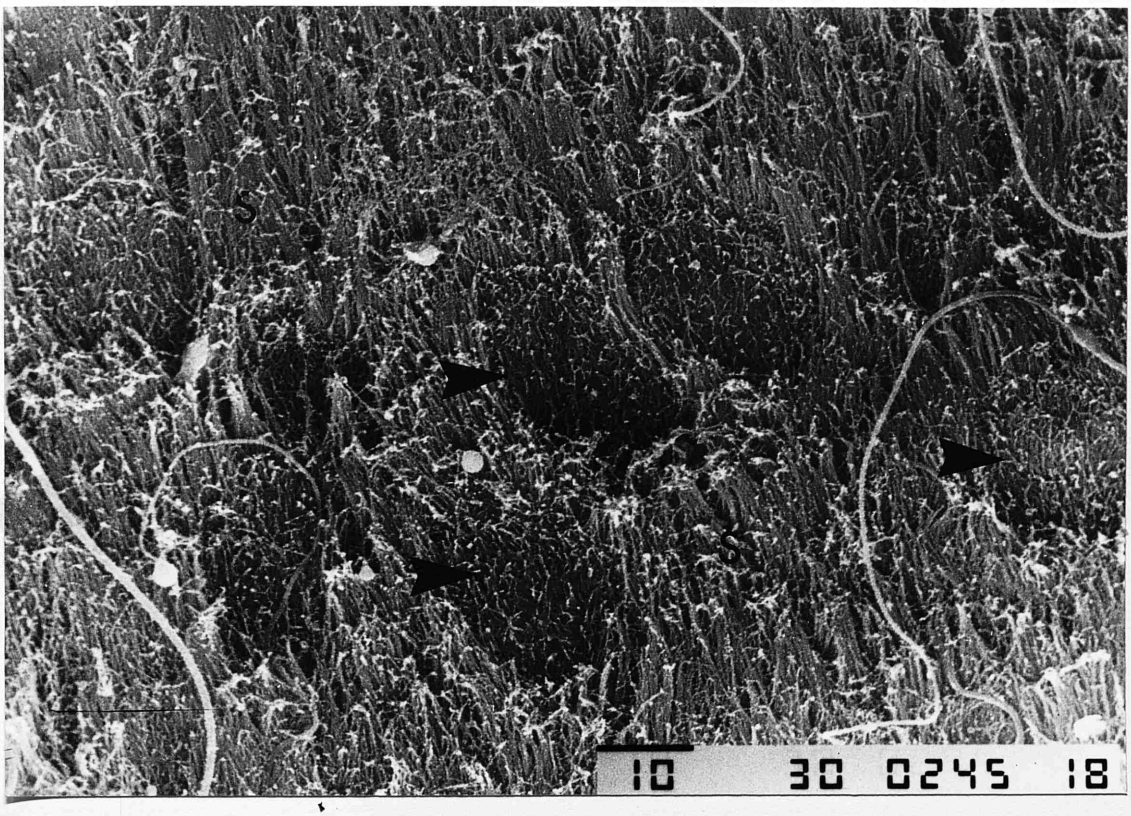

G1
G2

Fig. 82: The epididymal epithelium at zone III, seen by scanning electron microscope. The principal cells exhibited numerous long stereocilia (S), which cover its luminal surfaces. On the contrary, the clear cells (arrows) had fewer and shorter microvilli.

(Sham 42 x 1300)

Fig. 83: The luminal surface of the epithelium of the cauda epididymidis as seen with the scanning electron microscope. Note the large number of clear cells (arrows), scattered throughout the epithelium.

(Sham 42 x 650)


10 30 0245 18

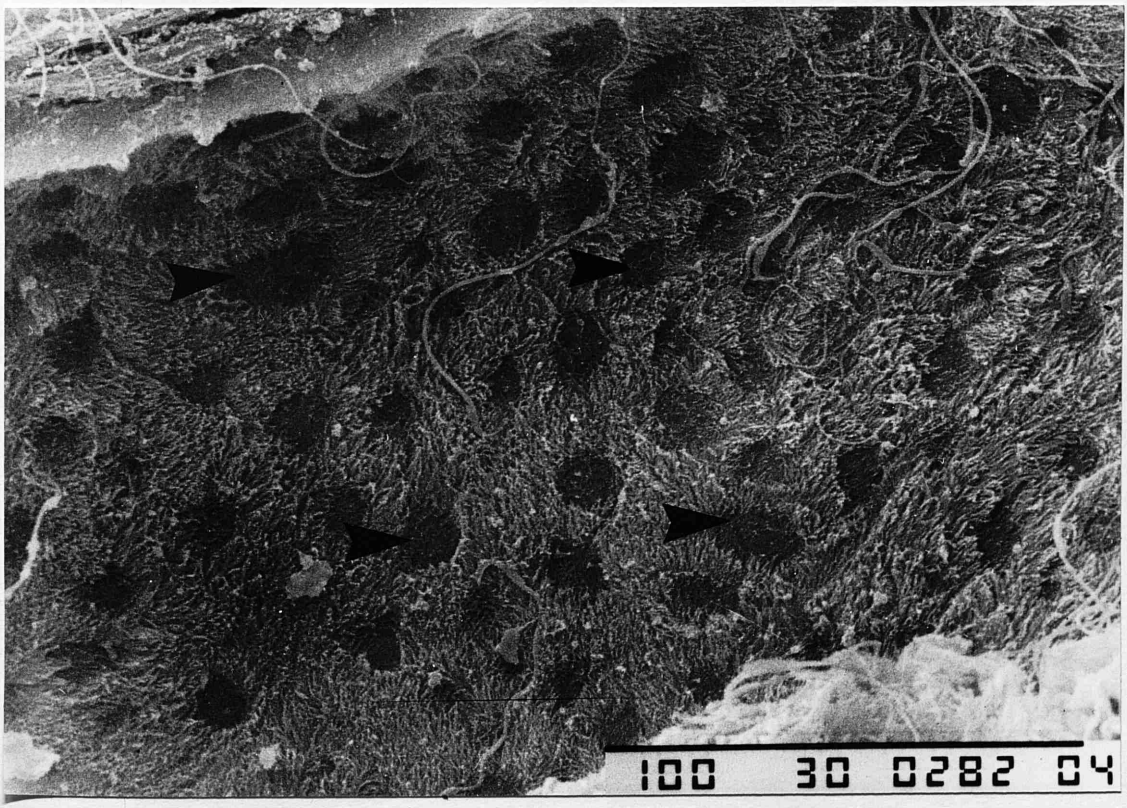
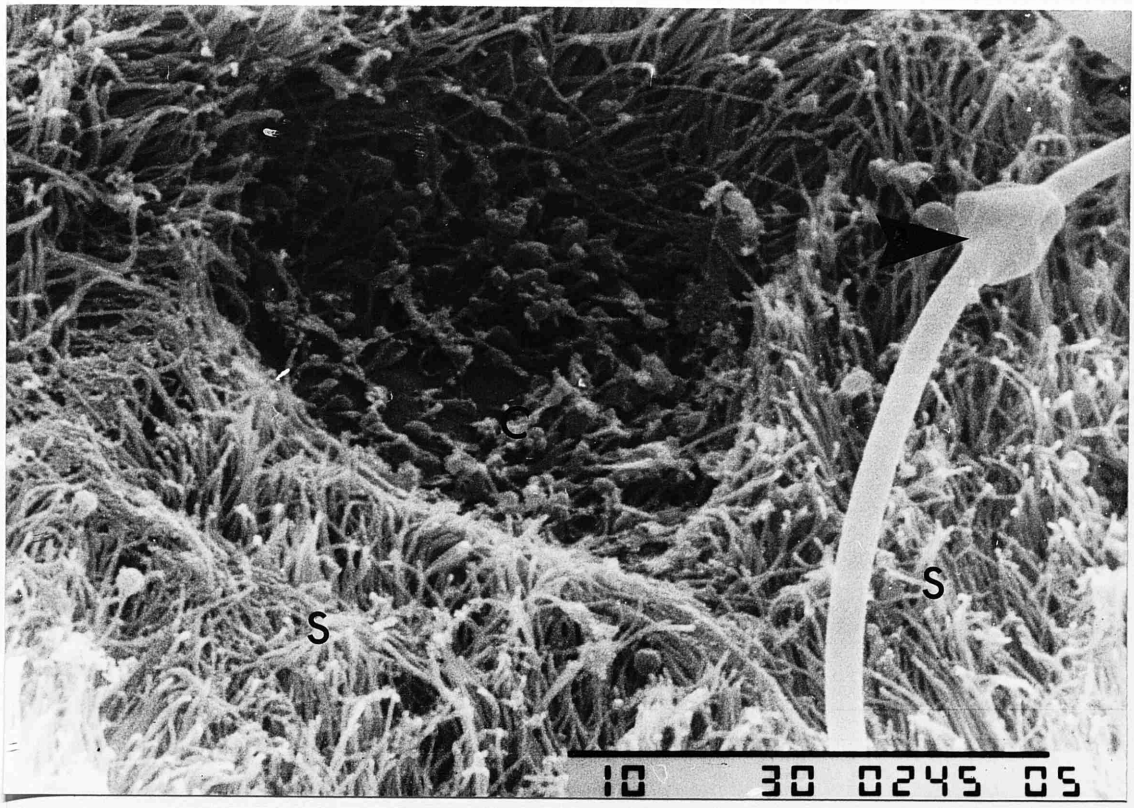

100 30 0282 04

Fig. 84: High magnification scanning electron micrograph of a clear cell (C), in the cauda epididymidis. The luminal surface was below the normal epithelial surfaces, and exhibited less numerous and shorter microvilli than principal cells. The stereocilia (S) which cover the principal cells appeared relatively shorter than those seen in the caput region. Note the spermatozoa present with its cytoplasmic droplet (arrow) on the surface of the principal cell.

(Sham 41 x 6500)

Fig. 85: This high magnification scanning electron micrograph, showing a clear cell (arrows) in the cauda epididymidis, with a dome-like surface that raised above the adjacent principal cells surfaces.

(Sham 43 x 6500)


C
S
S
10 30 0245 05

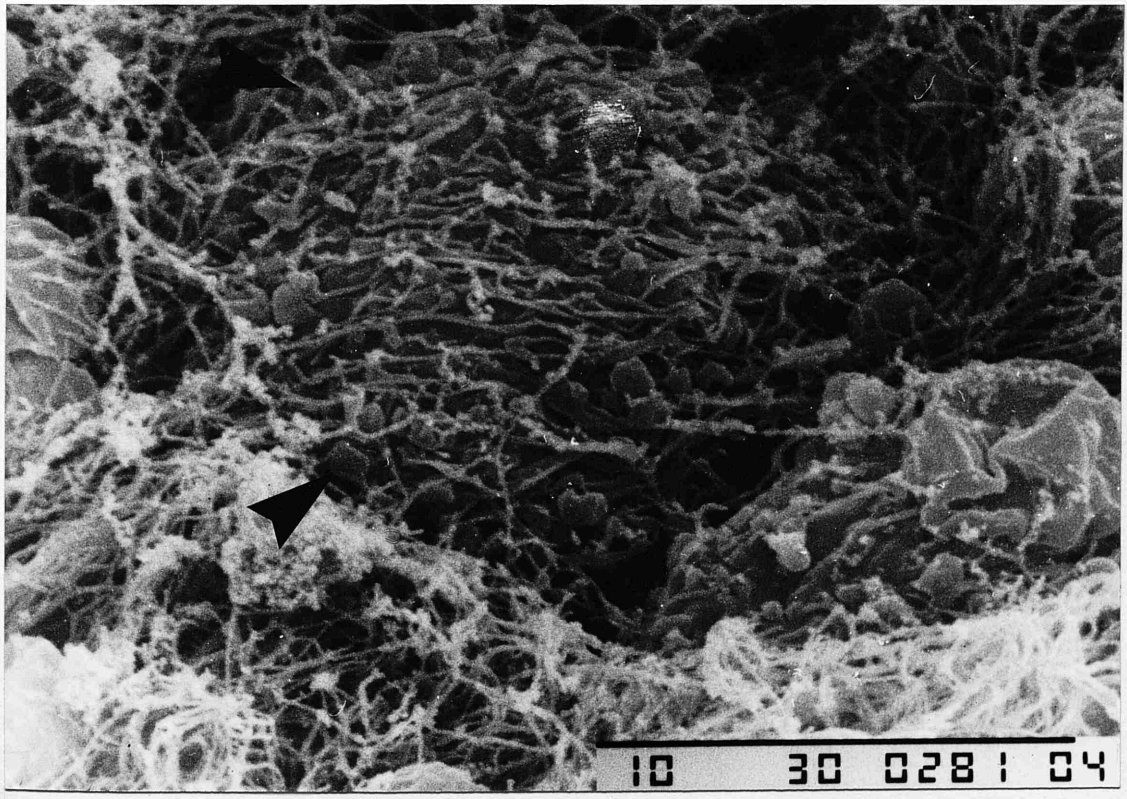
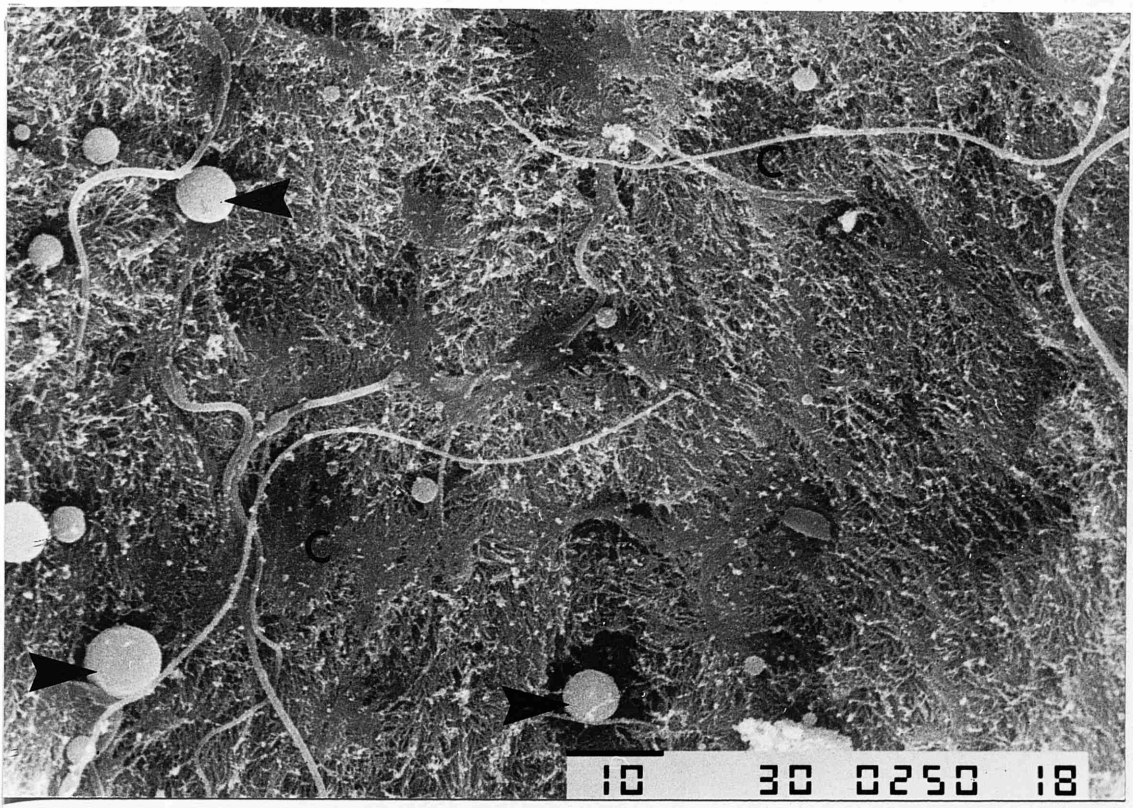

10 30 0281 04

Fig. 86: SEM of the epithelial surface of Zone III of the epididymal duct. Several clear cells (C) were scattered throughout the epithelium. Note the various size of secretory droplets (arrows) lying on the surface.

