

VASECTOMY: LONG-TERM EFFECTS IN THE RHESUS MONKEY*

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Summary. Long-term vasectomy of rhesus monkeys causes many morphological and functional changes in the epididymis, particularly in the ductuli efferentes. In intact animals, the epithelium of the ductuli efferentes consists of about equal numbers of ciliated and non-ciliated cells. Normally, in their apical portions, the ciliated cells contain lipid complexes associated with mitochondria. Spermatozoa are less frequently found in the lumen of the ductuli but are packed together and stored in large numbers in the caudal portion of the epididymis. In vasectomized animals, the ductuli enlarge as much as four times in diameter, and the epithelial basal lamina (normally 500 to 700 Å) thickens considerably (up to 3.2 µm). The number of ciliated cells is greatly reduced and in those remaining, the characteristic lipid complexes are absent. Spermatozoa become agglutinated in the lumen of the ductuli where they are ingested by macrophages. Fluorescein-labelled antibody and electron microscopy show the thickened basal lamina to be in the site of antigen-antibody complexing. It is postulated that long-term vasectomy results in an autoimmune response to spermatozoa that may aid in the disposal of the many spermatozoa still being produced by the testes but lacking an exit passage.

INTRODUCTION

Vasectomy, a widespread birth control measure, was first attempted in 1823 by Astley Cooper on dogs (Jhaver & Ohri, 1960). Examination of the sperm-filled testes and epididymides 6 years later demonstrated that vasectomy had not prevented spermatogenesis. It has been repeatedly demonstrated since that after vasectomy, spermatozoa are still produced although the exit passage is no longer present. The morphological effects of obstruction of the vas deferens have not been adequately investigated, interest having been largely centred on the effect of ligation of the vasa deferentia on the testes. However, after vasectomy, changes occur throughout the entire male duct system but, in the rhesus monkey at least, are most prevalent in the ductuli efferentes, a site well removed from the area of the surgical ligation. This study is concerned with the long-term rather than the immediate structural changes of the ductuli efferentes

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associated with vasectomy, and the fate of the spermatozoa produced by the testes is discussed.

METHODS

Electron microscopy

Biopsies of the ductuli efferentes, and of caput, mid- and cauda epididymidis were taken from normal rhesus, *Macaca mulatta*, and stump-tailed, *Macaca speciosa*, monkeys and from rhesus monkeys that had been vasectomized 1 month, 3 months, and 3, 5 and 7 years previously. During surgery, both ends of the vas deferens in the region cranial to the neck of the scrotum were ligated and a piece of the vas was removed. The tissues were fixed for 2 hr in 0.75% glutaraldehyde, 4% formalin, 3.5% sucrose, and 0.05% CaCl_2 in s-collidine buffer at pH 7.4, washed in buffer, post-fixed in 1% osmium tetroxide, dehydrated in alcohol, and embedded in Araldite. Thick sections were stained with 1% toluidine blue in 1% borax. Thin sections were stained with uranyl acetate in alcohol and lead citrate.

Acid phosphatase

Tissue was prepared for both light and electron microscopy by being fixed in 4.6% glutaraldehyde in cacodylate buffer for 2 hr and washed in buffer overnight. Sections were cut at 40 μm on a freezing microtome, and incubated in Gomori's substrate. Sections for light microscopy were rinsed in acetate buffer, washed in acetic acid, ammonium sulphide and water, and mounted in glycerine jelly. For electron microscopy, sections were put in acetate buffer, then post-fixed in osmium tetroxide, dehydrated and embedded in Araldite.

Fluorescein-labelled antibody

For the fluorescein-labelled antibody study, samples of ductuli efferentes, epididymis and vas deferens from intact and vasectomized animals were placed in n-hexane cooled by acetone and dry ice. The biopsies were stored in liquid nitrogen. Sections were cut at 10 μm on a cryostat and, after drying, were fixed for 5 min in 95% ethanol, rinsed in phosphate-buffered saline, dried, and stained overnight while chilled. They were then rinsed in phosphate-buffered saline, dried and mounted with buffered glycerol. The rabbit anti-human immunoglobulins were purchased from Melpar, Springfield, Virginia, the goat anti-human C'3 was from the Cordis Laboratories, Miami, Florida. The sections were stained with anti-C'3 and anti-IgG conjugated with fluorescein isothiocyanate which had been adsorbed with kidney powder to avoid non-specific staining. Sections were viewed with a Zeiss Ultraphot fluorescence microscope with barrier 53 and exciter 12 filters.