(Sham 40 x 800)

Fig. 87: Intact rat spermatozoa seen with the scanning electron microscope.

(Sham 41 x 6500)


10 30 0250 18

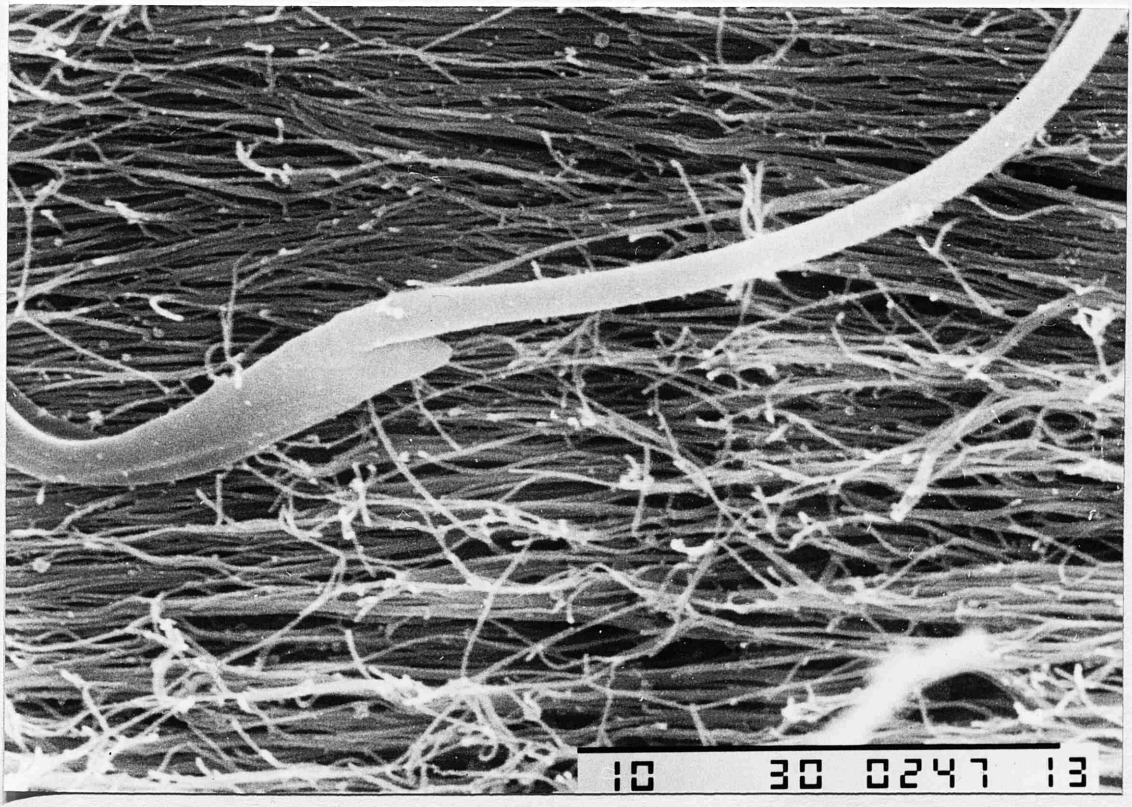
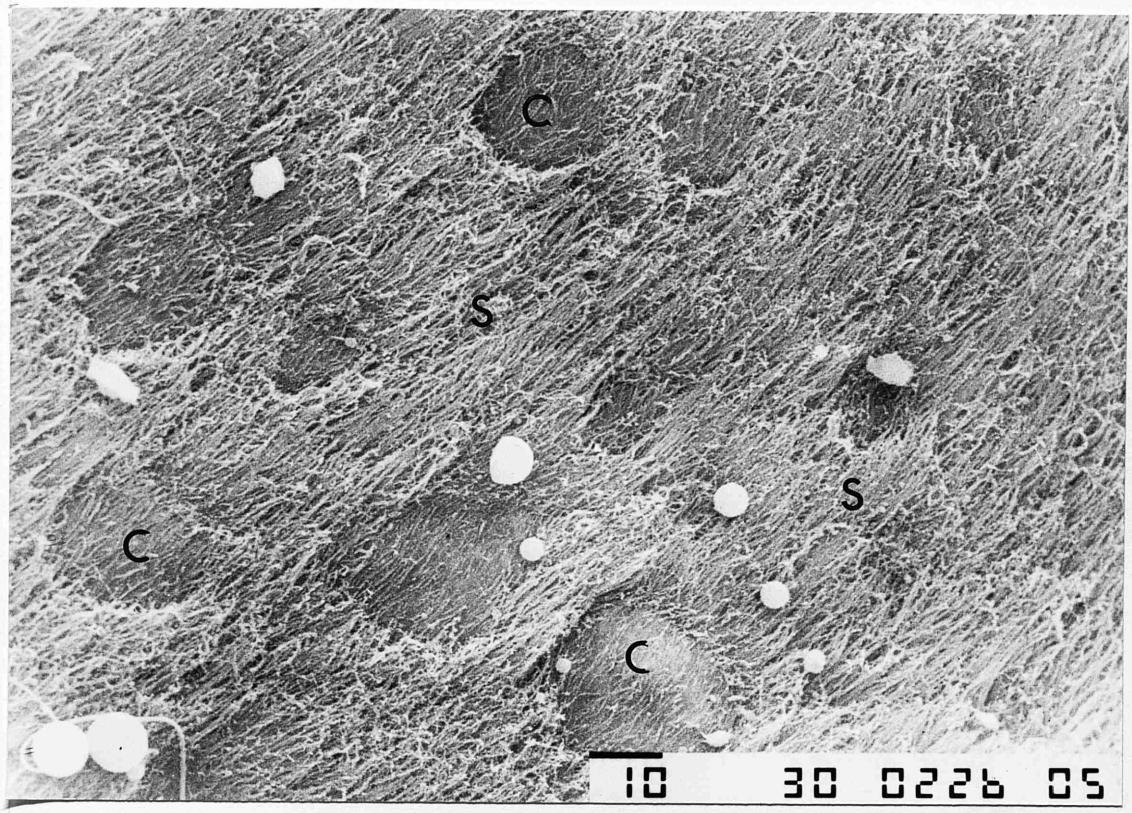

10 30 0247 13

Fig. 88: SEM of the luminal surface of the epididymal epithelium at Zone III from a rat one week after vasectomy. The principal cells were covered by long stereocilia (S); several clear cells (C) were scattered throughout the epithelium. Variable sizes of secretory droplets (arrows) were also seen.

(Vas 39 x 500)

Fig. 89: SEM of a clear cell (C) at Zone IV of the epididymis one week after vasectomy. Note the long stereocilia (S) covering the surrounding surfaces of principal cells.

(Vas 39 x 4500)


C
S
S
C
C
10 30 0226 05

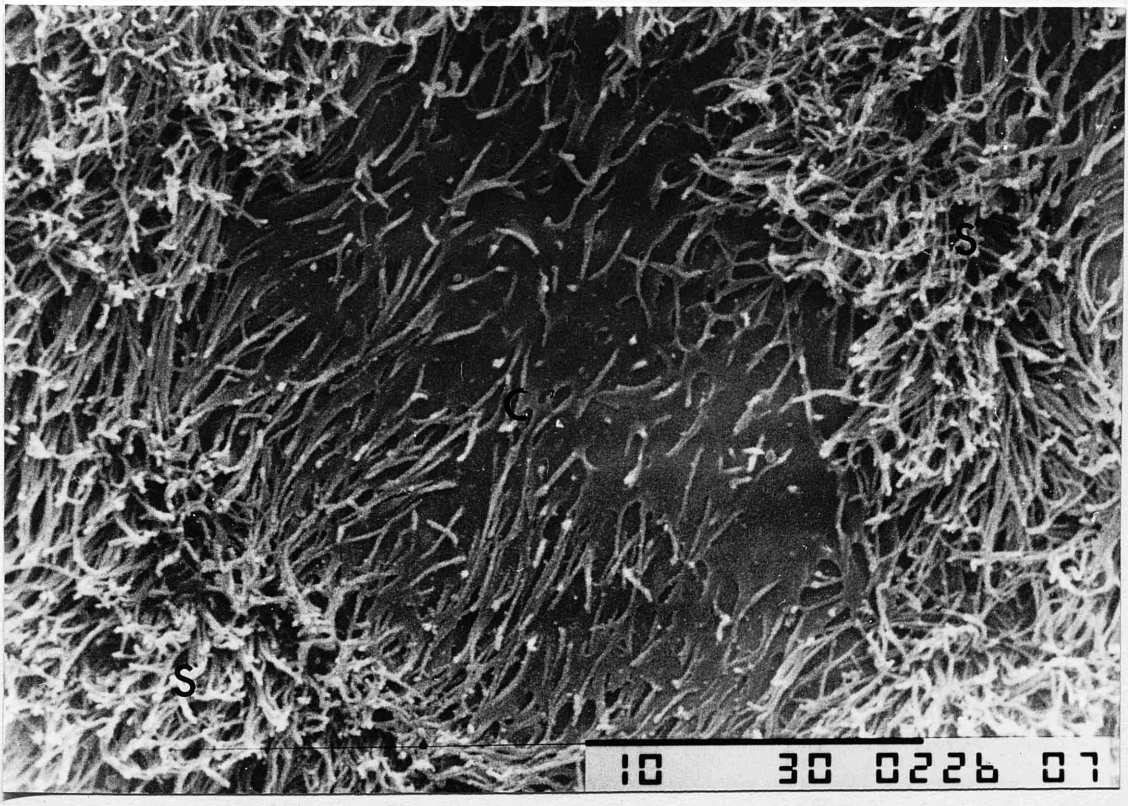
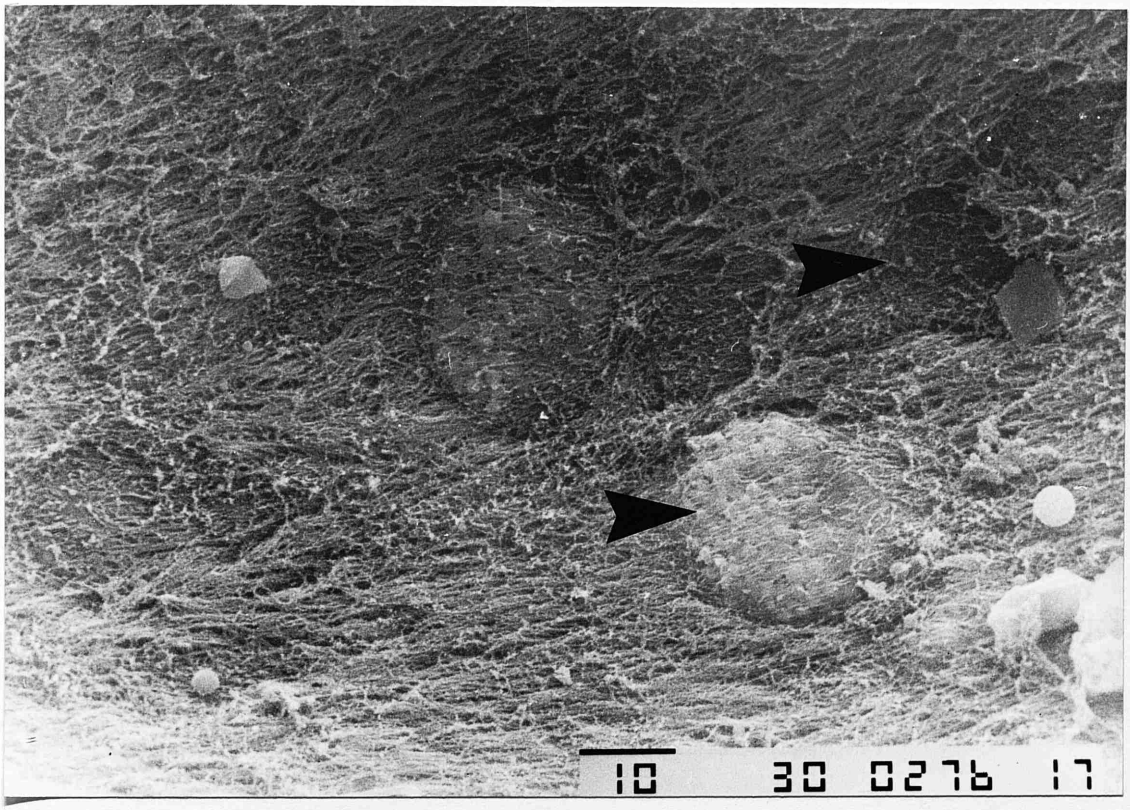

S
C
S
10 30 0226 07

Fig. 90: SEM of the epididymal epithelium at Zone III of a rat six weeks after vasectomy, illustrating the two physiological forms of clear cell (arrows).

(Vas 44 x 800)

Fig. 91: SEM showing the long delicate stereocilia (S) which cover the luminal surface of the epithelial cells of the epididymis at Zone II of a rat three weeks after vasectomy. Note the intact spermatozoon present on the surface.

(Vas 41 x 6500)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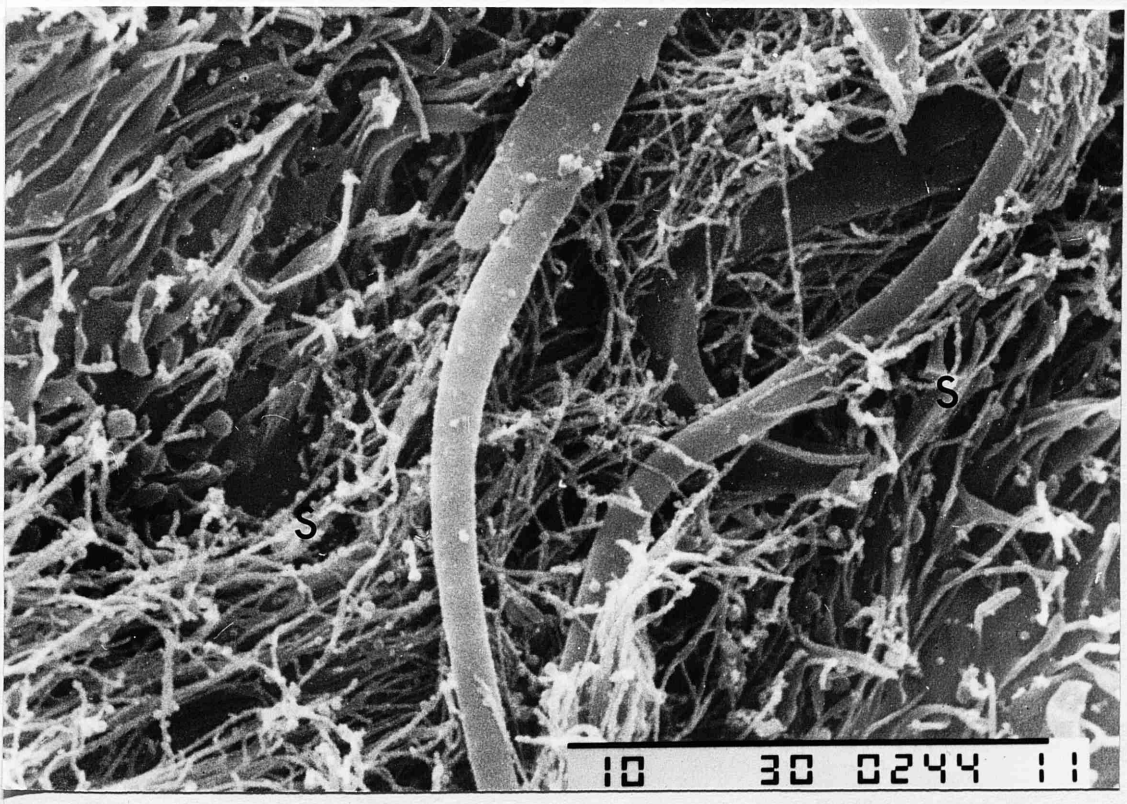
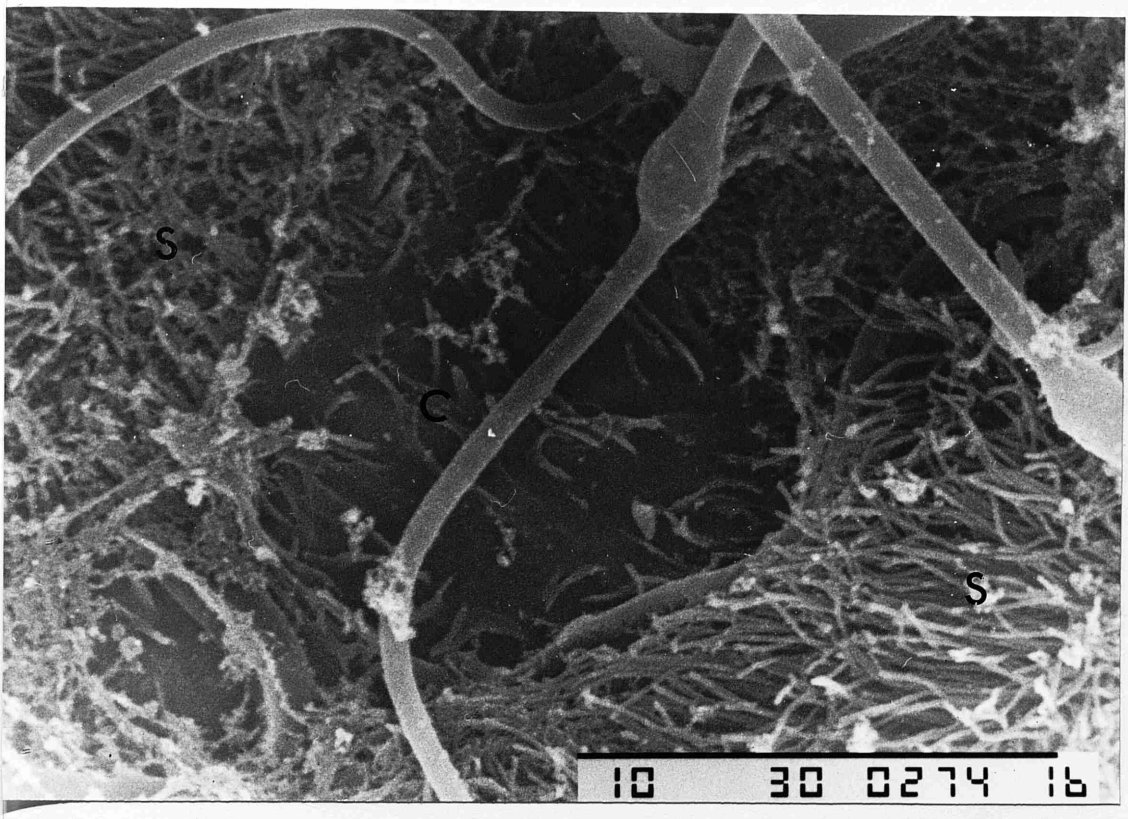

S
S
10 30 0244 11

Fig. 92: High magnification SEM of the luminal surface of the epididymal epithelium at Zone V of a rat six weeks after vasectomy, showing a clear cell (C). The surrounding principal cells were covered by numerous stereocilia (S). Note the spermatozoal tail present on the surface with its cytoplasmic droplet (arrows).

(Vas 43 x 6500)

Fig. 93: Illustrates lymphatics in the interlobular connective tissue of the cauda epididymidis in a sham-operated rat six weeks after operation.

(Sham 4 x 287.5)


S
C
S
10 30 0274 16

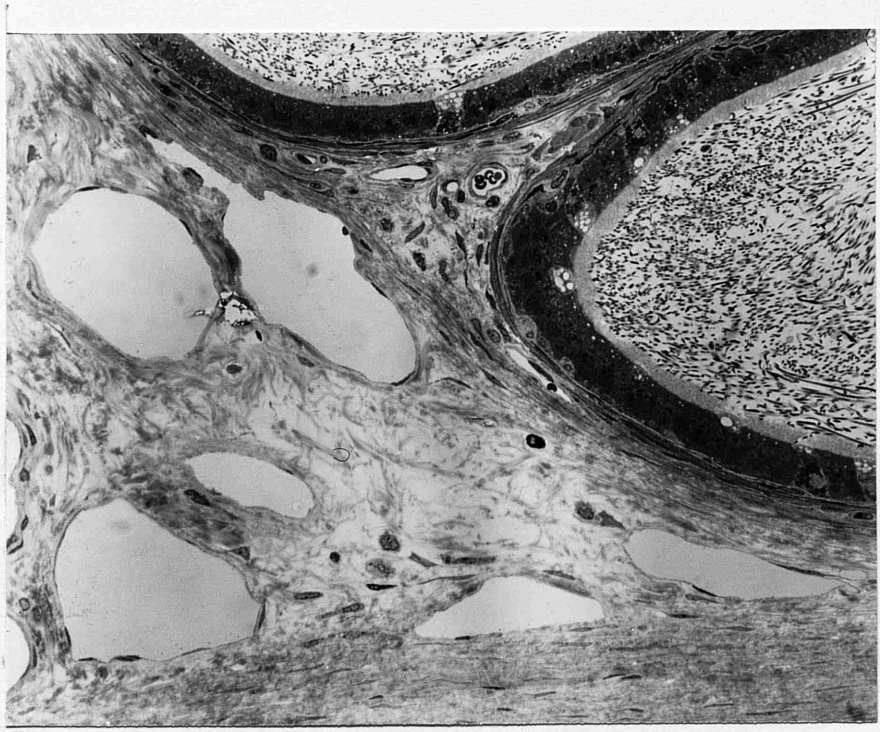
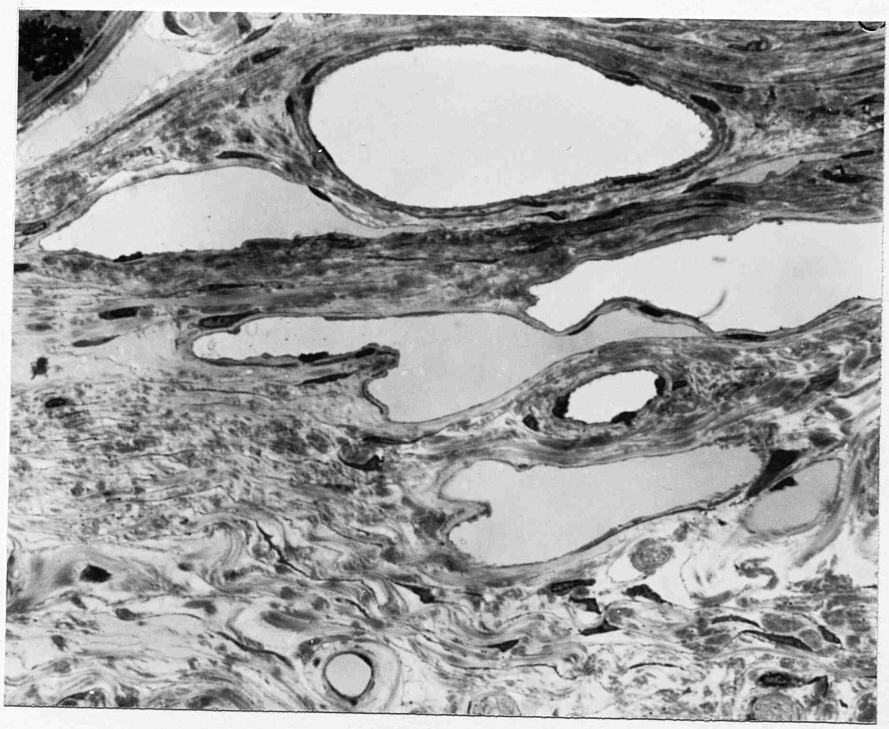


Fig. 94: Lymphatics in the coarse inter-lobular connective tissue of the cauda epididymidis of sham-operated rat nine months after operation.

(Sham 4 × 500)

Fig. 95: Section through the caput epididymidis of a sham-operated rat six weeks after operation. Note the large collecting lymphatic vessel with valve (L) present within the fibrous capsule.

(Sham 2 × 312.5)



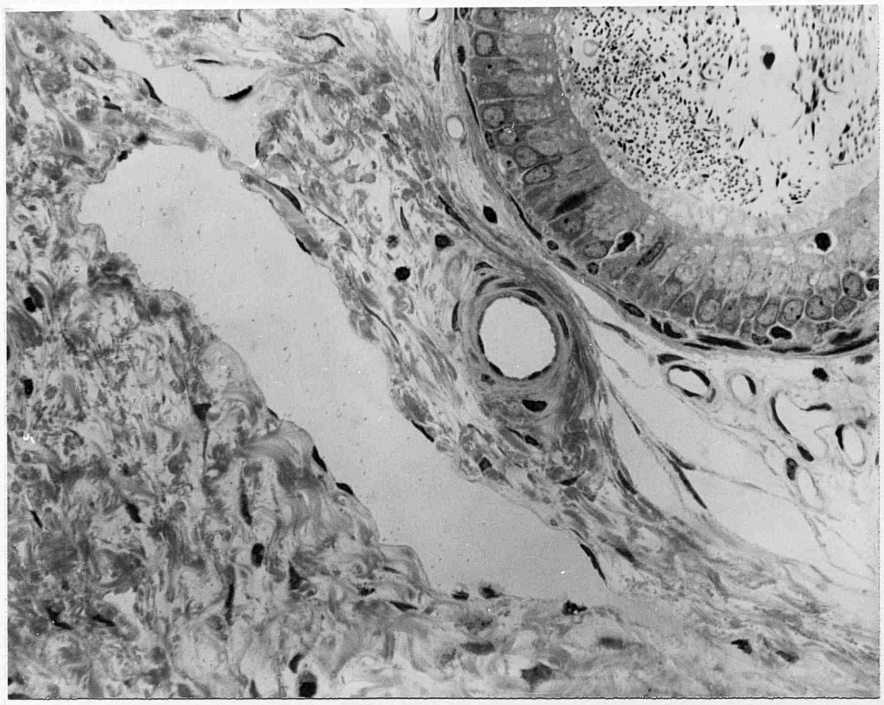

L

Fig. 96: The inter lobular lymphatics of the caput epididymidis of a vasectomized rat, in which an epididymal granuloma had not developed.

(Vas 7 x 400)

Fig. 97: Light micrograph showing inter lobular lymphatics of the cauda epididymidis of a rat six weeks after vasectomy. In this animal, an epididymal granuloma had not formed.

(Vas 4 x 500)



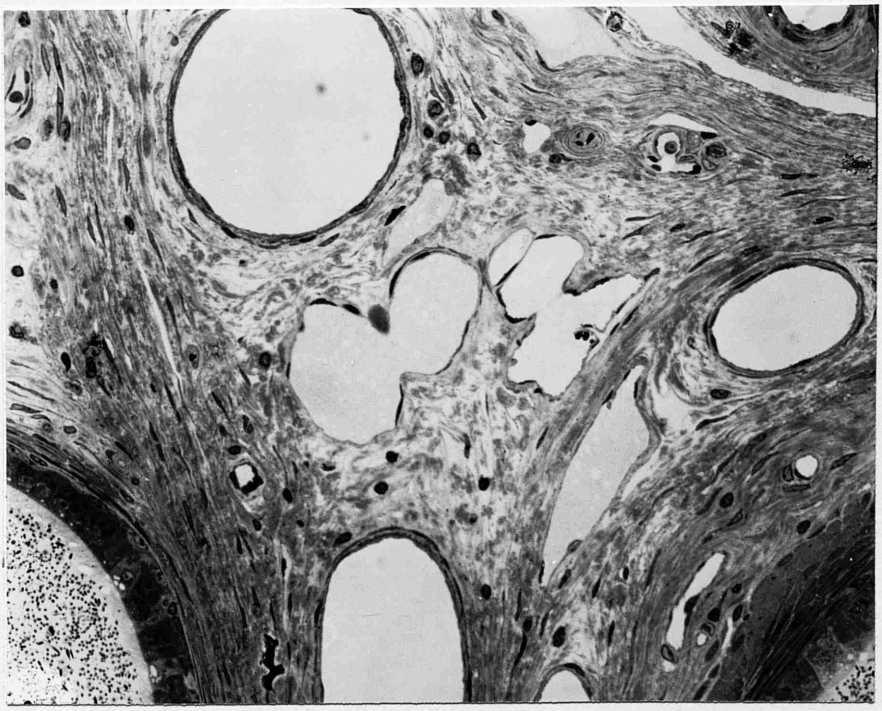
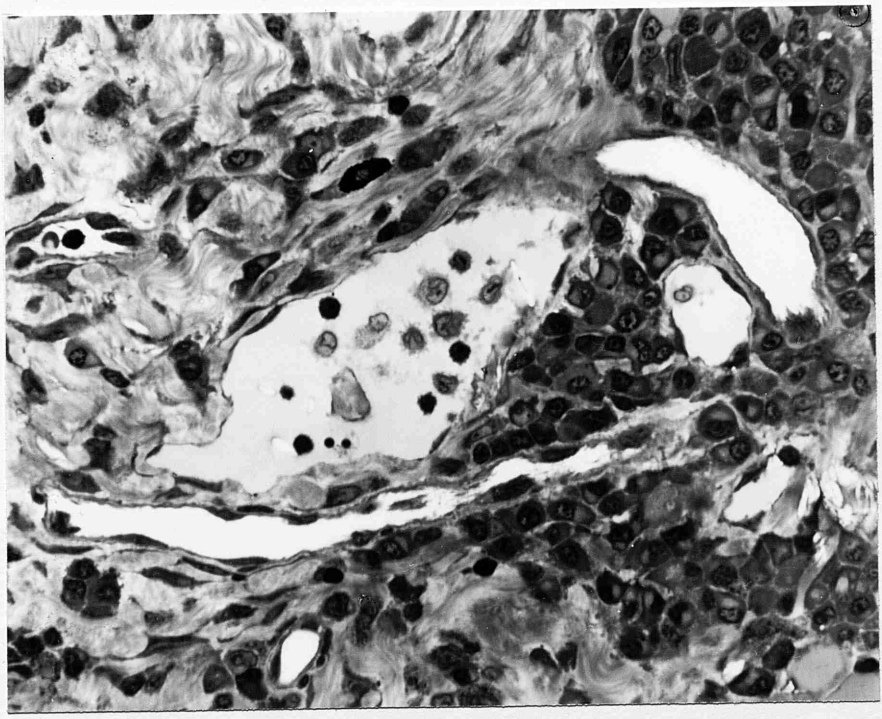


Fig. 98: Light micrograph of the loose connective tissue in the wall of an epididymal spermatic granuloma. Note the lymph vessel contains many lymphocytes and macrophages.

(Vas 27 x 500)

Fig. 99: Light micrograph of the edge of epididymal spermatic granuloma, showing a lymphatic vessel containing several mononuclear cells.