Daily human sperm output

A rough estimate of the daily sperm output in man was needed to determine the amount of sperm resorption after vasectomy. The procedure of Freund (1968) was followed. Two men ejaculated at least twice daily for a week before collection to eliminate any spermatozoa stored in the epididymis. The next

five ejaculates on the next 5 consecutive days were collected and allowed to liquefy for 45 min at 37°C, and a sample was counted on a haemocytometer. The total counts of each day were averaged. The monkey was not a good model for this study because of the infeasibility of repeated frequent electroejaculations and because of the possibility of masturbation.

RESULTS

The ductuli efferentes of rhesus and stump-tailed macaques consist of twelve tubules about 1 cm long that connect the rete testis with the coiled caput epididymidis. Numerous blood vessels interweave within the surrounding connective tissue. The ductuli range in diameter from 275 μm to 350 μm . Each ductule is composed of a single layer of columnar epithelial cells and is encircled by a 500- to 800-Å basal lamina. In thin sections, spermatozoa are relatively infrequently encountered within the lumen. The ductule epithelial cells consist of ciliated and non-ciliated cells, at least 50% of them being ciliated (Pl. 1, Fig. 1). In all respects, the epithelial cells of the rhesus monkey and of the stump-tailed macaque are similar. The cytoplasm of the ciliated cells usually stains lightly in thick sections (Pl. 2, Fig. 2). The basal half of these cells is filled with the nucleus; the apical part contains many mitochondria and dense membrane-bounded granules (Pl. 3, Fig. 4) that resemble lysosomes but are not acid-phosphatase-positive.

The non-ciliated cells are columnar (25 μm high \times 1 μm wide), extending from the base of the ductule epithelium to the lumen (Pl. 1, Fig. 1). An elongated nucleus fills the basal part of the cell; many dense granules (0.06 to 0.11 μm in diameter) are located in the apical part. The non-ciliated cells have apical microvilli with much side-to-side anastomosis (stereocilia).

In intact (non-vasectomized) animals, macrophages are rare throughout the duct system.

The ductuli efferentes of animals vasectomized for 2 or more years have a two- to fourfold increase in diameter, undoubtedly because of being packed with spermatozoa. Ciliated cells remain the same size as in normal animals, although they are greatly reduced in number; in some regions, they disappear entirely. The apical dense granules so common in ciliated cells have vanished. The non-ciliated cells become shorter (15 μm) as the duct becomes stretched, and their long stereocilia become reduced to simple microvilli. In a vasectomized animal, the basal lamina, normally around 700 Å thick, undergoes a threefold increase (up to 3.2 μm thick) (Pl. 2, Fig. 3). The apical part of all the cells, whether non-ciliated or ciliated, of the ductuli become laden with dense homogeneous granules (Pl. 3, Fig. 5).

Within the ductuli efferentes of the monkeys vasectomized 3, 5 and 7 years previously, the spermatozoa become agglutinated as soon as they leave the testis (Pl. 3, Fig. 5 and Pl. 5, Fig. 7) and increase in number in the lumen. Numerous macrophages are present in the ductuli efferentes and can be seen engulfing and digesting spermatozoa (Pl. 4, Fig. 6). These macrophages are not found in the lumina of animals vasectomized only 1 or 3 months previously. In the cauda epididymidis of monkeys vasectomized 3, 5 and 7 years previously,

few spermatozoa are seen. Macrophages are abundant in this region of the duct, but the remnants of spermatozoa within the macrophages are no longer recognizable (Pl. 5, Fig. 8).

Because of the agglutinated appearance of the spermatozoa and because previous work (Alexander, 1972) has demonstrated high titres of spermagglutinin antibodies in animals with long-term vasectomies, a fluorescein-labelled antibody was used to pinpoint the site of antigen-antibody complexing. Both C'3 and IgG antibodies were conjugated with fluorescein and tested on tissue slices from an intact animal and from one vasectomized 3 years before. Some orange autofluorescent granules were seen in the apical cytoplasm of cells lining the duct in tissue from both the experimental and control animals; however, no green-yellow fluorescein label was seen in the non-vasectomized control animal. In the vasectomized animal, the basement membrane surrounding the sections of ductuli fluoresced green-yellow. In sections from the cauda epididymidis, the spermatozoa themselves exhibited some fluorescence which

EXPLANATION OF PLATES 1 TO 5

PLATE 1

FIG. 1. Electron micrograph of part of a cross-section of an efferent duct from an intact adult stump-tailed macaque, showing a ciliated cell and an adjacent non-ciliated cell that contains dense granules (SG). All cells rest upon a basal lamina (BL). $\times 10,000$.

PLATE 2

FIG. 2. Light micrograph showing part of a cross-section of an efferent duct from an adult rhesus monkey. Note the large proportion of ciliated cells. $\times 950$.