(Vas 29 x 500)



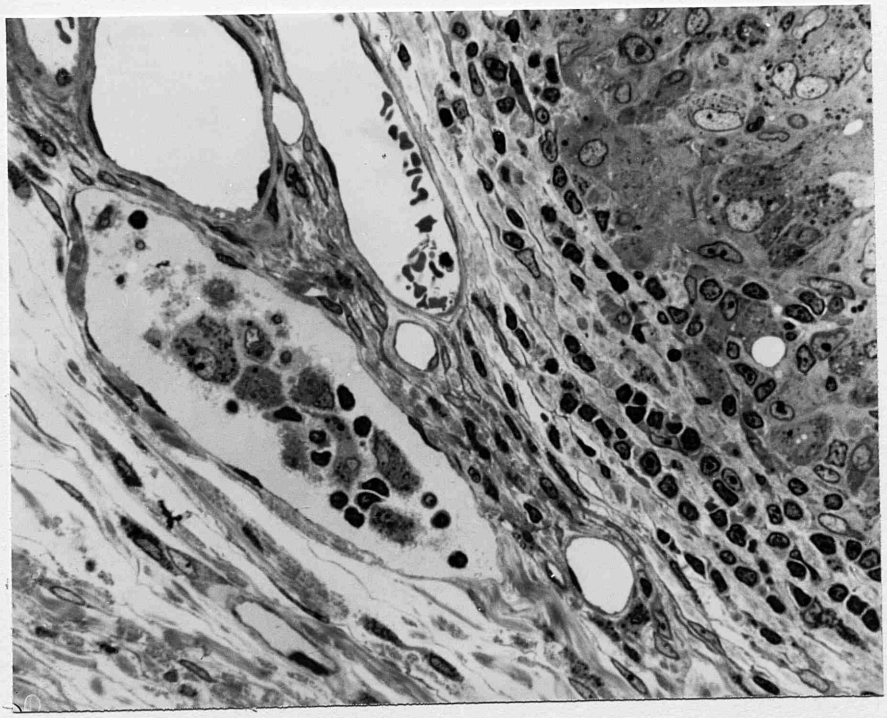
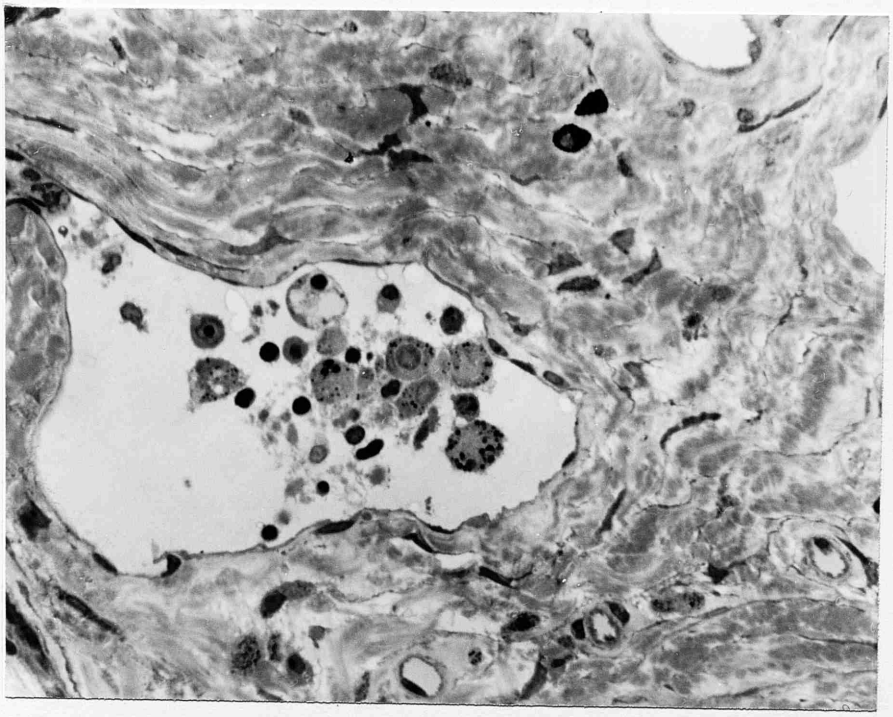


Fig. 100: Light micrograph of a lymphatic vessel immediately adjacent to a caudal granuloma; it contains several lymphocytes and macrophages.

(Vas 21 x 500)

Fig. 101: Light micrograph of epididymal spermatic granuloma, showing lymphatic vessel with numerous lymphocytes and macrophages.

(Vas 29 x 500)



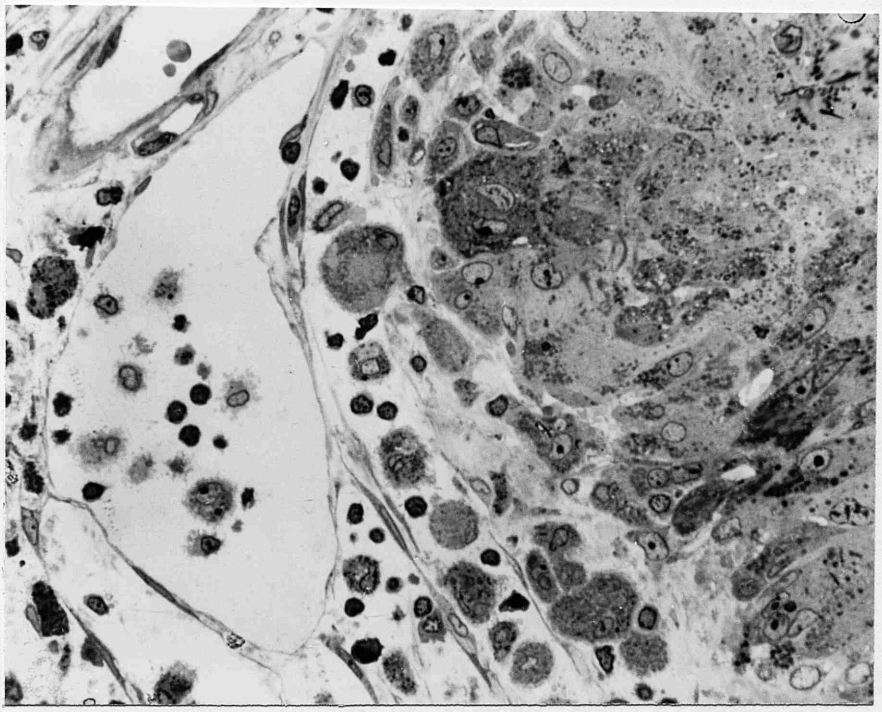
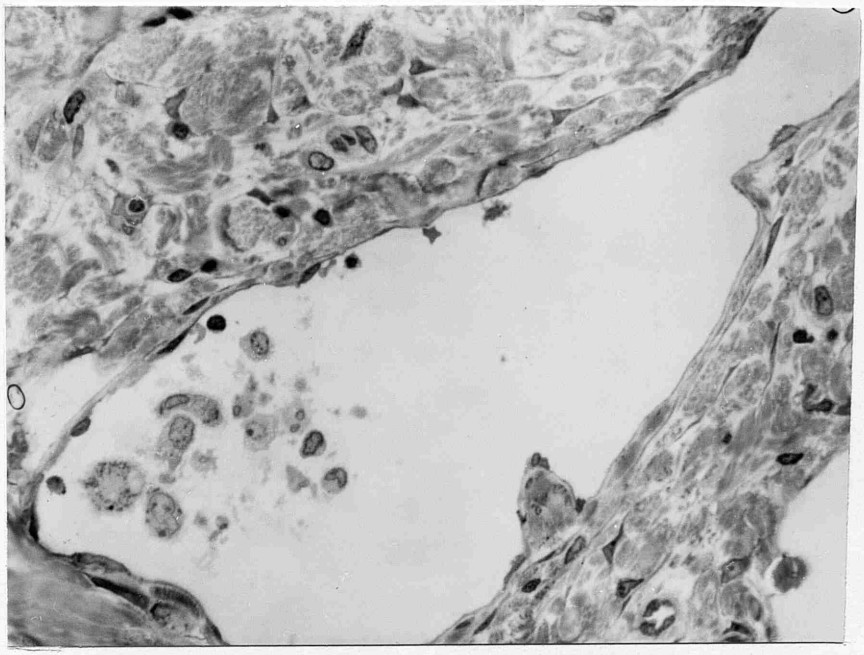


Fig. 102: High power view of a lymphatic vessel present within the immediate neighborhood of an epididymal granuloma. It contains several mononuclear cells.

(Vas 25 x 500)

Fig. 103: Shows some of the lymphatic vessels in the vicinity of an epididymal spermatic granuloma. Note the mononuclear cells present within the lymphatic lumen.

(Vas 23 x 480)



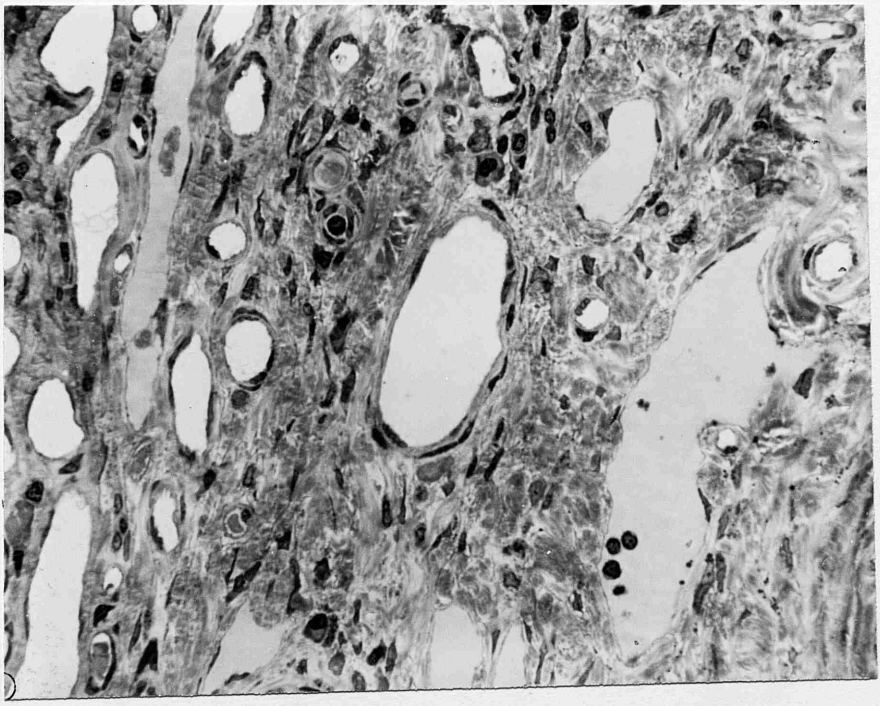
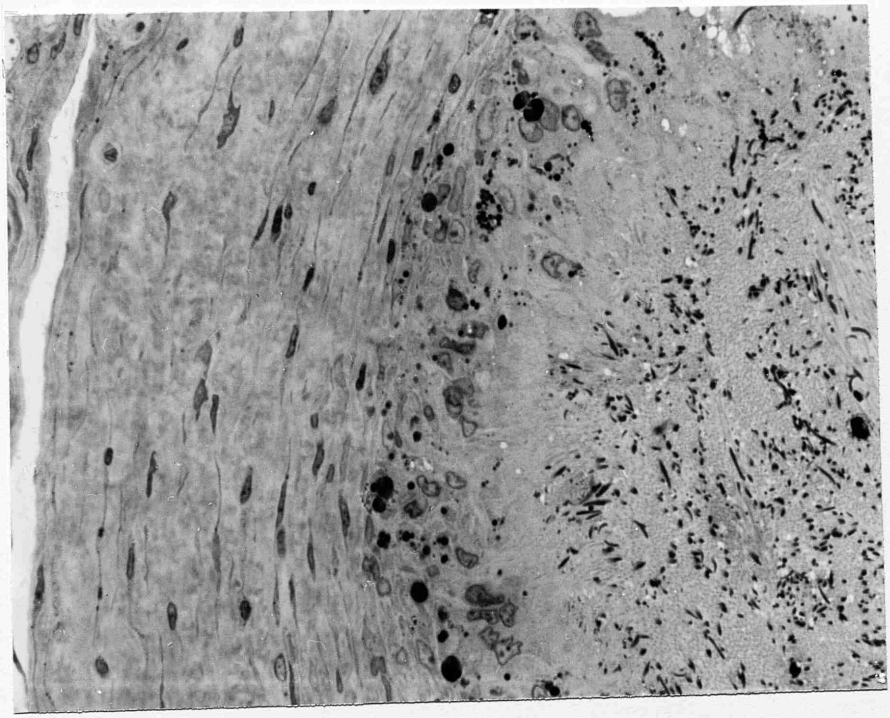


Fig. 104: Shows the wall and content of a long-standing epididymal spermatic granuloma.

(Vas 20 x 480)

Fig. 105: Lymphatic vessel at site away from the epididymal spermatic granuloma.

(Vas 27 x 480)



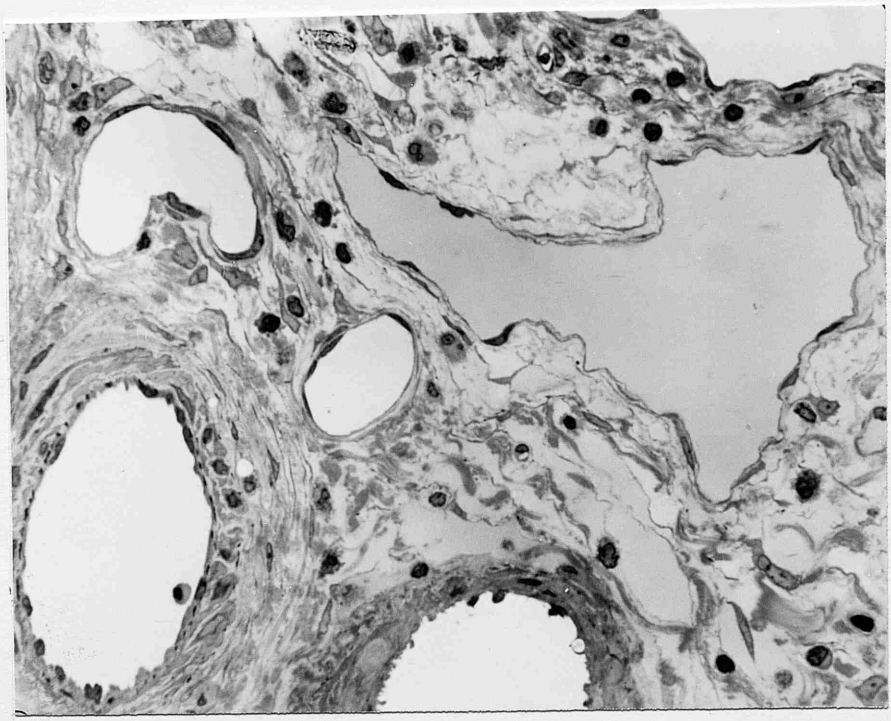
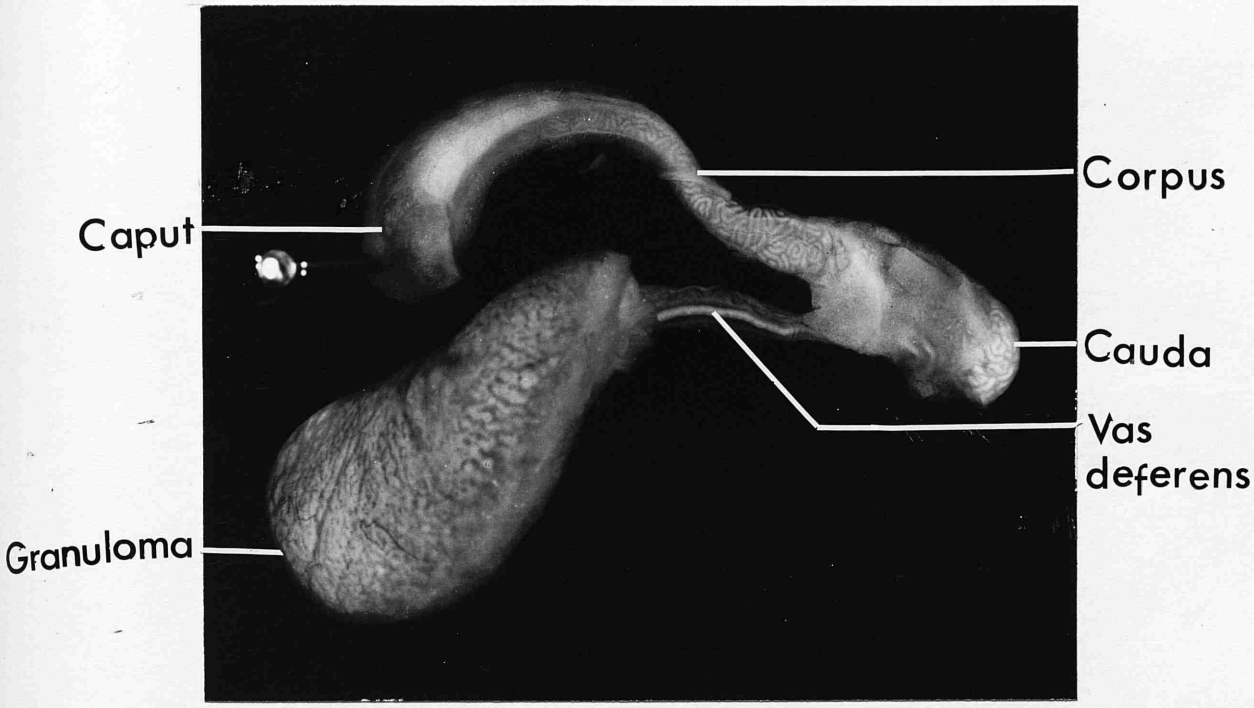


Fig.106: Example of large granuloma at the proximal end of the vas deferens.