FIG. 3. Electron micrograph of the basal part of an efferent duct from a rhesus monkey vasectomized 7 years previously. The duct has taken on a pseudostratified appearance. The underlying basal lamina (BL) is considerably thickened (compare with Pl. 1, Fig. 1). $\times 7400$.

PLATE 3

FIG. 4. The apical area of a ciliated cell from the efferent ductules of a normal rhesus monkey. Basal bodies (BB) and a ciliary rootlet (CR) are visible in the upper right corner. The dense conglomerate granules (DCG) which appear to have a lipid component are a hallmark of ciliated cells in the normal animal, but these granules disappear after vasectomy. In the lower right corner is the apical edge of a secretory cell. Mitochondria (M), nucleus (N). $\times 18,000$.

FIG. 5. Apical area of cells from the efferent duct of a rhesus monkey vasectomized 5 years previously. All cells, whether non-ciliated or ciliated, contain dense amorphous granules (DG). The elaborations in the apical cytoplasm (Fig. 4) are much reduced and the stereocilia take the form of stubby microvilli. In the lumen, the spermatozoa (S) are agglutinated and abnormal. $\times 10,000$.

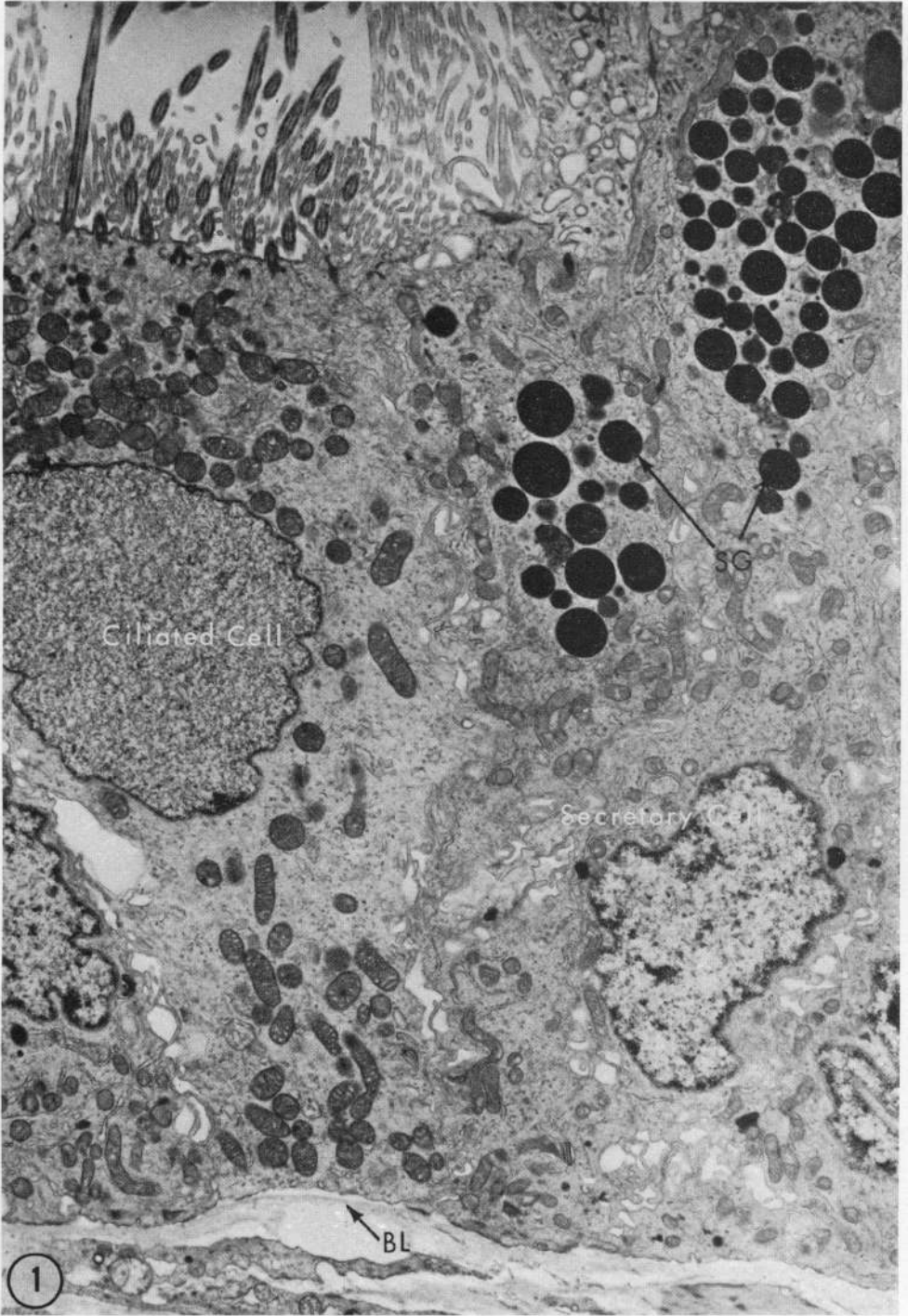
PLATE 4

FIG. 6. Numerous macrophages are present throughout the lumina of the ductuli efferentes and caput epididymidis of rhesus monkeys with a long-term vasectomy. This micrograph shows a macrophage containing many newly engulfed and partially digested spermatozoa. Nucleus (N). $\times 14,600$.

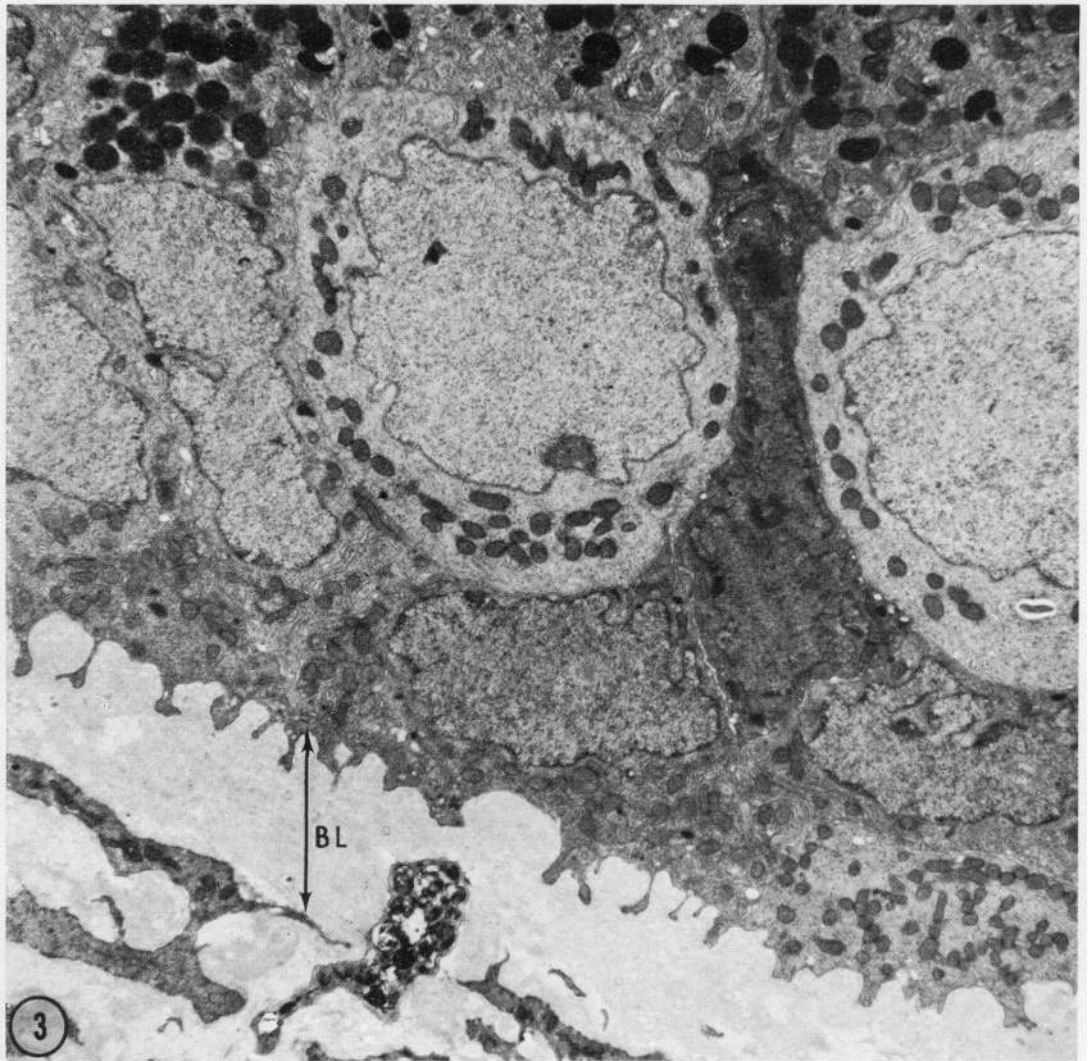
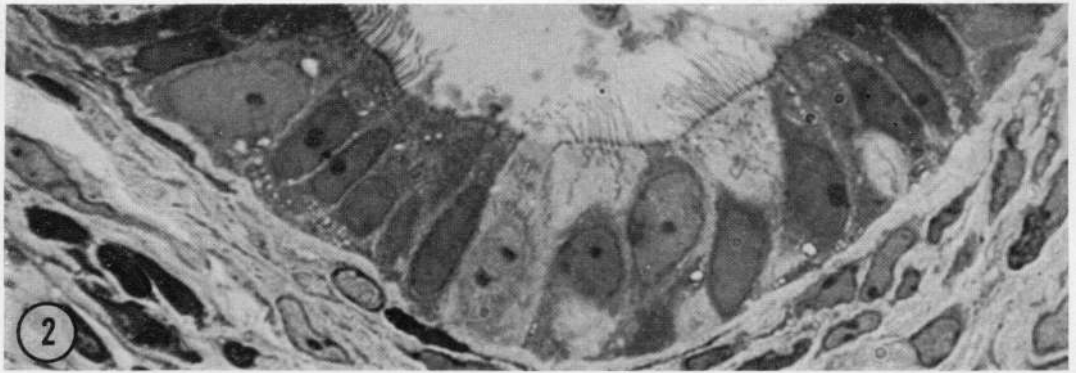
PLATE 5