(Vas 31 x 2.5)

Fig.107: Example of granulomas at site of vasectomy and within cauda epididymidis.

(Vas 38 x 2.5)


Caput
Corpus
Cauda
Vas deferens
Granuloma

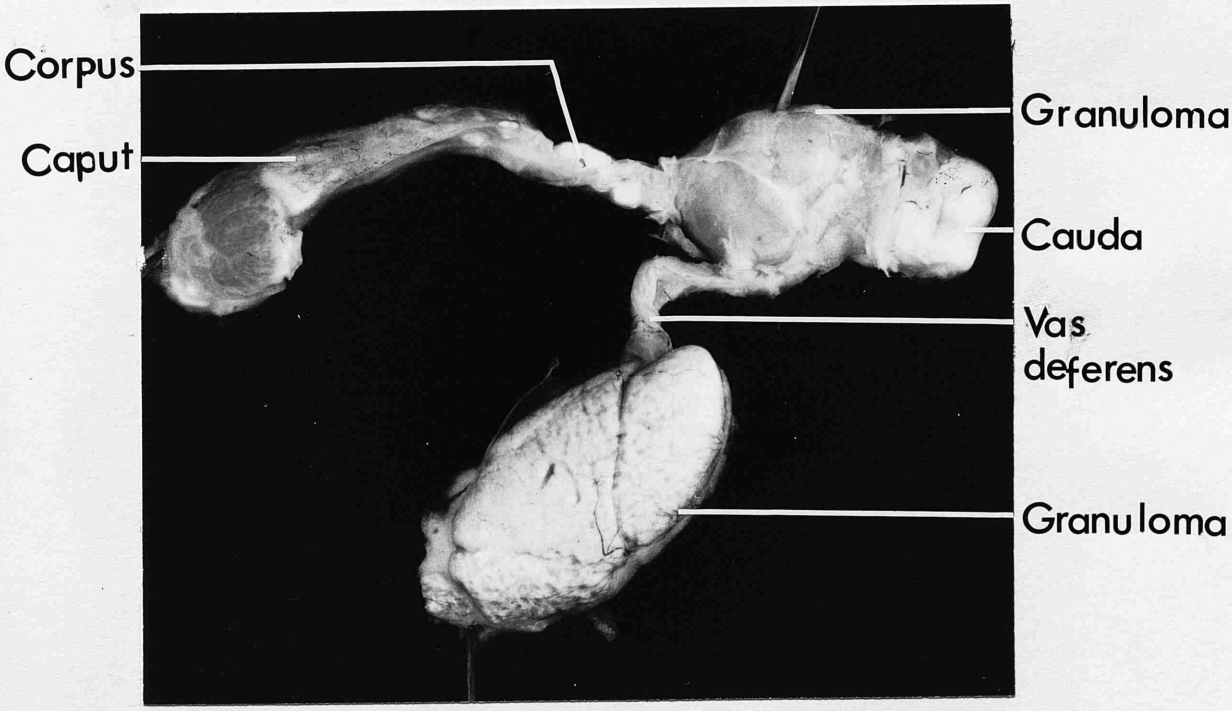
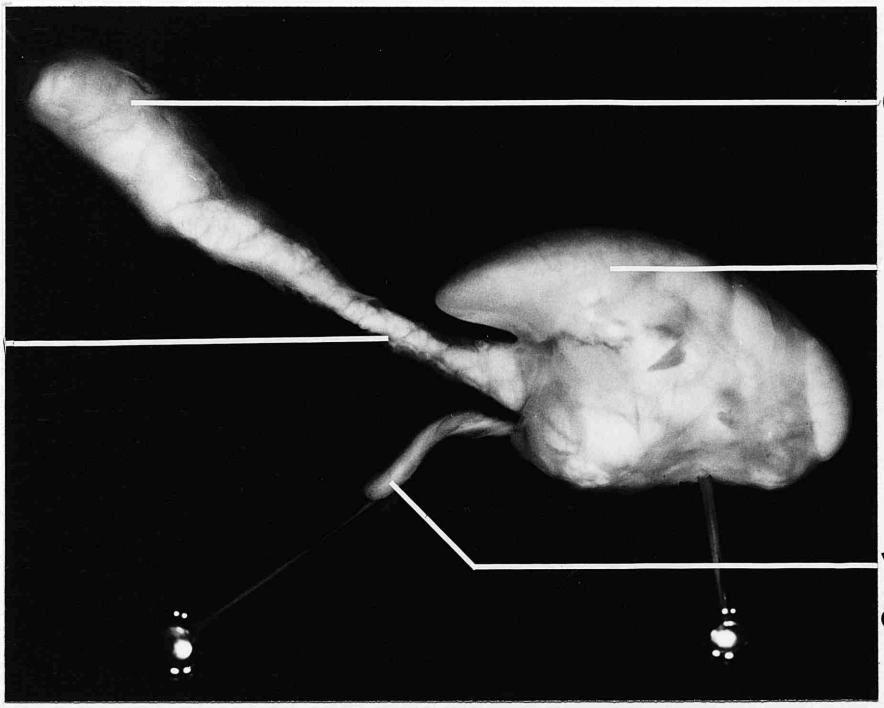

Corpus
Caput
Granuloma
Cauda
Vas deferens
Granuloma

Fig.108: Example of large spermatic granuloma in the cauda epididymidis.

(Vas 33 x 2.5)

Fig.109: Section of a spermatic granuloma at the cauda epididymidis showing the highly vascular connective tissue layer (C), cellular wall (B), and the centre (A), which is occupied by sperm and phagocytes.

(Vas 9 x 125)


Caput
Granuloma
Corpus
Vas
deferens

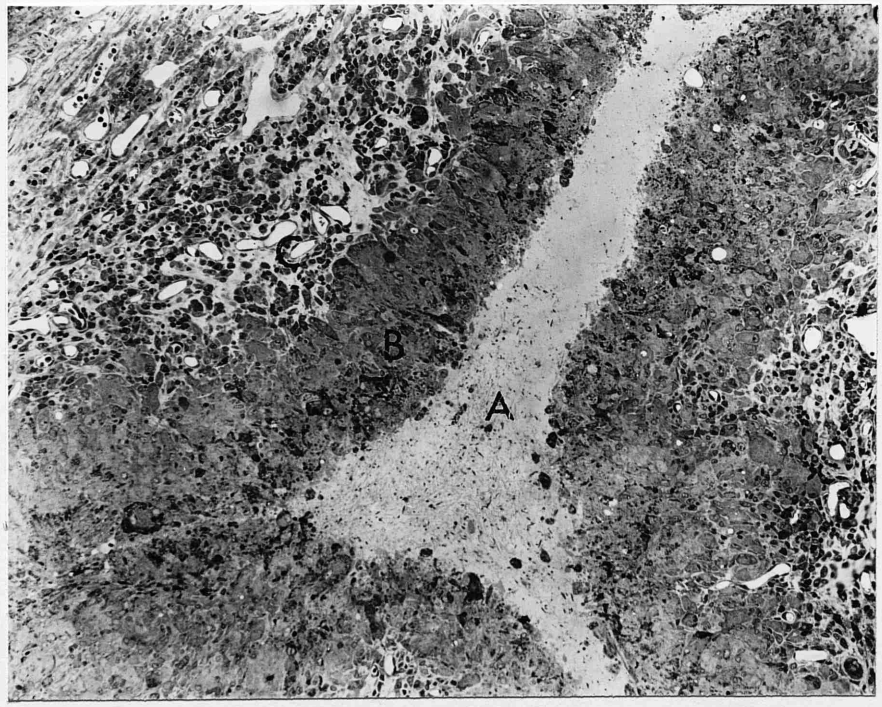
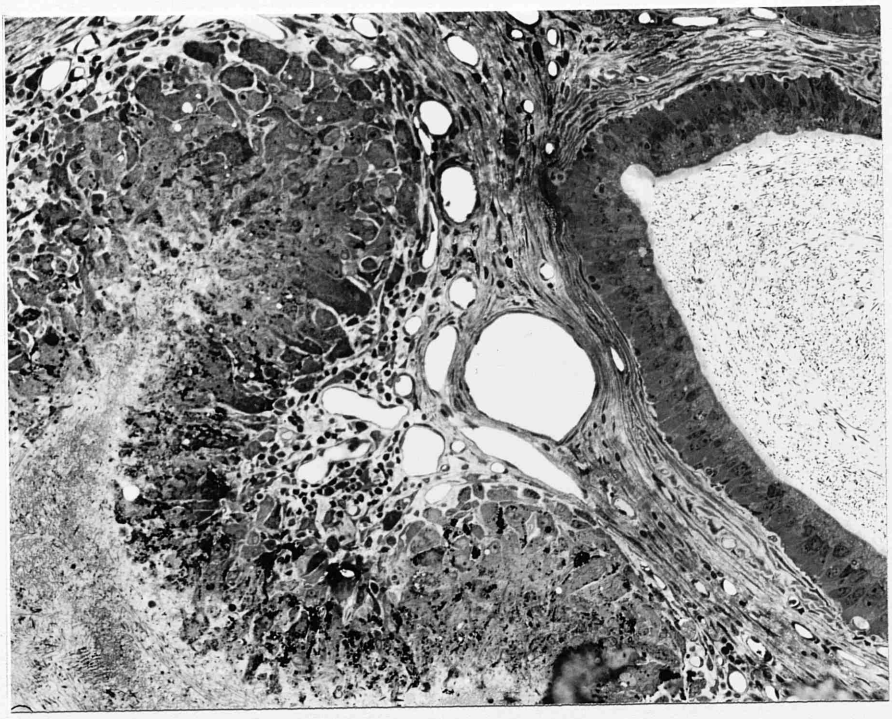

B
A

Fig. 110: Cross section through the cauda epididymidis, illustrating morphological features of the spermatic granuloma.

(Vas 23 x 200)

Fig. 111: Spermatic granuloma of the cauda epididymidis. The well organized granuloma wall consisted of an inner epithelioid layer (B), a mid portion of loosely organized connective tissue (C), and the outer fibrous capsule (D), surrounding a central sperm mass (A).

(Vas 18 x 312.5)



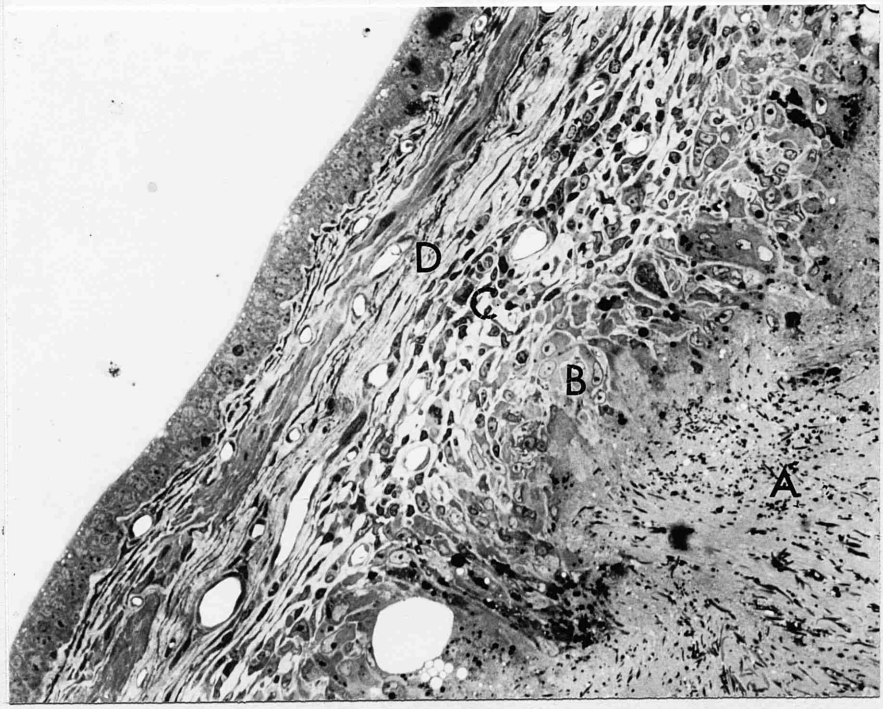
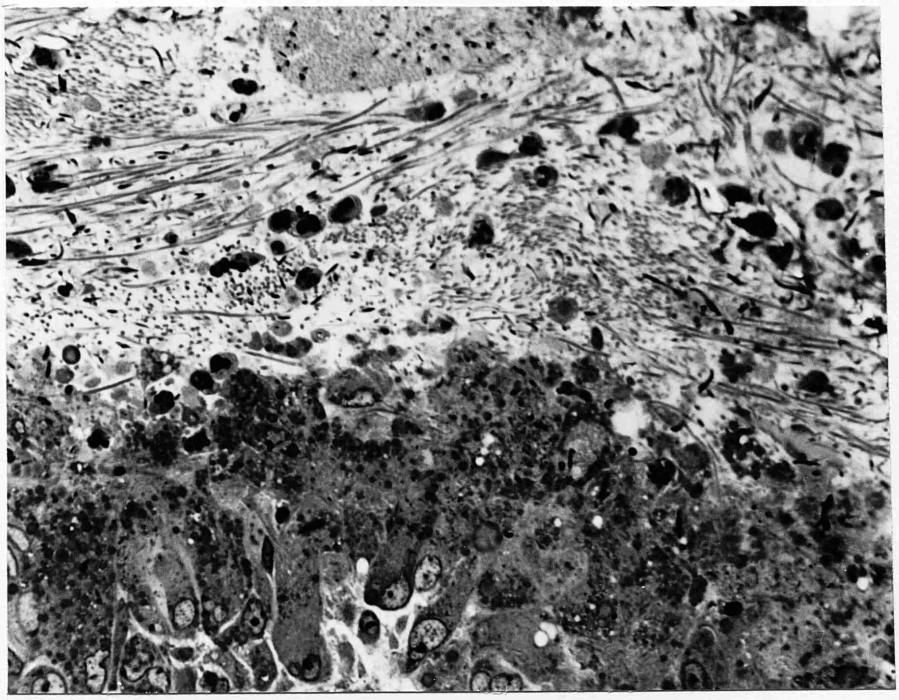

D
C
B
A

Fig. 112: High power of edge of a caudal spermatic granuloma. Pool of sperm and spermiophages is walled off by epithelioid macrophages.