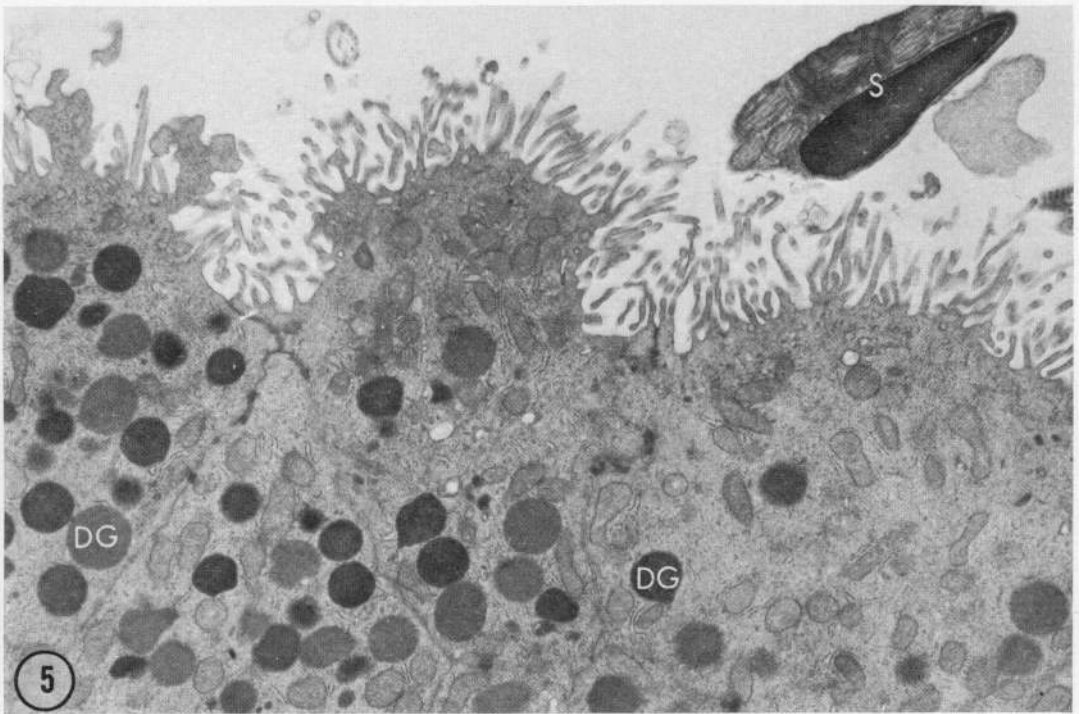
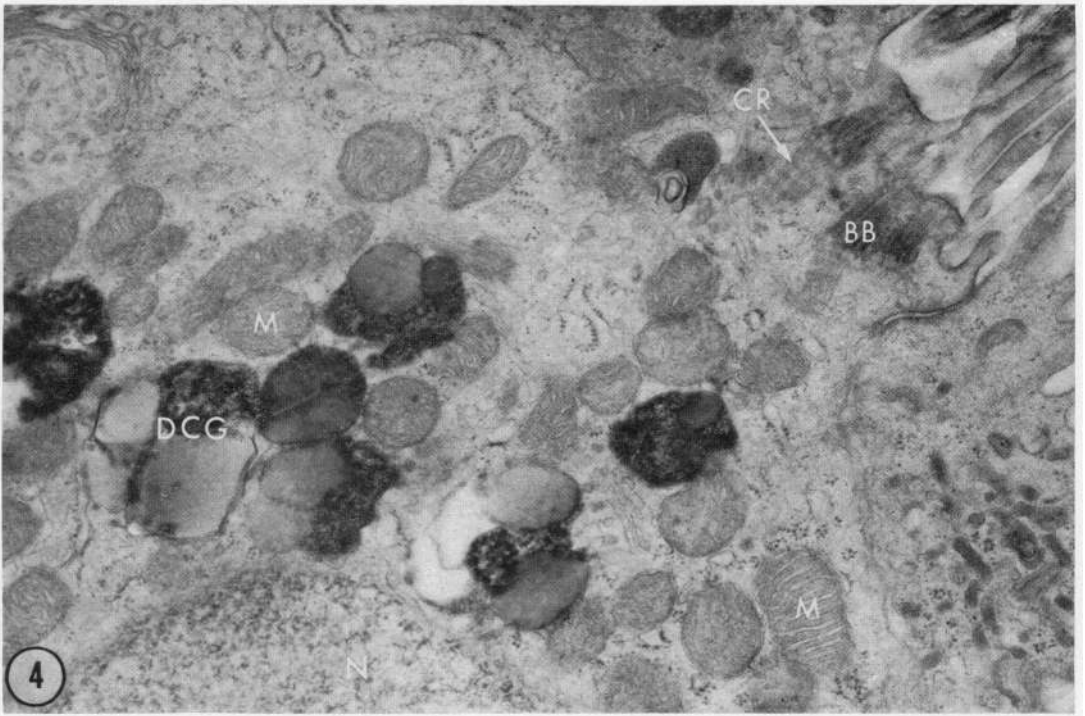
FIG. 7. Electron micrograph of the lumen of an efferent duct of a rhesus monkey with a long-term vasectomy. The spermatozoa are all clumped or agglutinated. This phenomenon may be caused by spermagglutinin titres in the serum that rise after vasectomy. $\times 18,500$.

FIG. 8. In the cauda epididymidis of rhesus monkeys with a long-term vasectomy, no recognizable spermatozoa are present, even in macrophages. This macrophage from the caudal region contains only residues. $\times 24,000$.

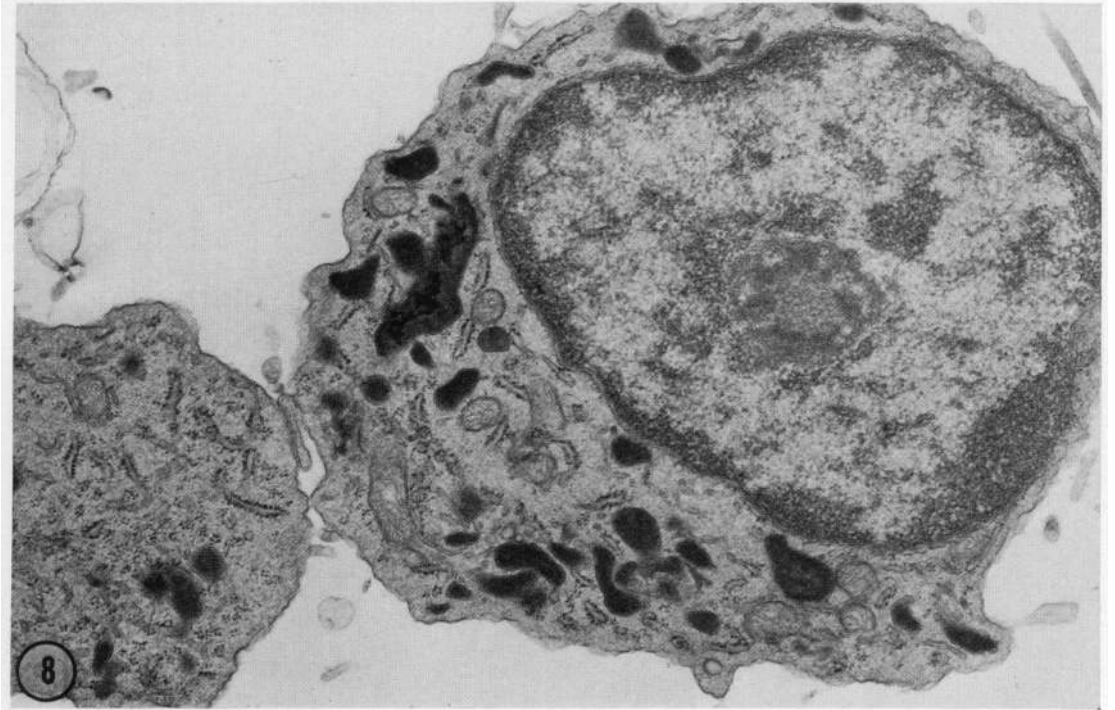
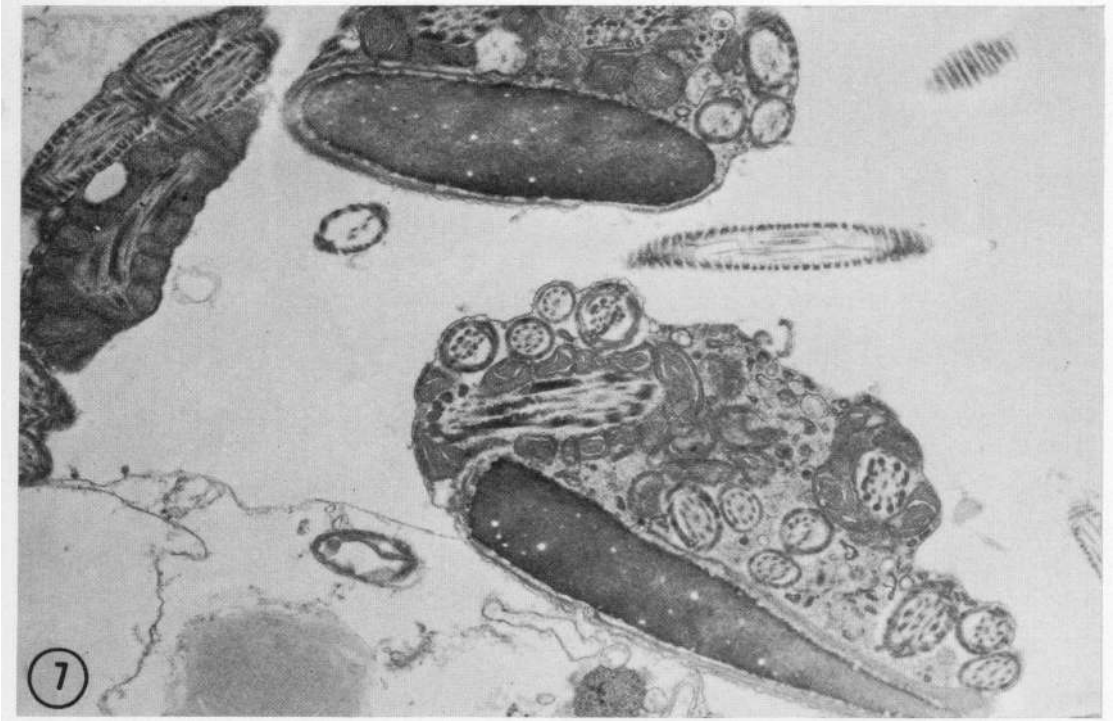