(Vas 29 x 500)

Fig. 113: The loosely-organized connective tissue region of the granuloma wall (region C, Fig. 109). Note the dense accumulation of mononuclear cells.

(Vas 9 x 500)



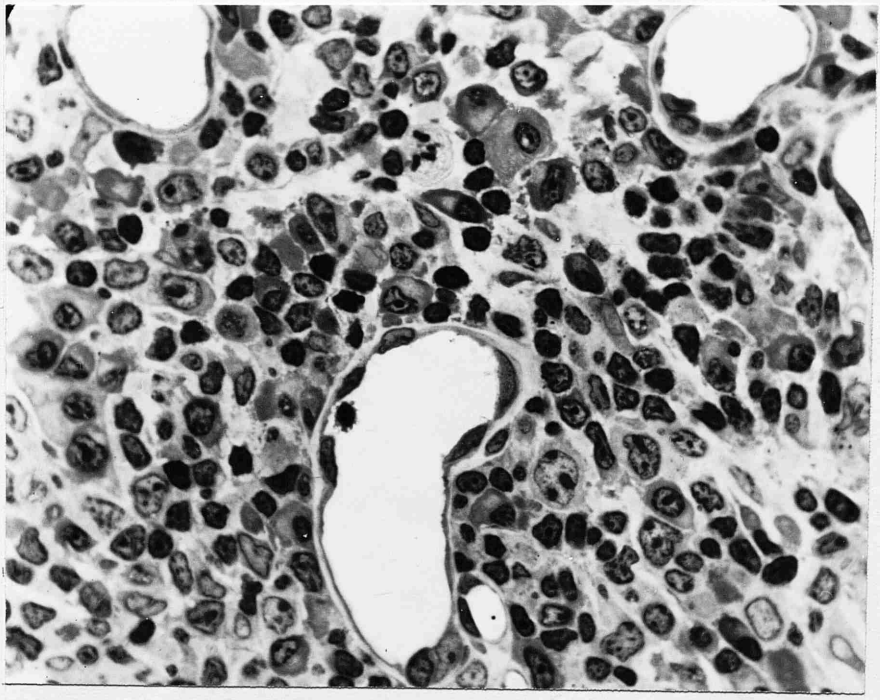
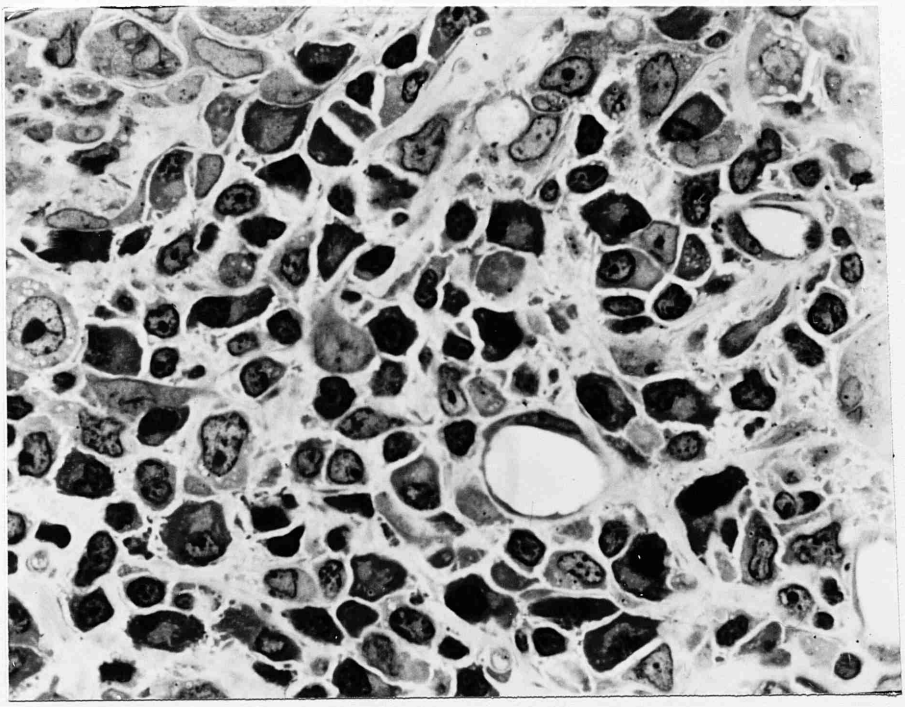


Fig.114: High power view of the highly vascularized connective tissue layer of the granuloma. Showing the mononuclear cells infiltration which were found associated with this layer.

(Vas 9 x 900)

Fig.115: The connective tissue portion of the wall of a spermatic granuloma at the cauda epididymidis. Two zones can be distinguished, an inner highly vascular loose connective tissue (A), and outer fibrous capsule (B).

(Vas 21 x 500)



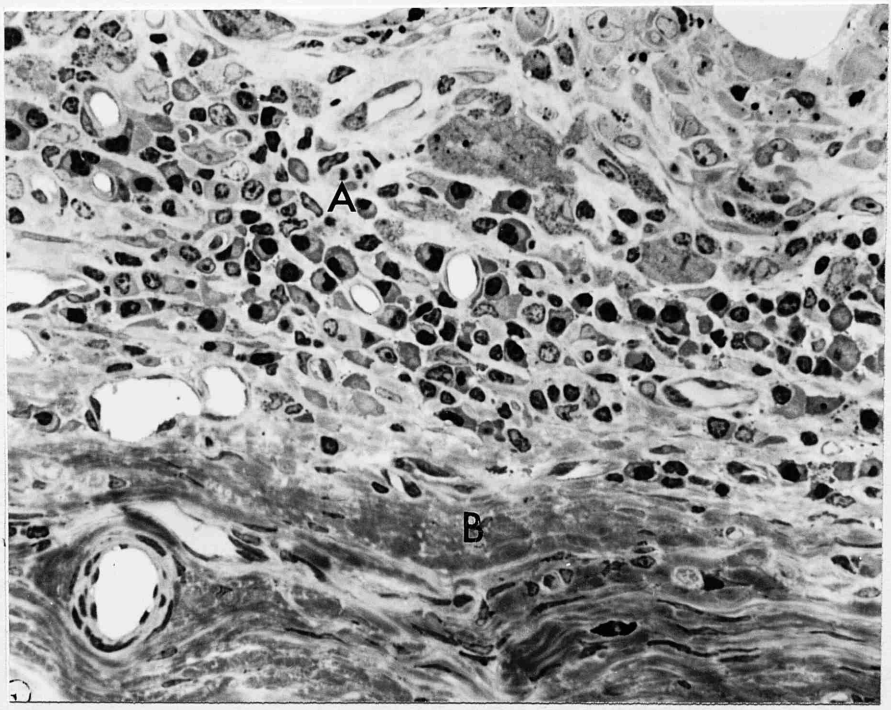

A
B

Fig.116: Mass of sperm has broken through the epithelial wall of the caput epididymidis into the adjacent interstitial tissue and surrounded by epithelioid macrophages. Several polymorphonuclear neutrophils and macrophages invade the sperm mass and ascend into the lumen of the duct.

(Vas 32 x 252)

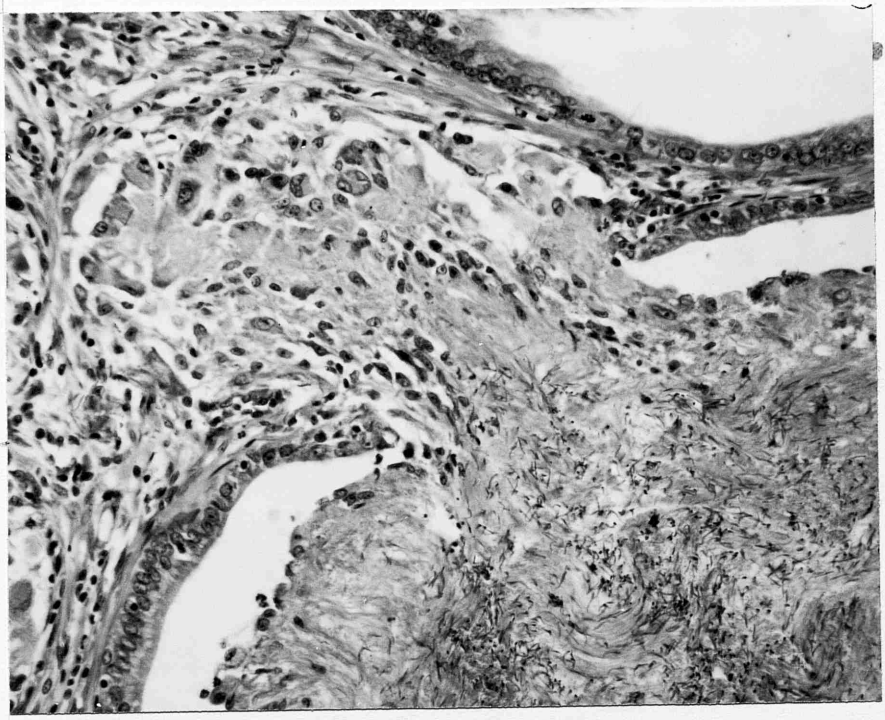
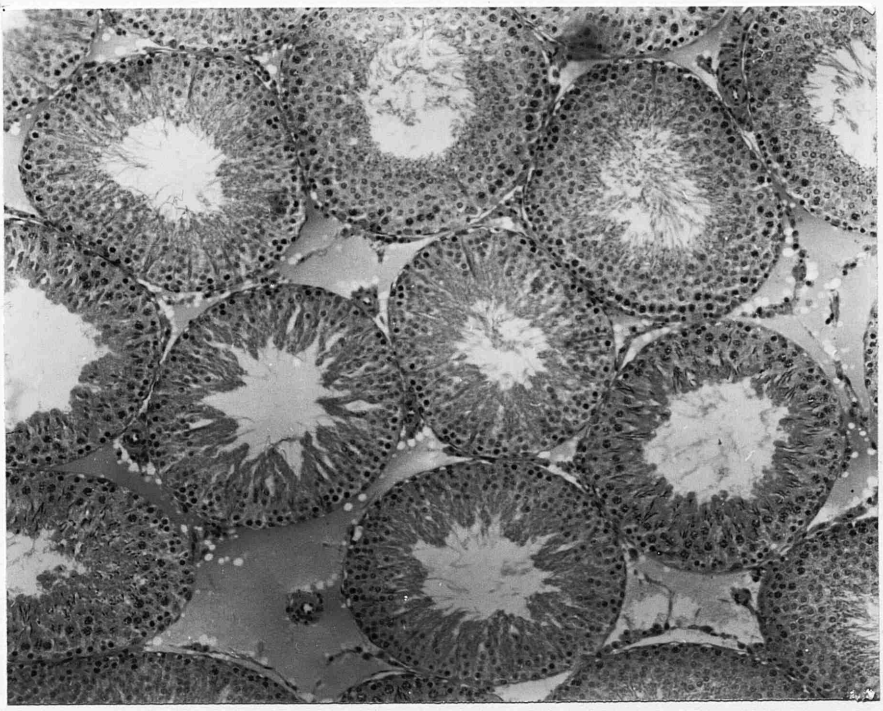


Fig. 117: Section of rat testis, six weeks after vasectomy, illustrating normal spermatogenesis.

(Vas 5 x 100)

Fig. 118: Higher power micrograph of the seminiferous epithelium of Figure 25.

(Vas 5 x 400)



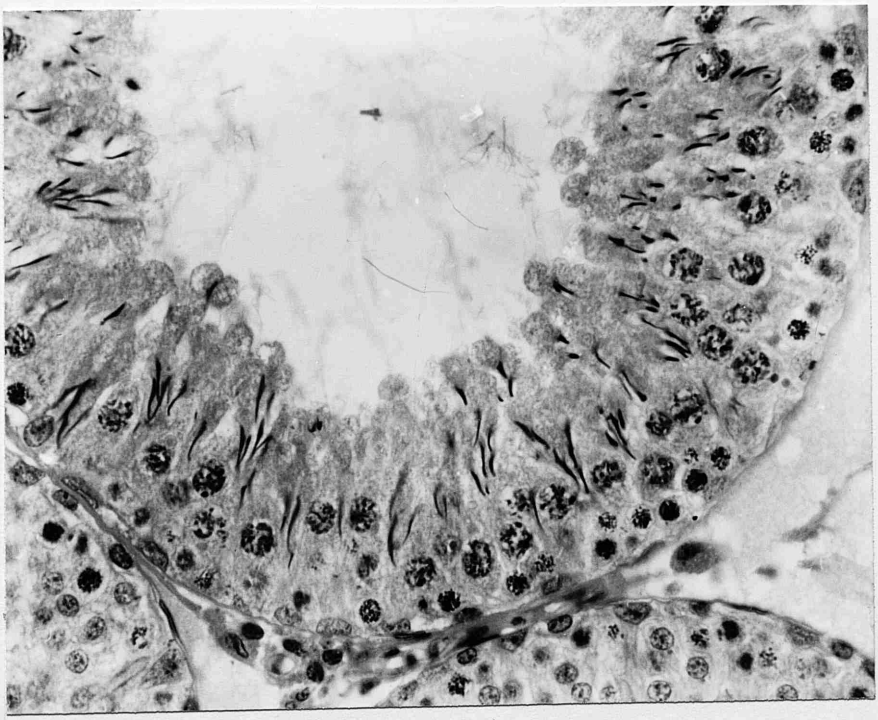
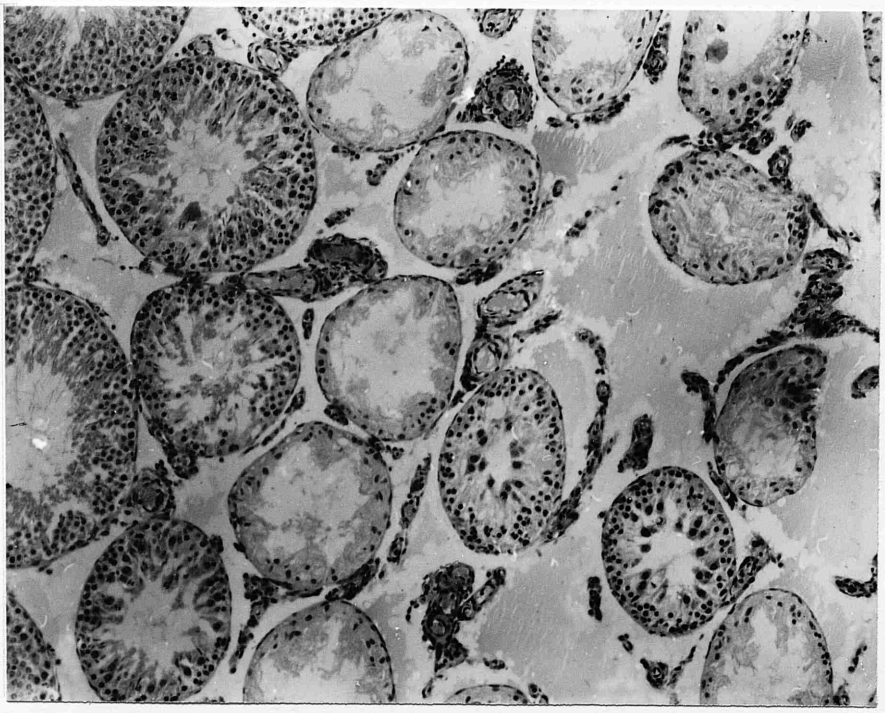


Fig.119: The seminiferous tubules of a rat testis, eighteen months after vasectomy. Note the patchy degenerative changes occurring in its epithelium.