(Facing p. 402)









was lacking in the control animal. The fluorescence study with antihuman IgG showed that significant levels of IgG were present in the area; the non-vasectomized animal also showed some IgG present but much less. However, only the vasectomized animal showed staining with antihuman C'3. This strongly indicates (1) the formation of immune complexes, (2) a local Arthus reaction, and (3) the autoimmune nature of the changes observed.

Since the spermatozoa from a vasectomized animal must be entirely absorbed, an estimate of sperm output had to be determined. In man, the daily sperm output varies considerably but, generally speaking, about 50,000 spermatozoa are produced every minute. Comparisons of single ejaculates from men and monkeys indicated that the number of spermatozoa per ejaculate for monkeys is about four times that of men (average number of spermatozoa per monkey ejaculate is 5.3425×10^8). This estimate does not take into account those spermatozoa that are resorbed *en route*.

DISCUSSION

Spermatozoa are neither motile (Gaddum, 1968) nor fertile (Igboeli & Foote, 1969) when they leave the testis but become so only after passage through the ductuli efferentes and the epididymis. The ductuli are more than a passage-way for the transportation of spermatozoa; they probably push the spermatozoa (1) by muscular contraction, (2) by fluid production and flow from within the seminiferous tubules (Toothill & Young, 1931), or (3) by means of the many ciliated cells that are thought to have a vortical flushing action (Zawisch-Ossenitz, 1933; Kwiet, 1966). The millions of spermatozoa produced daily pass rapidly through this region; hence the low number seen in a thin section of lumen.

The ductuli efferentes and caput epididymidis are active sites of absorption (Young, 1933); in the ram, 99% of testicular fluids are resorbed in this region (Crabo, 1965). In other species, colloidal particles and peroxidase have been shown to be selectively incorporated into the non-ciliated cells of the ductuli efferentes by micropinocytosis (Sedar, 1966; Montorzi & Burgos, 1967) as is trypan blue (Shaver, 1954). Moreover, since spermatozoa are always moved in a fluid medium, the ductuli efferentes and caput epididymidis must add some secretion products to the seminal plasma.