(Vas 32 x 100)

Fig.120: Light micrograph of the testis of a rat vasectomized for six months. Note the patchy tubular degeneration, the reduction in the diameter of the seminiferous tubules, and the massive increase in the extent of interstitial fluid.

(Vas 11 x 100)



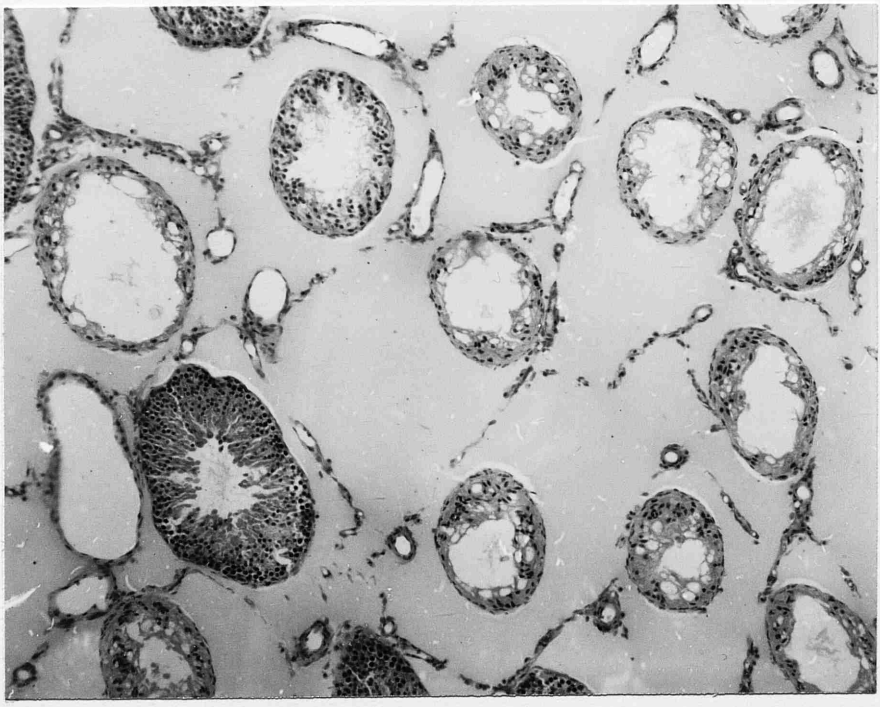
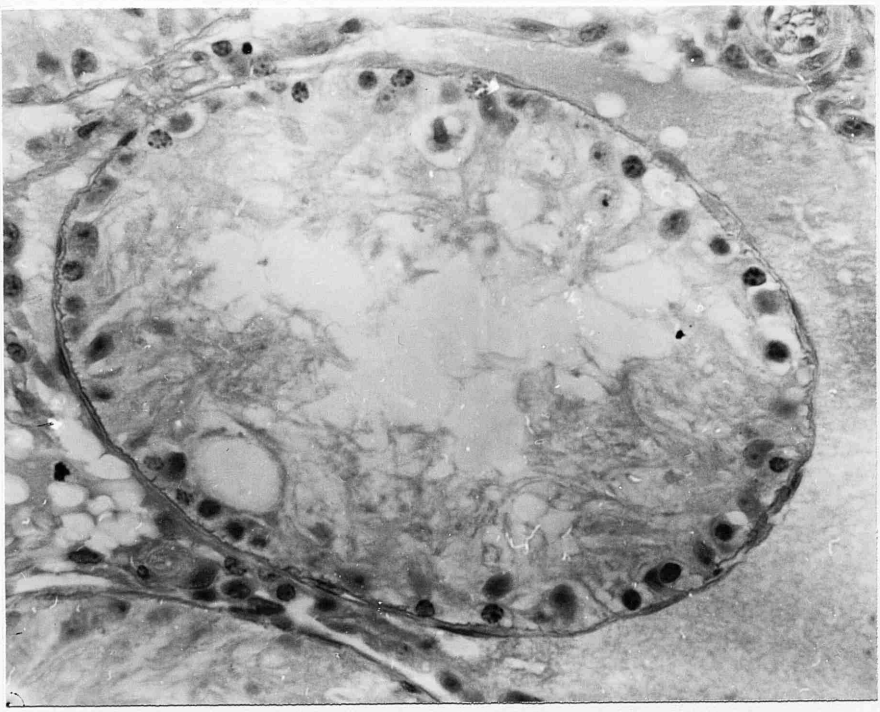


Fig.121: High power micrograph of a seminiferous tubule, six months after vasectomy. The epithelium contains only Sertoli cells and scattered spermatogonia.

(Vas 11 x 440)

Fig.122: High power micrograph of a seminiferous tubule six months after vasectomy. The epithelium contains mainly Sertoli cells, spermatogonia, and spermatocytes.

(Vas 11 x 440)



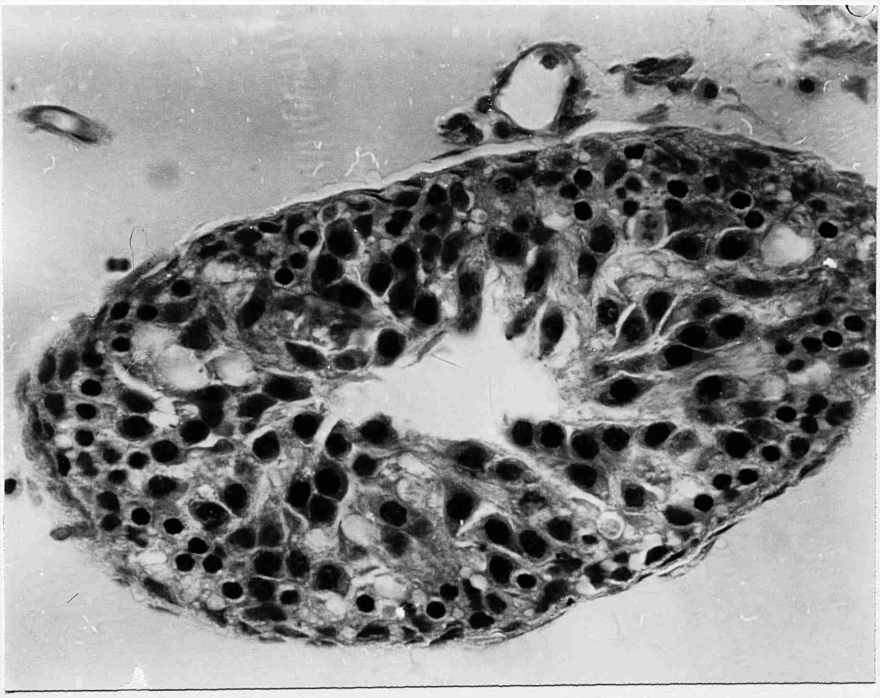
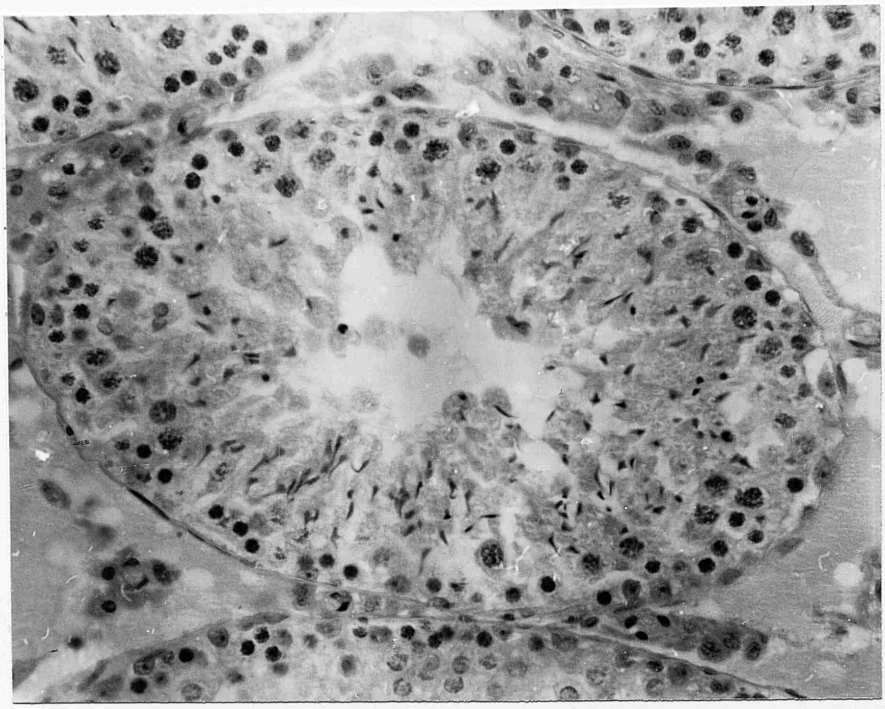


Fig.123: High power micrograph of the testis of a rat vasectomized for six months, showing a seminiferous tubule at Stage V of the cycle.

(Vas 11 x 340)

Fig.124: High power micrograph of a seminiferous tubule of a rat, six months after vasectomy, showing a degenerative change in its epithelium. The lining epithelium consists of Sertoli cells, spermatogonia, round spermatids and a few spermatocytes. Note the shedding of multinucleated cells (FS).

(Vas 11 x 500)



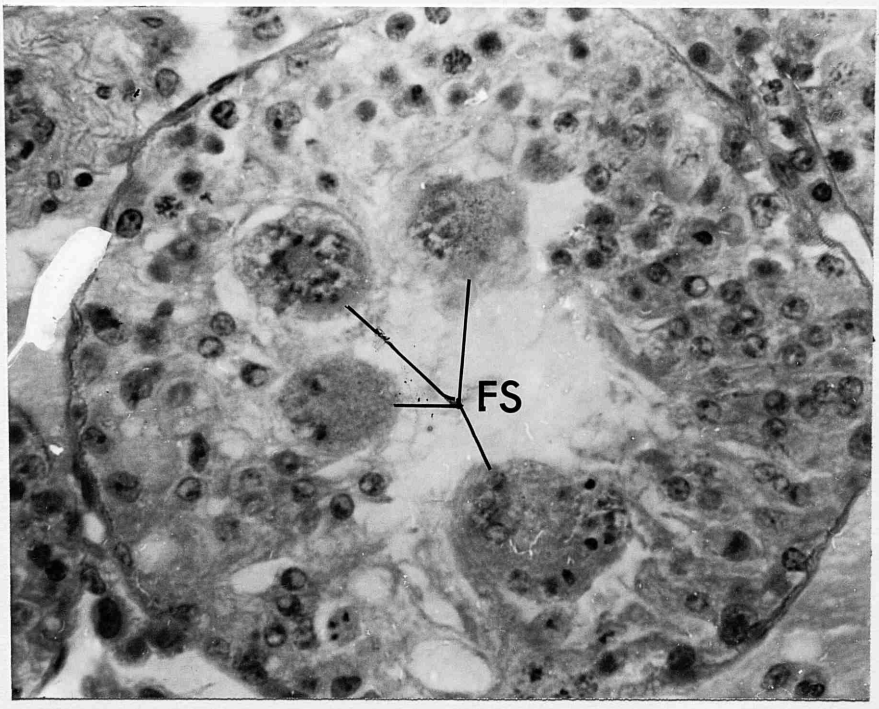
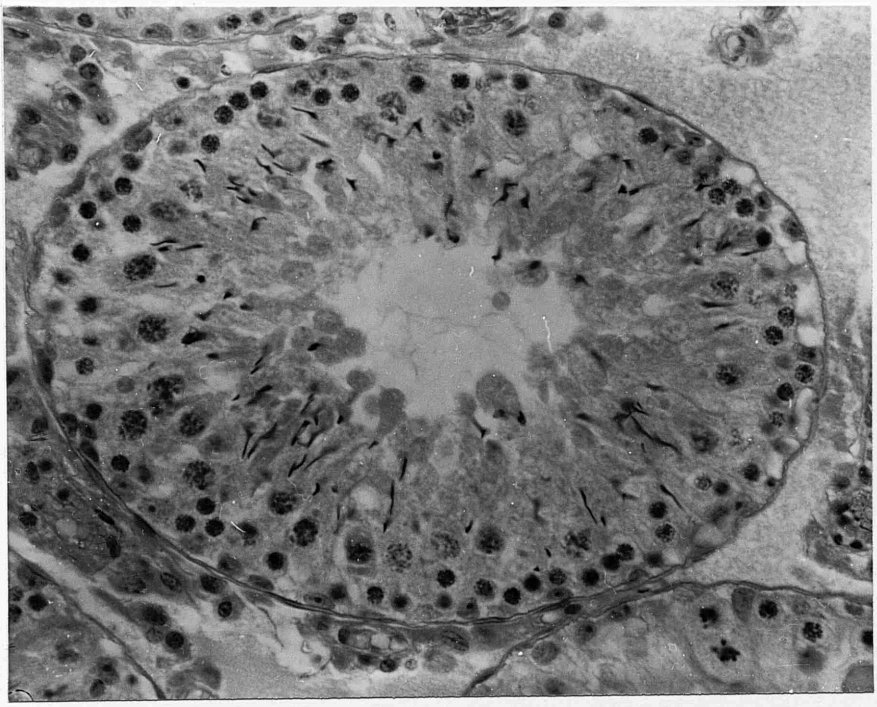

FS

Fig. 125: High power micrograph of a seminiferous tubule of a rat, six months after vasectomy. The lining epithelium is at Stage VI of the cycle.

(Vas 11 x 380)

Fig. 126: Light micrograph of atrophic testis from a rat, fifteen months after vasectomy. Some tubules are almost completely fibrotic.

(Vas 26 x 250)



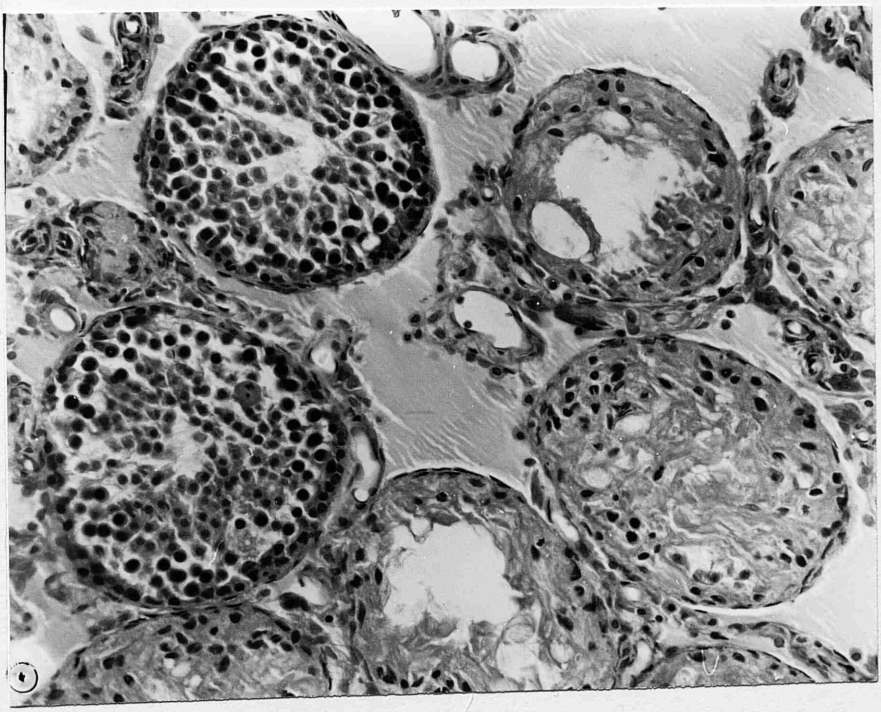
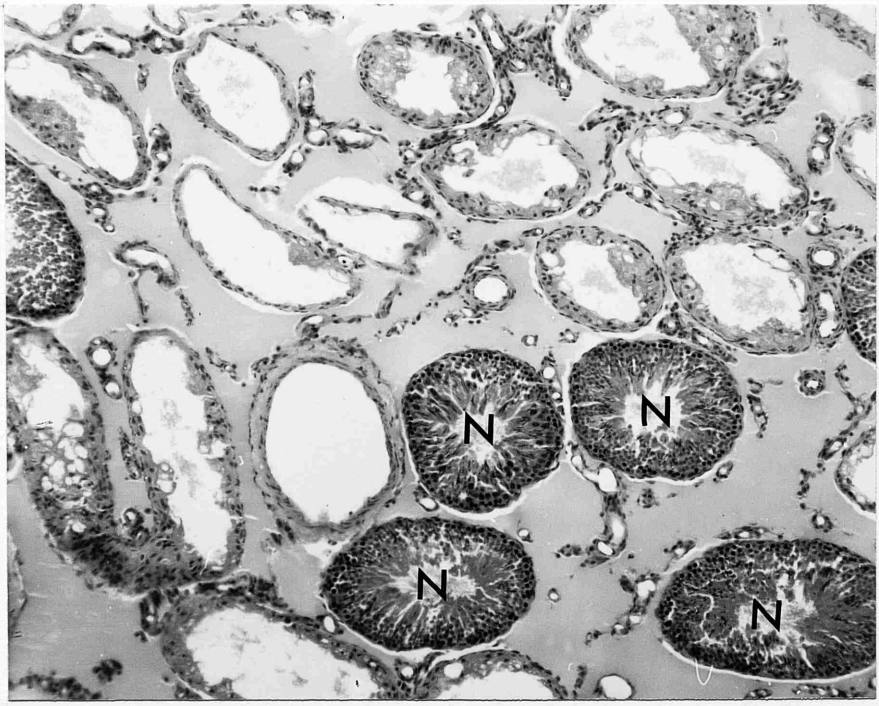


Fig.127: Seminiferous tubules from a rat, nine months after vasectomy, showing severe degenerative changes in the germinal epithelium. Note the presence of a normal tubule (N).