With vasectomy, these same processes continue but the spermatozoa produced by the seminiferous tubules now lack an exit passage. Although the ligated duct increases two to four times in diameter, spermatogenesis itself is not affected by vasectomy (Moore & Quick, 1924; Amann, 1962; Glover, 1969) except for a possible slight decrease in sperm production (Swanson & Hafs, 1969; Igboeli & Rakha, 1970). All studies on testicular tissue indicate that little or no change is associated with long-term vasectomy (Wheelon, 1921; Smith, 1962; Kubota, 1969). After vasectomy, since thousands of spermatozoa are still being produced every minute, they must be reabsorbed somewhere between the testis and the vas deferens. The ductuli efferentes and the epididymis are the most probable site of resorption.

In intact animals, spermatozoa are absorbed as part of a normal process.

Orgebin-Crist (1968) estimated that at least 50% of all spermatozoa produced by the rabbit were resorbed in the epididymis. Similarly, Amann (1970) stated that, in the rabbit, daily sperm output is only 60% of daily sperm production. Resorption occurs primarily in the caudal portion of the epididymis. The fact that broken spermatozoa are most common in the body of the epididymis (Glover, 1961; Amann & Almquist, 1962) and relatively uncommon in the ejaculate, supports this assumption. The rate of sperm resorption is thought to depend on the number of spermatozoa in the cauda epididymidis (Amann & Almquist, 1962); therefore resorption would occur more rapidly in sexually rested animals. The actual mode of resorption is unknown, since there appear to be no partially degenerated spermatozoa in the epididymis, and epididymal cells do not appear to be actively involved in phagocytosis (Fulka, Kopečný & Koefoed-Johnsen, 1971). Normally, there are not many phagocytic cells in the lumen of the epididymis.

After vasectomy, however, the number of macrophages in the epididymis increases markedly, particularly in the ductuli efferentes and caput epididymidis. These macrophages actively engulf and degrade spermatozoa and, thus, are instrumental in the rapid removal of the trapped spermatozoa. This process is essentially completed in the caput epididymidis of vasectomized animals; few whole spermatozoa are contained in the cauda epididymidis where macrophages have digested rather than newly ingested sperm residues. Phagocytosis of spermatozoa is not limited to the monkey but occurs in man after ligation of the vas deferens (Phadke, 1964). Thus, the main method of sperm removal after ligation seems to be phagocytosis by macrophages.

After vasectomy, the level of circulating spermagglutinins rises (Alexander, 1972) and increases with time. Sera of animals vasectomized 7 years ago have an antibody titre which is several times higher than that of animals vasectomized more recently. The following sequence probably occurs in vasectomized animals. Macrophages invade the epididymis, ingest the spermatozoa, and then transfer either an antigen or information about the antigen to those cells destined to make antibodies. These antibodies may then aid the macrophages to recognize the cells to be ingested. As the antibody titre increases, the spermatozoa probably undergo a greater degree of agglutination in the ductuli efferentes and caput epididymidis. This hypothesis is supported by the fact that biopsies from monkeys with long-term vasectomies showed that all the spermatozoa within the lumina were agglutinated; biopsies of the same region from animals recently vasectomized (1 and 3 months) showed no agglutination. Antigen-antibody complexing revealed by fluorescent-labelled antibody occurs in the basement membrane, indicating that immunoglobulin complexes are precipitating in this area. The changes following vasectomy may be initiated by a local inflammatory reaction which causes an infiltration of polymorphonuclear granulocytes. The presence of C'3 in the basement membrane indicates that this antigen-antibody precipitate binds complement and therefore the immune reaction involved is an Arthus reaction. Electron microscopy shows that, after vasectomy, the basal lamina gradually becomes thickened, an indication of an autoimmune antibody reaction to spermatozoa resulting from vasectomy. Thickening of the basal lamina may be directly related to the amount of anti-

body present. This is supported by the fact that the spermagglutinin titre in the serum of monkeys rises with the time elapsing after a vasectomy (Alexander, 1972) and, likewise, basement membrane thickening is not apparent until several years after vasectomy. These changes in the epididymis are certainly of an autoimmune nature and may become more progressive with time, possibly involving other areas of the body.

Another area of consideration brought up by this study concerns the possibility of successful surgical re-anastomosis after long-term vasectomy in man. High spermagglutinin titres have been demonstrated more frequently in sterile men (Fjällbrant, 1968), causing reduced motility and agglutination of spermatozoa (Fjällbrant & Obrant, 1968). These same agglutinins are present in vasectomized animals (Alexander, 1972). Cases of pregnancy after repair of vasectomies in man are lower than might be expected (Dubin & Amelar, 1971). The autoimmune changes both in the epididymis and in the serum after long-term vasectomy may function in reducing the chance for successful pregnancy after surgical re-anastomosis of the vas deferens.

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