(Vas 19 x 100)

Fig.128: Seminiferous tubules from a rat,nine months after vasectomy, showing normal (N) and abnormal (A) tubules.

(Vas 18 x 125)


N
N
N
N

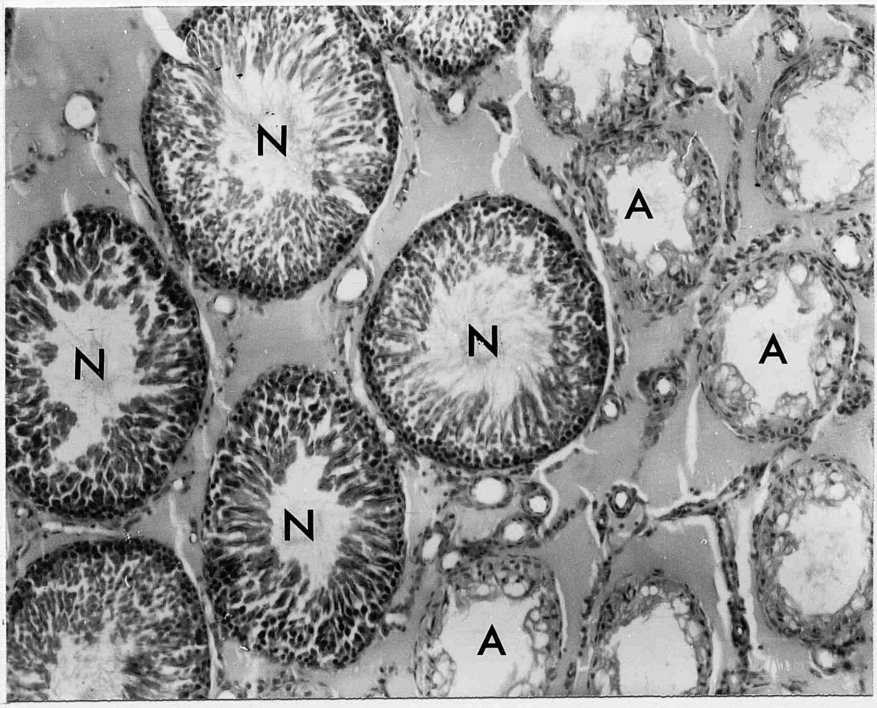
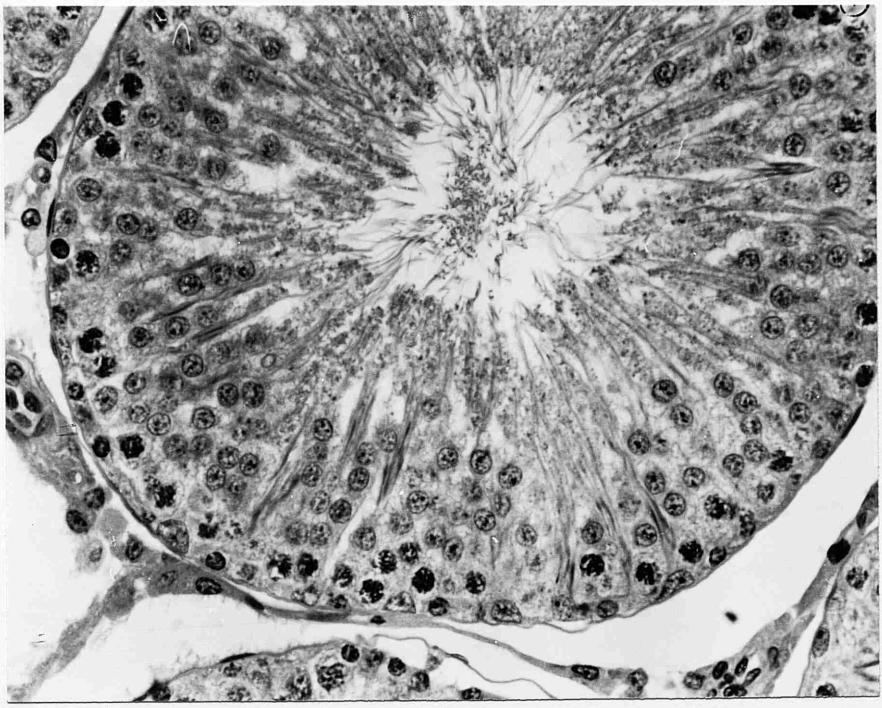

N
A
N
A
N
N
A

Fig.129: High magnification of normal seminiferous tubule from atrophied testes of a rat, six months after vasectomy.

(Vas 11 x 250)

Fig.130: Seminiferous tubules from a vasectomised rat, fifteen months after surgery, showing the degenerative changes occurring in its epithelium. Note the sloughing of abnormal germinal cells (arrows).

(Vas 26 x 250)



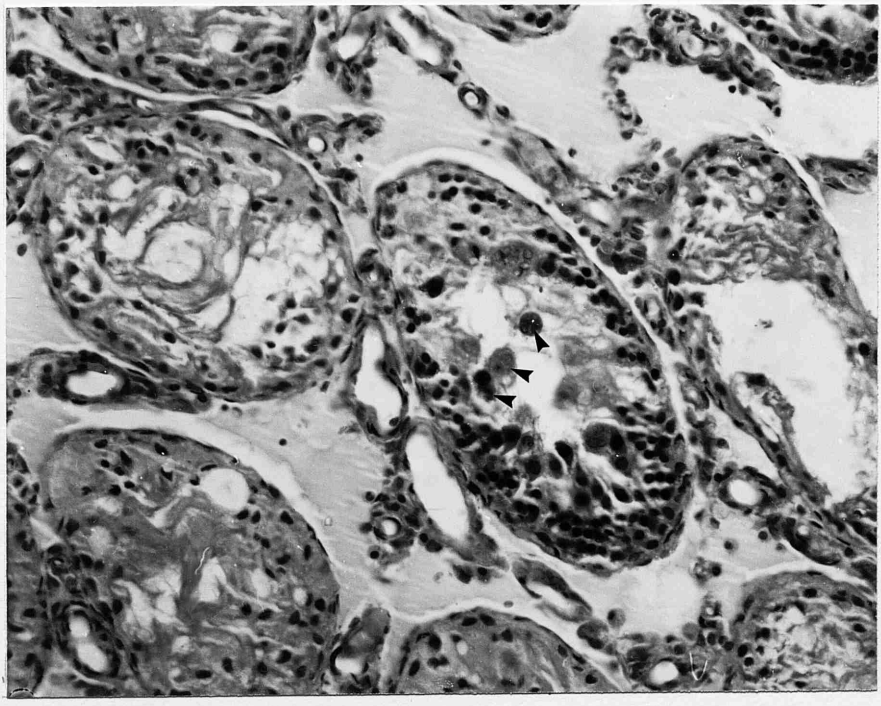
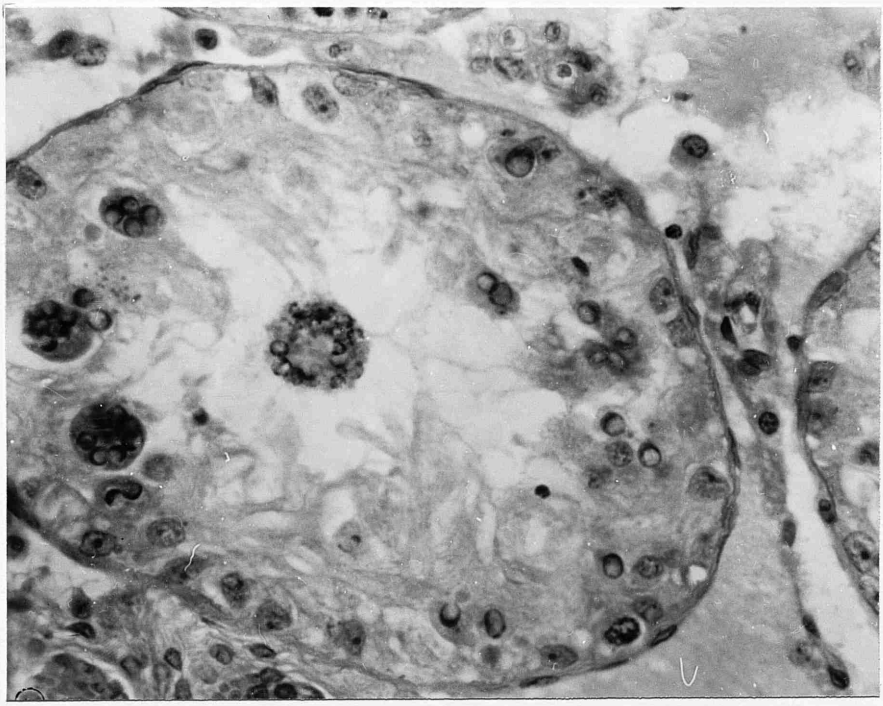


Fig. 131: Seminiferous tubule from a rat,nine months after vasectomy. Note the multinucleate cells present within its lumen.

(Vas 18 x 500)

Fig.132: Seminiferous tubule from a rat, fifteen months after vasectomy, showing fused spermatids (FS).

(Vas 26 x 500)



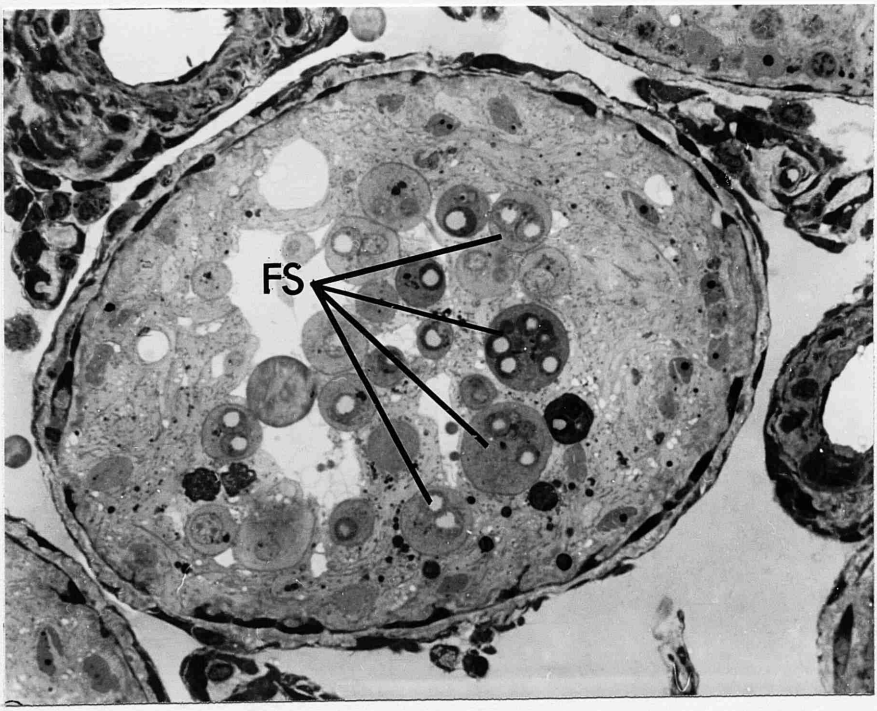

FS

Fig.133: Light micrograph of the testis of a rat vasectomized for nine months. The seminiferous epithelium of the affected tubules is greatly reduced in thickness. Note the normal seminiferous tubules (N), in which spermatogenesis appeared unaltered.

(Vas 18 x 50)

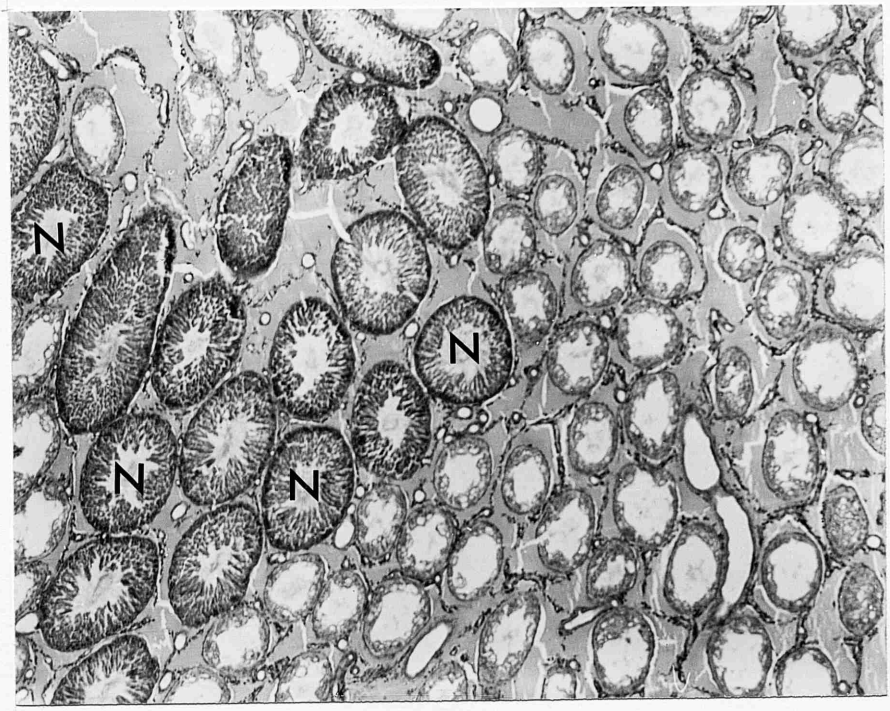

N
N
N
N

Fig. 134: In section, the rat spleen is roughly triangular in shape. The white pulp masses are scattered throughout the red pulp (R), and consist of the dense lymphatic tissue (D) surrounded by the marginal zone (M). At the hilum, the fibrous capsule is traversed by the splenic arteries and veins (V).